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(54) Title: T CELL RETARGETING HETERO-DIMERIC IMMUNOGLOBULINS

(57) Abstract: The present invention describes novel hetero-dimeric immunoglobulins or fragments thereof which bind to CD3 and a disease associated antigen. These hetero-dimeric immunoglobulins have been engineered to promote hetero-dimer formation during expression and can be purified to a high degree using a Protein A differential purification technique.

T cell retargeting hetero-dimeric immunoglobulins

Field of the Invention

The present invention relates to hetero-dimeric immunoglobulins that target both a component of the human CD3 antigen and a disease associated antigen and methods of making the same.

Background of the invention

T cell redirected killing is a desirable mode of action in many therapeutic areas. Various bispecific antibody formats have been shown to mediate T cell redirection both in pre-clinical and clinical investigations (May C *et al.*, (2012) Biochem Pharmacol, 84(9): 1105-12; Frankel SR & Baeuerle PA, (2013) Curr Opin Chem Biol, 17(3): 385-92). All T cell retargeting bispecific antibodies or fragments thereof are engineered to have at least two antigen binding sites wherein one site binds a surface antigen on a target cell and the other site binds a T cell surface antigen. Amongst T cell surface antigens, the human CD3 epsilon subunit from the TCR protein complex has been the most targeted to redirect T cell killing.

Many bispecific antibody formats have been used to redirect T cell killing, these mainly include tandem of scFv fragments and diabody based formats with only few examples of Fc-based bispecific antibody formats reported (Moore PA *et al.*, (2011) Blood, 117(17): 4542-51; May C *et al.*, (2012) *supra*; Frankel SR & Baeuerle PA, (2013) *supra*) . Bispecific formats that will encompass a human Fc region will have longer circulation half-lives which may result in enhanced efficacy and/or less frequent dosing regimens. Among possible Fc-based bispecific formats, one preferred format to redirect T cell killing is the so-called heavy chain hetero-dimer format. This format is of particular interest as it does not allows aggregation of multiple copies of human CD3 molecules at the T cell surface thereby preventing any T cell inactivation (Klein C *et al.*, (2012) MAbs, 4(6): 653-63).

The first described method to engineer heavy chain hetero-dimers is a method known as the "knob-into-hole" method (PCT Publication No: WO199627011; Merchant AM *et al.*, (1998) Nat Biotechnol, 16(7): 677-81). Recently a chemical method known as the FAB-arm exchange method wherein two antibodies are combined into one bispecific antibody via reduction and *in vitro* reshuffling of half-immunoglobulins has been reported (PCT

Publication Nos: WO2008119353 (Schuurman J *et al.*) and WO2013060867 (Gramer M *et al.*); Labrijn AF *et al.*, (2013) Proc Natl Acad Sci USA, 110(13): 5145-50).

Both methods and derivatives thereof are currently inadequate to produce Fc-based bispecific antibody formats in mammalian cell hosts. When expressing "knob-into-hole" heavy chain hetero-dimers in mammalian cell hosts, bispecific antibody recovery is impaired by the presence of homo-dimers (Jackman J *et al.*, (2010) J Biol Chem, 285(27): 20850-9; Klein C *et al.*, *supra*). The FAB-arm exchange method and derivatives thereof suffers from the same drawback with the added problem of having first to produce the two "monospecific" antibodies separately.

When developing bispecific antibodies that redirect T cell killing via the engagement of a CD3 subunit, it is essential that no homo-dimers specific for the CD3 subunit are present in the final drug product. In the case of targeting the CD3 epsilon subunit, traces of anti-human CD3 epsilon antibody species (monospecific and bivalent for the human CD3 epsilon antigen) may trigger transient T cell activation and cytokine release before leading to T cell apoptosis thereby interfering with the goal of a controlled and specific T cell activation. Production of stable and safe Fc-based bispecific antibodies that efficiently redirect T cell killing remains a challenge to the pharmaceutical industry with respect to purity and yields.
Accordingly there remains a need for a technology to efficiently produce anti-human CD3 based heavy chain hetero-dimers free of anti-human CD3 homo-dimers wherein the secreted bispecific antibody product is readily isolated from the cell culture supernatant from a recombinant mammalian host cell line.

Techniques to purify heavy chain hetero-dimers over homo-dimers based on a differential affinity for a reagent have been described. The first example of known differential affinity purification technique involved the use of two different heavy chains from two different animal species, wherein one of which does not bind the affinity reagent Protein A (Lindhofer H *et al.*, (1995) J Immunol, 155(1): 219-225). The same authors also described the use of two different heavy chains originating from two different human immunoglobulin isotypes (IGHG1 and IGHG3), one of which does not bind the affinity reagent Protein A (IGHG3; see US6,551,592 Lindhofer H *et al.*). More recently, a variation of this technique was reported by Davis S *et al.* (PCT Publication No: WO2010151792) and made use of the two amino acid

substitutions H435R and Y436F described by Jendeberg (1997) (Jendeberg L. *et al.* (1997) J Immunol Methods, 201(1): 25-34) to abrogate the affinity for the reagent Protein A in one of the hetero-dimer heavy chains.

The preferred known differential Protein A affinity purification technique of the present invention corresponds to a technique wherein all three species i.e. the two homo-dimeric species and the hetero-dimer of interest differ in their total number of Protein A binding sites by at least one site and wherein one of the two homo-dimeric species has no Protein A binding site and therefore does not bind Protein A (as shown in FIG.1).

Drug stability is an important aspect of successful pharmaceutical development and VH3 based immunoglobulins or fragments thereof are of major importance to the biological drug industry. Therapeutic antibodies based on the VH3 subclass have been extensively developed as these frameworks bind Protein A and facilitate the testing of antibody fragments before their formatting into immunoglobulins; for example, many synthetic antibody phage display libraries used for antibody discovery are based on the VH3 subclass. In addition VH3 based antibodies are often selected for their good expression and stability over other known heavy chain variable domain subclasses.

Although a VH3 domain has only one Protein A binding site with a weaker affinity when compared to a Fc region which has two sites with a stronger affinity (Roben PW *et al.*, (1995) J Immunol, 154(12): 6437-45), there is enough affinity to interfere with the known differential Protein A affinity purification techniques. When dealing with the purification of hetero-dimers of heavy chains wherein the heavy chain engineered in its Fc region to have no binding for Protein A encompasses one VH3 based antigen binding site, then Protein A binding is restored via the VH3 domain and the preferred technology described in FIG. 1 and above is no longer useful (FIG. 2A). In this instance, abrogating Protein A binding in the VH3 based antigen binding site provides a straightforward solution and allows to keep the initial architecture of the desired hetero-dimer (FIG. 2B). Alternatively, the heavy chain hetero-dimer can be re-engineered to have the VH3 based antigen binding site located on the heavy chain that binds Protein A in its Fc region (FIG. 2C; note that a VH3 domain has a weaker affinity for Protein A compared to a Fc monomer hence the hetero-dimer of interest still elutes at a separate pH value from the other homo-dimeric species, typically at pH 4, while the

homo-dimeric species that binds Protein A now encompasses two additional Protein A binding sites and elutes at a pH value ≤ 3).

More importantly, when dealing with the purification of hetero-dimers of heavy chains wherein both heavy chains encompass a VH3 based antigen binding site, then the relocation strategy described above may only be partially helpful (FIG. 2D and FIG. 15B). Protein A based differential purification is only enabled when Protein A binding in at least one (FIG. 2E) or both (FIG. 2F) VH3 based antigen binding sites is abrogated .

Accordingly, there remains a need to abrogate Protein A binding within VH3 domains when undertaking the production of hetero-dimers of heavy chains encompassing this variable domain subclass.

Summary of the Invention

The present invention provides new anti-human CD3 bispecific antibodies comprising a second binding arm which can recognise and bind to a disease associated antigen.

In the context of the present invention a disease associated antigen means any antigen or epitope associated with a pathological state such as an oncogenic marker or a marker of some other metabolic or immunological dysfunction. In addition a disease marker my also relate to an infectious disease such as a pathogenic virus or bacteria.

In accordance with the present invention the target binding portion of the anti-human CD3 binding arm comprises a single chain variable fragment (scFv) of SP34. The scFv in particular has a better expression profile in comparison to a scFv-Fc comprising the heavy and light variable regions encoded by SEQ ID NOs: 60 and 61, whilst maintaining its CD3 binding properties.

In particular when expressed as a scFv-Fc it has at least a twofold improvement in expression in comparison to a SP34 chimera formatted as a scFv-Fc comprising the heavy and light variable regions encoded by SEQ ID NOs: 60 and 61. Preferably the improved SP34 scFv

has at least a sixfold improvement in expression in comparison to a SP34 chimera formatted as an scFv comprising the heavy and light variable regions encoded by SEQ ID NOs: 60 and 61 and most preferably a twelvefold improvement in expression in comparison to a SP34 chimera formatted as an scFv comprising the heavy and light variable regions encoded by SEQ ID NOs: 60 and 61.

In accordance with another aspect of the present invention when expressed as a scFv in a bispecific antibody comprising a FAB arm in the BEAT format, it has at least a twofold improvement in expression in comparison to a SP34 chimera formatted as an scFv comprising the heavy and light variable regions encoded by SEQ ID NOs: 60 and 61. Preferably the improved SP34 scFv as a component of a BEAT bispecific antibody has at least a fivefold improvement in expression in comparison to a SP34 chimera formatted as an scFv, comprising the heavy and light variable regions encoded by SEQ ID NOs: 60 and 61, as a component of a BEAT bispecific antibody.

In accordance with the present invention the two binding arms of the anti-human CD3 bispecific antibody each comprise an immunoglobulin constant region and wherein the first arm or polypeptide binds to protein A and the second arm or polypeptide does not bind to protein A.

According to the present invention the binding of the first polypeptide to protein A and the lack of binding of the second polypeptide to protein A, is not intended to mean that the second polypeptide may not have some residual binding to protein A and it is instead intended that the second polypeptide binds less well to protein A in comparison to the first arm.

According to the present invention the first and second polypeptides of the hetero-dimeric immunoglobulin or fragment thereof, comprise an engineered immunoglobulin constant region with a modified CH3 region having a protein-protein interface that favours hetero-dimer formation over homo-dimer formation. In a preferred embodiment, the present invention provides a hetero-dimeric immunoglobulin or fragment thereof wherein the first and second polypeptides comprise an engineered immunoglobulin constant region with a modified CH3 domain having a protein-protein interface, wherein the protein-protein interface of the first polypeptide comprises an amino acid substitution at a position selected from the group

consisting of: 3, 5, 7, 20, 22, 26, 27, 79, 81, 84, 84.2, 85.1, 86, 88 and 90 (IMGT® numbering), and wherein the protein-protein interface of the second polypeptide comprises an amino acid substitution at a position selected from the group consisting of: 3, 5, 7, 20, 22, 26, 27, 79, 81, 84, 84.2, 84.4, 85.1, 86, 88 and 90 (IMGT® numbering).

Preferably wherein the protein-protein interface of the second polypeptide comprises an amino acid substitution at position 84.4 and at least one further substitution at a position selected from the group consisting of: 3, 5, 7, 20, 22, 26, 27, 79, 81, 84, 84.2, 85.1, 86, 88 and 90 (IMGT® numbering).

In a further embodiment, the present invention provides a hetero-dimeric immunoglobulin or fragment thereof, wherein the first and second polypeptides comprise an engineered immunoglobulin constant region with a modified CH3 domain having a protein-protein interface, wherein the protein-protein interface of the first polypeptide comprises an amino acid substitution at position 88 and at a position selected from the group consisting of: 3, 5, 7, 20, 22, 26, 27, 79, 81, 84, 84.2, 85.1, 86 and 90 (IMGT® numbering), and wherein the protein-protein interface of the second polypeptide comprises an amino acid substitution at position 85.1 and/or 86 and at a position selected from the group consisting of 3, 5, 7, 20, 22, 26, 27, 79, 81, 84, 84.2, 84.4, 88 and 90 (IMGT® numbering).

According to a further aspect of the present invention the epitope binding region of the first polypeptide binds the CD3 protein complex and the epitope binding region of the second polypeptide binds a disease associated antigen or wherein the epitope binding region of the first polypeptide binds a disease associated antigen and the epitope binding region of the second polypeptide binds the CD3 protein complex; and wherein the epitope binding region that binds the CD3 protein complex comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 200, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 201 and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 202, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 203, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 204 and a light chain CDR3 comprising the amino acid sequences of: SEQ ID NO: 205; or
wherein the epitope binding region that binds the CD3 protein complex comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 352, a heavy chain CDR2

comprising the amino acid sequence of SEQ ID NO: 353 and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 354, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 355, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 356 and a light chain CDR3 comprising the amino acid sequences of SEQ ID NO: 357.

Use of these new anti-human CD3 bispecific antibodies is not limited to but includes treatments of various human cancers and autoimmune and inflammatory diseases. The specific destruction of cancer cells over healthy cells and tissues represents a primary objective in oncology. Therapeutics that could safely redirect T cell killing against tumour associated cell surface antigens may offer improved clinical efficacy. Known areas of clinical unmet needs in oncology include but are not limited to breast cancer, metastatic breast cancer, ovarian cancer, pancreatic cancer, lung cancer, lymphomas and multiple myeloma. Elimination of disease-causing T cells could be more beneficial than inhibiting T cell differentiation in treating autoimmune and inflammatory diseases such as psoriasis, multiple sclerosis and diabetes.

A preferred set of disease associated antigens come from the gene products CCR3, CCR6, CRTH2, PDL1, BLUT1, PirB, CD33, TROP2, CD105, GD2, GD3, CEA, VEGFR1, VEGFR2, NCAM, CD133, CD123, ADAM17, MCSP, PSCA, FOLR1, CD19, CD20, CD38, EpCAM, HER2, EGFR, PSMA, IgE, Integrin a4b1, CCR5, LewisY, FAP, MUC-1, Wue-1, MSP, EGFRvIII, P glycoprotein, AFP, ALK, BAGE proteins, CD30, CD40, CTLA4, ErbB3, ErbB4, Mesothelin, OX40, CA125, CAIX , CD66e, cMet, EphA2, HGF/SF , MUC1 , Phosphatidylserine , TAG-72 , TPBG, β-catenin, brc-abl, BRCA1, BORIS, CA9, caspase-8, CDK4, Cyclin-B1, CYP1B1, ETV6-AML, Fra-1, FOLR1, GAGE-1, GAGE-2, GloboH, glypican-3, GM3, gp100, HLA/B-raf, HLA/k-ras, HLA/MAGE-A3, hTERT, LMP2, MAGE1, MAGE2, MAGE3, MAGE4, MAGE6, MAGE12, MART-1, ML-IAP, Muc2, Muc3, Muc4, Muc5, Muc16, MUM1, NA17, NY-BR1, NY-BR62, NY-BR-85, NY-ESO1, p15, p53, PAP, PAX3 PAX5, PCTA-1, PLAC1, PRLR, PRAME, RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, Steap-1, Steap-2, survivin, TAG-72, TGF-β, TMPRSS2, Tn, TRP-1, TRP-2, tyrosinase, uroplakin-3.

A hetero-dimeric immunoglobulin or fragment thereof according to the invention, wherein the epitope binding region that binds a disease associated antigen comprises heavy chain CDR1, CDR2 and CDR3 amino acid sequences and light chain CDR1, CDR2 and CDR3 amino acid sequences, respectively, selected from the group consisting of:

i) SEQ ID NOs: 206 – 211;
ii) SEQ ID NOs: 212 – 217;
iii) SEQ ID NOs: 218 – 223;
iv) SEQ ID NOs: 224 – 229;
v) SEQ ID NOs: 230 – 235;
vi) SEQ ID NOs: 236 – 241;
vii) SEQ ID NOs: 242 – 247;
viii) SEQ ID NOs: 248 – 253;
ix) SEQ ID NOs: 254 – 259;
x) SEQ ID NOs: 260 – 265;
xi) SEQ ID NOs: 266 – 271; and
xii) SEQ ID NOs: 272 – 277;

In accordance with a further aspect of the present invention the constant region of the second polypeptide of the hetero-dimeric immunoglobulin or fragment thereof, comprises an IgG3 CH3 region.

In accordance with a further aspect of the present invention the constant region of the second polypeptide of the hetero-dimeric immunoglobulin or fragment thereof, comprises a CH3 region other than that from IgG, and the non-IgG3 CH3 region comprises at least one substitution so as to decrease/abolish protein A binding.

According to a further aspect of the present invention the epitope binding region of second polypeptide of the hetero-dimeric immunoglobulin or fragment thereof comprises a VH3 region comprising at least one modification that reduces protein A binding.

The inventors have shown that VH3 based antigen binding sites can be readily produced and purified with a high degree of purity in a single Protein A chromatography step. These

antibodies may exhibit higher efficacy over current therapies in addition to their ease of production.

The present invention also provides a method to produce anti-human CD3 bispecific heavy chain hetero-dimers having at least one VH3 based antigen binding site from a recombinant mammalian host cell line wherein the bispecific antibody product is readily isolated after a single Protein A chromatography step with a high degree of purity.

In particular the modified VH3 region comprises an amino acid substitution selected from the group consisting of: 57, 65, 81, 82a and combination 19/57/59 (Kabat numbering) and even more preferably wherein the modified VH3 region comprises an amino acid substitution selected from the group consisting of: 57A, 57E, 65S, 81E, 82aS and combination 19G/57A/59A (Kabat numbering).

According to a further aspect of the present invention the hetero-dimeric immunoglobulin or fragment thereof, may comprise further substitutions wherein the heavy chain variable framework region comprises an amino acid substitution selected from the group consisting of: I34M, V48I, A49G, R58N/Y, I69L, A71T and T73K (Kabat numbering) and the light chain variable framework region comprises an amino acid substitution selected from the group consisting of: M4L, V33M, A34N, L46R, L47W, T51A, R66G, F71Y and P96F (Kabat numbering); or wherein the heavy chain variable framework region comprises the amino acid substitutions I34M, A49G and A71T (Kabat numbering) and the light chain variable framework region comprises the amino acid substitutions M4L, L46R, L47W and F71Y (Kabat numbering).

In a further embodiment, the epitope binding region that binds to the CD3 protein complex comprises a heavy chain variable framework region that is the product of or derived from the human VH3 subclass. Preferably the heavy chain variable framework region is the product of or derived from human IGHV3-23. More preferably, the heavy chain variable framework region is the product of or derived from human IGHV3-23*04 (SEQ ID NO: 22). The heavy chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the heavy chain variable region of the corresponding murine antibody comprising the amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 60.

In a preferred embodiment, the epitope binding region of the first polypeptide that binds to the CD3 protein complex comprises a light chain variable framework region that is the product of or derived from the human VK1 subclass or the human VK3 subclass. Preferably the light chain variable framework region is the product of or derived from human VK1-39 or VK3-20. More preferably the light chain variable framework region is the product of or derived from human IGKV1-39*01 (SEQ ID NO: 23) or IGKV3-20*01 (SEQ ID NO: 24). The light chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the light chain variable region of the corresponding murine antibody comprising the amino acid sequence of SEQ ID NO: 19 or SEQ ID NO: 61.

In a preferred embodiment, the epitope binding region that binds to the CD3 protein complex comprises a humanized heavy chain variable domain having the back mutations selected from the group consisting of: I34M, V48I, A49G, R58N/Y, I69L, A71T and T73K (Kabat numbering) and a humanized light chain variable domain having the back mutations selected from the group consisting of: M4L, V33M, A34N, L46R, L47W, R66G, F71Y and P96F (Kabat numbering). More preferably, the epitope binding region that binds to the CD3 protein complex comprises a humanized heavy chain variable domain having the back mutations I34M, A49G and A71T (Kabat numbering) and a humanized light chain variable domain having the back mutations M4L, L46R, L47W and F71Y (Kabat numbering).

According to a further aspect of the present invention the epitope binding region that binds the CD3 protein complex of the hetero-dimeric immunoglobulin or fragment thereof,
wherein the epitope binding region that binds the CD3 protein complex comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 101, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 105; or
wherein the epitope binding region that binds the CD3 protein complex comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 103, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 106; or
wherein the epitope binding region that binds the CD3 protein complex comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 104, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 106; or

wherein the epitope binding region that binds the CD3 protein complex comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 104, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 401; or wherein the epitope binding region that binds the CD3 protein complex comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 104, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 402.

The CD3 protein complex comprises a number of subunits, for example, delta, epsilon and gamma. In a preferred embodiment, the epitope binding region that binds to the CD3 protein complex binds to the CD3 epsilon subunit.

An epitope binding region as described herein includes the combination of one or more heavy chain variable domains and one or more complementary light chain variable domains which together form a binding site which permits the specific binding of the hetero-dimeric immunoglobulin or fragment thereof to one or more epitopes. In an embodiment of the present invention, the epitope binding region of the first poly peptide comprises a FAB and the epitope binding region of the second polypeptide comprises a scFv. Alternatively, the epitope binding region of the first poly peptide comprises a scFv and the epitope binding region of the second polypeptide comprises a FAB.

In one embodiment, the epitope binding region that binds a disease associated antigen binds to HER2. The epitope binding region comprises a heavy chain variable framework region that is the product of or derived from the human VH3 subclass, preferably human VH3-23, more preferably human IGHV3-23*04 (SEQ ID NO: 22), and a light chain variable framework region that is the product of or derived from the human VK1 subclass, preferably human VK1-39, more preferably human IGKV1-39*01 (SEQ ID NO: 23).

In a preferred embodiment, the epitope binding region that binds the disease associated antigen HER2 comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 20 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 21. In a further preferred embodiment, the epitope binding region that binds HER2 may comprise a heavy chain variable domain and a light chain variable domain joined by a G_4S linker forming a scFv fragment comprising the amino acid sequence of SEQ ID NO:

107. Preferably, the variable domain of the scFv fragment comprises a modification to abrogate binding to Protein A, wherein the amino acid substitution is 65S (Kabat numbering) and wherein the scFv fragment comprises the amino acid sequence of SEQ ID NO: 109 or wherein the amino acid substitution is 82aS (Kabat numbering) and wherein the scFv fragment comprises the amino acid sequence of SEQ ID NO: 111.

In particular wherein said Herceptin binding arm comprises a heavy chain variable region encoded by SEQ ID NO: 20 and a light chain variable region encoded by SEQ ID NO: 21.

In another embodiment, the epitope binding region that binds a disease associated antigen binds to CD38. The epitope binding region comprises a heavy chain variable framework region that is the product of or derived from the human VH3 subclass, preferably human VH3-23, more preferably human IGHV3-23*04 (SEQ ID NO: 22). The heavy chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the heavy chain variable region of the corresponding murine antibody comprising the amino acid sequence of SEQ ID NO: 112 or 114 or 122. The epitope binding region further comprises a light chain variable framework region that is the product of or derived from the human VK1 subclass, preferably human VK1-39, more preferably human IGKV1-39*01 (SEQ ID NO: 23). The light chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the light chain variable region of the corresponding murine antibody comprising the amino acid sequence of SEQ ID NO: 113 or 115 or 123.

In particular the CD38 binding polypeptide comprises variable heavy chain domain and variable light chain domain pair encoded by SEQ ID NOs: 116/117, 129/130, 133/134 and 135/136.

In one embodiment, the epitope binding region that binds a disease associated antigen binds to OX40. The epitope binding region comprises a heavy chain variable framework region that is the product of or derived from the human VH3 subclass, preferably human VH3-23, more preferably human IGHV3-23*04 (SEQ ID NO: 22). The heavy chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the heavy chain variable region of the corresponding murine antibody comprising

the amino acid sequence of SEQ ID NO: 139. The epitope binding region further comprises a light chain variable framework region that is the product of or derived from the human VK1 subclass, preferably human VK1-39, more preferably human IGKV1-39*01 (SEQ ID NO: 23). The light chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the light chain variable region of the corresponding murine antibody comprising the amino acid sequence of SEQ ID NO: 140.

Most preferably, the humanized heavy chain variable domain comprises a modification to abrogate binding to Protein A comprising the substitution G65S or the substitution N82aS (Kabat numbering).

In particular the OX40 binding polypeptide comprises variable heavy chain domain and variable light chain domain pair encoded by SEQ ID NOs: 141/142, 278/280 and 279/281.

In one embodiment, the epitope binding region that binds a disease associated antigen binds to CD19. The epitope binding region comprises a heavy chain variable framework region that is the product of or derived from the human VH3 subclass, preferably human VH3-23, more preferably human IGHV3-23*04 (SEQ ID NO: 22) and most preferably comprises the amino acid sequence of SEQ ID NO: 296. The epitope binding region further comprises a light chain variable framework region that is the product of or derived from the human VK1 subclass, preferably human VK1-39, more preferably human IGKV1-39*01 (SEQ ID NO: 23) and most preferably comprises the amino acid sequence of SEQ ID NO: 297. In a preferred embodiment, the heavy chain variable domain comprises a modification to abrogate binding to Protein A comprising the substitution G65S or the substitution N82aS (Kabat numbering).

In particular the CD19 binding polypeptide comprises variable heavy chain domain and variable light chain domain pair encoded by SEQ ID NOs: 296/297.

In one embodiment, the epitope binding region that binds a disease associated antigen binds to CD20. The epitope binding region comprises a heavy chain variable framework region that is the product of or derived from the human VH3 subclass, preferably human VH3-23, more preferably human IGHV3-23*04 (SEQ ID NO: 22). The heavy chain variable framework region comprises at least one amino acid modification from the corresponding framework

region of the heavy chain variable region of the corresponding murine antibody comprising the amino acid sequence of SEQ ID NO: 143. The epitope binding region further comprises a light chain variable framework region that is the product of or derived from the human VK1 subclass, preferably human VK1-39, more preferably human IGKV1-39*01 (SEQ ID NO: 23). The light chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the light chain variable region of the corresponding murine antibody comprising the amino acid sequence of SEQ ID NO: 144.

Most preferably, the humanized heavy chain variable domain comprises a modification to abrogate binding to Protein A comprising the substitution G65S or the substitution N82aS (Kabat numbering).

In particular the CD20 binding polypeptide comprises variable heavy chain domain and variable light chain domain pair encoded by SEQ ID NOs: 143/144, 282/284, 283/285.

In one embodiment, the epitope binding region that binds a disease associated antigen binds to EGFR. The epitope binding region comprises a heavy chain variable framework region that is the product of or derived from the human VH3 subclass, preferably human VH3-23, more preferably human IGHV3-23*04 (SEQ ID NO: 22). The heavy chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the heavy chain variable region of the corresponding murine antibody comprising the amino acid sequence of SEQ ID NO: 145. The epitope binding region further comprises a light chain variable framework region that is the product of or derived from the human VK1 subclass, preferably human VK1-39, more preferably human IGKV1-39*01 (SEQ ID NO: 23). The light chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the light chain variable region of the corresponding murine antibody comprising the amino acid sequence of SEQ ID NO: 146.

Most preferably, the humanized heavy chain variable domain comprises a modification to abrogate binding to Protein A comprising the substitution G65S or the substitution N82aS (Kabat numbering).

In particular the EGFR binding polypeptide comprises variable heavy chain domain and variable light chain domain pair encoded by SEQ ID NOs: 145/146, 286/288, 287/289, 290/291, 292/294.

In one embodiment, the epitope binding region that binds a disease associated antigen binds to IgE. The epitope binding region comprises a heavy chain variable framework region that is the product of or derived from the human VH3 subclass, preferably human VH3-23, more preferably human IGHV3-23*04 (SEQ ID NO: 22). The heavy chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the heavy chain variable region of the corresponding humanized antibody comprising the amino acid sequence of SEQ ID NO: 298 or the corresponding murine antibody comprising the amino acid sequence of SEQ ID NO: 304. The epitope binding region further comprises a light chain variable framework region that is the product of or derived from the human VK1 subclass, preferably human VK1-39, more preferably human IGKV1-39*01 (SEQ ID NO: 23). The light chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the light chain variable region of the corresponding humanized antibody comprising the amino acid sequence of SEQ ID NO: 299 or the corresponding murine antibody comprising the amino acid sequence of SEQ ID NO: 305.

Most preferably, the heavy chain variable domain comprises a modification to abrogate binding to Protein A comprising the substitution G65S or the substitution N82aS (Kabat numbering).

In particular the IgE binding polypeptide comprises variable heavy chain domain and variable light chain domain pair encoded by SEQ ID NOs:, 298/299, 300/302, 301/303, 304/305, 306/308, 307/309.

Anti-CD3 antibodies have been found to trigger toxicity by both direct and indirect mechanisms. Indirect mechanisms are mediated by the Fc region of the CD3 antibody which acts with the Fc receptor expressing immune cells and lead to transient T cell activation and cytokine release. Therefore in order to improve the safety of the hetero-dimeric

immunoglobulins or fragment thereof as described herein, the immunoglobulin constant region of the first and/or second polypeptide has reduced or no binding for effector immune cells and/or complement C1q. Preferably, the immunoglobulin constant region is engineered to abrogate Fc receptor binding in the lower hinge region. More preferably the immunoglobulin constant region of the first and/or second polypeptide comprises the substitution(s) L234A and/or L235A (EU numbering). Most preferably, the immunoglobulin constant region of the first and/or second polypeptide comprises the substitutions L234A and L235A (EU numbering).

In another aspect, the disclosure of the present invention also describes a hetero-dimeric immunoglobulin or fragment thereof wherein the epitope binding region binds to the CD3 epsilon subunit of the CD3 protein complex and comprises a FAB having a FAB thermo-stability superior to the FAB thermo-stability of the SP34 chimera comprising a heavy chain variable domain of amino acid sequence of SEQ ID NO: 60 and a light chain variable domain of amino acid sequence of SEQ ID NO: 61, as measured by Differential Scanning Calorimetry (DSC) as in Table 1. This increased thermostability will mean that these improved SP34 binding arms will have increased *in vivo* and *in vitro* stability, meaning better performance as a therapeutic and also in terms of its stability/storage/shelf life.

In accordance with a further aspect of the present invention there is provided a method to improve the expression of an scFv comprising the variable heavy and light domain sequences SEQ ID NO: 60 and 61 of SP34, including the steps:

a) Modifying at least one of the residues in the heavy or light variable domains encoded by SEQ ID NO: 60 and 61;
b) Expressing the expression of the modified construct from step a);
c) Comparing the expression level of the modified construct to an scFv comprising the heavy/light variable domains encoded SEQ ID NO: 60 and SEQ ID NO: 61 or SEQ ID NO: 62 and SEQ ID NO: 63 or SEQ ID NO: 64 and SEQ ID NO: 69;
d) Selecting the modified construct if it is more expressed than the an scFv comprising the heavy/light variable domains encoded SEQ ID NO: 60 and SEQ ID NO: 61 or SEQ ID NO: 62 and SEQ ID NO: 63 or SEQ ID NO: 64 and SEQ ID NO: 69.

In accordance with the present invention the heavy variable domain are modified at least at position 100e.

In particular residue 100e is substituted for an amino acid with a hydrophobic side chain and in particular for a phenylalanine or tyrosine residue.

In accordance with the present invention the light variable domain are modified at least at one of positions 29, 30, 91, 95.

In accordance with the present invention the residues are randomly mutated.

In accordance with the present invention the residues are mutated by site directed mutagenesis for all canonical amino acids and/or non-canonical amino acids or subsets thereof.

In accordance with a preferred aspect of the present invention the residues are mutated by site directed mutagenesis to other residues present in the same position in other characterised versions of the variable heavy and light domains.

In particular residue 29 is substituted for a alanine, glutamic acid or serine residue; residue 30 is substituted for a alanine or aspartic acid residue; residue 91 is substituted for a phenylalanine residue and residue 95 is substituted for a glycine or threonine residue.

In further aspect, the present invention provides a hetero-dimeric immunoglobulin or fragment thereof as described herein wherein one epitope binding region binds to the CD3 epsilon subunit of the CD3 protein complex and the other epitope binding region that binds a disease associated antigen, binds HER2. The potency of such a hetero-dimeric immunoglobulin or fragment thereof to redirect T-cell killing can be measured in an *in vitro* assay using a flow cytometry method (RDL-FACS) or a colorimetric based method (RDL-MTS) on cell lines expressing HER2 such as JIMT-1, BT-474 and MDA-MB-231, as described in the Examples.

In a preferred embodiment, the present invention provides hetero-dimeric immunoglobulin or fragment thereof binding to:

i) the CD3 protein complex and HER2, wherein the first polypeptide has an amino acid sequence selected from the group comprising SEQ ID NO: 359and is assembled with a light chain of amino acid sequence of SEQ ID NO: 399 or 400 and binds CD3 epsilon, and wherein the second polypeptide has an amino acid sequence of SEQ ID NO: 167 and binds HER2;

ii) the CD3 protein complex and HER2, wherein the first polypeptide has an amino acid sequence selected from the group comprising SEQ ID NO: 359 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 399 or 400 and binds CD3 epsilon, and wherein the second polypeptide has an amino acid sequence of SEQ ID NO: 167 and binds HER2;

iii) the CD3 protein complex and CD38, wherein the first polypeptide has an amino acid sequence selected from the group comprising SEQ ID NO: 359and is assembled with a cognate light chain of amino acid sequence of SEQ ID NO: 399 or 400 and binds CD38, and wherein the second polypeptide has an amino acid sequence of SEQ ID NO: 162 and binds CD3 epsilon;

iv) the CD3 protein complex and CD38, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 170 and is assembled with a cognate light chain of amino acid sequence of SEQ ID NO: 138 and binds CD38, and wherein the second polypeptide has an amino acid sequence of SEQ ID NO: SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

v) the CD3 protein complex and CD38, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 176 and is assembled with a cognate light chain of amino acid sequence of SEQ ID NO: 119 and binds CD38, and wherein the second polypeptide has an amino acid sequence of SEQ ID NO: SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

vi) the CD3 protein complex and CD38, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 178 and is assembled with a cognate light chain of amino acid sequence of SEQ ID NO: 128 and binds CD38, and wherein the second polypeptide has an amino acid sequence of SEQ ID NO: SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

vii) the CD3 protein complex and OX40 wherein the first polypeptide has an amino acid sequence selected from the group comprising SEQ ID NO: 359and is assembled with a cognate light chain of amino acid sequence of SEQ ID NO: 399 or 400 and binds OX40, and wherein the second polypeptide has an amino acid sequence of SEQ ID NO: 162 and binds CD3 epsilon;

viii) the CD3 protein complex and EGFR wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 174 and is assembled with a cognate light chain of amino acid sequence of SEQ ID NO: 175 and binds EGFR, and wherein the second polypeptide has an amino acid sequence of SEQ ID NO: SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396 and binds CD3 epsilon;

ix) the CD3 protein complex and CD20, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 180 and is assembled with a cognate light chain of amino acid sequence of SEQ ID NO: 181 and binds CD20, and wherein the second polypeptide has an amino acid sequence of SEQ ID NO: SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon.

In a further embodiment, the present invention provides hetero-dimeric immunoglobulin or fragment thereof binding to:

the CD3 protein complex and HER2, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 310 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 3 and binds HER2, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396 and binds CD3 epsilon;

the CD3 protein complex and CD38, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 312 or 404 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 132 and binds CD38, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and CD38, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 313 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 138 and binds CD38, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396 and binds CD3 epsilon;

the CD3 protein complex and OX40, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 314 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 315 and binds OX40, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396 and binds CD3 epsilon;

the CD3 protein complex and OX40, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 316 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 317 and binds OX40, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and CD20, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 318 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 319 and binds CD20, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and CD20, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 320 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 321 and binds CD20, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and EGFR, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 322 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 323 and binds EGFR, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396 and binds CD3 epsilon;

the CD3 protein complex and EGFR, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 324 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 325 and binds EGFR, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and EGFR, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 326 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 327 and binds EGFR, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396 and binds CD3 epsilon;

the CD3 protein complex and EGFR, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 328 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 329 and binds EGFR, and wherein the second polypeptide has an amino acid

sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396 and binds CD3 epsilon;

the CD3 protein complex and CD19, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 330 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 331 and binds CD19, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and IgE, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 332 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 333 and binds IgE, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and IgE, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 334 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 335 and binds IgE, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and IgE, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 336 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 337 and binds IgE, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and IgE, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 338 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 339 and binds IgE, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and OX40, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 340 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 173 and binds OX40, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396 and binds CD3 epsilon;

the CD3 protein complex and CD20, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 341 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 181 and binds CD20, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and EGFR, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 342 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 175 and binds EGFR, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and EGFR, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 343 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 344 and binds EGFR, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and IgE, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 345 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 346 and binds IgE, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon;

the CD3 protein complex and IgE, wherein the first polypeptide has an amino acid sequence of SEQ ID NO: 347 and is assembled with a light chain of amino acid sequence of SEQ ID NO: 348 and binds IgE, and wherein the second polypeptide has an amino acid sequence selected from the group comprising of SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396and binds CD3 epsilon.

In accordance with a further aspect of the present invention the hetero-dimeric immunoglobulin or fragment thereof wherein said CD3 binding polypeptide comprises at least one or a combination of a heavy and light chain variable regions selected from the group: SEQ ID NOs: 101/105, 103/106, 104/106, 104/401, 104/402.

As discussed above for bispecific antibody generation, there is a need to efficiently produce anti-human CD3 based heavy chain hetero-dimers free of anti-human CD3 homo-dimers

wherein the secreted bispecific antibody product is readily isolated from the cell culture supernatant from a recombinant mammalian host cell line. To this effect, a Protein A based differential purification technique can be used to isolate hetero-dimeric immunoglobulins or fragments thereof encompassing the variable domain subclass of VH3, wherein the Protein A binding site in at least one but preferably both VH3 based epitope binding regions is abrogated. Therefore, in another aspect, the present invention provides an *in vitro* method for the production of a hetero-dimeric immunoglobulin or fragment thereof as described herein, comprising the following steps:

ia) preparing a DNA vector encoding a heavy chain of the first polypeptide and a DNA vector encoding a heavy chain of the second polypeptide wherein one or both DNA vectors or a third DNA vector optionally encode a common light chain or a light chain that assembles with a heavy chain of the first or second polypeptide; or

ib) preparing one DNA vector encoding heavy chains of the first and second polypeptides wherein the DNA vector optionally encodes a common light chain or a light chain that assembles with a heavy chain of the first or second polypeptide; and

wherein said DNA vectors are suitable for transient or stable expression in a mammalian host cell;

ii) transfecting or co-transfecting the DNA vector(s) from (i) in a mammalian host cell line;

iii) culturing the transfected cell line or stably selected clone therefrom and harvesting the cell culture supernatant;

iv) contacting the cell culture supernatant on a Protein A affinity chromatography resin;

v) eluting and collecting the hetero-dimeric immunoglobulin of interest.

Preferably the hetero-dimeric immunoglobulin or fragment thereof found in the purified material from step (v) is at least 95% pure. More preferably the hetero-dimeric immunoglobulin or fragment thereof found in the purified material from step (v) is at least 96% pure. Even more preferably the hetero-dimeric immunoglobulin or fragment thereof found in the purified material from step (v) is at least 97%. Purity of the hetero-dimeric immunoglobulin or fragment thereof found in the purified material can be measured by capillary electrophoresis.

Brief Description of the Figures

FIG. 1: Schematic diagram of the preferred differential affinity purification technique using Protein A. None of the heavy chains encompass a VH3 based antigen binding site. Legend: [(A+)] means a functional Protein A binding site and [(A-)] means a nonfunctional Protein A binding site. pH of elution is indicated.

FIG. 2A-F: Schematic diagrams illustrating the problems faced when purifying hetero-dimers of heavy chains encompassing one or more VH3 domains using differential protein A chromatography. Examples of solutions based on mutating the Protein A binding site within at least one VH3 domain of the hetero-dimer are shown. **FIG. 2A:** Problem faced when the hetero-dimer of heavy chains encompasses a VH3 domain within the heavy chain that does not bind Protein A in its Fc region. **FIG. 2B:** Solution to the purification problem described in FIG.2A, the heavy chain of the hetero-dimer that does not bind Protein A in its Fc region encompasses a VH3 domain which has been mutated to abrogate its Protein A binding site. **FIG. 2C:** Alternative solution to the problem described in FIG. 2A, the hetero-dimer encompasses only one VH3 domain and the hetero-dimer is engineered to have its VH3 domain located on the heavy chain that binds Protein A in its Fc region (VH3 domain relocation strategy as a solution). **FIG. 2D:** Problem faced when both heavy chains of the hetero-dimer encompass a VH3 domain. **FIG. 2E:** Solution to the purification problem described in FIG.2D, the heavy chain of the hetero-dimer that does not bind Protein A in its Fc region encompasses a VH3 domain which has been mutated to abrogate its Protein A binding site. **FIG. 2F:** Alternative solution to the purification problem described in FIG.2D, each VH3 domain has its Protein A binding site abrogated. Boxed species indicated that these species co-elute during the differential Protein A chromatography process. pH values A and B differ by about one pH unit and allow efficient separation of the species that binds Protein A. Typically pH values for pH A and pH B are 4 and 3, respectively. Legend for all figures: [(A+)] means a functional Protein A binding site and [(A-)] means a nonfunctional Protein A binding site.

FIG. 3: Protein A gradient mode chromatography traces for Fc 133 (HiTrap™ MabSelect SuRe™ Protein A column). Plots of absorbance at 280 nm *vs.* total volume of mobile phase

are shown as solid line. Plots of mobile phase pH and percentage of eluent buffer (B) present in mobile phase are shown as dashed and dotted-dashed lines, respectively.

FIG. 4A-C: Protein A gradient mode chromatography traces. Plots of absorbance at 280 nm *vs.* total volume of mobile phase are shown as solid line. Plots of mobile phase pH and percentage of eluent buffer (B) present in mobile phase are shown as dashed and dotted-dashed lines, respectively. **FIG. 4A:** Anti-HER2 FAB-Fc 133 (HiTrap™ MabSelect SuRe™ Protein A column). **FIG. 4B:** Anti-HER2 scFv-Fc 133 (HiTrap™ MabSelect SuRe™ Protein A column). **FIG. 4C:** Anti-HER2 FAB (HiTrap™ MabSelect SuRe™ Protein A column and HiTrap™ MabSelect™ Protein A column).

FIG. 5: Representative amino acid sequences for each of the seven known human VH framework subclasses. Sequences were aligned according to the Kabat numbering. Positions in the human VH3-23 framework subclass that interact with the domain D of Protein A are shown in bold.

FIG. 6A-I: Protein A gradient mode chromatography traces (HiTrap™ MabSelect™ Protein A column). Plots of absorbance at 280 nm *vs.* total volume of mobile phase are shown as solid line. Plots of mobile phase pH and percentage of eluent buffer (B) present in mobile phase are shown as dashed and dotted-dashed lines, respectively. **FIG. 6A:** Anti-HER2 FAB. **FIG. 6B:** Anti-HER2 FAB T57A. **FIG. 6C:** Anti-HER2 FAB T57E. **FIG. 6D:** Anti-HER2 FAB G65S. **FIG. 6E:** Anti-HER2 FAB R66Q. **FIG. 6F:** Anti-HER2 FAB T68V. **FIG. 6G:** Anti-HER2 FAB Q81E. **FIG. 6H:** Anti-HER2 FAB N82aS. **FIG. 6I:** Anti-HER2 FAB R19G/T57A/Y59A.

FIG. 7: Equilibrium dissociation constants (KD) of selected anti-HER2 FAB variants for the HER2 antigen.

FIG. 8A-D: Protein A gradient mode chromatography traces (HiTrap™ MabSelect SuRe™ Protein A column). Plots of absorbance at 280 nm *vs.* total volume of mobile phase are shown as solid line. Plots of mobile phase pH and percentage of eluent buffer (B) present in mobile phase are shown as dashed and dotted-dashed lines, respectively. **FIG. 8A:** Anti-HER2

scFv(G65S)-Fc 133. **FIG. 8B:** Anti-HER2 scFv(N82aS)-Fc 133. **FIG. 8C:** Anti-HER2 FAB(G65S)-Fc 133. **FIG. 8D:** Anti-HER2 FAB(N82aS)-Fc 133.

FIG. 9A-F: These figures all relate to OKT3 humanization on stable human frameworks. **FIG. 9A-C:** Summary of humanized candidates formatted as human IgG1 antibodies. HPB-ALL staining relative to the chimeric OKT3 antibody: (-) indicates no binding, (+) weaker binding, (++) moderate binding and (+++) similar binding. **FIG. 9D:** DSC profiles of selected antibodies of candidates. **FIG. 9E:** Summary of humanized candidates formatted as scFv-Fc fusions. HPB-ALL staining relative to the chimeric OKT3 antibody: (-) indicates no binding, (+) weaker binding, (++) moderate binding and (+++) similar binding. **FIG. 9F:** DSC profiles of selected scFv-Fc candidates.

FIG. 10A-B: These figures all relate to SP34 humanization on stable human frameworks. **FIG. 10A:** Summary of humanized candidates formatted as human IgG1 antibodies. **FIG. 10B:** Summary of humanized candidates formatted as scFv-Fc fusion proteins (Fc of human IgG1 isotype). SPR data relative to the chimeric SP34 antibody for human and cynomolgus monkey CD3 epsilon 1-26_Fc fusion proteins: (-) indicates no binding, (+) weaker binding, (++) moderate binding, strong but not similar binding (+++), and (++++) similar binding.

FIG. 11A-J: These figures all relate to anti-human CD38 antibodies.
FIG. 11A: Antibody-antigen interaction measured by SPR between the chimeric HB-7 antibody and the human CD38 antigen. A CM5 sensor chip was covalently coupled with protein G and 200 RUs of chimeric HB-7 antibody were captured. Human CD38 protein (human CD38 extracellular domain with a poly-histidine tag) was injected at 125, 31, 7.8, 3.9, 1.9, 1 and 0.5 nM at a flow rate of 30 µl/min in HBS-P. **FIG. 11B:** Antibody-antigen interaction measured by SPR between the humanized HB-7 best-fit antibody and the human CD38 antigen. A CM5 sensor chip was covalently coupled with protein G and 200 RUs of humanized HB-7 best-fit antibody were captured. Human CD38 protein (human CD38 extracellular domain with a poly-histidine tag) was injected at 50, 25, 12.5, 6.25 and 0.39 nM at a flow rate of 30 µl/min in HBS-P. **FIG. 11C:** Antibody-antigen interaction measured by SPR between the humanized 9G7 best-fit antibody and the human CD38 antigen. A CM5 sensor chip was covalently coupled with protein G and 200 RUs of humanized 9G7 best-fit antibody were captured. Human CD38 protein (human CD38 extracellular domain with a

poly-histidine tag) was injected at 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, and 0.1 nM at a flow rate of 30 µl/min in HBS-P. **FIG. 11D:** Antibody-antigen interaction measured by SPR between the humanized 9G7 best-framework antibody and the human CD38 antigen. A CM5 sensor chip was covalently coupled with protein G and 200 RUs of humanized 9G7 best-framework antibody were captured. Human CD38 protein (human CD38 extracellular domain with a poly-histidine tag) was injected at 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, and 0.1 nM at a flow rate of 30µl/min in HBS-P. **FIG. 11E:** Antibody-antigen interaction measured by SPR between the human 767 antibody and the human CD38 antigen. A CM5 sensor chip was covalently coupled with protein G and 200 RUs of human 767 antibody were captured. Human CD38 protein (human CD38 extracellular domain with a poly-histidine tag) was injected at 500, 250, 125, 62.5, 31.25, and 15.6 nM at a flow rate of 30 µl/min in HBS-P. Affinity was obtained from a plot of the equilibrium response (Req) vs. analyte concentration (C) according to the following equation: Req=KA*C*Rmax/(KA*C*n+1), concentration at 50% saturation is KD. All SPR data are expressed as number of response units (abbreviated RU; Y axis) vs. time (X axis). **FIG. 11F:** DSC profiles of chimeric HB-7 and humanized HB-7 best-fit antibodies. **FIG. 11G:** DSC profiles of chimeric 9G7 and humanized 9G7 best-fit antibodies. **FIG. 11H:** DSC profiles of humanized 9G7 best-framework antibody. **FIG. 11I:** DSC profiles of human clone 767 antibody. **FIG. 11J:** summary table for the 9G7 humanized antibodies.

FIG. 12A-C: Schematic diagram of the BEAT HER2/CD3 antibodies in alternative formats. **FIG.12A:** BEAT HER2/CD3-1 (format A) and BEAT HER2/CD3-2 (format B) antibodies. **FIG.12B:** BEAT HER2/CD3-3 (format C) and BEAT HER2/CD3(SP34) (format D) antibodies. **FIG.12C:** BEAT HER2/CD3(SP34-Kappa1) (format E) antibody. Legend: [(A+)] means functional Protein A binding site. [(A-)] means nonfunctional Protein A binding site.

FIG. 13: Protein A purification profile of BEAT HER2/CD3-1 antibody (Absorbance trace at 280 nm). Column: 1ml MabSelect SuRe. Flow rate: 1 ml/min. Running buffer: 0.2 M NaH_2PO_4 pH 6. Elution buffer No 1: 20 mM Na Acetate pH 4 (20 ml). Elution buffer No 2: 0.1 M Glycine pH 3 (20ml). Neutralization: 1/10 vol. of 1M Tris pH 8.

FIG. 14: Capillary Electrophoresis profile of BEAT HER2/CD3-1 antibody preparations.

FIG. 15A: SDS-PAGE analysis of N82aS substituted BEAT HER2/CD3-1 antibody. **FIG. 15B:** SDS-PAGE analysis of N82aS non substituted BEAT HER2/CD3-1 antibody variant. Legend: [(A+)] means a functional Protein A binding site and [(A-)] means a nonfunctional Protein A binding site. pH of elution is indicated.

FIG. 16A: Antibody-antigen interaction measured by SPR between the BEAT HER2/CD3-1 antibody and the human CD3 epsilon antigen. A CM5 sensor chip was covalently coupled with 7400 RUs of the human CD3 gamma-epsilon-Fc fusion protein. BEAT HER2/CD3-1 antibody was injected at 5000, 2500, 1250, 625, 312.5 and 156.25 nM at a flow rate of 10 µl/min in HBS-P. Data are expressed as number of response units (abbreviated RU; Y axis) vs. time (X axis). Affinity was obtained from a plot of the equilibrium response (Req) vs. analyte concentration (C) according to the following equation: Req=KA*C*Rmax/(KA*C*n+1), concentration at 50% saturation is KD. **FIG. 16B:** Antibody-antigen interaction measured by SPR between the BEAT HER2/CD3-1 antibody and the human HER2 antigen. A CM5 sensor chip was covalently coupled protein G and 150 RUs of BEAT HER2/CD3-1 antibody were captured. HER2-his was injected at 1000, 333, 111, 37, 12, 4.1, 1.4, 0.5 and 0.15 nM at a flow rate of 30µl/min in HBS-P. Data are expressed as number of response units (abbreviated RU; Y axis) vs. time (X axis). **FIG. 16C:** DSC profiles of BEAT HER2/CD3-1 and -2 antibodies shown in profiles A and B, respectively.

FIG. 17A-G: Examples of T cell redirected killing by the BEAT HER2/CD3 antibodies. Readout: RDL-MTS method. Effector cells: human PBMCs. Effector cells-to-targeted cells ratio of 10:1. Means of three donors with 48h incubation. Antibody concentrations are shown in nM. **FIG. 17A:** BEAT HER2/CD3-1 and BEAT HER2/CD3-2 antibodies, target cells: BT-474. **FIG. 17B:** BEAT HER2/CD3-1 and BEAT HER2/CD3-2 antibodies, target cells: JIMT-1. **FIG 17C:** BEAT HER2/CD3-1 and BEAT HER2/CD3-2 antibodies, target cells: MDA-MB-231. **FIG. 17D:** BEAT HER2/CD3(SP34) antibody, target cells: NCI-N87. **FIG. 17E:** BEAT HER2/CD3(SP34) antibody, target cells: HT-1080. **FIG. 17F:** BEAT HER2/CD3(SP34-Kappa1) antibody, target cells: NCI-N87. **FIG. 17G:** BEAT HER2/CD3(SP34-Kappa1) antibody, target cells: HT-1080.

FIG. 18A-C: JIMT-1 xenografts with human PBMC supplementation. **FIG. 18A:** Human PBMCs do not interfere with tumor growth. **FIG. 18B-C:** Tumor volumes (mm^3) for BEAT HER2/CD3-1 treated and non-treated mice, four human PBMC donors, cohorts of five mice.

FIG. 19: Schematic diagram of the BEAT CD38-HB7bestfit/CD3 (format A) and BEAT CD38-767/CD3 (format B) antibodies. [(A+)] means functional Protein A binding site. [(A-)] means nonfunctional Protein A binding site.

FIG. 20A: Antibody-antigen interaction measured by SPR between the BEAT CD38-HB7bestfit/CD3 antibody and the human CD38 antigen. A CM5 sensor chip was covalently coupled with protein G and 200 RUs of BEAT CD38-HB7bestfit/CD3 antibody were captured. Human CD38 protein (poly-histidine tagged protein) was injected at 50, 25, 12.5, 6.25 and 0.39 nM at a flow rate of 30µl/min in HBS-P. Data are expressed as number of response units (abbreviated RU; Y axis) vs. time (X axis). **FIG. 20B:** BEAT CD38-HB7bestfit/CD3 antibody DSC profile.

FIG. 21: Example of T cell redirected killing by the BEAT CD38-HB7bestfit/CD3 antibody. Readout: RDL-FACS method. Effector cells: purified human T cells. Effector cells-to-targeted cells ratio of 10:1. Mean of two donors with 48h incubation. Target cells: RPMI 8226. Antibody concentration is shown in nM.

FIG. 22: Example of T cell redirected killing by the BEAT CD38-767/CD3(SP34) antibody. Readout: RDL-FACS method. Effector cells: human PBMCs. Effector cells-to-targeted cells ratio of 10:1. Mean of three donors with 24h incubation. Target cells: Daudi. Antibody concentration is shown in nM.

FIG. 23: Schematic diagram of the BEAT OX40/CD3antibody. Legend: [(A+)] means functional Protein A binding site. [(A-)] means nonfunctional Protein A binding site.

FIG. 24: Example of T cell redirected killing by the BEAT OX40/CD3antibody. Readout: RDL-MTS method. Effector cells: Human PBMCs. Effector cells-to-targeted cells ratio of 20:1. Mean of three donors with 48h incubation. Target cells: recombinant stable CHO[OX40] cells. Antibody concentration is shown in nM.

FIG. 25: Schematic diagram of the BEAT EGFR/CD3 antibody. Legend: [(A+)] means functional Protein A binding site. [(A-)] means nonfunctional Protein A binding site.

FIG. 26: Example of T cell redirected killing by the BEAT EGFR/CD3antibody. Readout: RDL-MTS method. Effector cells: Human PBMCs. Effector cells-to-targeted cells ratio of 10:1. Mean of four donors with 48h incubation. Target cells: HT-29 cells. Antibody concentration is shown in nM.

FIG. 27: Schematic diagram of the BEAT CD38-HB7bestfit/CD3(SP34) (format A) and BEAT CD38-9G7bestfit/CD3(SP34-Kappa2) (format B) antibodies. [(A+)] means functional Protein A binding site.

FIG. 28: Example of T cell redirected killing by the BEAT CD38-HB7bestfit/CD3(SP34) antibody. Readout: RDL-FACS method. Effector cells: Human PBMCs. Effector cells-to-targeted cells ratio of 10:1. Mean of three donors with 24h incubation. Target cells: Daudi cells. Antibody concentration is shown in nM.

FIG. 29: Antibody-antigen interaction measured by SPR between the BEAT CD38-9G7bestfit/CD3(SP34-Kappa2) antibody and the human CD3 epsilon 1-26_Fc fusion protein. A CM5 sensor chip was covalently coupled with 500 RUs of the human CD3 epsilon 1-26_Fc fusion protein. BEAT CD38-9G7bestfit/CD3(SP34-Kappa2) antibody was injected at 50, 25, 12.5, 6.2, 3.1, 0.8 and 0.4 nM at a flow rate of 30 µl/min in HBS-P. Data are expressed as number of response units (abbreviated RU; Y axis) vs. time (X axis).

FIG. 30: Example of T cell redirected killing by the BEAT CD38/CD3(SP34-Kappa2) antibody. Readout: RDL-FACS method. Effector cells: Human PBMCs. Effector cells-to-

targeted cells ratio of 10:1. Mean of three donors with 24h incubation. Target cells: Daudi cells. Antibody concentration is shown in nM.

FIG. 31: Schematic diagram of the BEAT CD20/CD3(SP34) antibody. [(A+)] means functional Protein A binding site.

FIG. 32: Example of T cell redirected killing by the BEAT CD20/CD3(SP34) antibody. Readout: RDL-FACS method. Effector cells: Human PBMCs. Effector cells-to-targeted cells ratio of 10:1. Means of three donors with 24h incubation. Target cells: Daudi cells. Antibody concentration is shown in nM.

FIG. 33 Shows the relative expression levels after reformatting from IgG1 to scFv-Fc for the SP34 chimera as well as Sp34 H1L21, wherein a dramatic loss of expression was observed.

FIG. 34 Shows the effects on expression level of an SP34 H1L21 ScFv-Fc by Alanine scan in positions: T27, G27a, V27c, T28, T29, S30, N31, Y32, N52, K53, R54, P56, L90, Y92, S93, N94, and L95.

FIG. 35a Shows the effects on expression level of random mutation at position 29 of a SP34 H3L23 ScFv-Fc; **b** Shows the effects on expression level of random mutation at position 30 of a SP34 H3L23 ScFv-Fc; c Shows the effects on expression level of random mutation at position 95 of a SP34 H5L23 ScFv-Fc.

FIG. 36 shows the normalised expression level for several humanized SP34.

FIG. 37 shows the performance in an RDL assay of several CD38/CD3 bispecific antibodies, in which the CD3 binding arm comprises the original mouse SP34 reformatted as an scFv (SEQ ID NO: 403), or modified humanised SP34 scFv's comprising the heavy/light chain combinations H1/L21 (SEQ ID NO: 361), H5/L32 (SEQ ID NO: 311), H5/L65 (SEQ ID NO: 394) and H5/L67 (SEQ ID NO: 396).

FIG. 38 shows the effect of a CD3-Rituximab BEAT bispecific on a RAJI cell population.

FIG. 39 shows the effect in a RDL assay of Erbitux on the MCF-7, HCT116 and A549 cell populations.

FIG. 40 shows the effect in a RDL assay of a CD3-Erbitux BEAT bispecific antibody on the MCF-7, HCT116 and A549 cell populations.

FIG. 41 shows the EGFR status of the cell lines determined using the EGFR PharmDx immunohistochemistry kit (Dako, Cambridge, UK).

FIG. 42 shows the effect in a RDL assay of Vectibix on the MCF-7, HCT116 and A549 cell populations.

FIG. 43 shows the effect in a RDL assay of a CD3-Vectibix BEAT bispecific antibody on the MCF-7, HCT116 and A549 cell populations

Detailed description of the invention

The present invention relates generally to novel hetero-dimeric immunoglobulins that bind to the CD3 protein complex and a disease associated antigen. Furthermore, these hetero-dimeric immunoglobulins have reduced or eliminated binding to protein A and therefore can be purified to a very high degree of purity using affinity chromatography.

For the purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

The terms "polypeptide" and "protein" refer to a polymer of amino acid residues wherein amino acids are combined via peptide bonds to form a chain of amino acids that have been linked together via dehydration synthesis. Polypeptides and proteins can be synthesized through chemical synthesis or recombinant expression and are not limited to a minimum amino acid length.

In accordance with the invention, the group of polypeptides comprises "proteins" as long as the proteins consist of a single polypeptide chain. Polypeptides may further form multimers such as dimers, trimers and higher oligomers, i.e. consisting of more than one polypeptide molecule. Polypeptide molecules forming such dimers, trimers etc. may be identical or non-identical. The corresponding higher order structures of such multimers are, consequently, termed homo- or hetero-dimers, homo- or hetero-trimers etc. An example for a hetero-multimer is an antibody molecule, which, in its naturally occurring form, consists of two identical light polypeptide chains and two identical heavy polypeptide chains. The terms "polypeptide" and "protein" also refer to naturally modified polypeptides/proteins wherein the modification is effected e.g. by post-translational modifications like glycosylation, acetylation, phosphorylation and the like. Such modifications are well known in the art. Furthermore, for purposes of the present invention, a "polypeptide" refers to a protein which includes modifications, such as deletions, additions and substitutions (which can be conservative in nature) to the native sequence. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

The term "CD3 complex" as used herein refers to the protein complex known as the CD3 (cluster of differentiation 3) T-cell co-receptor (Wucherpfennig KW *et al.*, (2010) Cold Spring Harb Perspect Biol, 2(4): a005140). The CD3 protein complex is composed of four distinct chains. In mammals, the complex contains a CD3γ chain, a CD3δ chain, and two CD3ε chains. These chains associate with a molecule known as the T-cell receptor (TCR) and the ζ-chain to generate an activation signal in T lymphocytes (van der Merwe PA & Dushek O (2011) Nat Rev Immunol, 11(1): 47-55). The TCR, ζ-chain, and CD3 molecules together comprise the TCR complex. The CD3γ, CD3δ, and CD3ε chains are highly related cell-surface proteins of the immunoglobulin superfamily containing a single extracellular immunoglobulin domain. The intracellular tails of the CD3 molecules contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif or ITAM for short, which is essential for the signalling capacity of the TCR. Since CD3 is required for T-cell activation, drugs (often monoclonal antibodies) that target CD3 have and are being investigated as immunosuppressant therapies.

The term "disease associated antigen" as used herein refers to molecules that are involved in a disease process. Examples of disease associated antigens are found in a broad range of therapeutic areas such as inflammation, cancer and autoimmune diseases. In oncology, disease associated antigens are molecules that can broadly be used for the screening and/or monitoring and/or therapeutic targeting of cancers within a patient population, for example EpCAM antigen in prostate cancer. Tumour antigens can be produced directly by the tumour or by non-tumour cells as a response to the presence of a tumour and preferred tumour antigens are cell-surface molecules. Inflammatory disease associated antigens are known, which include but are not limited to, pro-inflammatory cytokines such as TNF-α and IL-1. Autoimmune disease associated antigens are also known; examples of these include but are not limited to antibodies against double-stranded DNA in systemic lupus erythematosus and amyloid beta peptide in Alzheimers disease.

The term "immunoglobulin" as referred to herein can be used interchangeably with the term "antibody". Immunoglobulin includes full-length antibodies and any antigen binding fragment or single chains thereof. Immunoglobulins can be homo-dimeric or hetero-dimeric. Immunoglobulins and specifically naturally occurring antibodies are glycoproteins which exist as one or more copies of a Y-shaped unit, composed of four polypeptide chains. Each "Y" shape contains two identical copies of a heavy (H) chain and two identical copies of a light (L) chain, named as such by their relative molecular weights. Each light chain pairs with a heavy chain and each heavy chain pairs with another heavy chain. Covalent interchain disulfide bonds and non-covalent interactions link the chains together. Immunoglobulins and specifically naturally occurring antibodies contain variable regions, which are the two copies of the antigen binding site. Papain, a proteolytic enzyme splits the "Y" shape into three separate molecules, two so called "Fab" or "FAB" fragments (Fab = fragment antigen binding) and one so called "Fc" fragment or "Fc region" (Fc = fragment crystallizable). A Fab fragment consists of the entire light chain and part of the heavy chain. The heavy chain contains one variable region (VH) and either three or four constant regions (CH1, CH2, CH3 and CH4, depending on the antibody class or isotype). The region between the CH1 and CH2 regions is called the hinge region and permits flexibility between the two Fab arms of the Y-shaped antibody molecule, allowing them to open and close to accommodate binding to two antigenic determinants separated by a fixed distance. The "hinge region" as referred to herein is a sequence region of 6-62 amino acids in length, only present in IgA, IgD and IgG, which

encompasses the cysteine residues that bridge the two heavy chains. The heavy chains of IgA, IgD and IgG each have four regions, i.e. one variable region (VH) and three constant regions (CH1-3). IgE and IgM have one variable and four constant regions (CH1-4) on the heavy chain. The constant regions of the immunoglobulins may mediate the binding to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the complement system classical pathway. Each light chain is usually linked to a heavy chain by one covalent disulfide bond. Each light chain contains one variable region (VL) and one light chain constant region. The light chain constant region is a kappa light chain constant region designated herein as IGKC or is a lambda light chain constant region designated herein as IGLC. IGKC is used herein equivalently to Cκ or CK and has the same meaning. IGLC is used herein equivalently to Cλ or CL and has the same meaning. The term "an IGLC region" as used herein refer to all lambda light chain constant regions e.g. to all lambda light chain constant regions selected from the group consisting of IGLC1, IGLC2, IGLC3, IGLC6 and IGLC7. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR or FW). Each VH and VL is composed of three CDRs and four FRs, arranged from amino- terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain an epitope- binding region that interacts with an antigen. Engineered immunoglobulins can encompass different epitope binding region formats such as scFv, FAB or dAb fragments. These fragments are usually assembled in an antibody-like structure by genetic fusion to a IgG Fc region. Engineered immunoglobulins can be constructed as homo or hetero-dimers with or without the use of hetero-dimerization enhancing techniques, and can have mono- or bispecific binding properties.

The term "full length antibody" as used herein includes the structure that constitutes the natural biological form of an antibody, including variable and constant regions. For example, in most mammals, including humans and mice, the full length antibody of the IgG class is a tetramer and consists of two identical pairs of two immunoglobulin chains, each pair having one light and one heavy chain, each light chain comprising immunoglobulin regions VL and a light chain constant region, and each heavy chain comprising immunoglobulin regions VH, CH1 (Cγ1), CH2 (Cγ2), CH3 (Cγ3) and CH4 (Cγ4), depending on the antibody class or isotype). In some mammals, for example in camels and llamas, IgG antibodies may consist of

only two heavy chains, each heavy chain comprising a variable region attached to the Fc region.

Antibodies are grouped into classes, also referred to as isotypes, as determined genetically by the constant region. Human constant light chains are classified as kappa (CK) and lambda (Cλ) light chains. Heavy chains are classified as mu (μ), delta (δ), gamma (γ), alpha (α), or epsilon (ε) and define the antibody's isotype as IgM, IgD, IgG, IgA and IgE, respectively. Thus, "isotype" as used herein is meant any of the classes and/or subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. The known human immunoglobulin isotypes are IGHG1 (IgG1), IGHG2 (IgG2), IGHG3 (IgG3), IGHG4 (IgG4), IGHA1 (IgA1), IGHA2 (IgA2), IGHM (IgM), IGHD (IgD) and IGHE (IgE). The so-called human immunoglobulin pseudo-gamma IGHGP gene represents an additional human immunoglobulin heavy constant region gene which has been sequenced but does not encode a protein due to an altered switch region (Bensmana M *et al.*, (1988) Nucleic Acids Res, 16(7): 3108). In spite of having an altered switch region, the human immunoglobulin pseudo-gamma IGHGP gene has open reading frames for all heavy constant regions (CH1-CH3) and hinge. All open reading frames for its heavy constant regions encode protein regions which align well with all human immunoglobulin constant regions with the predicted structural features. This additional pseudo-gamma isotype is referred herein as IgGP or IGHGP. Other pseudo immunoglobulin genes have been reported such as the human immunoglobulin heavy constant region epsilon P1 and P2 pseudo-genes (IGHEP1 and IGHEP2). The IgG class is the most commonly used for therapeutic purposes. In humans this class comprises subclasses IgG1, IgG2, IgG3 and IgG4. In mice this class comprises subclasses IgG1, IgG2a, IgG2b, IgG2c and IgG3.

The term "Immunoglobulin fragments" as used herein include, but is not limited to, (i) a region including for example a CH1, a CH2 or a CH3 region, (ii) the Fab fragment consisting of VL, VH, CL or CK and CH1 regions, including Fab' and Fab'-SH, (ii) the Fd fragment consisting of the VH and CH1 regions, (iii) the dAb fragment (Ward ES *et al.*, (1989) Nature, 341(6242): 544-6) which consists of a single variable region (iv) $F(ab')_2$ fragments, a bivalent fragment comprising two linked Fab fragments (v) single chain Fv fragments (scFv), wherein a VH region and a VL region are linked by a peptide linker which allows the two regions to associate to form an antigen binding site (Bird RE *et al.*, (1988) Science, 242(4877): 423-6;

Huston JS *et al.*, (1988) Proc Natl Acad Sci U S A, 85(16): 5879-83), (vi) "diabodies" or "triabodies", multivalent or multispecific fragments constructed by gene fusion (Holliger P *et al.*, (1993) Proc Natl Acad Sci U S A, 90(14): 6444-8; Tomlinson I & Holliger P, (2000) Methods Enzymol, 326:461-79), (vii) scFv, diabody or region antibody fused to an Fc region and (viii) scFv fused to the same or a different antibody.

The term "variable region" refers to the regions or domains that mediates antigen-binding and defines specificity of a particular antibody for a particular antigen. In naturally occurring antibodies, the antigen-binding site consists of two variable regions that define specificity: one located in the heavy chain, referred herein as heavy chain variable region (VH) and the other located in the light chain, referred herein as light chain variable region (VL). In humans, the heavy chain variable region (VH) can be divided into seven subgroups or subclasses: VH1, VH2, VH3, VH4, VH5, VH6 and VH7. In some cases, specificity may exclusively reside in only one of the two regions as in single-domain antibodies from heavy-chain antibodies found in camelids. The V regions are usually about 110 amino acids long and consist of relatively invariant stretches of amino acid sequence called framework regions (FRs or "non-CDR regions") of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are 7-17 amino acids long. The variable domains of native heavy and light chains comprise four FRs, largely adopting a beta-sheet configuration, connected by three hypervariable regions, which form loops. The hypervariable regions in each chain are held together in close proximity by FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen binding site of antibodies (see Kabat EA *et al.*, *supra.*). The term "hypervariable region" as used herein refers to the amino acid residues of an antibody which are responsible for antigen binding. The hypervariable region generally comprises amino acid residues from a "complementary determining region" or "CDR", the latter being of highest sequence variability and/or involved in antigen recognition. For all variable regions numbering is according to Kabat (Kabat EA *et al.*, *supra.*).

A number of CDR definitions are in use and are encompassed herein. The Kabat definition is based on sequence variability and is the most commonly used (Kabat EA *et al.*, *supra.*). Chothia refers instead to the location of the structural loops (Chothia & Lesk J. (1987) Mol Biol, 196: 901-917). The AbM definition is a compromise between the Kabat and the Chothia

definitions and is used by Oxford Molecular's AbM antibody modelling software (Martin ACR *et al.*, (1989) Proc Natl Acad Sci USA 86:9268–9272; Martin ACR *et al.*, (1991) Methods Enzymol, 203: 121–153; Pedersen JT *et al.*, (1992) Immunomethods, 1: 126–136; Rees AR *et al.*, (1996) In Sternberg M.J.E. (ed.), Protein Structure Prediction. Oxford University Press, Oxford, 141–172). The contact definition has been recently introduced (MacCallum RM *et al.*, (1996) J Mol Biol, 262: 732-745) and is based on an analysis of the available complex structures available in the Protein Databank. The definition of the CDR by IMGT®, the international ImMunoGeneTics information system® (http://www.imgt.org) is based on the IMGT numbering for all immunoglobulin and T cell receptor V-REGIONs of all species (IMGT®, the international ImMunoGeneTics information system®; Lefranc MP *et al.*, (1999) Nucleic Acids Res, 27(1): 209-12; Ruiz M *et al.*, (2000) Nucleic Acids Res, 28(1): 219-21; Lefranc MP (2001) Nucleic Acids Res, 29(1): 207-9; Lefranc MP (2003) Nucleic Acids Res, 31(1): 307-10; Lefranc MP *et al.*, (2005) Dev Comp Immunol, 29(3): 185-203; Kaas Q *et al.*, (2007) Briefings in Functional Genomics & Proteomics, 6(4): 253-64). All Complementarity Determining Regions (CDRs) as referred to in the present invention, are defined preferably as follows (numbering according to Kabat EA *et al.*, *supra*): LCDR1: 24-34, LCDR2: 50-56, LCDR3: 89-98, HCDR1: 26-35, HCDR2: 50-65, HCDR3: 95-102.

The "non-CDR regions" of the variable domain are known as framework regions (FR). The "non-CDR regions" of the VL region as used herein comprise the amino acid sequences: 1-23 (FRl), 35-49 (FR2), 57-88 (FR3) and 99-107 (FR4). The "non-CDR regions" of the VH region as used herein comprise the amino acid sequences: 1-25 (FRl), 36-49 (FR2), 66-94 (FR3) and 103-113 (FR4).

The CDRs of the present invention may comprise "extended CDRs" which are based on the aforementioned definitions and have variable domain residues as follows: LCDR1: 24-36, LCDR2: 46-56, LCDR3:89-97, HCDR1: 26-35, HCDR2:47-65, HCDR3: 93-102. These extended CDRs are numbered as well according to Kabat *et al., supra*. The "non-extended CDR region" of the VL region as used herein comprise the amino acid sequences: 1-23 (FR1), 37-45 (FR2), 57-88 (FR3) and 98- approximately 107 (FR4). The "non-extended CDR region" of the VH region as used herein comprise the amino acid sequences: 1-25 (FR1), 37-46 (FR2), 66-92 (FR3) and 103- approximately 113 (FR4).

The term "Fab" or "FAB" or "Fab region" or "FAB region" as used herein includes the polypeptides that comprise the VH, CH1, VL and light chain constant immunoglobulin regions. Fab may refer to this region in isolation, or this region in the context of a full length antibody or antibody fragment.

The term "Fc" or "Fc region", as used herein includes the polypeptide comprising the constant region of an antibody heavy chain excluding the first constant region immunoglobulin region. Thus Fc refers to the last two constant region immunoglobulin regions of IgA, IgD and IgG or the last three constant region immunoglobulin regions of IgE and IgM, and the flexible hinge N-terminal to these regions. For IgA and IgM, Fc may include the J chain. For IgG, Fc comprises immunoglobulin regions Cgamma2 and Cgamma3 (Cγ2 and Cγ3) and the hinge between Cgamma1 (Cγl) and Cgamma2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index. Fc may refer to this region in isolation or this region in the context of an Fc polypeptide, for example an antibody.

The term "immunoglobulin constant region" as used herein refers to immunoglobulin or antibody heavy chain constant regions from human or animal species and encompasses all isotypes. Preferably, immunoglobulin constant regions are of human origin and are selected from the group consisting of, but not limited to: IGHG1 CH1, IGHG2 CH1, IGHG3 CH1, IGHG4 CH1, IGHA1 CH1, IGHA2 CH1, IGHE CH1, IGHEP1 CH1, IGHM CH1, IGHD CH1, IGHGP CH1, IGHG1 CH2, IGHG2 CH2, IGHG3 CH2, IGHG4 CH2, IGHA1 CH2, IGHA2 CH2, IGHE CH2, IGHEP1 CH2, IGHM CH2, IGHD CH2, IGHGP CH2, IGHG1 CH3, IGHG2 CH3, IGHG3 CH3, IGHG4 CH3, IGHA1 CH3, IGHA2 CH3, IGHE CH3, IGHEP1 CH3, IGHM CH3, IGHD CH3, IGHGP CH3, IGHE CH4 and IGHM CH4. Prefered "immunoglobulin constant regions" are selected from the group consisting of human IGHE CH2, IGHM CH2, IGHG1 CH3, IGHG2 CH3, IGHG3 CH3, IGHG4 CH3, IGHA1 CH3, IGHA2 CH3, IGHE CH3, IGHM CH3, IGHD CH3 and IGHGP CH3. More prefered "immunoglobulin constant regions" are selected from the group consisting of human IGHG1 CH3, IGHG2 CH3, IGHG3 CH3, IGHG4 CH3, IGHA1 CH3, IGHA2 CH3, IGHE CH3,

IGHM CH3, IGHD CH3 and IGHGP CH3.

The term "epitope binding region" includes a polypeptide or a fragment thereof having minimal amino acid sequence to permit the specific binding of the immunoglobulin molecule to one or more epitopes. Naturally occurring antibodies have two epitope binding regions which are also known as antigen binding or combining sites or paratopes. Epitope binding regions in naturally occurring antibodies are confined within the CDR regions of the VH and/or VL domains wherein the amino acid mediating epitope binding are found. In addition to naturally occurring antibodies, artificial VH domains or VL domains or fragments thereof and combinations thereof can be engineered to provide epitope binding regions (Holt LJ *et al.*, (2003) Trends Biotechnol, 21(11): 484-490; Polonelli L *et al.,* (2008) PLoS ONE, 3(6): e2371). Examples of non-immunoglobulin based epitope binding regions can be found in artificial protein domains used as "scaffold" for engineering epitope binding regions (Binz HK *et al.,* (2005) Nat Biotechnol, 23(10): 1257-1268) or peptide mimetics (Murali R & Greene MI (2012) Pharmaceuticals, 5(2): 209-235). Preferably the term 'epitope binding region' includes the combination of one or more heavy chain variable domains and one or more complementary light chain variable domains which together forms a binding site which permits the specific binding of the immunoglobulin molecule to one or more epitopes. Examples of an epitope binding region as exemplified in the present invention include scFv and FAB.

As used herein, the term "epitope" includes a fragment of a polypeptide or protein or a non-protein molecule having antigenic or immunogenic activity in an animal, preferably in a mammal and most preferably in a human. An epitope having immunogenic activity is a fragment of a polypeptide or protein that elicits an antibody response in an animal. An epitope having antigenic activity is a fragment of a polypeptide or protein to which an antibody or polypeptide specifically binds as determined by any method well-known to one of skill in the art, for example by immunoassays. Antigenic epitopes need not necessarily be immunogenic. Preferably, the term "epitope" as used herein refers to a polypeptide sequence of at least about 3 to 5, preferably about 5 to 10 or 15 and not more than about 1,000 amino acids (or any integer there between), which define a sequence that by itself or as part of a larger sequence, binds to an antibody generated in response to such sequence. There is no critical upper limit to the length of the fragment, which may comprise nearly the full-length of the protein sequence,

or even a fusion protein comprising one or more epitopes. An epitope for use in the subject invention is not limited to a polypeptide having the exact sequence of the portion of the parent protein from which it is derived. Thus the term "epitope" encompasses sequences identical to the native sequence, as well as modifications to the native sequence, such as deletions, additions and substitutions (generally conservative in nature).The epitopes of protein antigens are divided into two categories, conformational epitopes and linear epitopes, based on their structure and interaction with the epitope binding site (Goldsby R *et al.*, (2003) "Antigens (Chapter 3)" Immunology (Fifth edition ed.), New York: W. H. Freeman and Company. pp. 57-75, ISBN 0-7167-4947-5). A conformational epitope is composed of discontinuous sections of the antigen's amino acid sequence. These epitopes interact with the paratope based on the 3-D surface features and shape or tertiary structure of the antigen. Most epitopes are conformational. By contrast, linear epitopes interact with the paratope based on their primary structure. A linear epitope is formed by a continuous sequence of amino acids from the antigen.

The term "hetero-dimeric immunoglobulin" or "hetero-dimeric fragment" or "hetero-dimer" or "hetero-dimer of heavy chains" as used herein includes an immunoglobulin molecule or part of comprising at least a first and a second polypeptide, like a first and a second region, wherein the second polypeptide differs in amino acid sequence from the first polypeptide. Preferably, a hetero-dimeric immunoglobulin comprises two polypeptide chains, wherein the first chain has at least one non-identical region to the second chain, and wherein both chains assemble, i.e. interact through their non-identical regions. More preferably the hetero-dimeric immunoglobulin, has binding specificity for at least two different ligands, antigens or binding sites, i.e. is bispecific. Hetero-dimeric immunoglobulin as used herein includes but is not limited to full length bispecific antibodies, bispecifc Fab, bispecifc $F(ab')_2$, bispecific scFv fused to an Fc region, diabody fused to an Fc region and domain antibody fused to an Fc region.

The term "homo-dimeric immunoglobulin" or "homo-dimeric fragment" or "homo-dimer" or "homo-dimer of heavy chains" as used herein includes an immunoglobulin molecule or part of comprising at least a first and a second polypeptide, like a first and a second region, wherein the second polypeptide is identical in amino acid sequence to the first polypeptide. Preferably, a homo-dimeric immunoglobulin comprises two polypeptide chains, wherein the

first chain has at least one identical region to the second chain, and wherein both chains assemble, i.e. interact through their identical regions. Preferably, a homo-dimeric immunoglobulin fragment comprises at least two regions, wherein the first region is identical to the second region, and wherein both regions assemble, i.e. interact through their protein-protein interfaces.

For all immunoglobulin constant regions included in the present invention, numbering can be according to the IMGT® (IMGT®; *supra*).

For all human CH1, CH2, CH3 immunoglobulin heavy chain constant regions selected from the group consisting of IGHG1, IGHG2, IGHG3 and IGHG4, numbering can be according to the "EU numbering system" (Edelman GM *et al.*, (1969) Proc Natl Acad Sci USA, 63(1): 78-85). A complete correspondence for the human CH1, hinge, CH2 and CH3 constant regions of IGHG1 can be found at the IMGT database (IMGT®; *supra*).

For the human kappa immunoglobulin light chain constant region (IGKC), numbering can be according to the "EU numbering system" (Edelman GM et al., *supra*). A complete correspondence for the human CK region can be found at IMGT database (IMGT®; *supra*).

For the human lambda immunoglobulin light chain constant regions (IGLC1, IGLC2, IGLC3, IGLC6 and IGLC7), numbering can be according to the "Kabat numbering system" (Kabat EA *et al.*, *supra*). A complete correspondence for human IGLC regions can be found at the IMGT database (IMGT®; *supra*).

The human IGHG1 immunoglobulin heavy chain constant regions as referred to herein have the following region boundaries: CH1 region (EU numbering: 118-215), Hinge γ1 region (EU numbering: 216-230), CH2 region (EU numbering: 231-340) and CH3 region (EU numbering: 341-447). The human CK region referred herein spans residues 108 to 214 (EU numbering). The human IGLC1, IGLC2, IGLC3, IGLC6 and IGLC7 regions referred herein span residues 108-215 (Kabat numbering).

The terms "amino acid" or "amino acid residue" as used herein includes natural amino acids as well as non-natural amino acids. Preferably natural amino acids are included.

The term "modification" or "amino acid modification" herein includes an amino acid substitution, insertion and/or deletion in a polypeptide sequence. The terms "substitution" or "amino acid substitution" or "amino acid residue substitution" as used herein refers to a substitution of a first amino acid residue in an amino acid sequence with a second amino acid residue, whereas the first amino acid residue is different from the second amino acid residue i.e. the substituted amino acid residue is different from the amino acid which has been substituted. For example, the substitution R94K refers to a variant polypeptide, in which the arginine at position 94 is replaced with a lysine. For example 94K indicates the substitution of position 94 with a lysine. For the purposes herein, multiple substitutions are typically separated by a slash or a comma. For example, "R94K/L78V" or "R94K, L78V" refers to a double variant comprising the substitutions R94K and L78V. By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid at a particular position in a parent polypeptide sequence. For example, insert -94 designates an insertion at position 94. By "amino acid deletion" or "deletion" as used herein is meant the removal of an amino acid at a particular position in a parent polypeptide sequence. For example, R94- designates the deletion of arginine at position 94.

In certain embodiments, the terms "decrease", "reduce", or "reduction" in binding to Protein A refers to an overall decrease of at least 25%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, or 99% up to 100% (elimination) in the binding of a modified immunoglobulin or fragment thereof to Protein A detected by standard art known methods such as those described herein, as compared to a parental i.e. unmodified immunoglobulin or wild-type IgG or an IgG having the wild-type human IgG Fc region. In certain embodiments these terms alternatively may refer to an overall decrease of 10-fold (i.e. 1 log), 100-fold (2 logs), 1,000-fold (or 3 logs), 10,000-fold (or 4 logs), or 100,000-fold (or 5 logs).

The terms "eliminate", "abrogate", "elimination" or "abrogation" of binding to Protein A refers to an overall decrease of 100% in the binding of a modified immunoglobulin or fragment thereof to Protein A i.e. a complete loss of the binding of a modified immunoglobulin or fragment thereof to Protein A, detected by standard art known methods

such as those described herein, as compared to a parental i.e. unmodified immunoglobulin or wild-type IgG or an IgG having the wild-type human IgG Fc region.

Similarly, the terms "decrease", "reduce", or "reduction" in binding to an affinity reagent refers to an overall decrease of at least 25%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, or 99% up to 100% (elimination) in the binding of a modified immunoglobulin or fragment thereof to the affinity reagent detected by standard art known methods such as those described herein, as compared to a parental, i.e. unmodified immunoglobulin or wild-type IgG or an IgG having the wild-type human IgG Fc region. In certain embodiments these terms alternatively may refer to an overall decrease of 10-fold (i.e. 1 log), 100-fold (2 logs), 1,000-fold (or 3 logs), 10,000-fold (or 4 logs), or 100,000-fold (or 5 logs).

The terms "eliminate" , "abrogate", "elimination" or "abrogation" of binding to an affinity reagent refers to an overall decrease of 100% in the binding of a modified immunoglobulin or fragment thereof to the affinity reagent i.e. a complete loss of the binding of a modified immunoglobulin or fragment thereof to the affinity reagent detected by standard art known methods such as those described herein, as compared to a parental, i.e. unmodified immunoglobulin or wild-type IgG or an IgG having the wild-type human IgG Fc region.

"Bispecific antibodies" are monoclonal antibodies that have binding specificities for at least two different antigens. In certain embodiments, the bispecific antibodies are bispecific antibodies with one or more amino acid modifications in the VH region relative to the parental antibody. In certain embodiments, bispecific antibodies may be human or humanized antibodies. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a target antigen. These antibodies possess a target-antigen-binding arm and an arm which binds a cytotoxic agent, such as, e.g., saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten. Bispecific antibodies can be prepared as full length antibodies or antibody fragments. Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, (1983) Nature, 305: 537-40). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of different antibody molecules, of

which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome and the product yields are low. Similar procedures are disclosed in WO1993/08829 and in Traunecker *et al.*, (1991) EMBO J, 10: 3655-9. According to a different approach, antibody variable regions with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant region sequences. The fusion, for example, is with an immunoglobulin heavy chain constant region, comprising at least part of the hinge, CH2 and CH3 regions. In certain embodiments, the first heavy-chain constant region (CH1), containing the site necessary for light chain binding, is present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors and are co-transfected into a suitable host organism. This provides for flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US4,676,980) and for treatment of HIV infection (WO1991/00360, WO1992/00373 and EP03089). Heteroconjugate antibodies may be made using any convenient cross-linking method. Suitable cross-linking agents are well known in the art (see US4,676,980), along with a number of cross-linking techniques. Antibodies with more than two valencies are also contemplated. For example, trispecific antibodies can be prepared (see Tutt A *et al.* (1991) J. Immunol. 147: 60-9).

In some embodiments the present disclosure provides a bispecific hetero-dimeric immunoglobulin or fragment thereof or a bispecific full-length antibody which binds to CD3 and a disease associated antigens selected from within the groups of: tumor antigens, cytokines, vascular growth factors and lympho-angiogenic growth factors. Preferably the bispecific hetero-dimeric immunoglobulin or fragment thereof or the bispecific antibody

binds to CD3 and a disease associated antigen selected from the group consisting of: CCR3, CCR6, CRTH2, PDL1, BLUT1, PirB,CD33, TROP2, CD105, GD2, GD3, CEA, VEGFR1, VEGFR2, NCAM, CD133, CD123, ADAM17, MCSP, PSCA, FOLR1, CD19, CD20, CD38, EpCAM, HER2, HER3, EGFR, PSMA, IgE, Integrin a4b1, CCR5, LewisY, FAP, MUC-1, Wue-1, MSP, EGFRvIII, P glycoprotein, AFP, ALK, BAGE proteins, CD30, CD40, CTLA4, ErbB3, ErbB4, Mesothelin, OX40, CA125, CAIX , CD66e, cMet, EphA2, HGF/SF , MUC1 , Phosphatidylserine , TAG-72 , TPBG, β-catenin, brc-abl, BRCA1, BORIS, CA9, caspase-8, CDK4, Cyclin-B1, CYP1B1, ETV6-AML, Fra-1, FOLR1, GAGE-1, GAGE-2, GloboH, glypican-3, GM3, gp100, HLA/B-raf, HLA/k-ras, HLA/MAGE-A3, hTERT, LMP2, MAGE1, MAGE2, MAGE3, MAGE4, MAGE6, MAGE12, MART-1, ML-IAP, Muc2, Muc3, Muc4, Muc5, Muc16, MUM1, NA17, NY-BR1, NY-BR62, NY-BR-85, NY-ESO1, p15, p53, PAP, PAX3 PAX5, PCTA-1, PLAC1, PRLR, PRAME, RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, Steap-1, Steap-2, survivin, TAG-72, TGF-β, TMPRSS2, Tn, TRP-1, TRP-2, tyrosinase, uroplakin-3, PSMA. Preferably the bispecific hetero-dimeric immunoglobulin or fragment thereof or the bispecific antibody binds to CD3 and HER2 or CD3 and CD38 or CD3 and OX40 or CD3 and CD19 or CD3 and CD20 or CD3 and Erbitux or CD3 and Vectibix..

Protein A: Protein A is a cell wall component produced by several strains of *Staphylococcus aureus* which consists of a single polypeptide chain. The Protein A gene product consists of five homologous repeats attached in a tandem fashion to the pathogen's cell wall. The five domains are approximately 58 amino acids in length and denoted EDABC, each exhibiting immunoglobulin binding activity (Tashiro M & Montelione GT (1995) Curr. Opin. Struct. Biol., 5(4): 471-481). The five homologous immunoglobulin binding domains fold into a three-helix bundle. Each domain is able to bind proteins from many mammalian species, most notably IgGs (Hober S *et al.,* (2007) J. Chromatogr. B Analyt. Technol. Biomed. Life Sci., 848(1): 40-47). Protein A binds the heavy chain of most immunoglobulins within the Fc region but also within the Fab region in the case of the human VH3 family (Jansson B *et al,* (1998) FEMS Immunol. Med. Microbiol., 20(1): 69-78). Protein A binds IgG from various species including human, mouse, rabbit and guinea pig but does not bind human IgG3 (Hober S *et al.,* (2007) *supra*). The inability of human IgG3 to bind Protein A can be explained by the H435R and Y436F substitutions in the human IgG3 Fc region (EU numbering, Jendeberg *et*

al., (1997) J. Immunol. Methods, 201(1): 25-34). Besides IgG, Protein A also interacts with IgM and IgA.

Amongst human VH subclasses, VH3 is the only subclass to bind Protein A (Graille M *et al.*, (2000) Proc. Natl. Acad. Sci. USA 97(10): 5399-5404), and all five domains of Protein A are known to bind this variable domain subclass (Jansson B et al, (1998) FEMS Immunol. Med. Microbiol., 20(1): 69-78. VH3 based immunoglobulins or fragments thereof are of major importance to the biotechnology industry. VH3 based molecules have been extensively developed since their ability to bind Protein A facilitates their functional pre-screening, and as such many synthetic or donor based phage display libraries or transgenic animal technologies used for antibody discovery are based on the VH3 subclass. In addition VH3 based molecules are often selected for their good expression and stability over other known heavy chain variable domain subclasses.

The capacity of Protein A to bind antibodies with such high affinity is the driving motivation for its industrial scale use in biologic pharmaceuticals. Protein A used for production of antibodies in bio-pharmaceuticals is usually produced recombinantly in *E. coli* and functions essentially the same as native Protein A (Liu HF *et al.,* (2010) MAbs, 2(5): 480-499). Most commonly, recombinant Protein A is bound to a stationary phase chromatography resin for purification of antibodies. Optimal binding occurs at pH8.2, although binding is also good at neutral or physiological conditions (pH 7.0-7.6). Elution is usually achieved through pH shift towards acidic pH (glycine-HCl, pH2.5-3.0). This effectively dissociates most protein-protein and antibody-antigen binding interactions without permanently affecting protein structure. Nevertheless, some antibodies and proteins are damaged by low pH and it is best to neutralize immediately after recovery by addition of 1/10th volume of alkaline buffer such as 1 M Tris-HCl, pH 8.0 to minimize the duration of time in the low-pH condition.

There are various commercially available Protein A chromatography resins. The main differences between these media are the support matrix type, Protein A ligand modification, pore size and particle size. The differences in these factors give rise to differences in compressibility, chemical and physical robustness, diffusion resistance and binding capacity of the adsorbents (Hober S *et al.,* (2007), *supra*). Examples of Protein A chromatography

resins include but are not limited to the MabSelect SuRe™ Protein A resin and MabSelect™ Protein A resin from GE Healthcare as used in examples.

The term "chromatography" refers to protein liquid chromatography and includes fast protein liquid chromatography (FPLC) which is a form of liquid chromatography that is often used to analyze or purify mixtures of proteins. As in other forms of chromatography, separation is possible because the different components of a mixture have different affinities for two materials, a moving fluid (the mobile phase) which passes through a porous solid (the stationary phase). In FPLC, the mobile phase is an aqueous solution, or "buffer". The buffer flow rate can be operated under gravity flow or controlled by a positive-displacement pump which is normally kept at a constant rate, while the composition of the buffer can be varied by drawing fluids in different proportions from two or more external reservoirs. The stationary phase is a resin composed of beads, usually of cross-linked agarose, packed into a cylindrical glass or plastic column. FPLC resins are available in a wide range of bead sizes and surface ligands depending on the application.

The process of "affinity chromatography" involves the use of an affinity reagent as ligands which are cross-linked to the stationary phase and that have binding affinity to specific molecules or a class of molecules. Ligands can be bio-molecules, like protein ligands or can be synthetic molecules. Both types of ligand tend to have good specificity. The most commonly used protein ligand in production is the affinity reagent Protein A. In affinity chromatography when the solution (for example a crude cell supernatant containing a protein of interest) is loaded onto to the column the target protein is usually adsorbed while allowing contaminants (other proteins, lipids, carbohydrates, DNA, pigments, etc.) to pass through the column. The adsorbent itself is normally packed in a chromatography column; though the adsorption stage can be performed by using the adsorbent as a stirred slurry in batch binding mode. The next stage after adsorption is the wash stage, in which the adsorbent is washed to remove residual contaminants. The bound protein is then eluted in a semi-pure or pure form. Elution is normally achieved by changing the buffer or salt composition so that the protein can no longer interact with the immobilized ligand and is released. In some instances the protein of interest may not bind the affinity resin and affinity chromatography is directed at binding unwanted contaminants and the unbound fraction is therefore collected to isolate the

protein of interest. Affinity chromatography can be performed in a fixed bed or a fluidised bed.

The term "gradient mode chromatography" refers to a chromatography method wherein the proportion of the "elution" buffer (buffer B) is increased from 0% to 100% in a gradual or stepwise manner.

The terms "capture-elution mode chromatography" or "capture-elution purification mode" or "capture-elution purification" refers to a chromatography method wherein the proportion of the "elution" buffer (buffer B) is not increased from 0% to 100% in a gradual or stepwise manner but rather directly applied at a 100% after capture and optionally a wash step with running buffer (buffer A).

Development of hetero-dimeric immunoglobulins targeting CD3

The present invention provides an epitope binding region that binds the CD3 protein complex comprising the heavy and light chain CDRs as described *supra* and further comprising a heavy chain variable framework region that is the product of or derived from human gene IGHV3-23*04 (SEQ ID NO: 22). The heavy chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the heavy chain variable region of the corresponding murine antibody OKT3 comprising the amino acid sequence of SEQ ID NO: 18. Preferably the amino acid modification is an amino acid substitution. Typically, no more than seven, preferably no more than six, preferably no more than five, preferably no more than four, more preferably no more than three, even more preferably no more than two, most preferably no more than one amino acid modifications are performed within a framework region. In some embodiments the present disclosure provides an epitope binding region that binds to the CD3 protein complex, wherein the amino acid modification of the framework regions of the heavy chain variable region comprise an amino acid substitution at amino acid position selected from the group consisting of: 34, 48, 49, 58, 69, 71 and 73 and wherein the amino acid position of each group member is indicated according to the Kabat numbering. Preferably, amino acid substitutions of the framework regions of the heavy chain variable region are selected from the group consisting of: I34M, V48I, A49G, R58N, R58Y, I69L, A71T and T73K. Preferred amino acid substitution of the framework regions of the heavy chain variable region are at amino acid positions selected

from the group consisting of 34, 49 and 71. More preferred amino acid substitutions of the framework regions of the heavy chain variable region are selected from the group consisting of I34M, A49G and A71T.

In a further aspect, the epitope binding region of the first polypeptide that binds the CD3 protein complex comprises a light chain variable framework region that is the product of or derived from a human gene selected from the group consisting of: IGKV1-39*01 (SEQ ID NO: 23) and IGKV3-20*01 (SEQ ID NO: 24). The light chain variable framework region comprises at least one amino acid modification from the corresponding framework region of the light chain variable region of the corresponding murine antibody OKT3 comprising the amino acid sequence of SEQ ID NO: 19. Preferably the amino acid modification is an amino acid substitution. Typically, no more than eight, preferably no more than seven, preferably no more than six, preferably no more than five, preferably no more than four, more preferably no more than three, even more preferably no more than two, most preferably no more than one amino acid modifications are performed within a framework region. In some embodiments the present disclosure provides an epitope binding region that binds to the CD3 protein complex, wherein the amino acid modification of the framework regions of the light chain variable region sequence comprises an amino acid substitution at amino acid position selected from the group consisting of: 4, 33, 34, 46, 47, 66, 71 and 96. Preferably, amino acid substitutions of the framework regions of the light chain variable region are selected from the group consisting of: M4L, V33M, A34N, L46R, L47W, R66G, F71Y and P96F. Preferred amino acid substitution of the framework regions of the light chain variable region are at amino acid positions selected from the group consisting of 4, 46 and 47. More preferred amino acid substitutions of the framework regions of the light chain variable region are selected from the group consisting of M4L, L46R, L47W and F71Y. In some embodiments the epitope binding region of the first polypeptide that binds to the CD3 protein complex may comprise amino acid modifications of the framework regions of the heavy chain variable region sequence as set out above and amino acid modifications of the framework regions of the light chain variable region sequence as set out above.

The present disclosure also provides an antibody or fragment thereof that binds to the CD3 protein complex that comprises a heavy chain sequence selected from the group consisting of SEQ ID NOs: 27 to 38, 64-68 and 359, preferably selected consisting of SEQ ID NO: 359.

The present disclosure also provides an antibody or fragment thereof that binds to the CD3 protein complex that comprises a light chain sequence selected from the group consisting of SEQ ID NOs: 39 to 47, 69 to 90 360, 399 and 400 preferably consisting of SEQ ID NO: 360.

Given that each of these heavy and light chain variable region sequences can bind to the CD3 protein complex, the heavy and light chain variable region sequences can be "mixed and matched" to create anti-CD3 binding molecules of the invention. CD3 binding of such "mixed and matched" antibodies can be tested using the binding assays described e.g. in the Examples.

Engineering of the immunoglobulin constant region to promote hetero-dimer formation over homo-dimer formation

Methods to produce hetero-dimeric immunoglobulins are known in the art and one of the simplest methods relies on expressing the two distinct immunoglobulin chains in a single cell (WO95/33844, Lindhofer H & Thierfelder S). Without engineering, this straightforward method is limited by the formation of homo-dimeric species over the hetero-dimer of interest (Kufer P *et al.*, (2004) Trends Biotechnol., 22(5): 238-244). When using complementary technologies that will enhance heavy chain hetero-dimerization (Merchant AM *et al.*, (1998) Nat. Biotechnol., 16(7): 677-681), greater hetero-dimer production can be achieved but still results in the production of a significant amount of undesirable homo-dimers (Jackman J *et al.*, (2010) J Biol Chem., 285(27):20850-9, Klein C *et al.*, (2012) MAbs, 4(6):653-63). The present invention therefore utilises the BEAT® technology described method (PCT publication No: WO2012/131555), which is based on a unique concept of bio-mimicry that exhibit superior hetero-dimerisation over prior art methods. The BEAT technology is based on an interface exchange between naturally occurring homo or hetero-dimeric immunoglobulin domain pairs to create new hetero-dimers which can be used as building blocks for Fc-based bispecific antibodies.

In one aspect, the present invention provides a hetero-dimeric immunoglobulin or fragment thereof comprising first and second polypeptides comprising an engineered immunoglobulin constant region with a modified CH3 domain having a protein-protein interface, wherein the protein-protein interface of the first polypeptide comprises an amino acid substitution at a position selected from the group consisting of: 3, 5, 7, 20, 22, 26, 27, 79, 81, 84, 84.2, 85.1,

86, 88 and 90 (IMGT® numbering), and wherein the protein-protein interface of the second polypeptide comprises an amino acid substitution at position 84.4 and at a position selected from the group consisting of 3, 5, 7, 20, 22, 26, 27, 79, 81, 84, 84.2, 85.1, 86, 88 and 90 (IMGT® numbering).

In a further embodiment, the present invention provides a hetero-dimeric immunoglobulin or fragment thereof, wherein the first and second polypeptides comprise an engineered immunoglobulin constant region with a modified CH3 domain having a protein-protein interface, wherein the protein-protein interface of the first polypeptide comprises an amino acid substitution at position 88 and at a position selected from the group consisting of: 3, 5, 7, 20, 22, 26, 27, 79, 81, 84, 84.2, 85.1, 86 and 90 (IMGT® numbering), and wherein the protein-protein interface of the second polypeptide comprises an amino acid substitution at position 85.1 and/or 86 and at a position selected from the group consisting of 3, 5, 7, 20, 22, 26, 27, 79, 81, 84, 84.2, 84.4, 88 and 90 (IMGT® numbering), wherein the amino acid residue substituted at position 88 in the first engineered immunoglobulin constant region is interacting with the amino acid residue substituted at position 85.1 and/or 86 in the second engineered immunoglobulin constant region,
wherein the amino acid position of each group member is indicated according to the IMGT® numbering.

Preferably the amino acid residue which is substituted in the protein-protein interface of the first engineered immunoglobulin constant region at position 88 is 88W and conservative amino acid substitutions thereof, wherein the amino acid position is indicated according to IMGT® numbering. More preferably, the amino acid residue which is substituted in the protein-protein interface of the first engineered immunoglobulin constant region at position 88 is 88W and wherein the further amino acid residue substituted in the protein-protein interface of the first engineered immunoglobulin constant region is selected from the group consisting of: 3A, 20V, 20T, 20A, 20N, 20Q, 20E, 20S, 20K, 20W, 22A, 22G, 22T, 22L, 22I, 22V, 26R, 26Q, 26T, 26K, 26V, 26S, 26N, 26E, 79Y, 85.1T, 85.1M, 85.1A, 85.1S, 85.1R, 85.1H, 85.1K, 85.1F, 85.1C, 85.1N, 85.1W, 86S, 86I, 86T, 86H, 86Q, 86V, 86W, 86Y, 86F and 90N, wherein the amino acid position is indicated according to the IMGT® numbering.

Preferably the amino acid residue which is substituted at position 85 and 86 in the protein-protein interface of the second engineered immunoglobulin constant region is selected from the group consisting of: 85.1A, 85.1S, 85.1C and 86S and conservative amino acid substitutions thereof (IMGT® numbering). More preferably the amino acid residue which is substituted in the protein-protein interface of the second engineered immunoglobulin constant region is selected from the group consisting of: 85.1A, 85.1S, 85.1C and 86S and wherein the further amino acid residue substituted in the protein-protein interface of the second engineered immunoglobulin constant region is selected from the group consisting of: 3E, 5A, 7F, 20T, 22V, 26T, 81D, 84L, 84.2E, 88R and 90R and conservative amino acid substitutions thereof (IMGT® numbering).

In a preferred embodiment the amino acid residue which is substituted in the protein-protein interface of the first engineered immunoglobulin constant region at position 88 is 88W and wherein the further amino acid residue substituted in the protein-protein interface of the first engineered immunoglobulin constant region is: 3A, 20K, 22V, 26T, 79Y, 85.1S, 86V and 90N and, wherein the amino acid residues which are substituted in the protein-protein interface of the second engineered immunoglobulin constant region at positions 85.1 and 86 are 85.1A, 85.1S or 85.1A and 86S and wherein the further amino acid residue substituted in the protein-protein interface of the second engineered immunoglobulin constant region is: 3E, 5A, 7F, 20T, 22V, 26T, 81D, 84L, 84.2E, 84.4Q, 88R and 90R (IMGT® numbering).

In an alternative embodiment, the present invention provides a hetero-dimeric immunoglobulin or fragment thereof, wherein the first and second polypeptides comprise an engineered immunoglobulin constant region with a modified CH3 domain having a protein-protein interface, wherein the protein-protein interface of the first polypeptide comprises an amino acid substitution at position 20, and at a position selected from the group consisting of: 3, 5, 7, 22, 26, 27, 79, 81, 84, 84.2, 85.1, 86, 88 and 90 and, wherein the protein-protein interface of the second polypeptide comprises an amino acid substitution at position 26 and at a position selected from the group consisting of: 3, 22, 27, 79, 81, 84, 85.1, 86, and 88, wherein the amino acid residue substituted at position 20 in the first engineered immunoglobulin constant region is interacting with the amino acid residue substituted at position 26 in the second engineered immunoglobulin constant region,

wherein the amino acid position of each group member is indicated according to the IMGT® numbering.

Preferably the amino acid residues which are substituted in the protein-protein interface of the first engineered immunoglobulin chain comprise the amino acid residues at positions 20 and 22, and optionally a further amino acid residue at a position selected from the group consisting of: 3, 5, 7, 26, 27, 79, 81, 84, 84.2, 84.4, 85.1, 86, 88 and 90 and, wherein the amino acid residues which are substituted in the protein-protein interface of the second engineered immunoglobulin chain comprise the amino acid residues at positions 26 and at a further position selected from the group consisting of: 3, 5, 7, 20, 22, 27, 79, 81, 84, 84.2, 84.4, 85.1, 86, 88 and 90, wherein the amino acid position of each group member is indicated according to the IMGT® numbering. Preferably the amino acid residues which are substituted in the protein-protein interface of the first engineered immunoglobulin chain comprise the amino acid residues at positions 20 and 22, and optionally a further amino acid residue at a position selected from the group consisting of: 3, 5, 7, 26, 27, 79, 81, 84, 84.2, 84.4, 85.1, 86, 88 and 90 and, wherein the amino acid residues which are substituted in the protein-protein interface of the second engineered immunoglobulin chain comprise the amino acid residues at positions 26 and 86 and optionally at a further position selected from the group consisting of 3, 5, 7, 20, 22, 27, 79, 81, 84, 84.2, 84.4, 85.1, 88 and 90, wherein the amino acid position of each group member is indicated according to the IMGT® numbering.

More preferably the amino acid residue which is substituted at position 20 in the protein-protein interface of the first engineered immunoglobulin constant region is selected from the group consisting of 20V, 20T, 20A, 20N, 20Q, 20K, 20S, 20W and 20E and wherein the further amino acid residue substituted in the protein-protein interface of the first engineered immunoglobulin constant region is selected from the group consisting of 3A, 22A, 22G, 22L, 22I, 22V, 22T, 26K, 26R, 26Q, 26T, 26V, 26S, 26N, 26E, 79Y, 85.1W, 85.1F, 85.1T, 85.1M, 85.1A, 85.1S, 85.1R, 85.1H, 85.1K, 85.1C, 85.1N, 86W, 86Y, 86S, 86I, 86H, 86Q, 86V, 86T, 86F, 88Q, 88L, 88V, 88R, 88E, 88T, 88I, 88Y, 88K, 88W and 90N, and wherein the amino acid residue which is substituted at position 26 in the protein-protein interface of the second engineered immunoglobulin constant region is selected from the group consisting of 26T and 26E and conservative amino acid substitutions thereof, wherein the amino acid position is indicated according to the IMGT® numbering.

In a most preferred embodiment the amino acid residue which is substituted in the protein-protein interface of the first engineered immunoglobulin constant region at position 20 is 20K and wherein the further amino acid residue substituted in the protein-protein interface of the first engineered immunoglobulin constant region is 3A, 22V, 26T, 79Y, 85.1S, 86V, 88W and 90N and, wherein the amino acid residues which are substituted in the protein-protein interface of the second engineered immunoglobulin constant region at position 26 is 26T and wherein the further amino acid residue substituted in the protein-protein interface of the second engineered immunoglobulin constant region is 3E, 5A, 7F, 20T, 22V, 81D, 84L, 84.2E, 84.4Q, 85.1C/S/A, 86S, 88R and 90R (IMGT® numbering).

Development of hetero-dimeric immunoglobulins targeting CD3 and a disease associated antigen

As a first step (Example 1), the substitutions that reduce or abrogate binding to Protein A were assayed in homo-dimeric immunoglobulins based on FAB or scFv fragments. It was found that the presence of a variable heavy chain domain of the VH3 subclass within the heavy chain which has substitutions for reduced or no binding to Protein A, hampers any differential affinity methods based on Protein A. Solutions to these major impediments were found in the forms of framework substitutions that reduce or abrogate Protein A binding to the VH3 subclass for the differential affinity methods based on Protein A.

In a second step (Example 2.1), a humanised antibody targeting the human CD3 (epsilon subunit) was generated by grafting the CDRs of a murine anti-CD3 antibody onto IGVH3-23 and IGVK1 or IGVK3 human germline frameworks. The best humanised variants had the Protein A binding site present in their VH domain abrogated using a G65S or N82aS substitution (Kabat numbering). These variants were formatted as FAB or scFv fragments.

In a third step, antigen binding sites of antibodies targeting disease associated antigens were generated. CDRs of murine antibodies could be grafted onto the human germline frameworks IGVH3-23 and IGVK1 (Examples 2.3, 2.4 and 2.6-2.10). Alternatively CDRs of antibodies isolated from phage display libraries could be based on the VH3 variable domain subclass or grafted onto the human germline frameworks IGVH3-23 and IGVK1 (Examples 2.5 and 2.6).

The Protein A binding site in the VH domain of the epitope binding region was abrogated using the G65S or N82aS substitutions (Kabat numbering).

In a fourth step, hetero-dimeric antibodies were produced based on the BEAT® technology (as described in WO2012/131555) in which the anti-CD3 antibody from Example 2.1 2 2 and the epitope binding region of the disease associated antigen as described in Examples 2.2-2.10 were used in an scFv-FAB format or vice versa (Example 3.1). Since a difference in the number of Protein A binding sites between homo- and hetero-dimeric species can be used to isolate the hetero-dimeric species by Protein A chromatography, the bispecific antibodies of the present invention were engineered to result in one of the two homo-dimeric species having no Protein A binding site and therefore no binding to Protein A resin. Furthermore, in order to improve the safety profile of the BEAT antibodies, the Fc receptor binding was reduced or eliminated by engineering the two substitutions L234A and L235A (EU numbering) into the lower hinge region of the Fc region.

Examples

Materials and Methods

Construction of expression vectors for transient mammalian cell expression

cDNAs encoding the different polypeptide chains in part or in full were first gene synthetized by GENEART AG (Regensburg, Germany) and modified using standard molecular biology techniques. PCR products were digested with appropriate DNA restriction enzymes, purified and ligated in a modified pcDNA3.1 plasmid (Invitrogen AG, Zug, Switzerland) carrying a CMV promoter and a bovine hormone poly-adenylation (poly(A)) previously digested with the same DNA restriction enzymes . All polypeptide chains were independently ligated in this expression vector where secretion was driven by the murine VJ2C leader peptide.

Expression of recombinant proteins

Antibodies, ScFv-Fc fusion proteins, BEAT antibodies and antigens were expressed as described below unless otherwise indicated. For transient expression, equal quantities of each engineered chains vectors were co-transfected into suspension-adapted HEK293-EBNA cells (ATCC-LGL standards, Teddington, UK; Cat. No: CRL-10852) using Polyethyleneimine (PEI; Sigma, Buchs, Switzerland). Typically, 100ml of cells in suspension at a density of 0.8-

1.2 million cells per ml is transfected with a DNA-PEI mixture. When recombinant expression vectors encoding each engineered chain genes are introduced into the host cells, the immunoglobulin construct is produced by further culturing the cells for a period of 4 to 5 days to allow for secretion into the culture medium (EX-CELL 293, HEK293-serum-free medium (Sigma), supplemented with 0.1% pluronic acid, 4mM glutamine and 0.25µg/ml geneticin). Cell-free culture supernatants containing the secreted immunoglobulins were prepared by centrifugation followed by sterile filtration and used for further analysis.

Differential Protein A affinity chromatography (Example 1)

Purification of Fc 133 fragment and homo-dimeric scFv-Fc immunoglobulins

Capture-elution mode chromatography

Supernatants were conditioned with 0.1 volume (V) of 1M Tris-HCl pH 8.0 prior purification. Protein G Sepharose™ 4 Fast Flow (GE Healthcare Europe GmbH, Glattbrugg, Switzerland; catalogue number 17-0618-01) was added to conditioned supernatants. Mixtures were incubated overnight at 4°C. After incubation, bound proteins were washed with 10CVs of PBS pH 7.4, eluted with 4 column volumes (CVs) of 0.1M Glycine pH 3.0 and neutralised with 0.1V of 1M Tris-HCl pH8.0. Supernatant, flow through and elution fractions were analysed under non-reduced conditions by SDS-PAGE (NuPAGE Bis-Tris 4-12% acrylamide, Invitrogen AG, Basel, Switzerland).

Gradient mode chromatography

Post production, cell-culture supernatants containing the Fc 133 fragment were first purified in capture-elution mode chromatography using Protein G Sepharose™ 4 Fast Flow (above). Eluted material from capture-elution mode chromatography were subsequently loaded onto a 1ml HiTrap™ MabSelect SuRe™ Protein A column (Protein A binding site mutants). The column was pre-equilibrated in 0.2M phosphate citrate buffer pH 8.0 and operated on an ÄKTApurifier™ chromatography system (both column and instrument from GE Healthcare Europe GmbH; column catalogue No: 11-0034-93) at a flow rate of 1ml/min. Elution was performed with a pH linear gradient combining various amounts of two buffers (running buffer (A): 0.2M phosphate citrate buffer pH 8.0 and elution buffer (B): 0.04M phosphate citrate buffer pH 3.0. The linear gradient went from 0% B to 100% B in five column volumes (CVs). Eluted fractions were neutralised with 0.1V of 1M Tris-HCl pH 8.0. Supernatant, flow

through and elution fractions were analysed under non-reduced conditions by SDS-PAGE (NuPAGE Bis-Tris 4-12% acrylamide, Invitrogen AG, Basel, Switzerland).

Purification of homo-dimeric FAB-Fc immunoglobulins and FAB fragments.

Post production, cell culture supernatants were conditioned with 0.1V of 1M Tris-HCl pH 8.0. Protein L resin (Genescript, Piscataway, USA) was added to the conditioned supernatant and incubated overnight at 4°C. After incubation, bound proteins were washed with ten CVs of PBS pH7.4, eluted with 4CVs of 0.1M Glycine pH 3.0, and finally neutralised with 0.1V of 1M Tris-HCl pH 8.0. To assess Protein A binding, Protein L purified FAB were injected on a 1ml HiTrap MabSelect™ column (GE Healthcare Europe GmbH, Glattbrugg, Switzerland) at pH8.0 (Citric acid/Na_2HPO_4 buffer). Elution was performed with a pH linear gradient combining various amounts of two buffers (running buffer (A): 0.2 M phosphate citrate buffer pH8.0 and elution buffer (B): 0.04 M phosphate citrate buffer pH3.0). The linear gradient went from 0% B to 100% B in 5CVs. Eluted fractions were neutralised with 0.1V of 1M Tris-HCl pH8.0. Supernatant, flow through and elution fractions were analysed under non-reduced conditions by SDS-PAGE (NuPAGE Bis-Tris 4-12% acrylamide, Invitrogen AG, Basel, Switzerland).

Purification and testing of VH3 based homo-dimeric FAB-Fc and scFv-Fc immunoglobulins abrogated for Protein A binding in their Fc and VH3 domains.

Purification scheme included a capture-elution mode chromatography followed by a gradient mode chromatography according to the procedure described above.

Differential Protein A affinity chromatography (Examples 1 & 3)

Post production, cell-free supernatants were loaded onto a 1ml HiTrap™ MabSelect SuRe™ Protein A column pre-equilibrated in 0.2M phosphate citrate buffer pH 6.0 and operated on an ÄKTApurifier™ chromatography system (both from GE Healthcare Europe GmbH; column Cat. No: 11-0034-93) at a flow rate of 1ml/min. Running buffer was 0.2 M phosphate citrate buffer pH 6. Elution of the hetero-dimer of interest was performed using 20 mM sodium citrate buffer pH 4 whilst homo-dimeric species were eluted with 0.1 M glycine, pH3.0. Elution was followed by OD reading at 280 nm; fraction containing the hetero-dimer of interest were pooled and neutralized with 0.1 volume of 1M Tris pH 8.0 (Sigma).

Supernatant, flow through and elution fractions were analysed under non-reduced conditions by SDS-PAGE (NuPAGE Bis-Tris 4-12% acrylamide, Invitrogen AG, Basel, Switzerland).

Differential Scanning Calorimetry (DSC)

The thermal stabilities of antibodies were compared using calorimetric measurements. Calorimetric measurements were carried out on a VP-DSC differential scanning microcalorimeter (MicroCal-GE Healthcare Europe GmbH, Glattbrugg, Switzerland). The cell volume was 0.128 ml, the heating rate was 1°C/min and the excess pressure was kept at 64 p.s.i. All protein fragments were used at a concentration of 1-0.5 mg/ml in PBS (pH 7.4). The molar heat capacity of each protein was estimated by comparison with duplicate samples containing identical buffer from which the protein had been omitted. The partial molar heat capacities and melting curves were analysed using standard procedures. Thermograms were baseline corrected and concentration normalized before being further analysed using a Non-Two State model in the software Origin v7.0.

The expected melting profiles for the human IgG subclasses are known (Garber E & Demarest SJ (2007) Biochem Biophys Res Commun, 355(3): 751-7) and all profiles have been shown to contain three unfolding transitions corresponding to the independent unfolding of the CH2, CH3 and FAB domains. Of the four human IgG subclasses, IGHG1 has the most stable CH3 domain (~85°C); while other subclasses CH3 domains are less stable, although none are known to melt below 70°C. Similarly, all subclasses are known to have a melting temperature of ~70°C for the CH2 domain.

Purity assessment by capillary gel electrophoresis (Example 3.2)

Non-reduced sample preparation

40 µg of desalted protein sample was buffered in SDS sample buffer (Beckman Coulter International S.A., Nyon, Switzerland; IgG Purity Kit, Cat. No: A10663) containing 5 mM Iodoacetamide (Sigma). A 10-kDa internal standard was added to the samples. The sample-mixtures were heated at 70°C for 10 min.

Capillary gel electrophoresis

Following sample preparation, samples were run on a ProteomeLab PA 800 (Beckman Coulter International S.A., Nyon, Switzerland) fitted with a photodiode array detector (DAD)

set at 220 nm. Bare-fused silica capillaries of 50 μm ID × 30.2 cm (20.2 cm effective length to detector) were used as separation medium. Sample injection and separation were performed at constant voltages of 5 and 15 kV, respectively, with reverse polarity in SDS-molecular weight gel buffer. The data were recorded at a rate of 2 Hz and current was stable during separation. Capillary and samples were thermo-stated at 25°C.

Affinity measurements by SPR (Example 1)

SPR testing of FAB fragments abrogated for Protein A binding

cDNA encoding the human HER2 extracellular region fused to an IGHG1 Fc fragment was cloned into an expression vector similar to the heavy and light expression vectors described above and transiently transfected in HEK293E cells using the PEI method (see PCT Publication No: WO2012131555). Supernatants were conditioned with 0.1V of 1 M Tris-HCl pH8.0 and the antigen purified by Protein A capture-elution chromatography as described in Example 1. For SPR experiments, a monoclonal mouse anti-human IgG (Fc) antibody sensor chip was used, this allowed for the capture the Fc fused recombinant HER2 antigen in the correct orientation (Human Antibody Capture Kit, catalogue number BR-1008-39, GE Healthcare Europe GmbH). Measurements were recorded on a BIAcore™ 2000 instrument (GE Healthcare Europe GmbH, Glattbrugg, Switzerland). Different dilutions of anti-HER2 FAB (50, 25, 12.5, 6.25, 3.13, 1.57, 0.78, 0.39nM) were injected over the sensor chip for 4min at 30μl/min. For each measurement, after seven minutes of dissociation, a 3M $MgCl_2$ solution was injected for 1min at 30 μl/min for regeneration. Data (sensorgram: fc2-fc1) were fitted with a 1:1 Langmuir. To account for the experimental variations in captured HER2-Fc at the beginning of each measurement, the Rmax value was set to local in all fits. Measurements were performed in duplicate, and included zero-concentration samples for referencing. Both Chi2 and residual values were used to evaluate the quality of a fit between the experimental data and individual binding models

Affinity measurements by SPR (Examples 2 & 3)

SPR analysis was used to measure the association and dissociation rate constants for the binding kinetics of the different antibodies (murine and humanized antibodies). The binding kinetics of antibodies were measured on a BIAcore 2000 instrument (BIAcore-GE Healthcare Europe GmbH, Glattbrugg, Switzerland) at room temperature and analysed with the BiaEvaluation software (version 4.1, BIAcore-GE Healthcare Europe GmbH).

Measurements were performed on CM5 sensor chips (GE Healthcare Europe GmbH, Cat. No: BR-1000-14) individually coupled with the ligand of interest using a commercial amine coupling kit (GE Healthcare Europe GmbH, Cat. No: BR-1000-50). Protein G ligand was from Pierce (Thermo Fisher Scientific-Perbio Science S.A., Lausanne, Switzerland, Cat. No: 21193).

Data (sensorgram: fc2-fc1) were fitted with a 1:1 Langmuir model with or without mass transfer as indicated. In capture experiments, to account for the experimental variations in at the beginning of each measurement, the Rmax value was set to local in all fits. Dissociation times were of at least 350 seconds. Measurements were performed in triplicate and included zero-concentration samples for referencing. Both Chi2 and residual values were used to evaluate the quality of a fit between the experimental data and individual binding models.

Affinity measurements on HPB-ALL cells by FACS

HPB-ALL cells (DSMZ, Braunschweig, Germany, Cat. No: ACC483) were used as CD3 positive cell line for FACS staining. HPB-ALL were maintained in RPMI 1640 supplemented with 10% FCS and 100 U/ml Penicillin and 100ug/ml streptomycin. 100 µl dilution series of the chimeric OKT3 antibody and humanized variants were incubated with $4x10^5$ HPB-all cells in PBS supplemented with 1% BSA and 0.1% Sodium Azide (referred as FACS buffer) for 45 min on ice. An irrelevant human IgG1 was used as isotype control and the chimeric OKT3 antibody as positive control. After washing, cells were incubated with a 1/200 dilution of anti-Human Fc-PE (EBioscience, Vienna, Austria) for 45 min on ice. Cells were then washed again and resuspended in 200ul FACS buffer. The relative mean fluorescence of each sample was measured on FACSCalibur (BD Biosciences, Allschwil, Switzerland) Results are summarized in FIG. 9 as the relative staining of HBP-ALL compared to the chimeric OKT3 antibody.

Cell-lines for in vitro assays

Human HER2 positive cell lines

Human cells expressing HER2 antigen have been described in PCT Publication No: WO2010108127. HER2 positive human cell lines as used herein were as follows:

BT474 (ATCC-LGL standards; Cat. No: HTB-20)

Culture conditions: RPMI medium supplemented with 10% heat-inactivated FBS, 1% penicillin-streptomycin (Invitrogen AG, Cat. No: 10378-016), 1% sodium pyruvate solution (PAA Laboratories, Pasching, Austria; Cat. No: S11-003), 1% MEM Non-Essential Amino Acids (PAA Laboratories, Cat. No: M11-00dsmz3) and 1% GlutaMAX-1 (Invitrogen AG, Cat. No: 35050-038) in 150 cm^2 tissue culture flask (TPP, Trasadingen, Switzerland; Cat. No: 90150). Cells were passaged twice per week.

JIMT-1 (DSMZ, Braunschweig, Germany, Cat. No: ACC589)

Culture conditions: Dulbeco's modified essential medium (DMEM (1X)) + GlutaMAX-1 (Invitrogen AG, Cat. No: 31966-012), supplemented with 10% heat-inactivated FBS, 1% penicillin-streptomycin (Invitrogen AG, Cat. No: 10378-016), 1% sodium pyruvate solution (PAA Laboratories, Cat. No: S11-003), 1% MEM Non-Essential Amino Acids (PAA Laboratories, Cat. No: M11-003) and 1% GlutaMAX-1 (Invitrogen AG, Cat. No: 35050-038). Cells were passaged 2-3 times per week.

MDA-MB-231 (ATCC-LGL standards; Cat. No: HTB-26).

Culture conditions: same culture conditions as JIMT-1.

HT-1080 (ATCC-LGL standards; Cat. No: CCL-121).

Culture conditions: HT1080 cells are cultured in EMEM medium supplemented with 10% heat-inactivated FBS, 1% penicillin-streptomycin (Invitrogen AG, Cat. No: 10378-016), and 1% glutamine (Invitrogen AG, Cat. No: 25030-024). The cells are cultured at split three times a week (1 in 6 dilution).

NCI-N87 (ATCC-LGL standards; Cat. No: CRL-5822).

Culture conditions: NCI-N87 cells are cultured in RPMI 1640 medium with 10% heat-inactivated FBS, 1% penicillin-streptomycin (Invitrogen AG, Cat. No: 10378-016), 1% sodium pyruvate solution (PAA Laboratories, Pasching, Austria; Cat. No: S11-003), 1% MEM Non-Essential Amino Acids (PAA Laboratories, Cat. No: M11-00dsmz3), and 1% glutamine (Invitrogen AG, Cat. No: 25030-024). The cells are split twice a week (1 in 3 dilution).

Human CD38 positive cell lines

Human cells expressing CD38 antigen have been described in PCT Publication Nos: WO2005103083, WO2008047242, WO2011154453 and WO2012092612. CD38 positive human cell lines as used herein were as follows:

Stable recombinant CHO[CD38] cells

A gene coding for human CD38 was ordered at Source Biosciences (Berlin, Germany, Cat.-No.: IRAU37D11, 4309086). Human CD38 was amplified using primers adding a kozak sequence, a start codon followed by a signal peptide (murine V leader) to the 5' end and a NheI restriction site to the 3' end. The amplicon was cut using NheI and HindIII and cloned into the expression cassette of pT1, a pcDNA3.1 (Invitrogen AG) derived vector developed in-house. The expression cassette of pT1 links the expression of the gene of interest with expression of GFP and PAC (the gene for puromycin resistance) using two IRES (internal ribosome entry sites) on a polycistronic mRNA. A midiprep of the plasmid was prepared and the cloned CD38 open reading frame was confirmed by DNA sequencing. Suspension CHO-S cells (Invitrogen AG) were transfected using polyethyleneimine (JetPEI®, Polyplus-transfection, Illkirch, France) in 50 ml bioreactor format (TubeSpin 50 bioreactors, TPP, Trasadingen, Switzerland). For this purpose, exponential growing cells were seeded in OptiMEM medium (Invitrogen AG, Cat. No.: 31985-047). A JetPEI®:DNA complex was added to the cells. After 5 h incubation of the cells with the JetPEI®:DNA complex at 37°C under shaking (200 RPM) for endocytosis, one volume of culture medium PowerCHO2 (Lonza, distributor RUWAG Lifescience, Bettlach, Switzerland, Cat. No:BE12-771Q) supplemented with 4 mM Gln was added to the cell suspension. The cells were then incubated on a shaken platform at 37°C, 5% CO2 and 80% humidity. One day after transfection the cells were seeded in 96 well plates at different concentrations in selective medium containing puromycin (Sigma, Cat. No: P8833-25mg). After approximately 14 days of selection under static conditions, 46 high GFP expressing cell pools were expanded as suspension cultures using TubeSpin 50 bioreactors. Once successfully adapted to suspension, the cells were analysed for CD38 by FACS. Stable CHO[CD38] clones with a homogenous CD38 staining profile were selected and used herein.

Other CD38 positive cell lines included:

NCI-H929 (ATCC-LGL standards; Cat. No: CRL-9068).

Namalwa (ATCC-LGL standards; Cat. No: CRL-1432)

U266 (ATCC-LGL standards; Cat. No: TIB-196)

RPMI 8226 (ATCC-LGL standards; Cat. No: CCL-155)

Culture conditions: RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 1% penicillin-streptomycin (Invitrogen AG) and 1% GlutaMAX-1 (Invitrogen AG)
Raji (ATCC-LGL standards; Cat. No: CCL-86)
Daudi (ATCC-LGL standards; Cat. No: CCL-213)

Human OX40 positive cell lines

Human cells expressing OX40 antigen have been described in PCT Publication No: WO2013008171.

Peripheral blood mononuclear cells (PBMCs) and HBP-ALL are examples of human OX40 positive cell lines.

Stable recombinant CHO[OX40] cells were used herein. A recombinant CHO cell line carrying a synthetic cDNA coding for human OX40 was engineered using a similar protocol to that of the stable recombinant CHO[CD38] cell line described above.

Human CD20 positive cell lines

Human cells expressing CD20 antigen have been described in PCT Publications No: WO2010095031. An example of CD20+ cancer cells is the Daudi cancer cell-line (ATCC-LGL standards; Cat. No: CCL-213), these B lymphoblast cancer cells are cultured in RPMI 1640 medium (Sigma) supplemented with 20% FBS and 1%P/S; 1% L-Glut; 1% Na-Pyr and 1% NEAA. The cells are cultured at 37°C with 5% CO2 supplementation.

Human EGFR positive cell lines

Human cells expressing EGFR antigen have been described in PCT Publication No: WO2010108127. An example of EGFR+ cancer cells is the HT-29 cancer cell-line (ATCC-LGL standards; Cat. No: HTB-38), these colorectal cancer cells are cultured are cultured in McCoy's 5A medium (Sigma) supplemented with 10% FBS and 1%P/S; 1% L-Glut; 1% Na-Pyr and 1% NEAA. The cells are cultured at 37°C with 5% CO2 supplementation.

Human CD19 positive cell lines

Human cells expressing CD19 antigen have been described in PCT Publication No: WO2010/095031. Namalwa (ATCC-LGL standards; Cat. No: CRL-1432) and Raji (ATCC-LGL standards; Cat. No: CCL-86) are examples of human CD20 positive cell lines.

Human membrane IgE positive cell lines

PCT Publication No: WO2010/033736 on page 71 describes a method to class switch human PBMCs into IgE producing B cells by adding interleukin-4 (IL-4) and anti-CD40 antibody.

Recombinant target antigens

Human CD3 gamma-epsilon-Fc fusion protein

A cDNA encoding the human CD3 gamma extracellular region (UniProt accession No: P09693 residues 23-103 (SEQ ID NO: 184); UniProt Consortium (2013) Nucleic Acids Res., 41(Database issue): D43-7; http://www.uniprot.org/) fused to the human CD3 epsilon extracellular region (UniProt accession No: P07766, residues 22-118 (SEQ ID NO: 185)) by a 26-residue peptide linker (sequence: GSADDAKKDAAKKDDAKKDDAKKDGS; SEQ ID NO: 186) was first synthetized by GENEART AG (Regensburg, Germany). This synthetic gene was fused to a human IgG1 Fc portion using standard overlap PCR techniques and a human IgG1 Fc cDNA template also obtain from Geneart AG. The resulting cDNA was cloned in the modified pcDNA3.1 plasmid mentioned above.

For transient expression of the CD3 gamma-epsilon-Fc protein (SEQ ID NO: 187), the recombinant vector was transfected into suspension-adapted HEK-EBNA cells (ATCC-CRL-10852) using Polyethyleneimine (PEI) as described above. The CD3 gamma-epsilon-Fc construct was then purified from cell-free supernatant using recombinant Streamline rProtein A media (GE Healthcare Europe GmbH, Glattbrugg, Switzerland) and used for further analysis.

Human and cynomolgus monkey CD3 epsilon 1-26_Fc fusion proteins

A cDNA encoding the human CD3 epsilon peptide 1-26 (UniProt accession No: P07766, amino acids 23-48, SEQ ID NO: 188) and a cDNA encoding the cynomolgus CD3 epsilon peptide 1-26 (UniProt accession No: Q95LI5, amino acids 22-47, SEQ ID NO: 189) were PCR amplified from synthetic cDNAs obtained from GENEART A.G. for the human and cynomolgus monkey CD3 epsilon extracellular regions, respectively. The amplified products were subsquently fused to a human IgG1 Fc portion using standard overlap PCR techniques. The human IgG1 Fc cDNA template was obtained from Geneart AG. The resulting cDNA were cloned in the modified pcDNA3.1 plasmid mentioned above.

For transient expression of human and cynomolgus CD3 epsilon constructs (SEQ ID NO: 190 and 191, respectively), the recombinant vectors were transfected into suspension-adapted HEK-EBNA cells (ATCC-CRL-10852) using Polyethyleneimine (PEI) as described above. The CD3 epsilon fusion constructs were then purified from cell-free supernatant using recombinant Streamline rProtein A media (GE Healthcare Europe GmbH, Glattbrugg, Switzerland) and used for further analysis. These two fusion proteins are referred herein as the human and cynomolgus monkey CD3 epsilon 1-26_Fc fusion proteins.

Human HER2 extracellular region

Preparations of HER2 soluble extracellular region have been described in PCT Publication No: WO2012131555. Human HER2 soluble extracellular region fused to a poly-histidine tag (referred herein as HER2-his) or fused to a human IgG1 Fc region (referred herein as HER2-Fc) were prepared.

Human and cynomolgus monkey CD38 extracellular regions

A cDNA for human CD38 was obtained from Source Biosciences (Erwin-Negelein-Haus, Germany, Cat. No.: IRAU37D11, 4309086), its extracellular region (UniProt accession No: P28907 residues 43-300) was PCR amplified and cloned into an in-house expression vector derived from pcDNA3.1 (Invitrogen AG). This expression vector encompassed a kozak sequence and a start codon followed by the murine VJ2C leader peptide to the 5' end and a 6-His-tag to the 3' end of its multiple cloning site. The soluble extracellular region of human CD38 fused to a 6-His-tag (SEQ ID NO: 192) was expressed and purified as follows: one volume of RPMI 1640 medium (PAA Laboratories, Cat. No: E15-039) containing HEK cells, 0.1% pluronic acid (Invitrogen AG), expression vector and polyethylenimine (JetPEI®, Polyplus-transfection, Illkirch, France) was incubated in a shaker flask at 37°C, 5% CO_2 and 80% humidity. One volume of ExCell293 medium supplemented with 6 mM glutamine was added to the mixture after 4 hours and incubation continued further for a total of 5 days. Post production, cell-free supernatant was prepared by centrifugation and filtrated using 0.2 µm filters, pH was adjusted at 7.4 (4°C) using Tris 1 M pH 8.7. Ni-Sepharose Excell beads (GE Healthcare, Cat. No: 17-3712-03) were added to the solution and incubated overnight at 4°C under agitation. The solution was loaded on an Econo-Column (Bio-Rad Laboratories AG, Reinach, Switzerland, Cat. No: 737-4252) for gravity-flow purification. The beads were

washed in PBS (2x), 20 mM imidazole and the protein was eluted in PBS, 500 mM Imidazole. Eluted fractions were pooled and buffer exchanged for PBS with two dialysis steps at 4°C. The purified human CD38 extracellular region was filtrated using 0.22 µm syringe filters.

Using the methods as described above the soluble extracellular region of cynomolgus monkey CD38 antigen fused to a 6-His-tag (SEQ ID NO: 193) was cloned, expressed and purified.

Human OX40 extracellular region

A method to prepare the soluble extracellular region of human OX40 has been described in PCT Publication No: WO2013008171.

Human EGFR extracellular region

An example of EGFR soluble extracellular region antigen preparation has been described in PCT Publication No: WO2012131555.

In vitro T cell redirection killing assay

Preparation of peripheral blood mononuclear cells

To produce peripheral blood mononuclear cells (PBMCs), blood filters containing human leukocytes were collected from the Blood Collection Centre in La Chaux-de-Fonds, Switzerland (Centre de Transfusion Sanguine et Laboratoire de Sérologie, rue Sophie-Mairet 29, CH-2300). Cells were removed from the filters by back-flushing with 60 ml of PBS containing 10 U/ml of liquemin (Drossapharm AG, Lucern, Switzerland). PBMCs were then purified with 50 mL Blood-Sep-Filter Tubes (Brunschwig, Basel, Switzerland) following manufacturer's instructions. Tubes were centrifuged for 20 min at 800g at room temperature (without brake) and the cells were collected from the interface. Cells were washed 3x with Roswell Park Memorial Institute (RPMI, PAA Laboratories, Pasching, Austria) medium without FBS or phosphate buffered Saline (PBS). PBMCs were resuspended at 10e6 cells/mL in RDL medium (RPMI supplemented with 10% heat inactivated Fetal bovine serum (FBS) and penicillin/streptomycin) and were cultured overnight at 37°C in a 5% CO_2 incubator prior to the assay.

T cell preparations

T cell purification was performed directly after the PBMC isolation using pan-T cell isolation kit II (Myltenyi Biotec GmbH, Bergisch Gladbach, Germany, Cat. No: 130-091-156) following manufacturer's instructions. After purification, T cells were resuspended at 10e6 cells/mL in RDL medium and cultured overnight at 37°C in a 5% CO_2 incubator prior assay.

Assay readouts

Two different readouts which gave highly comparable results were used to quantify the redirected killing.

A flow cytometry method, referred herein as RDL-FACS method, based on fluorescence-cytometry as described in Schlereth B *et al.* ((2005) Cancer Res, 65: 2882-2889), Moore PA *et al.* ((2011) Blood, 117(17): 4542-51) and Friedrich M *et al.* ((2012) Mol Cancer Ther, 11: 2664-2673). Target cells were harvested, counted, washed once and resuspended at 5x10e6 cells/mL in PBS+1 µM Carboxyfluorescein succinimidyl ester (CFSE, Sigma). Cells were incubated 15 min at 37°C with gentle agitation every 5 min. CFSE loaded cells were washed 3x with RDL medium and resuspended at 2x10e5 cells/mL in RDL medium. PBMCs were harvested, counted and resuspended at 2x10e6 cells/mL in RDL medium. Antibodies serial dilutions (3x solutions) were prepared in RDL medium. Target cells (50 µl/well), T cells (50 µl/well) and 3x antibody solutions (50 µl/well) were distributed in flat-bottom 96-well plate (TPP, Trasadingen, Switzerland). The effector: target ratio was 10:1. The plates were incubated for 48h in a 5% CO_2 incubator at 37°C. After incubation the plates were centrifuged for 3 min at 300g, the supernatants were discarded by flicking the plates. The plates were washed once with 200 µl of PBS, centrifuged again and the PBS was discarded. A pre-warmed solution of accutase (Invitrogen AG) was added and the plates were incubated 10 min at 37°C. The detached adherent cells were resuspended by pipetting up and down after addition of 100 µL of RDL medium. The solution was transferred into a U-bottom 96-well plate (TPP). The U-bottom plates were centrifuged for 3 min at 300 g, the supernatants were discarded and the cells were resuspended in 200µl of cold FACS buffer (PBS + 2% FBS + 10% Versene) supplemented with 7-AAD (Becton Dickinson AG, Allschwil, Switzerland) at a 1/40 dilution. The plates were immediately acquired on a Guava easyCyte™ Flow Cytometer (Millipore AG, Zug, Switzerland). For each well, the absolute number of living target cells was determined by gating on CFSE positive 7ADD negative population using Flowjo® software (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). The percentage of specific cytotoxicity for each sample was determined using the condition in which only target

cells were incubated as baseline. The EC_{50} values were determined using nonlinear variable slope regression method with Prism software (GraphPad software, La Jolla, CA, USA). The percentage of specific re-directed lysis (RDL) was calculated by subtracting the percentage of specific cytotoxicity of the condition without antibody to the conditions where a test antibody was added.

A cell viability method, referred herein as RDL-MTS method based on a colorimetric method to assess cell viability as described in in Bühler P *et al.* ((2008) Cancer Immunol Immunother, 57: 43–52, Labrijn AF *et al.* ((2013) Proc Natl Acad Sci USA, 110(13): 5145-50) and PCT Publication No: WO2012143524. Target cells were harvested, counted, washed once and resuspended at 2x10e5 cells/ml in RDL medium. PBMCs were harvested, counted and resuspended at 2x10e6 cells/mL in RDL medium. Antibodies serial dilutions (3x solutions) were prepared in RDL medium. Target cells (50 µl/well), T cells (50 µl/well) and 3x antibody solutions (50 µl/well) were distributed in flat-bottom 96-well plate (TPP). The effector: target ratio was 10:1. The plates were incubated for 48 h in a 5% CO_2 incubator at 37°C. After incubation the supernatants were discarded and the plates were washed 3 times with 200 µL of PBS to remove the PBMCs and 100 µl of RDL medium was then added to each well. The readout was done using CellTiter 96® kit (Promega AG, Dübendorf, Switzerland) according to manufacturer's instructions. Briefly, 10-20 µl of MTS reagent was added to each well and the plates were incubated 2-6h in a 5% CO_2 incubator at 37°C. The 490 nm absorbance was then read on a BioTek synergy plate reader (BioTek AG, Luzern, Switzerland). The percentage of specific killing was calculated using this formula: Specific killing = 100 x [(SD-Sp)/(SD-MD)]. SD is the absorbance measured in spontaneous death condition where target cells were incubated alone. Sp is the absorbance measured in each test condition (target cells + PBMCs + antibody). MD is the absorbance measured in the maximum death condition in which target cells were lysed by 3 freeze and thaw cycles. The percentage of specific redirected lysis (RDL) was calculated by subtracting the percentage specific cytotoxicity of the condition without antibody to the conditions where a test antibody was added. The EC_{50} values were determined using nonlinear variable slope regression method with Prism software (GraphPad software).

Xenograft model

JIMT-1 xenografts

Cells lines and reagents

Breast carcinoma JIMT-1 cell line was obtained from DSMZ (Cat. No: ACC589). Cells were maintained in DMEM (1X) with GlutaMAX™-1 (Invitrogen AG, Cat. No: 31966-021) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (AMIMED, London, UK, Cat. No: Z10834P), 1% penicillin-streptomycin (Invitrogen AG, Cat. No: 10378-016), 1% sodium pyruvate solution (PAA Laboratories, Cat. No: S11-003), 1% MEM Non-Essential Amino Acids (PAA Laboratories, Cat. No: M11-003) and 1% GlutaMAX™-1 (Invitrogen AG, Cat. No: 35050-038). Cells were split twice a week with StemPro Accutase (Invitrogen AG, Cat. No: A11105-01).

Peripheral blood mononuclear cells (PMBC) were collected from blood filters containing human leukocytes from the Blood Collection Centre in La Chaux-de-Fonds, Switzerland (Centre de Transfusion Sanguine et Laboratoire de Sérologie, rue Sophie-Mairet 29, CH-2300). Cells were removed from the filters by back flushing with 60 ml of PBS containing 10 U/mL of liquemin (Drossapharm AG, Lucern, Switzerland). PBMCs were then isolated with 50 ml Blood-Sep-Filter Tubes (Brunschwig, Basel, Switzerland) following manufacturer's instructions: tubes were centrifuged 20 min at 800 g at RT (without brake) and the cells were collected from the interface. Cells were washed 3 times with Roswell Park Memorial Institute medium without FBS (RPMI, Invitrogen AG, Cat. No: 21875-091). PBMCs were resuspended at 10e6 cells/ml in RPMI medium supplemented with 10% FBS (AMIMED), 1% penicillin-streptomycin (Invitrogen AG) and were cultured overnight at 37°C under 5% CO_2. Target cells were harvested, counted, washed once and resuspended at 5x10e6 cells/ml in PBS.

Mice and experimental conditions

In vivo experiments were performed in 5-week-old immunodeficient NOD.CB17/AlhnRj-Prkdcscid/Rj (NOD/SCID) female mice characterized by T cell, B cell and natural killer cell deficiency (Janvier Labs, St Berthevin, France). The mice were maintained under sterile and standardized environmental conditions in standard rodent micro-isolator cages (20 +/- 1°C room temperature, 50 ± 10% relative humidity, 12 hours light dark rhythm). Mice received irradiated food, bedding and 0.22 µm-filtered drinking water. All experiments were done according to the Swiss Animal Protection Law with permission from the responsible cantonal authorities (Neuchatel Canton, Switzerland). In compliance with the Animal Protection Law, mice had to be euthanized when tumor volumes exceeded 2000 mm^3. Statistical analysis of

the mean tumor volume of the corresponding treatment groups versus the vehicle control group was done by ANOVA one way and Bonferroni parametric tests.

All mice were depilated before engraftment with VEET cream (Reckitt Benckiser AG, Wallisellen, Switzerland) on the right flank. Photographs and weight measurements of mice were performed on the day of engraftment and later once a week. For each animal, 5x10e6 human PBMC were mixed with 5x10e6 JIMT-1 breast carcinoma cells in a final volume of 0.2 ml PBS. Four different PBMC donors were included. The PBMC/JIMT-1 mixture was subcutaneously injected into the right flank of each NOD/SCID mouse. A control group with 5x10e6 JIMT-1 breast carcinoma cells in a final volume of 0.2 ml PBS without any human PBMC was included. For each group of ten JIMT-1/PBMC engrafted animals (one group per PBMC donor), five animals were intravenously treated with HER2/CD3-1 bispecific antibody at 0.05mg/kg 3 hours after engraftment using a volume of 100 µl. Treatment was repeated 3 times per week, every two days, during two weeks. Tumors were measured twice a week with a caliper in two perpendicular dimensions and tumor volumes were calculated according to the following formula: tumor volume = [(width2 x length) / 2].

Example 1: Determination of mutations that reduce or abrogate binding to Protein A in VH3 subclass

Methods to abrogate Protein A binding in immunoglobulin constant region are known (Lindhofer H. *et al.*, (1995) J Immunol, 155(1): 219-225; US6,551,592; Jendeberg L. *et al.*, (1997) J Immunol Methods, 201(1): 25-34; PCT Publication No: WO2010151792).To assess the use of Protein A abrogating methods in full-length homo-dimeric immunoglobulins, an anti-HER2 homo-dimeric immunoglobulin based on a mixed IGHG1-IGHG3 Fc format and the corresponding Fc 133 control fragment were prepared. The anti-HER2 homo-dimeric immunoglobulin was formatted similarly to a naturally occurring antibody and consisted of a FAB fragment with anti-HER2 specificity fused to a Fc 133 fragment (a Fc sequence originating from the naturally occurring human IGHG3 isotype wherein the hinge sequence was substituted for the entire hinge sequence from the naturally occurring human IGHG1 isotype, abbreviated Fc 133 with SEQ ID NO:1 - wherein the numerals in the name correspond to the immunoglobulin gamma isotype subclass of each domain in the order of: hinge/CH2/CH3; the corresponding full-length anti-HER2 immunoglobulin being referred herein as anti-HER2 FAB-Fc 133; heavy chain with SEQ ID NO: 2 and light chain with SEQ

ID NO: 3). Post transfection, the anti-HER2 FAB-Fc 133 homo-dimer and Fc 133 fragment were assayed for Protein A binding by gradient chromatography according to the protocol described in the Materials and Methods Section. As shown in FIG. 3 and FIG. 4A, the Fc 133 fragment did not bind the commercial MabSelect SuRe™ Protein A resin (GE Healthcare Europe GmbH) while the anti-HER2 FAB-Fc 133 homo-dimer was able to bind.

To assess the contribution of the FAB constant domains, the anti-HER2 homo-dimer described above was reformatted as an anti-HER2 scFv-Fc molecule wherein the scFv unit consisted of the parent immunoglobulin variable domains fused by a 15 amino-acid linker (abbreviated herein as anti-HER2 scFv-Fc 133; heavy chain with SEQ ID NO: 4). The resulting anti-HER2 scFv-Fc 133 homo-dimer was therefore identical to the parent anti-HER2 FAB-Fc 133 homo-dimeric immunoglobulin but lacked the CH1 and CK constant domains. As shown in FIG. 4B, the scFv-Fc 133 homo-dimer exhibited Protein A binding as observed with the parent anti-HER2 homo-dimeric immunoglobulin. This finding led to the conclusion that the variable domains of the anti-HER2 FAB fragment were responsible for hampering the efficacy of the methods abrogating Protein A binding in the Fc portion of homo-dimeric immunoglobulins. More importantly, it was concluded that Protein A binding within variable domains of homo-dimeric immunoglobulins will prevent the preparation of hetero-dimeric immunoglobulins based on Protein A differential purification techniques.

All five domains of Protein A are known to bind the variable heavy chain domains from the VH3 variable domain subclass (Jansson B *et al.*, (1998) FEMS Immunol. Med. Microbiol., 20(1): 69-78), a feature which is known to hamper the preparation of VH3 based FAB fragments following papain digestion of whole antibody molecules, since Protein A binds both VH3 based FAB and Fc fragments. The heavy chain variable domain found in the homo-dimeric anti-HER2 immunoglobulin or its scFv-Fc version belongs to the VH3-23 subclass, and explains why these homo-dimeric molecules bound Protein A in spite of having no Protein A binding site within their engineered Fc regions.

VH3 based immunoglobulins or fragments thereof are of major importance to the biotechnology industry. VH3 based molecules have been extensively developed since their ability to bind Protein A facilitates their functional pre-screening, and as such many synthetic or donor based phage display libraries or transgenic animal technologies used for antibody

discovery are based on the VH3 domain subclass. In addition VH3 based molecules are often selected for their good expression and stability over other known heavy chain variable domain subclasses. A recombinant version of Protein A which does not bind VH3 based FAB fragments has been developed and commercialized by GE Healthcare under the trade name MabSelect SuRe™.

Since the MabSelect SuRe™ resin was used herein for the Protein A binding assessment of the two homo-dimeric anti-HER2 immunoglobulins discussed above, it was concluded that the MabSelect SuRe™ resin was unsuitable for the preparation of hetero-dimeric immunoglobulins having at least one VH3 variable domain when using Protein A differential purification techniques - since homo-dimeric species having no Protein A binding in their Fc regions will still bind Protein A through their VH3 domains.

To investigate substitutions that would abrogate or reduce Protein A binding fromVH3 based homo-dimeric immunoglobulins or fragments thereof, VH3 based FAB variants will need to be assayed for Protein A binding. Although the MabSelect SuRe™ resin type is known to lack VH3 domain subclass binding, another commercial Protein A resin known as MabSelect™ does bind the VH3 domain subclass (also from GE healthcare) and was selected to analyse VH3 based FAB variants for Protein A binding.

The use of the MabSelect™ resin was validated by preparing a recombinant anti-HER2 FAB fragment derived from the parent anti-HER2 homo-dimeric immunoglobulin described earlier that is known to be of the VH3-23 variable domain subclass (abbreviated herein as anti-HER2 FAB; heavy chain with SEQ ID NO: 5 and light chain with SEQ ID NO: 3), and assaying the fragment onto the MabSelect™ and MabSelect SuRe™ columns (having a light chain based on the VK subclass I, the FAB fragment was first purified in capture-elution mode using protein L chromatography before Protein A gradient chromatography was performed on MabSelect™ or MabSelect SuRe™ columns, protocol for both columns followed the protocol described the Materials and Methods section). As shown in FIG. 4C, the VH3 based anti-HER2 FAB only bound to the MabSelect™ column, confirming that the MabSelect SuRe™ resin lacks binding to the VH3 based FAB fragments; at least as far as monomeric VH3 based FAB fragments are concerned, and further contrasted with the results observed earlier for the VH3 based homo-dimeric immunoglobulins with engineered Fc regions having no binding to

Protein A. Conversely, the anti-HER2 FAB showed strong binding to the MabSelect™ column which offered the possibility to assay for VH3 based FAB variants that would have no or reduced Protein A binding.

To abrogate Protein A binding in VH3 based FAB fragments, critical Protein A binding residues in VH3 domains were identified from the crystal structure of a human FAB fragment in complex with the D domain of Protein A (PDB code: 1DEE; www.pdb.org; Graille M *et al.*, (2000) Proc Natl Acad Sci USA, 97(10): 5399-5404). This analysis was used as a starting point for rational design wherein the nature of the substitutions undertaken was based on sequence comparison between Protein A binding and non-Protein A binding VH subclasses from human origin. FIG. 5 shows an alignment of one representative framework for each human heavy chain variable domain subclass. Amino acid positions 15, 17, 19, 57, 59, 64, 65, 66, 68, 70, 81, 82a, and 82b (Kabat numbering) were identified as part of the protein-protein interaction interface between the D domain of Protein A and the VH3 based FAB fragment in the 1DEE structure. Amongst human VH subclasses, VH3 is the only subclass to bind Protein A, and residues at equivalent amino acid sequence positions in other subclasses were selected to be the source of the substitutions with the view to abrogate or reduce Protein A binding while having potentially reduce immunogenicity - since these substitutions involved the replacement of one residue with another naturally occurring residue at the same equivalent amino acid position found in a non-Protein A binding human VH subclass.

Mutations were introduced in the sequence of the aforementioned anti-HER2 FAB fragment by standard PCR based mutagenesis techniques, the following substitutions were made: G65S (heavy chain with SEQ ID NO:6 and light chain with SEQ ID NO: 3), R66Q (heavy chain with SEQ ID NO: 7 and light chain with SEQ ID NO: 3), T68V (heavy chain with SEQ ID NO: 8 and light chain with SEQ ID NO: 3), Q81E (heavy chain with SEQ ID NO: 9 and light chain with SEQ ID NO: 3), N82aS (heavy chain with SEQ ID NO: 10 and light chain with SEQ ID NO: 3), and the combination R19G/T57A/Y59A (heavy chain with SEQ ID NO: 11 and light chain with SEQ ID NO: 3).

In addition, the T57A substitution (heavy chain with SEQ ID NO: 12 and light chain with SEQ ID NO: 3), and T57E substitution (heavy chain with SEQ ID NO: 13 and light chain with SEQ ID NO: 3) were made. T57A was previously shown to abrogate Protein A binding

in WO2010075548, and T57E was designed to introduce a charged residue that may disrupt the VH3-Protein A interaction. Having a light chain based on the VK subfamily I, FAB mutants were first purified in capture-elution mode using Protein L chromatography, and further assayed for Protein A binding using the MabSelect™ column operated under gradient mode as described in the Materials and Methods section. FIG. 6 shows that only T57A, T57E, G65S, Q81E, N82aS and the combination R19G/T57A/Y59A abrogated or reduced anti-HER2 FAB binding to the MAbSelect™ resin. Substitutions G65S, Q81E and N82aS are preferred when abrogating Protein A binding in VH3 based FAB fragments since these mutations substitute for the sequence equivalent residue found in non-Protein A binding VH subclasses thereby potentially reducing immunogenicity.

Antibody affinity and specificity is essentially confined to the CDR regions, however, framework substitutions may impact on antibody properties as shown in the case of several humanized antibodies. To assess if the above substitutions may impact the specificity and/or the affinity of VH3 derived antibodies, two of the preferred FAB mutants were assayed for HER2 antigen binding by Surface Plasmon Resonance (SPR). SPR measurements with recombinant HER2 antigen were performed as described in the Materials and Methods section. Both preferred mutants showed identical binding to the HER2 antigen when compared to the original FAB molecule (FIG. 7) demonstrating that the substitutions had not impact in terms of specificity or affinity. It is therefore expected that these substitutions could be broadly used to engineer out Protein A binding in VH3 derived antibody molecules without significant loss of antigen binding.

Two of these preferred substitutions were introduced in the anti-HER2 homo-dimeric immunoglobulin and anti-HER2 scFv-Fc molecule described earlier, and variants were assayed for binding onto the MabSelect SuRe™ resin. The following variants were prepared: anti-HER2 scFv(G65S)-Fc 133 (heavy chain with SEQ ID NO:14), anti-HER2 scFv(N82aS)-Fc 133 (heavy chain with SEQ ID NO: 15), anti-HER2 FAB(G65S)-Fc 133 (heavy chain with SEQ ID NO: 16 and light chain with SEQ ID NO: 3), and anti-HER2 FAB(N82aS)-Fc 133 (heavy chain with SEQ ID NO: 17 and light chain with SEQ ID NO: 3).

FIG. 8 shows the profiles from the MabSelect SuRe™ chromatography for all four mutants. All variants now displayed reduced to almost no binding to the MabSelect SuRe™ column

indicating a successful removal of Protein A binding with the previously identified substitutions. More importantly, it was concluded that when combined with Protein A differential purification techniques, substitutions which abrogate or reduce VH3 based FAB affinity for Protein A will allow the preparation of hetero-dimeric immunoglobulins wherein at least one VH3 variable domain is present.

Example 2: Antigen binding sites that target the human CD3 antigen, tumour associated antigens and inflammatory cell surface antigens

Antigen binding sites against the human CD3 antigen

The human CD3 epsilon subunit was selected to drive T cell redirect killing via bispecific engagement.

2.1A *Humanized variants of the mouse OKT3 antibody*

The anti-human CD3 epsilon antigen binding site used herein was derived from the mouse OKT3 antibody (Muromonab-CD3, trade name Orthoclone OKT3, marketed by Janssen-Cilag and subsequently discontinued; murine variable heavy chain and light chain domains with SEQ ID NO: 18 and 19, respectively). OKT3 murine variable domains were humanized and formatted as scFv and FAB fragments.

Humanization followed the method described by Jung S & Plückthun A (1997, Protein Eng, 10(8): 959-66) to produce a highly stable humanized variant that would be suitable for both FAB and scFv formatting. The method makes use of the highly stable pair of VH/VL domains found in the Herceptin® antibody (rhuMAbHER2, huMAB4D5-8, trastuzumab or trade name Herceptin®; US Patent publication No.5,821,337; variable heavy chain and light chain domains with SEQ ID NO: 20 and 21, respectively) and follows the workflow of a humanization process onto fixed frameworks (Almagro JC & Fransson J (2008), Front Biosci, 13: 1619-33). Since the Herceptin® antibody is originally derived from the highly stable human families of germline framework VH3 and VK1, germline frameworks from these two families can be equally used as a source of fixed frameworks. Alternatively, the human VK3 germline light chain framework family can be used instead of VK1 as it also has good stability properties (Ewert S *et al.*, (2003) J Mol Biol, 325: 531-553). In addition to mouse antibodies, human antibodies can be engineered using this fixed framework method to improve stability. Preferred is the use of the human germline framework IGHV3-23*04, IGKV1-39*01 and IGKV3-20*01 having SEQ ID NO: 22, 23 and 24, respectively (referenced according to IMGT® (the international ImMunoGeneTics information system (Lefranc MP *et al.* (1999) Nucleic Acids Res, 27(1): 209-12; Ruiz M *et al.* (2000) Nucleic Acids Res, 28(1): 219-21; Lefranc MP (2001) Nucleic Acids Res, 29(1): 207-9; Lefranc MP (2003) Nucleic Acids Res, 31(1): 307-10; Lefranc MP *et al.*, (2005) Dev Comp Immunol,

29(3): 185-203; Kaas Q *et al.*, (2007) Briefings in Functional Genomics & Proteomics, 6(4): 253-64; http://www.imgt.org).

To this aim a first humanized antibody was constructed wherein the CDRs in the variable domains of the Herceptin® antibody were respectively replaced with the CDRs from the mouse OKT3 antibody and benchmarked against a chimera of the mouse OKT3 antibody (variable heavy chain and light chain with SEQ ID NO: 25 and 26, and referred herein as the chimeric OKT3 antibody).

The prototype antibody (variable heavy chain and light chain with SEQ ID NO: 27 and 39, and abbreviated VH/VL) had increased production levels in transient expression tests and increased FAB stability as measured by differential scanning calorimetry but had no binding to HPB-ALL cells (assessed by median fluorescence intensity in FACS experiments, see Materials and Methods section), a human CD3 epsilon positive T cell tumour line (FIG. 9A).

Based on a 3D model of the first prototype pair of variable domains, a subset of back mutations (from CDR grafted Herceptin® prototype to mouse OKT3 sequence) were selected and tested: I34M, V48I, A49G, R58N, R58Y, I69L, A71T and T73K in the variable heavy chain domain and M4L, V33M, A34N, L46R, L47W, R66G, F71Y and P96F in the variable light chain (Kabat numbering). Note that the R58N substitution corresponds to a CDR grafted Herceptin® prototype-to-mouse OKT3 mutation while the R58Y substitution corresponds to a CDR grafted Herceptin® prototype-to-human IGHV3-23*04 germline substitution. The engineering strategy with regard to the combination of substitutions was based on the complementarity of the different substitutions in terms of their putative influence on CDR regions and/or variable domain packing and/or immunogenicity.

In a first approach, all candidates were formatted as human IgG1 antibodies. Best variants were selected according to expression levels, FAB fragment thermo-stability and ability to bind HPB-ALL cells by FACS. Best humanized variants had the Protein A binding site present within their VH domain abrogated using the G65S or N82aS substitution. This engineering step was needed to further produce safe T cell retargeting BEAT antibodies free of anti-CD3 homo-dimers.

Back mutations in the VH of: I34M, A49G and A71T along with back mutations in the VL of: M4L, L46R, L47W and F71Y restored affinity. Best combinations of variable domains were VH8/VL4, VH8/VL8, VH11/VL4 and VH11/VL8 as these retained most of parental cell binding (FIG. 9A-C). In addition, combinations VH8/VL8 (variable domains with SEQ ID NO: 48 and 51, respectively) and VH11/VL8 (variable domains with SEQ ID NO: 49 and 51, respectively) had enhanced FAB stability and (+2.8°C and +1.6°C, respectively, FIG. 9D).

Finally, best humanized variants were also reformatted as scFv-Fc fusions and transiently expressed. Variants were ranked in terms of their relative affinity, stability, expression levels in transient transfection in this format (FIG. 9E). Best combinations of variable domains in a scFv-Fc fusion format were similar to the combinations identified in an antibody format: VH8-VL4 (scFv fragment with SEQ ID NO: 58) and VH8-VL8 (scFv fragment with SEQ ID NO: 59). Both scFv fragments had good thermal stability with the scFv-Fc fusion format (FIG. 9F).

2.1B Humanized variants of the mouse SP34 antibody

The mouse antibody SP34 was first described in 1985 (Pessano S *et al.*, (1985) EMBO J, 4(2):337-44). It was produced by a hybridoma obtained from mice immunised with denatured protein extracts from HPB-ALL cells, the antibody has human specificity and cross-reactivity to cynomolgus monkey. SP34 epitopes on human and cynomolgus monkey CD3 epsilon subunit are known (SEQ ID NO: 195 and 196, respectively).

Following the methods and work flow described in this example *supra*, humanized VH and VL domains for the murine SP34 antibody having a VH domain with SEQ ID NO: 60 and a VL domain with SEQ ID NO: 61 were engineered via CDR grafting onto the VH3-23 and VK3 germline frameworks, respectively. The resulting VH3 based variable domains can be further abrogated for Protein A binding using the G65S or N82aS substitutions (Kabat numbering) depending on their usage in a BEAT antibody format.

To this aim a first humanized antibody was constructed wherein the CDRs in the variable domains of a human antibody having a germline VH3 heavy chain domain and a germline VK3 light chain domain were respectively replaced with the CDRs from the mouse SP34 antibody. The resulting humanized antibody was used a starting point for further affinity

improvement and benchmarked against a chimera of the SP34 antibody (heavy chain and light chain with SEQ ID NO: 62 and 63, respectively, and referred herein as the chimeric SP34 antibody).

The prototype antibody (variable heavy chain and light chain with SEQ ID NO: 64 and 69, and abbreviated VH1/VL1) had a low binding to human CD3 epsilon 1-26_Fc fusion protein (assessed by SPR, see Materials and Methods section and FIG. 10A).

Based on a 3D model of the first prototype pair of variable domains, a subset of substitutions that corresponded to either back mutations between the CDR grafted human germline VH3/VK3 prototype and mouse SP34 sequence (human-to-mouse or mouse-to-human substitutions) or rationally designed amino acid changes was selected. The following changes were made and tested in various combinations: W100eF, and W100eY in the variable heavy chain domain and A2I, S25A, T27A, G27aA, V27cA, T28A, T29A, S30A, N31A, Y32A, E38Q, F44P, G46L, T51A, N52A, K53A, R54A, P56A, L66G, D69T, F87Y, Q89A, W91F, Y92A, S93A, N94A, and Q100G in the variable light chain (Kabat numbering; see FIG. 10A). The engineering strategy with regard to the combination of substitutions was based on the complementarity of the different substitutions in terms of their putative influence on CDR regions and/or variable domain packing and/or immunogenicity and/or impact on transient expression in mammalian cells.

In a first approach, all candidates were formatted as human IgG1 antibodies and later further tested in a scFv-Fc fusion protein format (FIG. 10B) with some variants having the Protein A binding site present within their VH domain abrogated using the G65S. Best humanized candidates were selected according to expression levels and ability to bind the human and cynomolgus monkey CD3 epsilon 1-26_Fc fusion proteins by SPR.

Surprisingly, when reformatted as scFv, SP34 chimera and H1L21 lead to a dramatic loss of expression compare to the IgG1 format, (Figure 33). However, scFv-Fc expression was enhanced by the combination of substitutions W100eY in the VH and W91F in the VL (FIG.10B).

To further improve the manufacturability of CD3 based bispecifics, humanized SP34 scFv was therefore further engineered. To identify key positions impacting SP34 scFv-Fc expression, an Alanine scan was performed in VL21 CDR. The following changes were made: T27A, G27aA, V27cA, T28A, T29A, S30A, N31A, Y32A, N52A, K53A, R54A, P56A, L90A, Y92A, S93A, N94A, and L95A. Interestingly, only substitutions at position 29, 30 (VH1/VL26 SEQ NO: 351) and 95 (VH1/VL34 SEQ NO: 363) lead to significant increase in scFv-Fc expression (Figure 34), moreover these mutants were the only ones to retain full binding to human and Cynomologus monkey CD3 epsilon.

Based on these results, more substitutions were tested at position 29, 30 and 95 in the light chain.

As position 29 and 30 display hyper variability in all the different variable light chain families, these two positions were randomly mutated by site directed PCR. From all the mutants produced, only substitutions T29A, T29E, T29S, S30A and S30D improved significantly transient expression level Figures 35a and 35b while maintaining binding to human CD3 epsilon.

In a different approach, L95, which is known in the art as a canonical structure residue, was substituted with residues most frequently found at this very same position in the variable lambda families. The following changes were made L95A, L95G, L95T, L95S, L95D, and L95N. Unexpectedly, only L95G and L95T significantly improved expression while maintaining binding to the target (Figure 35c).

From this work, several humanized SP34 based BEAT were designed and tested for expression and binding to CD3 epsilon. Among all these constructs, bispecific containing H5L65 (SEQ ID NO: 394) and H5L67 (SEQ ID NO: 396) scFv, showed a 2 fold increase in expression over the H5L32 scFv based BEAT control, Figure 36.

H5L65 and H5L67 were further characterized by DSC either as IgG1 or scFv-Fc and compare to H5L32, H1L21 and SP34 chimera. H5L65 showed superior thermostability as IgG1 but more interestingly an increase of 5 to 2°C compared to other humanized variants in a scFv-Fc

format. (Table 1) The *in vivo* stability of the improved antibodies is therefore increased as it also is *in vitro*.

SP34 Versions	Fab Tm (°C)	IgG (Fab) Tm (°C)	scFvFc Tm (°C)
SP34 chimera	66.9	66.7	N/A
hSP34 H1/L21	75.1	73.1	56.7
hSP34 H5/L32	77.5	75.9	59.7
hSP34 H5/L65	**78.1**	**77.3**	**61.6**
hSP34 H5/L67	77.4	76.5	59.9

Table 1

Preferred combinations of heavy chain and light chain variable domains with regard to antigen binding and recombinant expression were as follows: VH1 (SEQ ID NO: 101) or VH2 (SEQ ID NO: 102) or VH3 (SEQ ID NO: 103) or VH5 (SEQ ID NO: 104) paired with light chains domains VL21 (SEQ ID NO: 105), VL32 (SEQ ID NO: 106), VL65 (SEQ ID NO: 401) and VL67 (SEQ ID NO: 402).

2.2 HER2

Bispecific antibodies that would redirect T cells to kill HER2 positive cancer cells are useful to treat different forms of human breast cancer. Anti-HER2 antibodies have been described (Blumenthal GM *et al.*, (2013) Clin Cancer Res, 19(18): 4911-6) with some being currently used in the clinic or currently under clinical investigations in humans (Tsang RY & Finn RS (2012) Br J Cancer, 106(1): 6-13).

The anti-HER2 antigen binding site as used herein was derived from the recombinant humanized anti-HER2 antibody Herceptin® (see section 1.1) formatted as a FAB fragment (FAB heavy chain fragment with SEQ ID NO: 5 and light chain SEQ ID NO: 3) or a scFv fragment (SEQ ID NO: 107). In some formats, the Protein A binding present in the VH

domain of the humanized anti-HER2 antibody 4D5 (VH3 domain subclass) was abrogated using the G65S substitution (FAB fragment with heavy chain having SEQ ID NO: 108 and light chain SEQ ID NO: 3 or scFv fragment with SEQ ID NO: 109) or the N82aS substitution (FAB fragment with heavy chain having SEQ ID NO: 110 and light chain SEQ ID NO: 3 or scFv fragment with SEQ ID NO: 111).

2.3 CD38

CD38 is a type II transmembrane glycoprotein which is normally found on hemopoietic cells and in solid tissues. CD38 is also expressed in a variety of malignant hematological diseases. Bispecific antibodies that would redirect T cells to kill CD38 positive cancer cells will be useful to treat a variety of malignant hematological diseases, including multiple myeloma, B-cell chronic lymphocytic leukaemia, B-cell acute lymphocytic leukaemia, Waldenström's macroglobulinemia, primary systemic amyloidosis, mantle-cell lymphoma, pro-lymphocytic/myelocytic leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, follicular lymphoma, NK-cell leukaemia and plasma-cell leukaemia. Several anti-CD38 antibodies have been described as research reagents or therapeutic candidates (PCT Publication No: WO2006099875). Amongst the best characterized anti-human CD38 antibodies are OKT-10 and HB-7 mouse hybridomas (Hoshino S *et al.*, (1997) J Immunol, 158(2): 741-7).

In a first approach, anti-human CD38 antigen binding sites can be derived from mouse hybridomas OKT10 (variable heavy chain and light chain with SEQ ID NO: 112 and 113, respectively) or HB-7 (variable heavy chain and light chain with SEQ ID NO: 114 and 115, respectively) and humanized versions thereof which can be further formatted as a FAB or scFv fragments. Following the methods and work flow described in Example 2.1, humanized VH and VL domains for the HB-7 hybridoma are can engineered via CDR grafting onto the VH3-23 and VK1 germline frameworks, respectively.

In a second approach, following the so-called best-fit humanization method described by Almagro JC & Fransson J (Front Biosci, (2008) 13: 1619-33), best-fit humanized VH and VL domains for the HB-7 hybridoma were engineered via CDR grafting onto the human IGHV4-59*03 and IGKV1-NL1*01 germline frameworks, respectively (referenced according to IMGT® *supra*). Humanized VH and VL variants with different degree of back mutations were

investigated *in silico* and one preferred selection of humanized VH and VL was transiently expressed as a human IgG1 format and referred herein as humanized HB-7 best-fit VH (SEQ ID NO: 116) and VL (SEQ ID NO: 117) domains. The following mouse back mutations were introduced: (VH) S35H, I37V, I48L, V67L, V71K, T73N, F78V, Y91F and (VL): M4L, L48I, Y49S, T69K (Kabat numbering).

The humanized HB-7 best-fit antibody (heavy chain with SEQ ID NO: 118 and light chain with SEQ ID NO: 119) stained CHO[CD38] recombinant cells by FACS (data not shown). The humanized HB-7 best-fit antibody had a binding affinity for the CD38 extracellular region similar to that of the chimeric HB-7 antibody (heavy chain with SEQ ID NO: 120 and light chain with SEQ ID NO: 121) when assayed by SPR (KDs of 3.6 and 2.5 nM, respectively; FIG. 11A (chimeric) and FIG. 11B (humanized)). Surprisingly, the humanized HB-7 best-fit antibody displayed a significant enhancement (+14.6°C) in FAB fragment stability compared to the chimeric HB-7 antibody as judged from calorimetry profiles (76.4°C (chimeric) vs 91.0°C (humanized), FIG. 11F).

In a third approach, mice immunized with the human CD38 extracellular domain and human CD38+ cells were used to generate novel hybridoma candidates against human CD38. Methods to generate hybridomas are known and the methods used herein were similar to methods disclosed in PCT Publication No: WO2013008171. The 9G7 mouse antibody candidate had a high affinity for both human and cynomolgus monkey CD38 (variable heavy chain and light chain with SEQ ID NO: 122 and 123, respectively). This mouse antibody was first humanized according the methods described in this example *supra*. Using the best-fit approach, the germline VH framework IGHV2-5*09 and VK framework IGKV1-33*01 (referenced according to IMGT® *supra*) were selected as a starting point for the humanization process. Post CDR grafting, the first antibody prototype (formatted as a human IgG1 isoptype, heavy chain SEQ ID NO: 124 and light chain with SEQ ID NO: 125) exhibited a strong binding to human CD38 only three fold lower than the mouse parental antibody as judged by SPR (chimeric 9G7 antibody with heavy chain SEQ ID NO: 126 and light chain with SEQ ID NO: 127; KD of 0.3 nM and 1 nM for the chimeric 9G7 antibody (data not shown) and first humanized prototype (data not shown), respectively). Affinity improved by two fold upon introduction of the F36Y back mutation in the variable light chain of the antibody (Kabat numbering) (the resulting antibody is referred herein as the humanized 9G7 best-fit antibody

with heavy chain SEQ ID NO: 124 and light chain with SEQ ID NO: 128; KD of 0.5 nM for human CD38, FIG. 11C). The humanized 9G7 best-fit antibody also exhibited a high affinity for the cynomolgus monkey CD38 antigen (KD of 3.2 nM, data not shown), and an enhanced FAB thermo-stability (FAB Tm from DSC scans) over the chimeric 9G7 antibody (94°C vs. 82.2°C for the humanized 9G7 best-fit antibody and the chimeric 9G7 antibody, respectively; see FIG. 11G). The humanized 9G7 best-fit antibody has heavy chain variable domain with SEQ ID NO: 129 and light chain variable domain with SEQ ID NO: 130.

In addition, the 9G7 mouse antibody was humanized following the best-framework approach via CDR grafting onto the VH3-23 and VK1 germline frameworks. Humanized VH and VL variants with different degree of back mutations were investigated *in silico* and one preferred selection of humanized VH and VL combination was transiently expressed as a human IgG1 antibody (the resulting antibody is referred herein as the humanized 9G7 best-framework antibody with heavy chain SEQ ID NO: 131 and light chain with SEQ ID NO: 132). The following mouse back mutations were introduced: (VH) A24F, V37I, V48L, S49A, F67L, R71K, N73T, L78V, and K94R, and (VL) F36Y (Kabat numbering). This antibody exhibited a strong binding to human CD38 and cynomolgus monkey CD38 with affinity constants similar to that of the humanized 9G7 best-fit antibody (KD of 0.4 and 1 nM for human and cynomolgus monkey CD38, respectively; FIG. 11D). FAB thermo-stability (FAB Tm from DSC scans) was also very similar to that of the 9G7 best-fit F36Y humanized variant (89.2°C, see FIG. 11H). FIG. 11J summarizes the different humanized 9G7 antibodies described above. The humanized 9G7 best-framework antibody has heavy chain variable domain with SEQ ID NO: 133 and light chain variable domain with SEQ ID NO: 134.

In a fourth approach, an antibody phage library was screened to generate additional scFv fragments against human CD38. The library had a diversity based on the naturally occurring human V genes. This donor derived antibody phage display library used cDNAs amplified from blood lymphocytes originating from 48 human donors of which 70% had an autoimmune disease (vasculitis, systemic lupus erythematosus, spondiloarthropathy, rheumatoid arthritis and scleroderma). Library construction followed the protocol described by Schofield et al. (2007, Genome Biol., 8(11): R254) with a total diversity of 2.53 x 10e10 clones. ScFv fragments recognizing human and/or cynomolgus monkey CD38 were isolated from this donor derived phage display library as follows. ScFv fragments were isolated in a

series of repeated selection cycles on recombinantly derived human and/or cynomolgus monkey CD38 antigens (see Materials and Methods section). Methods to screen antibody phage display libraries are known (Viti F et al., (2000) Methods Enzymol, 326: 480-505). Briefly, following incubation with the library, the immobilised antigen which had been previously coated on a plastic immunotube (overnight in PBS at a concentration of 20 μg/ml) or captured on streptavidin beads (when using a biotin labelled form of the antigen, antigen captured at a concentration of 50 nM throughout the selection process), bound phages were recovered whist unbound phages were washed away. Bound phages were rescued as described by Marks et al (Marks JD et al., (1991) J Mol Biol, 222(3): 581-97) and the selection process repeated three times. Over one thousand clones from the second and third round of panning were expressed and analysed by ELISA against the human and cynomolgus monkey CD38 antigens. Positive clones were subjected to DNA sequencing and some of the unique clones were further analysed for their ability to bind cell lines expressing human CD38. Following a first round of panning on a biotin labelled version of the human CD38 antigen immobilized on streptavidin beads and a second round of panning on a biotin labelled version of the cynomolgus monkey CD38 antigen immobilized on streptavidin beads, one preferred scFv fragment (clone No 767) having a variable heavy chain sequence with SEQ ID NO: 135 and a variable light chain with SEQ ID NO: 136 was selected for its ability to bind both human and cynomolgus monkey CD38. When formatted as a human IgG1 antibody, clone 767 had a KD of about 300 nM for human CD38 (FIG. 11E) and about 1.2 μM for cynomolgus monkey CD38 (data not shown) (clone 767 IgG1 antibody is referred herein as human 767 antibody with heavy chain SEQ ID NO: 137 and light chain with SEQ ID NO: 138). FAB thermo-stability (FAB Tm from DSC scans) was 70.2°C (FIG. 11I). Clone 767 VH domain belongs to the VH3 domain subclass.

2.4 OX40

A bispecific antibody targeting CD3 and OX40 will allow targeting and depletion of activated T lymphocytes. In this combination, the activated T lymphocytes, which express both CD3 and OX40 molecules, will engage into a mutual killing process resulting in rapid cell disappearance. Co-engagement of human CD3 and OX40 by a bispecific antibody may achieve an effective elimination of pathogenic T cells in a short time frame. OX40 is a member of the TNFR-superfamily of receptors and was first identified in 1987 as a 50 kDa

glycoprotein expressed on activated CD4+ T cells from the rat (Paterson DJ *et al.*, (1987) Mol. Immunol. 24: 1281-90). Unlike CD28, OX40 is not constitutively expressed on naïve T cells but is induced after engagement of the T-Cell Receptor (TCR). OX40 is a secondary costimulatory molecule, expressed after 24 to 72 hours following activation; its ligand, OX40L, is also not expressed on resting antigen presenting cells, but is expressed following their activation.

The mouse anti-human OX40 antibody disclosed in PCT Publication No: WO2013008171 (heavy chain and light chain domains with SEQ ID NO: 139 and 140, respectively) can be used as a source of anti-human OX40 antigen binding site. A humanized version of this antibody based on the best-fit humanization method is also disclosed in PCT Publication No: WO2013008171 (heavy chain and light chain domains with SEQ ID NO: 141 and 142, respectively and with both antibodies being amendable for reformatting into a BEAT format.

Following the methods and work flow described in Example 2.1, humanized VH and VL domains for the anti-human OX40 hybridoma are engineered via CDR grafting onto the VH3-23 and VK1 germline frameworks, respectively. The resulting VH3 based variable domains are further abrogated for Protein A binding using the G65S or N82aS substitutions (Kabat numbering) depending on their usage in a BEAT antibody format. Only two humanized VH and VL domains were investigated differing by their different degree of back mutations. Back mutations were identified from sequence alignments between the parent antibody variable domains and a CDR grafted VH3 and VK1 similar to the first prototype antibody and the approach described in Example 2.1. These CDR grafted variable domains have no back mutations and are referred to herein as mingrafts. These sequences were then further modified to include all the back mutations identified from the previous alignment and resulted in modified variable domain sequences referred to herein as maxgrafts. The resulting sequences are summarized below:

Humanized and stabilized anti-OX40 VH having no back mutations; abbreviated humanized anti-OX40/mingraft VH (SEQ ID NO: 278).

Humanized and stabilized anti-OX40 VH having all possible back mutations; abbreviated humanized anti-OX40/maxgraft VH (SEQ ID NO: 279).

Humanized and stabilized anti-OX40 VL having no back mutations; abbreviated humanized anti-OX40/mingraft VL (SEQ ID NO: 280).

Humanized and stabilized anti-OX40 VL having all possible back mutations; abbreviated humanized anti-OX40/maxgraft VL (SEQ ID NO: 281).

2.5 CD20

Bispecific antibodies that would redirect T cells to kill CD20 expressing B cells can be useful to treat different forms of human lymphomas cancers. Several anti-human CD20 antibodies have been described as research reagents or therapeutic candidates. Amongst the best characterized anti-human CD20 antibodies are the chimeric rituximab antibody and humanized variants thereof (chimeric rituximab antibody, trade name Rituxan®, PCT Publication No: WO1994011026; mouse VH domain of SEQ ID NO: 143 and VL domain of SEQ ID NO: 144).

Following the methods and work flow described in Example 2.1, humanized VH and VL domains for the rituximab chimeric antibody are engineered via CDR grafting onto the VH3-23 and VK1 germline frameworks, respectively. The resulting VH3 based variable domains are further abrogated for Protein A binding using the G65S or N82aS substitutions (Kabat numbering) depending on their usage in a BEAT antibody format. Two humanized VH and VL domains are investigated differing by their different degree of back mutations. Back mutations were identified from sequence alignments between the parent antibody variable domains and a CDR grafted VH3 and VK1 similar to the first prototype antibody and the approach described in Example 2.1. These CDR grafted variable domains have no back mutations and are referred to herein as mingrafts. These sequences were then further modified to include all the back mutations identified from the previous alignment and resulted in modified variable domain sequences referred to herein as maxgrafts. The resulting sequences are summarized below:

Humanized and stabilized Rituximab VH having no back mutations; abbreviated humanized Rituximab/mingraft VH (SEQ ID NO: 282).

Humanized and stabilized Rituximab VH having all possible back mutations; abbreviated humanized Rituximab/maxgraft VH (SEQ ID NO: 283).

Humanized and stabilized Rituximab VL having no back mutations; abbreviated humanized Rituximab/mingraft VL (SEQ ID NO:284).

Humanized and stabilized Rituximab VL having all possible back mutations; abbreviated humanized Rituximab/maxgraft VL (SEQ ID NO: 285).

2.6 EGFR

Bispecific antibodies that would redirect T cells to kill EGFR positive cancer cells can be useful to treat different forms of human cancers, preferably human pancreatic cancers and human colon cancers. Several anti-human EGFR antibodies have been described as research reagents or therapeutic candidates. Amongst the best characterized anti-human EGFR antibodies are the chimeric cetuximab antibody and humanized variants thereof. (chimeric cetuximab antibody, trade name Erbitux®, C225, IMC-C225; PCT Publication No: WO199640210; mouse VH domain with SEQ ID NO: 145 and mouse VL domain with SEQ ID NO: 146).

Following the methods and work flow described in Example 2.1, humanized VH and VL domains for the Erbitux® chimeric antibody are engineered via CDR grafting onto the VH3-23 and VK1 germline frameworks, respectively. The resulting VH3 based variable domains are further abrogated for Protein A binding using the G65S or N82aS substitutions (Kabat numbering) depending on their usage in a BEAT antibody format. Two humanized VH and VL domains are investigated differing by their different degree of back mutations. Back mutations were identified from sequence alignments between the parent antibody variable domains and a CDR grafted VH3 and VK1 similar to the first prototype antibody and the approach described in Example 2.1. These CDR grafted variable domains have no back mutations and are referred to herein as mingrafts. These sequences were then further modified to include all the back mutations identified from the previous alignment and resulted in modified variable domain sequences referred to herein as maxgrafts. The resulting sequences are summarized below:

Humanized and stabilized Erbitux VH having no back mutations; abbreviated humanized Erbitux/mingraft VH (SEQ ID NO: 286).

Humanized and stabilized Erbitux VH having all possible back mutations; abbreviated humanized Erbitux/maxgraft VH (SEQ ID NO: 287).

Humanized and stabilized Erbitux VL having no back mutations; abbreviated humanized Erbitux/mingraft VL (SEQ ID NO: 288).
Humanized and stabilized Erbitux VL having all possible back mutations; abbreviated humanized Erbitux/maxgraft VL (SEQ ID NO: 289).

Another well characterized anti-human EGFR antibody is the human panitumumab antibody and humanized variants thereof (human panitumumab antibody, trade name Vectibix®, PCT Publication No: WO2012138997; mouse VH domain with SEQ ID NO: 290 and mouse VL domain with SEQ ID NO: 291).

Following the methods and work flow described in Example 2.1, humanized VH and VL domains for the Vectibix ® chimeric antibody are engineered via CDR grafting onto the VH3-23 and VK1 germline frameworks, respectively. The resulting VH3 based variable domains are further abrogated for Protein A binding using the G65S or N82aS substitutions (Kabat numbering) depending on their usage in a BEAT antibody format. Two humanized VH and VL domains are investigated differing by their different degree of back mutations. Back mutations were identified from sequence alignments between the parent antibody variable domains and a CDR grafted VH3 and VK1 similar to the first prototype antibody and the approach described in Example 2.1. These CDR grafted variable domains have no back mutations and are referred to herein as mingrafts. These sequences were then further modified to include all the back mutations identified from the previous alignment and resulted in modified variable domain sequences referred to herein as maxgrafts. The resulting sequences are summarized below:
Humanized and stabilized Vectibix VH having no back mutations; abbreviated humanized Vectibix /mingraft VH (SEQ ID NO: 292).
Humanized and stabilized Vectibix VH having all possible back mutations; abbreviated humanized Vectibix /maxgraft VH (SEQ ID NO: 293).
Humanized and stabilized Vectibix VL having no back mutations; abbreviated humanized Vectibix /mingraft VL (SEQ ID NO: 294).

Humanized and stabilized Vectibix VL having all possible back mutations; abbreviated humanized Vectibix /maxgraft VL (SEQ ID NO: 295).

2.7 CD19

Bispecific antibodies that would redirect T cells to kill CD19 expressing B cells will be useful to treat different forms of human blood and myeloid cancers. The human CD19 molecule is a structurally distinct cell surface receptor expressed on the surface of human B cells, including, but not limited to, pre-B cells, B cells in early development (i.e., immature B cells), mature B cells through terminal differentiation into plasma cells and malignant B cells. CD19 is expressed by most pre-B acute lymphoblastic leukemias (ALL), non-Hodgkin's lymphomas, B cell chronic lymphocytic leukemias (CLL), pro-lymphocytic leukemias, hairy cell leukemias, common acute lymphocytic leukemias and some Null-acute lymphoblastic leukemias (Nadler LM *et al.* (1983) J Immunol, 131: 244-250; Anderson KC *et al.*, (1984) Blood, 63: 1424-1433; Loken MR *et al.* (1987) Blood, 70: 1316-1324; Uckun FM *et al.* (1988) Blood, 71: 13-29; Scheuermann RH & Racila E (1995) Leuk Lymphoma, 18: 385-397). The expression of CD19 on plasma cells further suggests it may be expressed on differentiated B cell tumors such as multiple myeloma, plasmacytomas, Waldenstrom's tumors (Grossbard ML *et al.* (1998) Br J Haematol, 102: 509-15; Treon SP *et al.* (2003) Semin Oncol, 30: 248-52).

Humanized anti-human CD19 antibodies described in PCT Publication No: WO2010/095031 utilise the VH3-23 and VK1 variable domain frameworks and can be used to produce bispecific antibodies as described in Example 2.1. The humanized anti-human CD19 antibody having a VH domain with SEQ ID NO: 296 and a VL domain with SEQ ID NO: 297 is used and further abrogated for Protein A binding using the G65S or N82aS substitutions (Kabat numbering) depending on its use in a BEAT antibody format.

2.8 IgE

Bispecific antibodies that would redirect T cells to kill membrane bound IgE positive B cells can be useful to treat different inflammatory disease such as asthma or fibrosis. Several anti-human IgE antibodies have been described as research reagents or therapeutic candidates. Amongst the best characterized anti-human IgE antibodies are the Omalizumab antibody

(trade name Xolair®, USPTO publication No: US6,761,889, US6,329,509 and US20080003218A1; Presta LG *et al.*, (1993) J Immunol, 151: 2623-2632; humanized VH domain with SEQ ID NO: 298 and VL domain with SEQ ID NO: 299) and variants thereof.

Following the methods and work flow described in Example 2.1, humanized VH and VL domains for the Omalizumab antibody are engineered via CDR grafting onto the VH3-23 and VK1 germline frameworks, respectively. The resulting VH3 based variable domains are further abrogated for Protein A binding using the G65S or N82aS substitutions (Kabat numbering) depending on their usage in a BEAT antibody format. Two stabilized VH and VL domains are investigated differing by their different degree of back mutations. Back mutations were identified from sequence alignments between the parent antibody variable domains and a CDR grafted VH3 and VK1 similar to the first prototype antibody and the approach described in Example 2.1. These CDR grafted variable domains have no back mutations and are referred to herein as mingrafts. These sequences were then further modified to include all the back mutations identified from the previous alignment and resulted in modified variable domain sequences referred to herein as maxgrafts. The resulting sequences are summarized below:

Stabilized Omalizumab VH having no back mutations; abbreviated stabilized Omalizumab/mingraft VH (SEQ ID NO: 300).

Stabilized Omalizumab VH having all possible back mutations; abbreviated stabilized Omalizumab/maxgraft VH (SEQ ID NO: 301).

Stabilized Omalizumab VL having no back mutations; abbreviated stabilized Omalizumab/mingraft VL (SEQ ID NO: 302).

Stabilized Omalizumab VL having all possible back mutations; abbreviated stabilized Omalizumab/maxgraft VL (SEQ ID NO: 303).

Another example of anti-human IgE antibody is the mouse antibody Bsw17 (Vogel M *et al.*, (2004) J Mol Biol, 341(2): 477-89; mouse VH domain with SEQ ID NO: 304 and mouse VL domain with SEQ ID NO: 305).

Following the methods and work flow described in Example 2.1, humanized VH and VL domains for the humanized Bsw17 antibody are engineered via CDR grafting onto the VH3-23 and VK1 germline frameworks, respectively. The resulting VH3 based variable domains

are further abrogated for Protein A binding using the G65S or N82aS substitutions (Kabat numbering) depending on their usage in a BEAT antibody format. Two stabilized VH and VL domains are investigated differing by their different degree of back mutations. Back mutations were identified from sequence alignments between the parent antibody variable domains and a CDR grafted VH3 and VK1 similar to the first prototype antibody and the approach described in Example 2.1. These CDR grafted variable domains have no back mutations and are referred to herein as mingrafts. These sequences were then further modified to include all the back mutations identified from the previous alignment and resulted in modified variable domain sequences referred to herein as maxgrafts. The resulting sequences are summarized below:

Stabilized Bsw17 VH having no back mutations; abbreviated stabilized Bsw17/mingraft VH (SEQ ID NO: 306).

Stabilized Bsw17 VH having all possible back mutations; abbreviated stabilized Bsw17/maxgraft VH (SEQ ID NO: 307).

Stabilized Bsw17 VL having no back mutations; abbreviated stabilized Bsw17/mingraft VL (SEQ ID NO: 308).

Stabilized Bsw17 VL having all possible back mutations; abbreviated stabilized Bsw17/maxgraft VL (SEQ ID NO: 309).

Example 3: Production of T cell retargeting hetero-dimeric immunoglobulins

3.1 BEAT® technology and built-in purification system

BEAT antibodies are heavy chain hetero-dimers based on a unique concept of bio-mimicry that exhibit superior hetero-dimerization over the "knob-into-hole" technology (PCT publication No: WO2012131555). The BEAT platform is based on an exchange of interface amino acids at 3D equivalent positions between naturally occurring homo or hetero-dimeric immunoglobulin domain pairs to create new hetero-dimers that can be used as building blocks for Fc-based bispecific antibodies. The technology allows for the design of Fc-based bispecific antibodies using any type of antigen binding scaffold. A scFv-FAB format is used herein to design Fc-based bispecific antibodies without the need to develop a common light chain for both antigen binding sites.

Since BEAT antibodies are heavy chain hetero-dimers, it is needed to distinguish between the two different heavy chains. These are referred herein as BTA and BTB chains. BTA and BTB chains as used herein encompass an antigen binding site, a human IgG1 hinge region, a CH2 domain originating from human IgG1 or IgG3 isotype and a modified CH3 domain originating from human IgG1 or IgG3 isotype. Some of the BTA and BTB CH3 domains were identical or modified variants of the domains described in PCT Publication No: WO2012131555. BTA and BTB CH3 domains were selected from the groups consisting of: (BTA) SEQ ID NO: 147, 148, 149, 153, 154, and 155, and (BTB) SEQ ID NO: 150, 151, 152, 156, 157, and 158. Preferred BTA-BTB CH3 domain pairings are selected from the group consisting of: SEQ ID NO: 147 with SEQ ID NO: 150, SEQ ID NO: 148 with SEQ ID NO: 150, SEQ ID NO: 149 with SEQ ID NO: 151, SEQ ID NO: 147 with SEQ ID NO: 152, and SEQ ID NO: 148 with SEQ ID NO: 152. Most preferred BTA-BTB CH3 domain pairings are selected from the group consisting of: SEQ ID NO: 147 with SEQ ID NO: 156, SEQ ID NO: 148 with SEQ ID NO: 156, SEQ ID NO: 154 with SEQ ID NO: 150, and SEQ ID NO: 154 with SEQ ID NO: 152.

As described above, BEAT heavy chain hetero-dimers with an asymmetrical binding to Protein A can be created using parental domains from immunoglobulin isotypes having no binding to Protein A (PCT publication No: WO2012131555). A difference in the number of

Protein A binding sites between homo- and hetero-dimeric species is particularly useful to resolve these molecular species by Protein A chromatography. To avoid a residual binding that will interfere with species separation by Protein A chromatography, it is necessary to abrogate any secondary Protein A binding sites which are naturally found within the VH3 subclass of human heavy chain variable domains. When antigen binding sites originate from the VH3 family, abrogation of their Protein A binding site can be achieved through the G65S or N82aS substitutions (Kabat numbering).

When preparing a bispecific antibody encompassed by the present invention, using one antigen binding site of VH3 origin and one antigen binding site from a non VH3 origin, the antigen binding site of VH3 origin needs to be located on the heavy chain that does bind Protein A in its Fc region. Alternatively, the antigen binding site of VH3 origin can be substituted with the N82aS substitution or G65S substitution or equivalent substitutions thereof to abrogate Protein A binding. When preparing a bispecific antibody from the present invention using a pair of antigen binding sites of VH3 origin, the only possibility is to abrogate Protein A binding in at least one of the VH3 based antigen binding sites through the amino acid substitutions described above. Preferably, bispecific antibodies from the present invention are engineered to create one of the two homo-dimer without Protein A binding site. More preferably, bispecific antibodies from the present invention are engineered to create one homo-dimer without Protein A binding site, and the other homo-dimer having a substantial difference in its number of Protein A binding sites (at least one Protein A binding site, preferably two Protein A binding sites) over the hetero-dimer of interest.

Mechanisms of toxicity triggered by monospecific anti- human CD3 epsilon antibodies have been under extensive investigation; direct mechanisms have been linked to affinity, epitope and valency of the antibodies but indirect mechanisms of toxicity have also been described. These indirect mechanisms of toxicity are mediated by the Fc region of the anti- human CD3 epsilon antibodies which interact with Fc receptor expressing immune cells and lead to transient T cell activation and cytokine release. With a goal to improve safety, BEAT antibodies targeting human CD3 epsilon were abrogated for Fc-receptor binding in their lower hinge region. Fc receptor binding was abrogated or reduced using the L234A and L235A substitutions (EU numbering; Strohl WR *et al.*, (2009) Curr Opin Biotechnol, 20(6): 685-91); which are often referred as the LALA substitutions.

Examples of BEAT antibodies encompassing at least one VH3 domain abrogated for Protein A binding

Examples of HER2/CD3 targeting BEAT antibodies

Anti-HER2 and anti-CD3 epsilon arms can be formatted either as a scFv-Fc type of heavy chains consisting of a scFv fragment fused to a BEAT chain or as a heavy chain consisting of a FAB fragment fused to a BEAT chain similar to that of a naturally occurring antibody. The FAB based heavy chain requires its association with its cognate light chain to assemble into a functional antigen binding site.

L234A and L235A substitutions were introduced in CH2 regions and residual Protein A binding was abrogated within using the G65S or N82aS substitutions (Kabat numbering) when appropriate. Examples of BEAT antibodies targeting both human HER2 antigen and human CD3 epsilon were formatted as follows:

A first BEAT HER2/CD3 antibody was engineered using a combination of antigen binding sites described in Example and 2.2 for the anti-human CD3 epsilon and the anti-human HER2 arms, respectively. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 159) encompassing a variable heavy chain region with the N82aS substitution (Kabat numbering), a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 47). This heavy chain encompassed part of a human IgG3 Fc region and therefore had no binding to Protein A but since the heavy chain used herein had its heavy chain variable domain originating from the VH3 domain subclass, the VH domain was mutated to include the N82aS substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human HER2 arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 160) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT HER2/CD3-1 antibody (FIG. 12A format A).

A second BEAT HER2/CD3 antibody was engineered using a combination of antigen binding sites described in Example 2.1 and 2.2 for the anti-human CD3 epsilon and the anti-human HER2 arms, respectively. The anti-human HER2 arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 161) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 3). The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 162) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain. This heavy chain encompassed part of a human IgG3 Fc region and therefore had no binding to Protein A but since the heavy chain used herein had its heavy chain variable domain originating from the VH3 domain subclass, the VH domain was mutated to include the N82aS substitution thereby removing any additional Protein A binding sites within the heavy chain. This bispecific antibody is referred herein as BEAT HER2/CD3-2 antibody (FIG. 12A format B).

A third BEAT HER2/CD3 antibody was engineered using a combination of antigen binding sites described in Example 2.1 and 2.2 for the anti-human CD3 epsilon and the anti-human HER2 arms, respectively. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 163) encompassing a variable heavy chain domain with the G65S substitution (Kabat numbering), a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 47). This heavy chain encompassed part of a human IgG3 Fc region and therefore had no binding to Protein A but since the heavy chain used herein had its heavy chain variable domain originating from the VH3 domain subclass, the VH domain was mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human HER2 arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 164) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. The scFv portion of the bispecific antibody was further stabilised using an engineered disulfide bond between the heavy and light chain domains at Kabat position heavy chain 44 (G44C) and light chain 100 (Q100C) as described in PCT publication No WO

1994029350. This bispecific antibody is referred herein as BEAT HER2/CD3-3 antibody (FIG. 12B format C).

A fourth BEAT HER2/CD3 antibody was engineered using a combination of antigen binding sites described in Example 2.1 and 2.2 for the anti-human CD3 epsilon and the anti-human HER2 arms, respectively. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 165) encompassing a variable heavy chain domain, a CH1 γ1 region, a γ1 hinge region, a γ 1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ 1 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 166). This heavy chain and light assembly encompassed a humanized version of the anti-human CD3 epsilon antibody (SP34) as described in PCT Publication No: WO2008119565. The anti-human HER2 arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 167) encompassing a scFv fragment, a CH1 γ 1 region, a γ 1 hinge region, a γ 3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ 3 based BEAT CH3 domain. This heavy chain encompassed part of a human IgG3 Fc region and therefore had no binding to Protein A but since the heavy chain used herein had its heavy chain variable domain originating from the VH3 domain subclass, the VH domain was mutated to include the N82aS substitution thereby removing any additional Protein A binding sites within the heavy chain. This bispecific antibody is referred herein as BEAT HER2/CD3(SP34) antibody (FIG. 12B format D).

A fifth BEAT HER2/CD3 antibody was engineered using a combination of antigen binding sites described in Example 2.1 and 2.2 for the anti-human CD3 epsilon and the anti-human HER2 arms, respectively. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 168) encompassing a variable heavy chain domain, a CH1 γ1 region, a γ1 hinge region, a γ 1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ 1 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 89). This arm of the bispecific antibody encompassed the variable domains of the humanized SP34 VH1/VL21 antibody described in Example 2.1. The anti-human HER2 arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 167) encompassing a scFv fragment, a CH1 γ 1 region, a γ 1 hinge region, a γ 3 CH2 region with L234A and L235A substitutions (EU numbering), and a

γ 3 based BEAT CH3 domain. This arm is equivalent to the BEAT HER2/CD3(SP34) anti-HER2 arm described above (see FIG. 12B format D). The heavy chain encompassed part of a human IgG3 Fc region and therefore had no binding to Protein A but since the heavy chain used herein had its heavy chain variable domain originating from the VH3 domain subclass, the VH domain was mutated to include the N82aS substitution thereby removing any additional Protein A binding sites within the heavy chain. This bispecific antibody is referred herein as BEAT HER2/CD3(SP34-Kappa1) antibody (FIG. 12C format E).

BEAT HER2/CD3-1, BEAT HER2/CD3-2, BEAT HER2/CD3-3, BEAT HER2/CD3(SP34), and BEAT HER2/CD3(SP34-Kappa1) antibodies were expressed transiently, purified and tested *in vitro* for their affinity towards the HER2 and CD3 epsilon antigens, their stability and their ability to redirect T cell killing. Transient expression yields were in the range of 5-15 mg/l of culture supernatant for all BEAT antibodies. Importantly, all bispecific antibodies exhibited very low level of homo-dimeric contaminants in their preparation after a single Protein A chromatography step.
Since all these BEAT antibodies were designed with both arm encompassing a VH3 domain, only abrogation of Protein A binding in at least one VH3 domain allowed to readily purify the hetero-dimer of interest using the one of the preferred differential purification method (see FIG. 2E). An example of differential Protein A purification trace for the BEAT HER2/CD3-1 antibody is shown in FIG.13, and FIG. 14 shows the capillary electrophoresis profile of the purified hetero-dimer. Only a marginal content of homo-dimeric contaminants can be identified from this profile. Homo-dimers of the heavy chain formatted to carry a FAB portion are not found since these do not bind Protein A. Homo-dimers of the heavy chain formatted to carry the scFv fragments are found in a marginal proportion (2.5%), resulting in a hetero-dimer content of 97% after a single Protein A chromatography step. BEAT HER2/CD3-2, BEAT HER2/CD3-3, BEAT HER2/CD3(SP34), and BEAT HER2/CD3(SP34-Kappa1) antibodies purified to similar levels of homogeneity and purity after a single Protein A chromatography step. The BEAT HER2/CD3-3 antibodies showed a proportion of disulfide bonded hetero-dimer aggregates after Protein A chromatography (27%) that were removed by cation exchange chromatography.

To further demonstrate that abrogation of Protein A binding within VH3 based heavy chain hetero-dimers greatly impacts on post Protein A chromatography purity, a BEAT HER2/CD3-

1 antibody was engineered without the aforementioned N82aS substitution. FIG. 15A and 15B show the SDS-PAGE analysis of eluted Protein A chromatography fractions for the BEAT HER2/CD3-1 and its non N82aS substituted version, respectively. At pH 4, the eluted fraction for the non N82aS substituted version exhibits an additional band corresponding to homo-dimers of the heavy chain formatted to carry a FAB arm (FIG. 15B) while the N82aS substituted BEAT HER2/CD3 version does not (FIG. 15A), since the heavy chain formatted to carry a FAB arm has no binding to Protein A in its Fc region (Fc region based on human IgG3 isotype), it can only be deduced that the VH3 based variable domains found in this homo-dimeric species are responsible for Protein A binding. This result clearly demonstrates the utility of abrogating Protein A binding within VH3 based heavy chain hetero-dimers.

Both BEAT HER2/CD3-1 and BEAT HER2/CD3-2 antibodies had similar KD values for the human HER2 and human CD3 epsilon antigens. KD values were in the range of 0.50 - 2 nM for the human HER2 antigen and 1-2 μM for the human CD3 epsilon antigen (measured by SPR using the human CD3gamma-epsilon-Fc construct (see Materials and Methods section; FIG. 16A and 16B). DSC profiles for the two bispecific antibodies were similar, in both case the scFv portions either engaging human HER2 or human CD3 epsilon had retained their good thermo-stability profiles with Tm in the range of 68°C. FAB portions in both antibodies had Tm in the range of 82-83°C (FIG. 16C).

Another example of BEAT antibodies targeting both human HER2 antigen and human CD3 epsilon using the humanized Herceptin VH and VL sequences is formatted as follows: a BEAT HER2/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.2 for the anti-human CD3 epsilon and the anti-human HER2 antigen binding sites, respectively.

The anti-human HER2 arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 310) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 3). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain.

The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT HER2/CD3(SP34-Kappa2) antibody.

In vitro T cell killing assays

The mechanism of action of BEAT HER2/CD3 antibodies is based on targeting cytotoxic T cell killing towards targeted cells by bridging the CD3 antigen on the cell surface of cytotoxic T cells and the HER2 antigen expressed on targeted cells.

The potency of BEAT HER2/CD3-1 and BEAT HER2/CD3-2 antibodies to redirect T cell killing was measured using a flow cytometry based method (referred herein as RDL-FACS method) or a colorimetric based method (referred herein as RDL-MTS method).
The high expressing HER2 cell line JIMT-1, a Herceptin® (trastuzumab) resistant breast carcinoma cell line, the high expressing HER2 cell line BT-474, a Herceptin® (trastuzumab) sensitive breast carcinoma cell line and the low HER2 expressing breast adenocarcinoma cell line MDA-MB-231 were individually cultured during 48 h in the presence of human PBMCs and serial dilutions of BEAT HER2/CD3-1 or -2 antibodies or control antibodies.
In these assays, human PBMCs from blood donations were used a source of cytotoxic T lymphocytes. An effector to target cells ratio of 10:1 was used in all assays. Negative controls were provided in the form of samples without antibody treatment (target cells and human PBMCs only). The cytotoxicity was determined using the RDL-FACS or RDL-MTS methods after the incubation period (see Materials and Methods section). The results showed that control antibodies did not trigger specific T cell-mediated cytotoxicity. In contrast BEAT HER2/CD3-1 and -2 antibodies induced a very potent, dose dependent, tumor target cell death. Maximum killing was close to 100%. Both readout methods methods gave close results. Donor-to-donor variability accounted for about a tenfold different in EC_{50} between the methods. Measured EC_{50}s correlated to the level of HER2 antigen expression by the target cell lines.

BT-474 cells express the most HER2 antigen and EC_{50}s for both BEAT HER2/CD3-1 and -2 antibodies were in the sub-picomolar to picomolar range (0.6 and 2 pM, respectively, FIG. 17A). JIMT-1 cells have masked HER2 antigen on their cell surface (Nagy P *et al.* (2005), Cancer Res, 65(2): 473-482) and consequently exhibit low Herceptin® binding in spite of having high HER2 expression. Surprisingly, both BEAT HER2/CD3-1 and -2 antibodies had EC_{50}s in the picomolar range against JIMT-1 cells as measured by the RDL-MTS method (21 and 16 pM, respectively; FIG. 17B). When measured with the RDL-FACS method, the BEAT HER2/CD3-1 antibody had an EC_{50} of 1.4 pM. Low HER2 expressing breast adenocarcinoma cell line MDA-MB-231 was less sensitive than the previous two cell lines with both antibodies exhibiting sub-nanomolar EC_{50}s (both values close to 0.2 nM; FIG. 17C). When measured with the RDL-FACS method, the BEAT HER2/CD3-1 antibody had an EC_{50} of 0.08 nM. Taken together, these results show that BEAT HER2/CD3-1 and -2 antibodies were highly potent at redirecting T cell killing against various HER2 expressing breast cancer cell lines.

The BEAT HER2/CD3(SP34) antibody encompassed a humanized version of the anti-human CD3 epsilon antibody (SP34) described in PCT Publication No: WO2008119565. The ability of this BEAT antibody format to redirect T cell killing towards HER2+ cells was investigated *in vitro*. Two different HER2+ cell lines were used in killing assays, a high HER2 expressing cell line (NCI-N87) and a low HER2 expressing cell line (HT-1080) (See Materials and Methods section). FIG. 17D-E show T cell redirected killing of NCI-N87 and HT-1080 cells by the BEAT HER2/CD3(SP34) antibody, respectively. The assays used an effector cells to target cells ratio of 10 to 1, and the RDL-MTS readout method after a 48h incubation period (see Materials and Methods section). The results show that the BEAT HER2/CD3(SP34) antibody was highly potent at redirecting T cell killing against HER2+ cell lines with EC_{50}s of 0.35 and 29 pM when targeting NCI-N87 and HT-1080 cells, respectively.

The BEAT HER2/CD3(SP34-Kappa1) antibody encompassed the humanized version of the anti-human CD3 epsilon antibody (SP34-Kappa1) VH1/VL21 described in Example 2.1. The ability of this BEAT antibody format to redirect T cell killing towards HER2+ cells was investigated *in vitro*. Two different HER2+ cell lines were used in killing assays, a high HER2 expressing cell line (NCI-N87) and a low HER2 expressing cell line (HT-1080) (See Materials and Methods section). FIG. 17F-G show T cell redirected killing of NCI-N87 and

HT-1080 cells by the BEAT HER2/CD3(SP34-Kappa1) antibody, respectively. The assays used an effector cells to target cells ratio of 10 to 1, and the RDL-MTS readout method after a 48h incubation period (see Materials and Methods section). The results show that the BEAT HER2/CD3(SP34-Kappa1) antibody was highly potent at redirecting T cell killing against HER2+ cell lines with EC_{50}s of 0.46 and 338 pM when targeting NCI-N87 and HT-1080 cells, respectively.

In vivo efficacy studies

JIMT-1 xenografts

The *in vivo* efficacy of the BEAT HER2/CD3-1 antibody was investigated using a JIMT-1/PBMC xenograft model. Human PBMCs from blood donations were used a source of cytotoxic T lymphocytes. Herceptin® resistant breast carcinoma JIMT-1 cells were mixed at a 1:1 ratio with non-stimulated human PBMCs (four different donors) and subsequently injected subcutaneously in immunodeficiency (NOD/SCID) mice. Following engraftment, animals were treated with the BEAT HER2/CD3-1 antibody intravenously three times per week during two weeks. Antibody treatment started 3 hours after engraftment and continued on day 2, 4, 7, 9 and 11 thereafter.

To assess tumour growth without PBMCs, one cohort out of five was inoculated subcutaneously with 5x10e6 JIMT-1 cells in the absence of human PBMCs, whereas the remaining cohorts were subcutaneously injected with mixtures of 5x10e6 JIMT-1 cells mixed with 5x10e6 non-stimulated human PBMCs from healthy donors.

Human PBMCs, in the absence of antibody did not show a negative effect on tumour growth (FIG. 18A). Treatment with the BEAT HER2/CD3-1 antibody, in the presence of human effector cells induced a complete suppression of tumour growth in most of the animals (18/20 tumours, FIG. 18B-C). Eighteen days after the last day of treatment, only 11% of tumours (2/18) started to grow again. These data show very clearly the potent antitumor efficacy of the BEAT HER2/CD3-1 antibody.

Examples of CD38/CD3 targeting BEAT antibodies

Anti-CD38 and anti-CD3 epsilon arms can be formatted either as a scFv-Fc type of heavy chains consisting of a scFv fragment fused to a BEAT chain or as a heavy chain consisting of a FAB fragment fused to a BEAT chain similar to that of a naturally occurring antibody. The FAB based heavy chain requires its association with its cognate light chain to assemble into a functional antigen binding site.

L234A and L235A substitutions were introduced in CH2 regions and residual Protein A binding was abrogated within using the G65S or N82aS substitutions (Kabat numbering) when appropriate. Examples of BEAT antibodies targeting both human CD38 antigen and human CD3 epsilon were formatted as follows:

A first example of BEAT antibodies targeting both human CD38 antigen and human CD3 epsilon using the humanized HB7 bestfit VH and VL sequences was formatted as follows: A BEAT CD38/CD3 antibody was engineered using a combination of antigen binding sites described in Example 2.1 and 2.3 for the anti-human CD3 epsilon and the anti-human CD38 arms, respectively. The anti-human CD38 arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 169) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 119). The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 162) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain. This heavy chain encompassed part of a human IgG3 Fc region and therefore had no binding to Protein A but since the heavy chain used herein had its heavy chain variable domain originating from a VH3 framework, the VH domain was mutated to include the N82aS substitution thereby removing any additional Protein A binding sites within the heavy chain. This arm is equivalent to the BEAT HER2/CD3-2 anti-CD3 epsilon arm described above (see FIG. 12A format B). The bispecific antibody is referred herein as BEAT CD38-HB7bestfit/CD3 antibody (FIG. 19 format A).

The BEAT CD38-HB7bestfit/CD3 antibody was expressed transiently, purified and tested *in vitro* for its affinity towards the CD38 and CD3 epsilon antigens, its stability and its ability to

redirect T cell killing. The KD value was 3.2 nM for the human CD38 antigen (measured by SPR; FIG. 20A). DSC profiles for the bispecific antibody showed good thermo-stability profiles with a Tm of approximately 68°C for the scFv portion. The FAB portion had a Tm of approximately 91°C (FIG. 20B).

CD38 expressing cell lines (see Materials and Methods section) were used to assess redirected T cell killing in assays similar to that of described in Example 3.2.1. FIG. 21 shows T cell redirected killing of RPMI 8226 myeloma cells using the BEAT CD38-HB7bestfit/CD3 antibody. Note that the assay used purified T cells as effector cells with an effector cells to target cells ratio of 10 to1. When measured with the RDL-FACS method, the BEAT CD38-HB7bestfit/CD3 antibody had an EC_{50} of 2.2 pM (mean of 2 donors, 48h incubation).

A second example of BEAT antibodies targeting both human CD38 antigen and human CD3 epsilon using the human clone 767 VH and VL sequences was formatted as follows: a BEAT CD38/CD3 antibody was engineered using a combination of antigen binding sites described in Example 2.1 and 2.3 for the anti-human CD3 epsilon and the anti-human CD38 arms, respectively. The anti-human CD38 arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 170) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 138). The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 171) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain. This heavy chain encompassed part of a human IgG3 Fc region and therefore had no binding to Protein A but since the heavy chain used herein had its heavy chain variable domain originating from a VH3 framework, the VH domain was mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. This bispecific antibody is referred herein as BEAT CD38-767/CD3 antibody (FIG. 19 format B).

The BEAT CD38-767/CD3 antibody was expressed transiently, purified and tested *in vitro* for its affinity towards the CD38 and CD3 epsilon antigens, its stability and its ability to redirect T cell killing. CD38 expressing cell lines (see Materials and Methods section) were

used to assess redirected T cell killing in assays similar to that of described in Example 3.2.1. FIG. 22 shows T cell redirected killing of Daudi cells using the BEAT CD38-767/CD3 antibody. Note that the assay used human PBMCs as effector cells with an effector cells to target cells ratio of 10:1. When measured with the RDL-FACS method, the BEAT CD38-767/CD3 antibody had an EC_{50} of 244 pM (mean of 3 donors, 24h incubation).

Another example of BEAT antibodies targeting both human CD38 antigen and human CD3 epsilon using the humanized 9G7 best-framework VH and VL sequences is formatted as follows: a BEAT CD38/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.3 for the anti-human CD3 epsilon and the anti-human CD38 antigen binding sites, respectively.

The anti-human CD38 arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 312 or 404) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 132). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT CD38-9G7bestframework/CD3(SP34-Kappa2) antibody.

Another example of BEAT antibodies targeting both human CD38 antigen and human CD3 epsilon using the human clone 767 VH and VL sequences is formatted as follows: a BEAT CD38/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.3 for the anti-human CD3 epsilon and the anti-human CD38 antigen binding sites, respectively.

The anti-human CD38 arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 313) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a

γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 138). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT CD38-767 /CD3(SP34-Kappa2) antibody.

Examples of OX40 /CD3 targeting BEAT antibodies

Anti-OX40 and anti-CD3 epsilon arms can be formatted either as a scFv-Fc type of heavy chains consisting of a scFv fragment fused to a BEAT chain or as a heavy chain consisting of a FAB fragment fused to a BEAT chain similar to that of a naturally occurring antibody. The FAB based heavy chain requires its association with its cognate light chain to assemble into a functional antigen binding site.

L234A and L235A substitutions were introduced in CH2 regions and residual Protein A binding was abrogated within using the G65S or N82aS substitutions (Kabat numbering) when appropriate. Examples of BEAT antibodies targeting both human OX40 antigen and human CD3 epsilon were formatted as follows:

An example of BEAT OX40/CD3 antibody was engineered using a combination of antigen binding sites described in Example 2.1 and 2.4 for the anti-human CD3 epsilon and the anti-human OX40 arms, respectively. The anti-human OX40 arm of the hetero-dimeric immunoglobulin used the variable domains of the humanized anti-human OX40 antibody disclosed in PCT Publication No: WO2013008171 (variable heavy chain and light chain domains with SEQ ID NO: 141 and 142, respectively) and consisted of a BEAT heavy chain (SEQ ID NO: 172) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 173). The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 162) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge

region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain. This heavy chain encompassed part of a human IgG3 Fc region and therefore had no binding to Protein A but since the heavy chain used herein had its heavy chain variable domain originating from a VH3 framework, the VH domain was mutated to include the N82aS substitution thereby removing any additional Protein A binding sites within the heavy chain. This arm is equivalent to the BEAT HER2/CD3-2 anti-CD3 epsilon arm described above (see FIG. 12A format B). The bispecific antibody is referred herein as BEAT OX40/CD3 antibody (FIG. 23).

The ability of the BEAT OX40/CD3 antibody to redirect T cell killing towards OX40+ cells was investigated *in vitro*. The stable recombinant CHO[OX40] cell line was used in killing assays. FIG. 24 show T cell redirected killing of stable recombinant CHO[OX40] cells by the BEAT OX40/CD3 antibody. The assays used human PBMCs as effector cells with an effector cells to target cells ratio of 20 to 1, and the RDL-MTS readout method after a 48h incubation period (see Materials and Methods section). The results show that the BEAT OX40/CD3 antibody was highly potent at redirecting T cell killing against the stable recombinant CHO[OX40] cells with an EC_{50} of 0.5 nM (mean of 3 donors).

Another example of BEAT antibodies targeting both human OX40 antigen and human CD3 epsilon using the humanized anti-OX40/maxgraft VH and VL sequences is formatted as follows: a BEAT OX40/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.4 for the anti-human CD3 epsilon and the anti-human OX40 antigen binding sites, respectively.

The anti-human OX40 arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 314) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 315). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge

region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT OX40maxgraft/CD3(SP34-Kappa2) antibody.

Another example of BEAT antibodies targeting both human OX40 antigen and human CD3 epsilon using the humanized anti-OX40/mingraft VH and VL sequences is formatted as follows: a BEAT OX40/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.4 for the anti-human CD3 epsilon and the anti-human OX40 antigen binding sites, respectively.
The anti-human OX40 arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 316) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 317). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain.
The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT OX40mingraft/CD3(SP34-Kappa2) antibody.

Examples of CD20 /CD3 targeting BEAT antibodies

An example of BEAT antibodies targeting both human CD20 antigen and human CD3 epsilon using the mouse rituximab antibody VH and VL sequences was formatted as follows:
A BEAT CD20/CD3 was engineered using a combination of antigen binding sites described in Example 2.1 and 2.5 for the anti-human CD3 epsilon and the anti-human CD20 arms, respectively.

An example of BEAT antibodies targeting both human CD20 antigen and human CD3 epsilon using the humanized rituximab/maxgraft VH and VL sequences is formatted as follows: a

BEAT CD20/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.5 for the anti-human CD3 epsilon and the anti-human CD20 antigen binding sites, respectively.

The anti-human CD20 arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 318) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 319). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT CD20maxgraft /CD3(SP34-Kappa2) antibody.

Another example of BEAT antibodies targeting both human CD20 antigen and human CD3 epsilon using the humanized rituximab/mingraft VH and VL sequences is formatted as follows: a BEAT CD20/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.5 for the anti-human CD3 epsilon and the anti-human CD20 antigen binding sites, respectively.

The anti-human CD20 arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 320) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 321). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1

based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT CD20mingraft/CD3(SP34-Kappa2) antibody.

Examples of EGFR/CD3 targeting BEAT antibodies

Anti-EGFR and anti-CD3 epsilon arms can be formatted either as a scFv-Fc type of heavy chains consisting of a scFv fragment fused to a BEAT chain or as a heavy chain consisting of a FAB fragment fused to a BEAT chain similar to that of a naturally occurring antibody. The FAB based heavy chain requires its association with its cognate light chain to assemble into a functional antigen binding site.

L234A and L235A substitutions were introduced in CH2 regions and residual Protein A binding was abrogated within using the G65S or N82aS substitutions (Kabat numbering) when appropriate. Examples of BEAT antibodies targeting both human EGFR antigen and human CD3 epsilon were formatted as follows:

An example of BEAT antibodies targeting both human EGFR and human CD3 epsilon antigens is formatted as follows: a BEAT EGFR/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.6 for the anti-human CD3 epsilon and the anti-human EGFR arms, respectively. The anti-human EGFR arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 174) based on the mouse Erbitux antibody variable domains (mouse variable heavy and light chain domains with SEQ ID NO: 145 and 146, respectively) that encompassed a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 175). The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 171) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain. This heavy chain encompassed part of a human IgG3 Fc region and therefore had no binding to Protein A but since the heavy chain used herein had its heavy chain variable domain originating from a VH3 framework, the VH domain was mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. This arm is equivalent to the BEAT CD38-767/CD3 anti-CD3 epsilon arm described above (see FIG. 19 format B). The bispecific antibody is referred herein as BEAT EGFR/CD3 antibody (FIG. 25).

The BEAT EGFR /CD3 antibody was transiently expressed, purified and tested *in vitro* for its ability to redirect T cell killing against human EGFR+ cell lines. The HT-29 cell line was used in killing assays. FIG. 26 show T cell redirected killing of HT-29 cells by the BEAT EGFR /CD3 antibody. The assays used human PBMCs as effector cells with an effector cells to target cells ratio of 10 to 1, and the RDL-MTS readout method after a 48h incubation period (see Materials and Methods section). The results show that the BEAT EGFR/CD3 antibody was highly potent at redirecting T cell killing against HT-29 cells with an EC_{50} of 70.6 pM (mean of 4 donors).

Another example of BEAT antibodies targeting both human EGFR antigen and human CD3 epsilon using the humanized Erbitux/maxgraft VH and VL sequences is formatted as follows: a BEAT EGFR/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.6 for the anti-human CD3 epsilon and the anti-human EGFR antigen binding sites, respectively.

The anti-human EGFR arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 322) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 323). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain.

The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT EGFRcetux-maxgraft/CD3(SP34-Kappa2) antibody.

Another example of BEAT antibodies targeting both human EGFR antigen and human CD3 epsilon using the humanized Erbitux/mingraft VH and VL sequences is formatted as follows: a BEAT EGFR/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.6 for the anti-human CD3 epsilon and the anti-human EGFR antigen binding sites, respectively.

The anti-human EGFR arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 324) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 325). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human CD3 epsilon part of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT EGFRcetux-mingraft/CD3(SP34-Kappa2) antibody.

Another example of BEAT antibodies targeting both human EGFR antigen and human CD3 epsilon using the humanized Vectibix/maxgraft VH and VL sequences is formatted as follows: a BEAT EGFR/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.6 for the anti-human CD3 epsilon and the anti-human EGFR antigen binding sites, respectively.

The anti-human EGFR arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 326) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 327). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain.

The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT EGFRpani-maxgraft/CD3(SP34-Kappa2) antibody.

Another example of BEAT antibodies targeting both human EGFR antigen and human CD3 epsilon using the humanized Vectibix /mingraft VH and VL sequences is formatted as follows: a BEAT EGFR/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.6 for the anti-human CD3 epsilon and the anti-human EGFR antigen binding sites, respectively.

The anti-human EGFR arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 328) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 329). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT EGFRpani-mingraft/CD3(SP34-Kappa2) antibody.

Examples of CD19/CD3 BEAT antibodies

Anti-CD19 and anti-CD3 heavy chains can be formatted either as a scFv-Fc type of heavy chains consisting of a scFv fragment fused to a first BEAT chain or as a heavy chain consisting of a FAB fragment fused to a first BEAT chain similar to that of a naturally occurring antibody. The FAB based heavy chain requires its association with its cognate light chain to assemble into a functional antigen binding site. L234A and L235A substitutions were introduced in CH2 regions and residual Protein A binding was abrogated within using the G65S or N82aS substitutions (Kabat numbering) when appropriate. An example of BEAT antibodies targeting both human CD19 antigen and human CD3 epsilon using anti-CD19 VH and VL sequences described in WO2010095031 is formatted as follows:

An example of BEAT CD19/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.7 for the anti-human CD3 epsilon and the anti-human CD19 antigen binding sites, respectively.

The anti-human CD19 arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 330) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 331). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT CD19/CD3(SP34-Kappa2) antibody.

CD19 expressing cell lines described in PCT Publication No: WO2010/095031 are used to assess redirected T cell killing in assays similar to that of described in Example 3.2.1.

Examples of IgE/CD3 BEAT antibodies

Anti-IgE and anti-CD3 heavy chains can be formatted either as a scFv-Fc type of heavy chains consisting of a scFv fragment fused to a first BEAT chain or as a heavy chain consisting of a FAB fragment fused to a first BEAT chain similar to that of a naturally occurring antibody. The FAB based heavy chain requires its association with its cognate light chain to assemble into a functional antigen binding site. L234A and L235A substitutions were introduced in CH2 regions and residual Protein A binding was abrogated within using the G65S or N82aS substitutions (Kabat numbering) when appropriate.

BEAT IgE/CD3 antibodies are engineered using a combination of antigen binding sites described in Example 2.1 and 2.8 for the anti-human CD3 epsilon and the anti-human IgE antigen binding sites, respectively.

Cell lines expressing IgE on their cell surface are described in PCT Publication No: WO2010/033736 and can used to assess redirected T cell killing in assays similar to that of described in Example 3.2.1.

An example of BEAT antibodies targeting both human IgE antigen and human CD3 epsilon using the stabilized omalizumab/maxgraft VH and VL sequences is formatted as follows: The anti-human IgE arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 332) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 333). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT IgEomali-maxgraft/CD3(SP34-Kappa2) antibody.

Another example of BEAT antibodies targeting both human IgE antigen and human CD3 epsilon using the stabilized omalizumab/mingraft VH and VL sequences is formatted as follows:

The anti-human IgE arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 334) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 335). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT IgEomali-mingraft/CD3(SP34-Kappa2)antibody.

Another example of BEAT antibodies targeting both human IgE antigen and human CD3 epsilon using the stabilized Bsw17/maxgraft VH and VL sequences is formatted as follows: The anti-human IgE arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 336) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 337). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT IgEbsw17-maxgraft/CD3(SP34-Kappa2) antibody.

Another example of BEAT antibodies targeting both human IgE antigen and human CD3 epsilon using the stabilized Bsw17/mingraft VH and VL sequences is formatted as follows: The anti-human IgE arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 338) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 339). This heavy chain encompasses part of a human IgG3 Fc region and therefore has no binding to Protein A but since the heavy chain used herein has its heavy chain variable domain originating from a VH3 framework, the VH domain is mutated to include the G65S substitution thereby removing any additional Protein A binding sites within the heavy chain. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT IgE bsw17-mingraft/CD3(SP34-Kappa2) antibody.

Examples of BEAT antibodies encompassing only one VH3 domain

Examples of CD38/CD3 targeting BEAT antibodies

An example of BEAT antibodies targeting both human CD38 antigen and human CD3 epsilon using the humanized HB7/bestfit VH and VL sequences was formatted as follows: a BEAT CD38/CD3 was engineered using a combination of antigen binding sites described in Example 2.1 and 2.3 for the anti-human CD3 epsilon and the anti-human CD38 arms, respectively. The anti-human CD38 arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 176) encompassing a variable heavy chain domain, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 119). This heavy chain had no binding to Protein A as it encompassed part of a human IgG3 Fc region and had its heavy chain variable domain originating from a non-VH3 domain subclass. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 177) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This heavy chain and light assembly encompassed a humanized version of the anti-human CD3 epsilon antibody (SP34) as described in PCT Publication No: WO2008119565. This BEAT antibody format is referred herein as BEAT CD38-HB7bestfit/CD3(SP34) antibody (FIG. 27 format A).

The ability of the BEAT CD38-HB7bestfit/CD3(SP34) antibody to redirect T cell killing towards CD38+ cells was investigated *in -vitro*. The CD38+ B lymphoblast cell line Daudi was used in killing assays. FIG. 28 show T cell redirected killing of Daudi cells by the BEAT CD38-HB7bestfit/CD3(SP34) antibody. The assays used human PBMCs as effector cells with an effector cells to target cells ratio of 10 to 1, and the RDL-FACS readout method after a 24h incubation period (see Materials and Methods section). The results show that the BEAT CD38-HB7bestfit/CD3(SP34) antibody was highly potent at redirecting T cell killing against the Daudi CD38+ cell line with an EC_{50} of 1.8 pM (mean of 3 donors).

A second example of BEAT antibodies targeting both human CD38 antigen and human CD3 epsilon using the humanized 9G7 best-fit VH and VL sequences (SEQ ID NO: 129 and 130,

respecitively) was formatted as follows: a BEAT CD38/CD3 was engineered using a combination of antigen binding sites described in Example 2.1 and 2.3 for the anti-human CD3 epsilon and the anti-human CD38 arms, respectively. The anti-human CD38 arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 178) encompassing a variable heavy chain domain, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 128). This heavy chain had no binding to Protein A as it encompassed part of a human IgG3 Fc region and had its heavy chain variable domain originating from a non-VH3 domain subclass. The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 179) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This arm of the bispecific antibody encompassed the variable domains of the humanized SP34 VH5/VL32 antibody described in Example 2.1. This BEAT antibody format is referred herein as BEAT CD38-9G7best-fit/CD3(SP34-Kappa2) antibody (FIG. 27 format B). CD38-9G7best-fit/CD3(SP34-Kappa2) antibody had a KD value of 18 nM for the human CD3 1-26_Fc fusion protein (FIG. 29).

The ability of the BEAT CD38-9G7best-fit/CD3(SP34-Kappa2) antibody to redirect T cell killing towards CD38+ cells was investigated *in vitro*. The CD38+ B lymphoblast cell line Daudi was used in killing assays. FIG. 30 show T cell redirected killing of Daudi cells by the BEAT CD38-9G7best-fit/CD3(SP34-Kappa2) antibody. The assays used human PBMCs as effector cells with an effector cells to target cells ratio of 10 to 1, and the RDL-FACS readout method after a 24h incubation period (see Materials and Methods section). The results show that the BEAT CD38-9G7best-fit/CD3(SP34-Kappa2) antibody was highly potent at redirecting T cell killing against the Daudi CD38+ cell line with an EC_{50} of 2 pM (mean of 3 donors).

Examples of OX40/CD3 targeting BEAT antibodies

An example of BEAT antibodies targeting both human OX40 antigen and human CD3 epsilon using the humanized anti-OX40 antibody VH and VL sequences (PCT Publication No: WO2013008171) is formatted as follows:

A BEAT OX40/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.4 for the anti-human CD3 epsilon and the anti-human OX40 antigen binding sites, respectively.

The anti-human OX40 arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 340) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 173). This heavy chain has no binding to Protein A as it encompasses part of a human IgG3 Fc region and has its heavy chain variable domain originating from a non-VH3 domain subclass.

The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT OX40/CD3(SP34-Kappa2) antibody.

Human OX40 expressing cell lines described above are used to assess redirected T cell killing in assays similar to that of described in Example 3.2.4.

Examples of CD20/CD3 targeting BEAT antibodies

An example of BEAT antibodies targeting both human CD20 antigen and human CD3 epsilon using the mouse rituximab antibody VH and VL sequences was formatted as follows:
A BEAT CD20/CD3 was engineered using a combination of antigen binding sites described in Example 2.1 and 2.5 for the anti-human CD3 epsilon and the anti-human CD20 arms, respectively.

The anti-human CD20 arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 180) based on the mouse rituximab antibody variable domains (mouse variable heavy and light chain domains with SEQ ID NO: 143 and 144, respectively) that encompassed a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 181). This heavy chain had no binding to Protein A as it encompassed part of a human IgG3 Fc region and had its heavy chain variable domain originating from a non-VH3 domain subclass.
The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consisted of a BEAT heavy chain (SEQ ID NO: 177) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This arm is equivalent to the BEAT CD38-HB7bestfit/CD3 anti-CD3 epsilon arm described above (see FIG. 27 format A). This scFv fragment encompassed a humanized version of the anti-human CD3 epsilon SP34 antibody as described in PCT Publication No: WO2008119565 (VH and VL domains with SEQ ID NO: 182 and 183, respectively). This BEAT antibody format is referred herein as BEAT CD20/CD3(SP34) antibody (FIG. 31).

The BEAT CD20/CD3(SP34) antibody was transiently expressed, purified and tested *in vitro* for its ability to redirect T cell killing against human CD20+ cell lines. The CD38+ B lymphoblast cell line Daudi was used in killing assays. FIG. 32 show T cell redirected killing of Daudi cells by the BEAT CD20/CD3(SP34) antibody. The assays used human PBMCs as effector cells with an effector cells to target cells ratio of 10 to 1, and the RDL-FACS readout method after a 24h incubation period (see Materials and Methods section). The results show that the BEAT CD20/CD3(SP34) antibody was highly potent at redirecting T cell killing against Daudi cells with an EC_{50} of 25 pM (mean of 3 donors).

Another example of BEAT antibodies targeting both human CD20 antigen and human CD3 epsilon using the chimeric rituximab antibody VH and VL sequences is formatted as follows: a BEAT EGFR/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.5 for the anti-human CD3 epsilon and the anti-human CD20 antigen binding sites, respectively.

The anti-human CD20 arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 341) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 181). This heavy chain has no binding to Protein A as it encompasses part of a human IgG3 Fc region and has its heavy chain variable domain originating from a non-VH3 domain subclass.

The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT CD20/CD3(SP34-Kappa2) antibody.

Examples of EGFR/CD3 targeting BEAT antibodies

An example of BEAT antibodies targeting both human EGFR antigen and human CD3 epsilon using the mouse Erbitux antibody VH and VL sequences is formatted as follows: a BEAT EGFR/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.6 for the anti-human CD3 epsilon and the anti-human EGFR antigen binding sites, respectively.

The anti-human EGFR arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 342) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 175). This heavy chain has no binding to Protein A as it encompasses part of a human IgG3 Fc region and has its heavy chain variable domain originating from a non-VH3 domain subclass.

The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT EGFRcetux/CD3(SP34-Kappa2) antibody.

Another example of BEAT antibodies targeting both human EGFR antigen and human CD3 epsilon using the human Vectibix antibody VH and VL sequences is formatted as follows: a BEAT EGFR/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.6 for the anti-human CD3 epsilon and the anti-human EGFR antigen binding sites, respectively.

The anti-human EGFR arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 343) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 344). This heavy chain has no binding to Protein A as it encompasses part of a human IgG3 Fc region and has its heavy chain variable domain originating from a non-VH3 domain subclass.

The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT EGFRpani/CD3(SP34-Kappa2) antibody.

Examples of IgE/CD3 targeting BEAT antibodies

An example of BEAT antibodies targeting both human IgE antigen and human CD3 epsilon using the humanized omalizumab antibody VH and VL sequences is formatted as follows: a BEAT IgE/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.8 for the anti-human CD3 epsilon and the anti-human IgE antigen binding sites, respectively.

The anti-human IgE arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 345) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 346). This heavy chain has no binding to Protein A as it encompasses part of a human IgG3 Fc region and has its heavy chain variable domain originating from a non-VH3 domain subclass.

The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT IgEomali/CD3(SP34-Kappa2) antibody.

Another example of BEAT antibodies targeting both human IgE antigen and human CD3 epsilon using the mouse Bsw17 antibody VH and VL sequences is formatted as follows: a BEAT IgE/CD3 is engineered using a combination of antigen binding sites described in Example 2.1 and 2.8 for the anti-human CD3 epsilon and the anti-human IgE antigen binding sites, respectively.

The anti-human IgE arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 347) encompassing a variable heavy chain region, a CH1 γ1 region, a γ1 hinge region, a γ3 CH2 region with L234A and L235A substitutions (EU numbering), and a γ3 based BEAT CH3 domain assembled with its cognate light chain (SEQ ID NO: 348). This heavy chain has no binding to Protein A as it encompasses part of a human IgG3 Fc region and has its heavy chain variable domain originating from a non-VH3 domain subclass.

The anti-human CD3 epsilon arm of the hetero-dimeric immunoglobulin consists of a BEAT heavy chain (SEQ ID NO: 311) encompassing a scFv fragment, a CH1 γ1 region, a γ1 hinge region, a γ1 CH2 region with L234A and L235A substitutions (EU numbering), and a γ1 based BEAT CH3 domain. This bispecific antibody is referred herein as BEAT IgEbsw17

/CD3(SP34-Kappa2) antibody.

Membrane IgE expressing cell lines described are used to assess redirected T cell killing in assays similar to that of described above.

Functional equivalence of improved SP34 in scFv format

- **Bispecific CD38xCD3 antibody**

In order to determine whether of the modifications made to the various SP34 scFv so as to improve their expression also affected the functional properties, namely the binding of CD3, they were tested in the context of a CD38xCD3 bispecific. The CD38 binding arm present as a FAB, comprises the heavy chain variable region encoded by SEQ ID NO: 133 and the light chain variable region encoded by SEQ ID NO: 134. The CD3 binding arm comprises the original mouse SP34 reformatted as an scFv (SEQ ID NO: 403), or modified humanised SP34 scFv's comprising the heavy/light chain combinations H1/L21 (SEQ ID NO: 361), H5/L32 (SEQ ID NO: 311), H5/L65 (SEQ ID NO: 394) and H5/L67 (SEQ ID NO: 396).

Each of the CD3/CD38 BEAT using the different version of SP34 were transiently expressed and purified. They were tested *in vitro* to compare their ability to redirect T cell killing. Raji CD38 expressing cell line (see Materials and Methods section) was used to assess redirected T cell killing. The assay used human PBMCs as effector cells with an effector cells to target cells ratio of 10:1.

When measured with the RDL-FACS method, all BEAT showed comparable EC50 between 6 and 10pM (the mean of 2 donors, 24h incubation) Figure 37.

- **Bispecific CD20xCD3 antibody**

The properties of a SP34 scFv comprising the H5/L65 heavy chain/light chain combinations (SEQ ID NO: 394) was also tested in conjunction with a CD20 FAB binding arm, Rituximab, VH and VL domains with SEQ ID NO: 282 and 283, respectively.

The CD3/CD20 BEAT was transiently expressed and purified, then tested *in vitro* to compare their ability to redirect T cell killing. Raji CD20 expressing cell line (see Materials and Methods section) was used to assess redirected T cell killing. The assay used human PBMCs as effector cells with an effector cells to target cells ratio of 5:1 incubated for 24 hours.

When measured with the RDL-FACS method, all BEAT showed a comparable EC50 of 37.56 pM (the mean of 4 donors, 24h incubation) Figure 38, to CD3xCD20 BEATs which comprised H1/L21 (SEQ ID NO: 361), see above.

- **Bispecific EGFRxCD3 antibody**
  - Erbitux/CD3

Patients carrying KRAS mutations show no benefit from treatment with the monoclonal antibody Cetuximab (trade name Erbitux) (Karapetis C et al., N Engl J Med 359: 1757 (2008).

To further evaluate the properties of the improved SP34 scFv and to establish that it is possible to kill high EGFR expressing cell lines and overcome the KRAS resistance, a bispecific Erbitux (Heavy Chain SEQ ID NO: 174 and Light Chain SEQ ID NO: 175) - hSP34 (SEQ ID NO: 394) BEAT was constructed, an *in vitro* redirected T-cell lysis assay was performed on the KRAS mutated cancer cell lines A549 (lung) and HCT116 (colorectal cancer). Additionally, to demonstrate that Erbitux -hSP34 kills only cells displaying high and medium expression levels of EGFR and not low EGFR expressing cells, the low EGFR expressing cell line MCF-7 was also included in the assay. The EGFR status of the cell lines was determined using the EGFR PharmDx immunohistochemistry kit (Table 2).

Cell line	Average of sABC
A549	182'454
HCT116	44'294
MCF-7	5'644

Table 2

IvIg was added at 2.5 mg/mL to each well to block Erbitux effector function to better mimic the mode of action of Erbitux, which is inhibition of EGFR signalling. As shown in figure 39. Erbitux has no effect on any of the cell lines (Not possible to assess any EC_{50}).

On the contrary, as shown in figure 40, Erbitux/hSP34 BEAT can effectively kill KRAS mutated A549 and HCT116 tumour cells with EC50's of 66.97 pM and 43.3 pM respectively while lower EGFR expressing MCF-7 cells are not efficiently killed.

- Vectibix

To further evaluate the properties of the improved SP34 scFv and to further establish that it is possible can kill high EGFR expressing cell lines and overcome the KRAS resistance mechanism with a bispecific Vectibix (VL SEQ ID NO: 291 and VH SEQ ID NO: 290) as a FAB -hSP34 BEAT (SEQ ID NO: 394) As ScFv, an *in vitro* redirected T-cell lysis assay was performed on the high EGFR expressing lung cancer cell line HCC827 as well as on the KRAS mutated lung cancer cell line A549 and colorectal cancer KRAS positive cell lines HCT116 and SW480.

To demonstrate that Vectibix/hSP34 BEAT kills only cells displaying high and medium expression levels of EGFR and not low EGFR expressing cells, the low EGFR expressing cell line MCF-7 was also included in the killing assay. The EGFR status of the cell lines (figure 41 was determined using the EGFR PharmDx immunohistochemistry kit (Dako, Cambridge, UK).

As shown in figure 42, Vectibix has no effect on any of the cell lines assessed (mean of 2 donors and effector:target cell ratio 20 to 1. Read out: after 48 hours with MTS.).

For the Vectibix/hSP34 BEAT an effector:target cell ratio of 10:1 was used and data are presented as the mean of 4 donors.

As shown in figure 43, the Vectibix/hSP34 BEAT can effectively kill high EGFR expressing cell line HCC827 and KRAS mutated A549, HCT116 and SW480 tumour cells with EC50's of 0.1557, 0.6127, 0.3406 and 6.986 pM respectively while lower EGFR expressing MCF-7 cells are not efficiently killed (EC50 = 627.4 pM).

EC_{50}s	HCC827	HCT116	SW480	MCF7
Vectibix-CD3	0.1457	0.3406	6.986	627.4
EC_{50} (X) / EC_{50} (HCC827) Vectibix-CD3	/	2.3	47.9	4303

Table3

In Table 3, the mean EC50's of 4 donors were determined and the therapeutic window calculated (EC_{50}(X)/EC_{50}(HCC827)Vectibix-CD3).

Claims

1. An epitope binding protein or a fragment thereof which binds to human CD3 and which comprises at least a heavy chain variable domain selected from the group comprising: SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103 and SEQ ID NO: 104 and a light chain variable domain selected from the group comprising: SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 401 and SEQ ID NO: 402.

2. The epitope binding protein or a fragment thereof according to claim 1, comprising a heavy chain variable domain and a light chain variable domain pair selected from the group comprising: SEQ ID NO: 101 and SEQ ID NO: 105, SEQ ID NO: 104 and SEQ ID NO: 106, SEQ ID NO: 104 and SEQ ID NO: 401, SEQ ID NO: 104 and SEQ ID NO: 402.

3. The epitope binding protein or fragment thereof according to claim 1, comprising a heavy chain and a light chain pair selected from the group comprising: SEQ ID NO: 359 and SEQ ID NO: 399, SEQ ID NO: 359 and SEQ ID NO: 400.

4. The epitope binding protein or a fragment thereof according to claim 1, 2 or 3, wherein said antigen binding protein or fragment is a heterodimeric immunoglobulin or a fragment thereof.

5. A heterodimeric immunoglobulin or a fragment thereof according to claim 4, wherein said heterodimeric immunoglobulin or fragment binds to a second epitope.

6. The heterodimeric immunoglobulin or a fragment thereof according to claim 4 or 5, wherein the epitope binding region of the first polypeptide is a FAB and the epitope binding region of the second polypeptide is a scFv or wherein the epitope binding region of the first polypeptide is a scFv and the epitope binding region of the second polypeptide is a FAB.

7. The heterodimeric immunoglobulin or a fragment thereof according to claim 6, wherein the scFv binds to human CD3 and comprises a sequence selected from the group SEQ ID NO: 361, SEQ ID NO: 311, SEQ ID NO: 394 and SEQ ID NO: 396.

8. The heterodimeric immunoglobulin or a fragment thereof according to claim 6 or 7, wherein said scFv has at least a twofold improvement in expression in comparison to a SP34 chimera formatted as a scFv-Fc comprising the heavy and light variable regions encoded by SEQ ID NOs: 60 and 61.

9. The heterodimeric immunoglobulin or a fragment thereof according to anyone of claims 6 to 8, wherein said scFv when expressed in a bispecific antibody comprising a FAB arm has at least a twofold improvement in expression in comparison to a SP34 chimera formatted as an scFv comprising the heavy and light variable regions encoded by SEQ ID NOs: 60 and 61 when expressed in a bispecific antibody comprising a FAB arm.

10. The epitope binding protein or a fragment thereof or a heterodimeric immunoglobulin or a fragment thereof or a heterodimeric immunoglobulin or a fragment thereof according to anyone of claims 1 to 9, wherein said epitope binding protein or a fragment thereof or a heterodimeric immunoglobulin or a fragment thereof has greater thermostability in comparison to an epitope binding protein or a fragment thereof or a heterodimeric immunoglobulin or a fragment thereof comprising the heavy and light variable regions encoded by SEQ ID NOs: 60 and 61.

FIG. 1

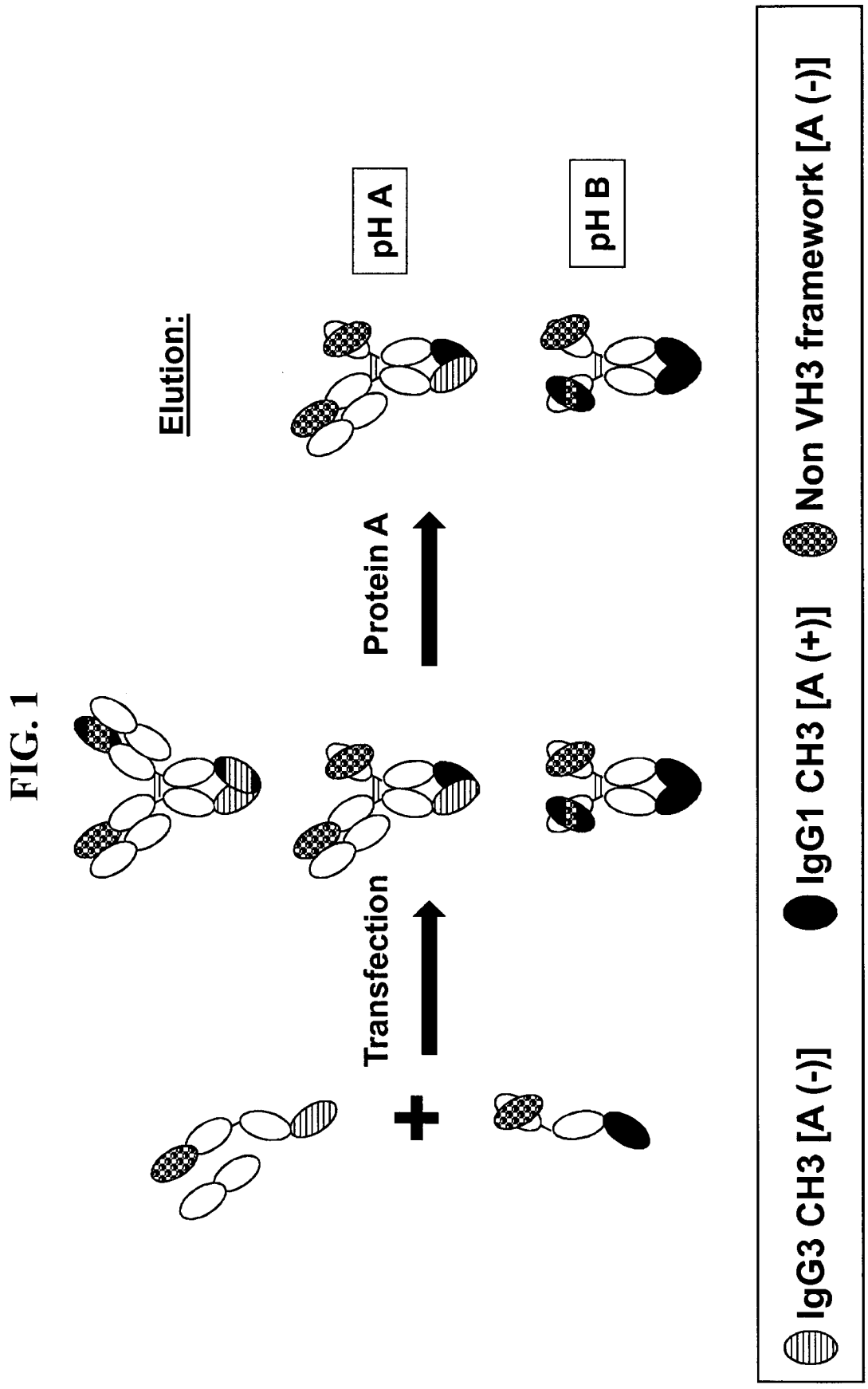
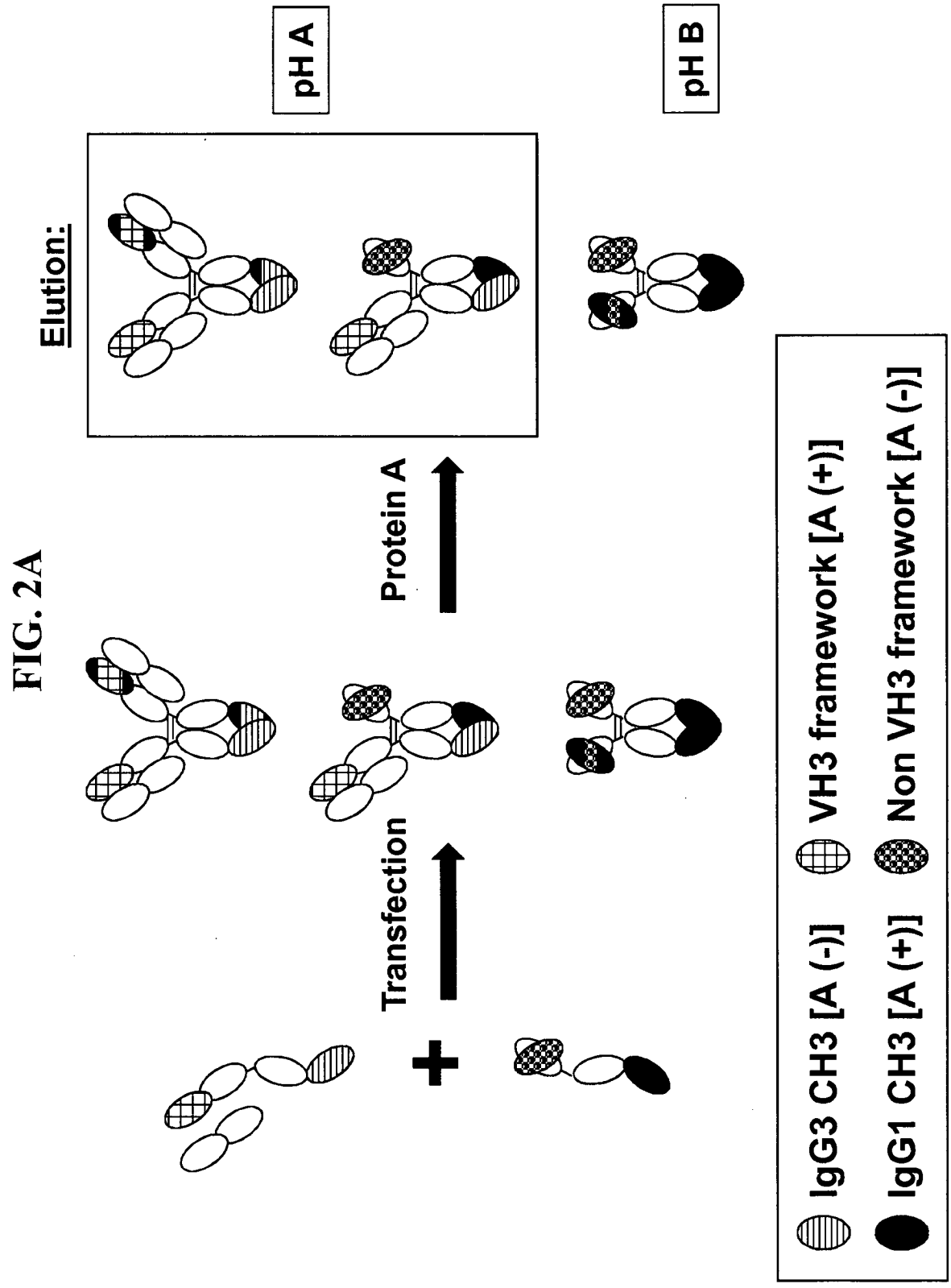

Transfection
Protein A
Elution:
pH A
pH B
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
Non VH3 framework [A (-)]

FIG. 2A


Elution:
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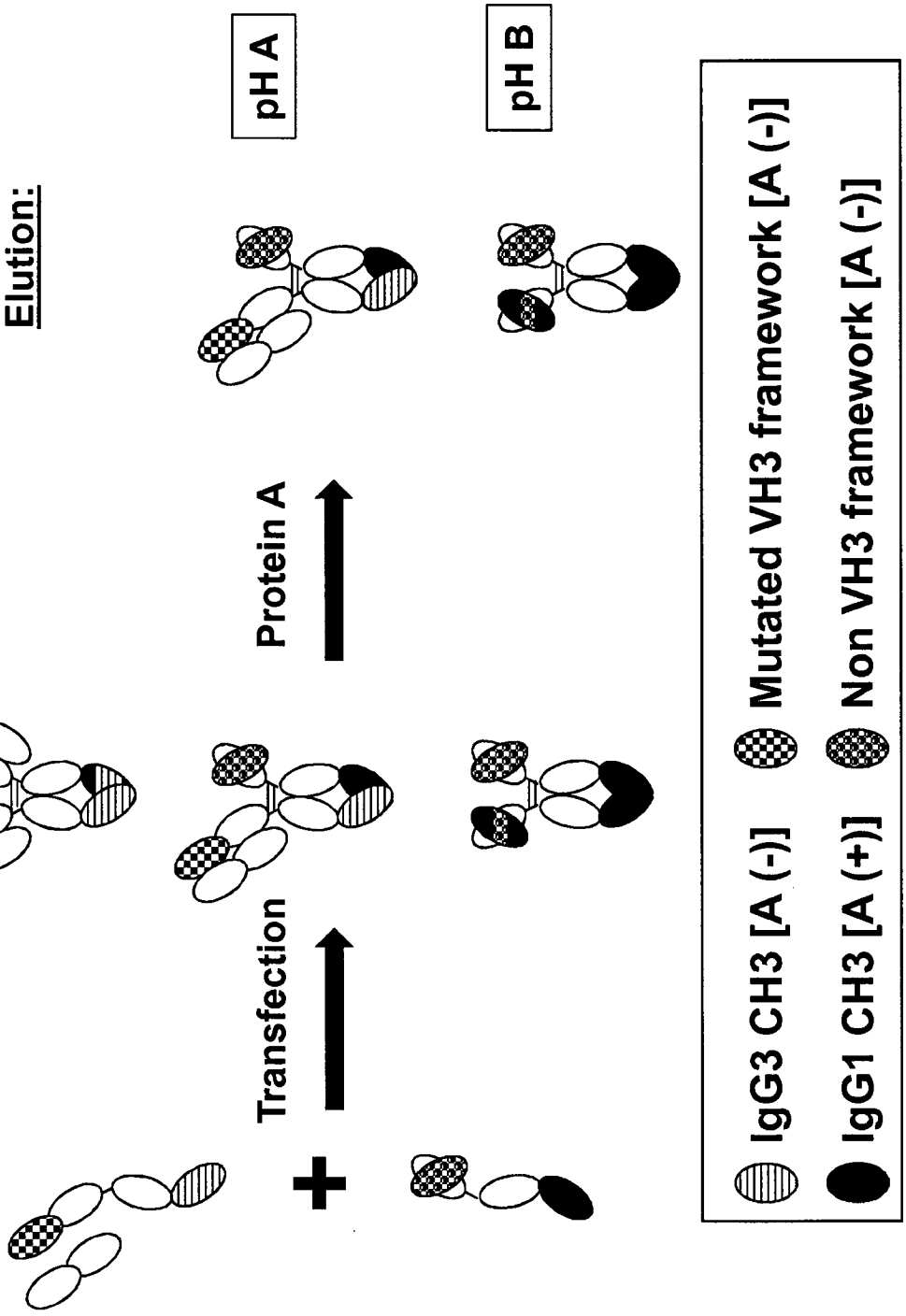
pH B
Protein A
Transfection
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 framework [A (+)]
Non VH3 framework [A (-)]

FIG. 2B


Elution:
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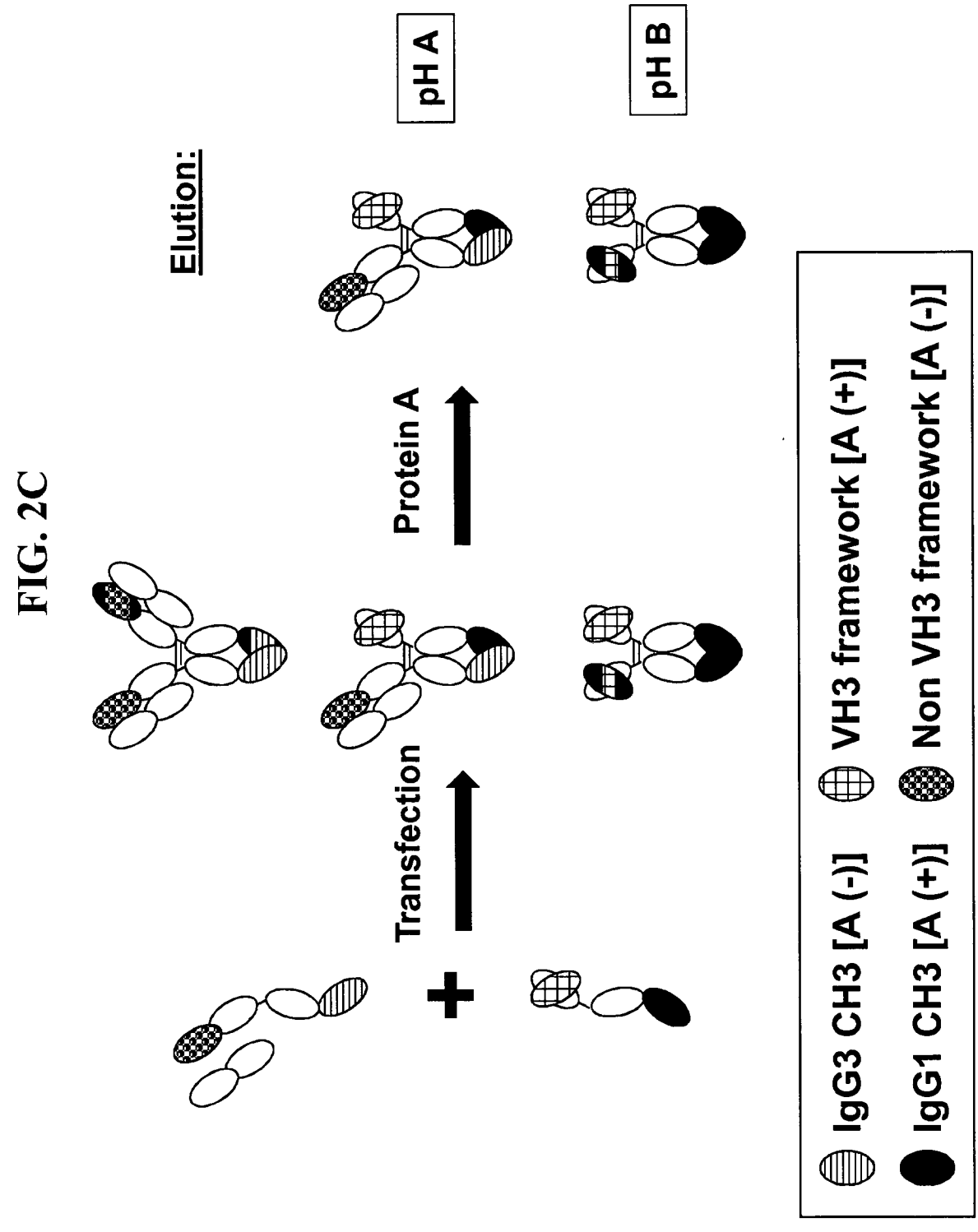
pH B
Protein A
Transfection
IgG3 CH3 [A (-)]
Mutated VH3 framework [A (-)]
IgG1 CH3 [A (+)]
Non VH3 framework [A (-)]

FIG. 2C


Elution:
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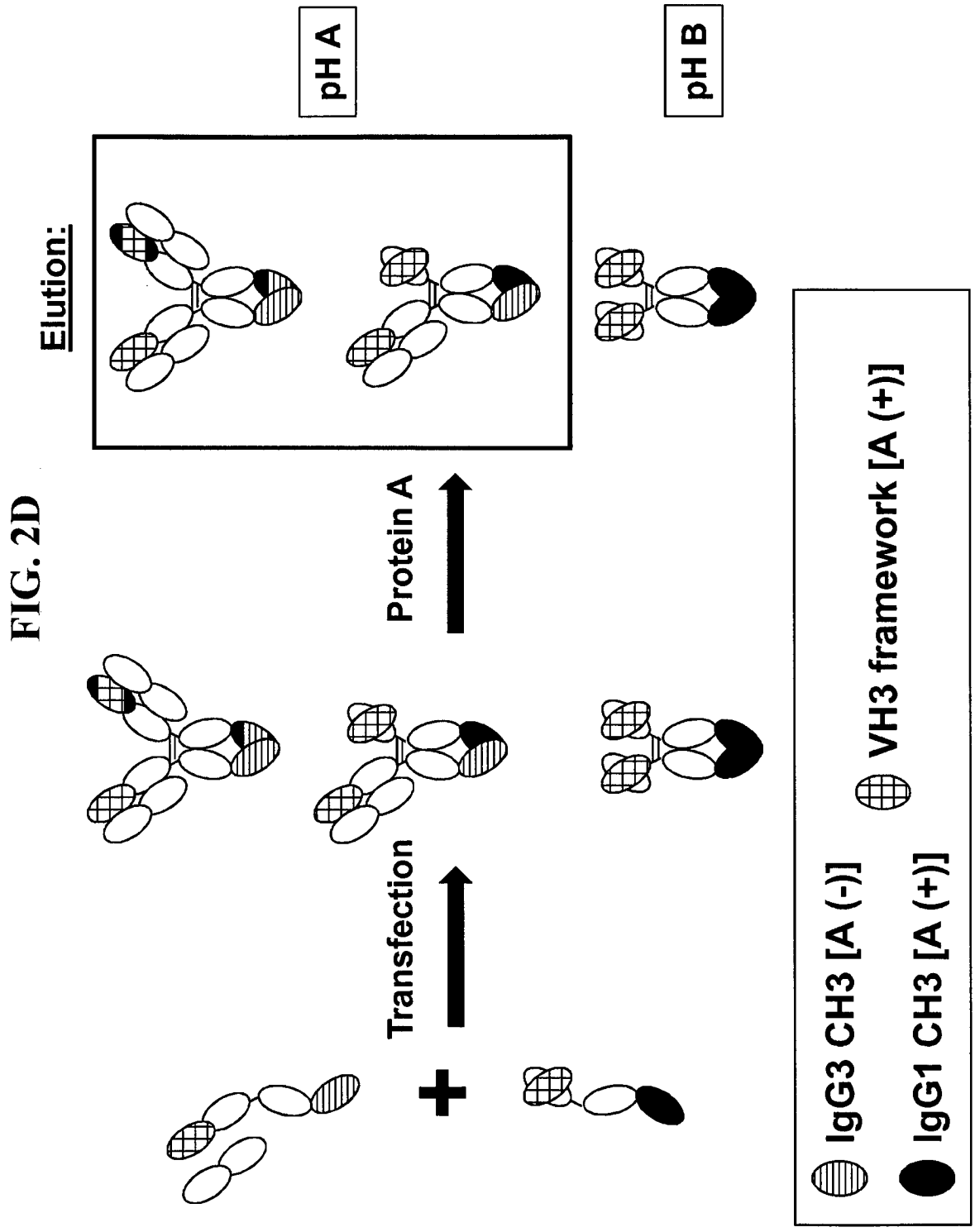
pH B
Protein A
Transfection
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 framework [A (+)]
Non VH3 framework [A (-)]

FIG. 2D


Elution:
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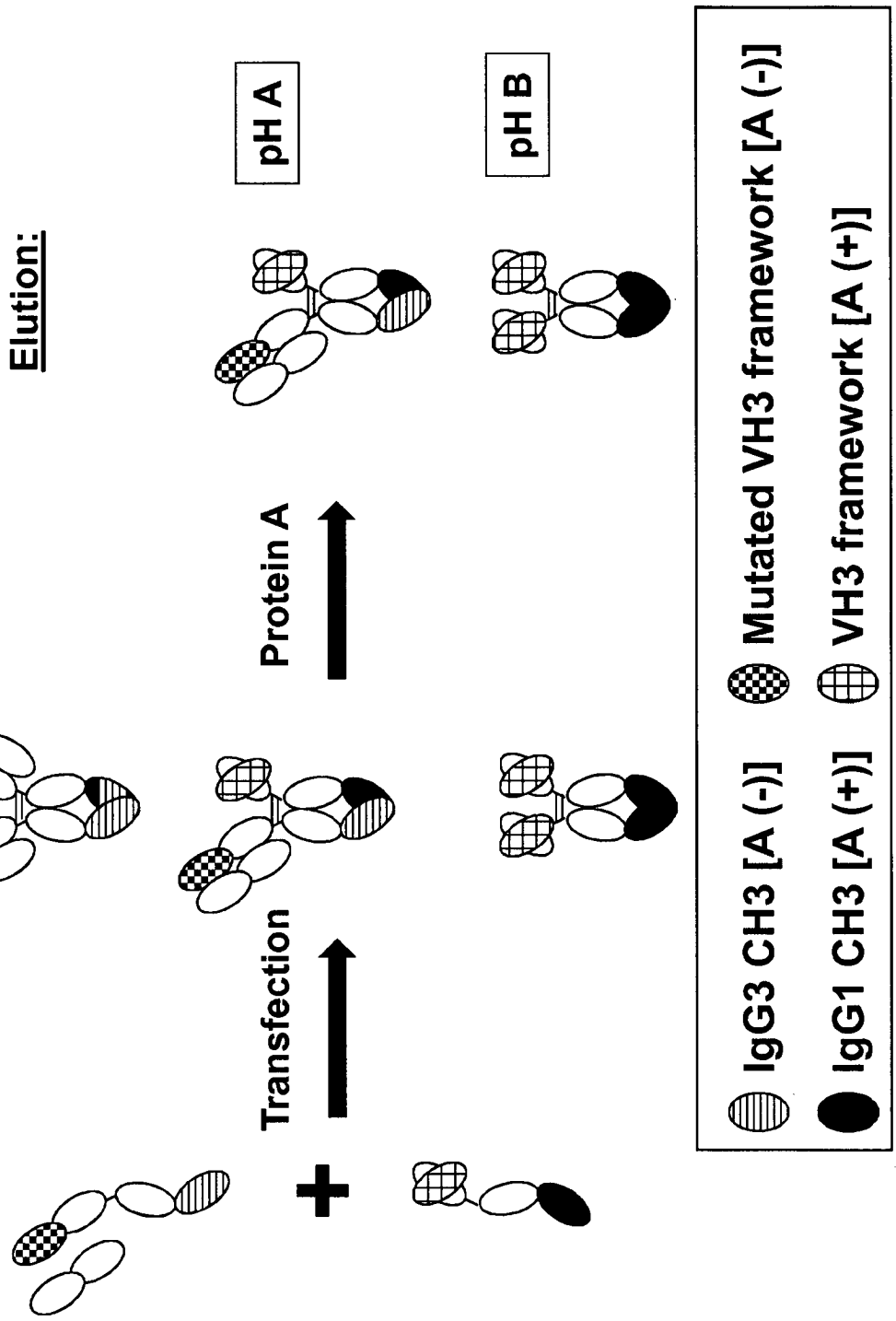
pH B
Protein A
Transfection
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 framework [A (+)]

FIG. 2E


Elution:
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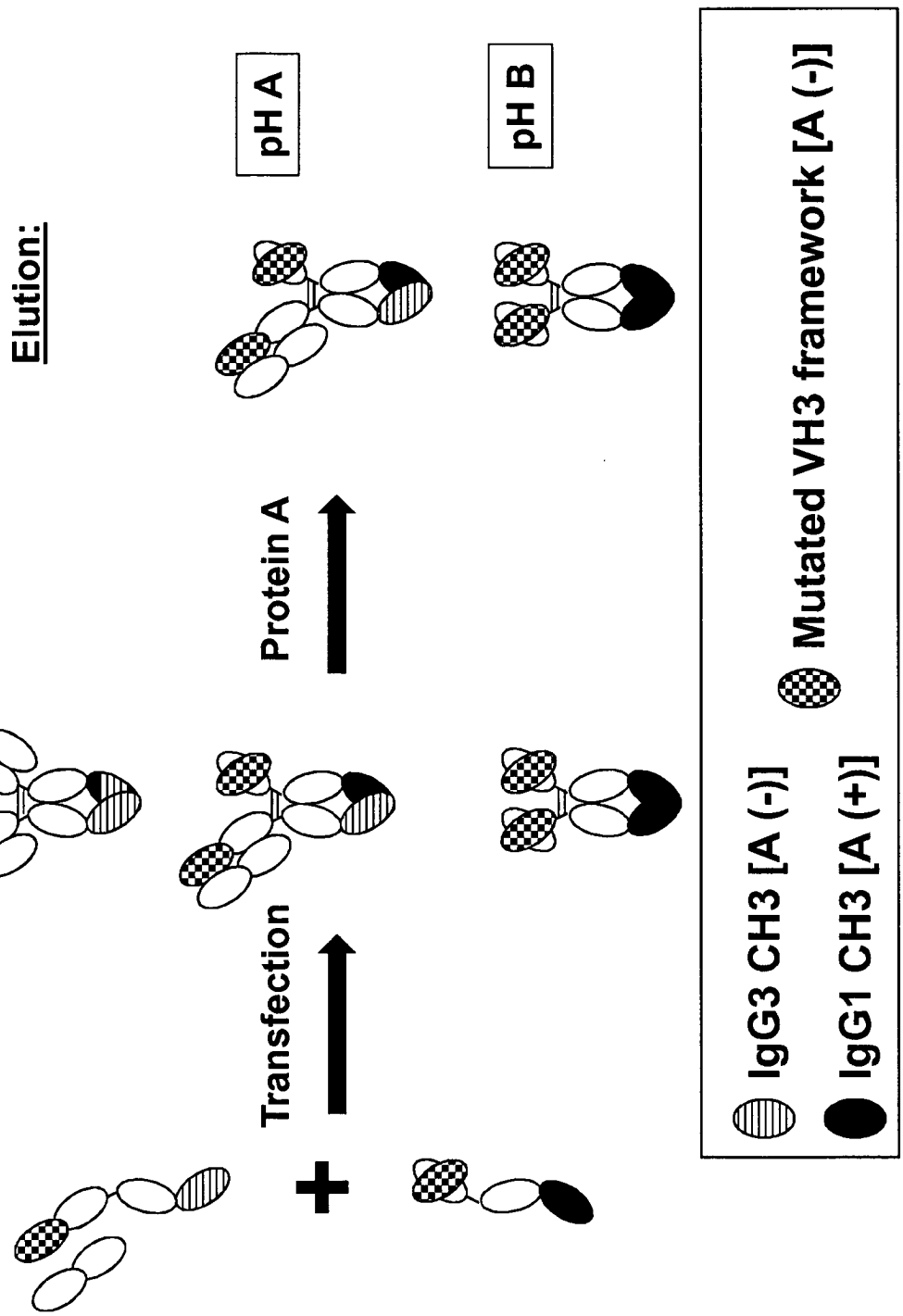
pH B
Protein A
Transfection
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
Mutated VH3 framework [A (-)]
VH3 framework [A (+)]

FIG. 2F


Elution:
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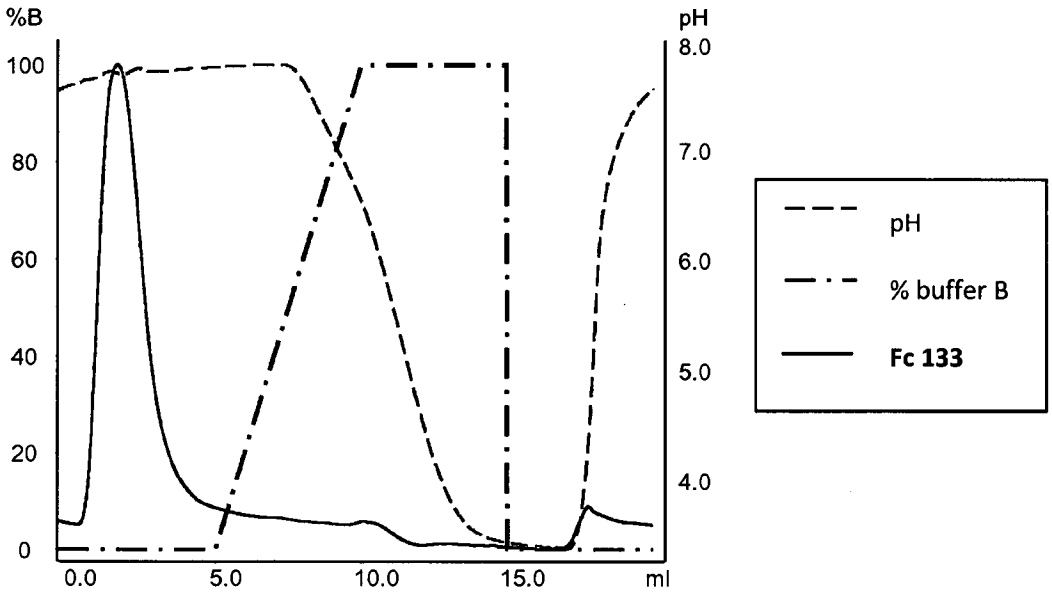
pH B
Protein A
Transfection
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
Mutated VH3 framework [A (-)]

FIG. 3


%B
pH
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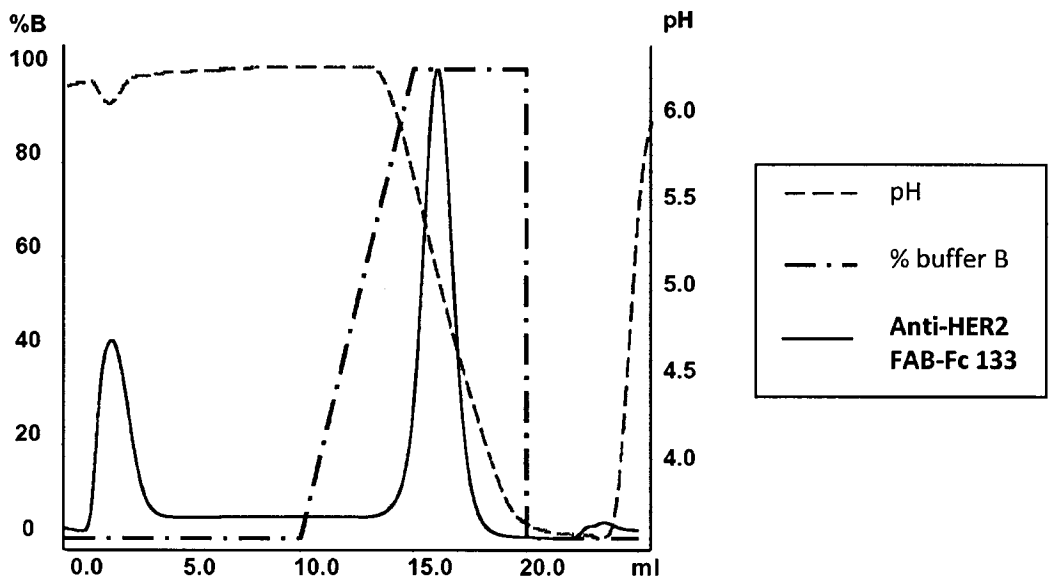
80
60
40
20
0
8.0
7.0
6.0
5.0
4.0
0.0
5.0
10.0
15.0
ml
pH
% buffer B
Fc 133

FIG. 4A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pH
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80
60
40
20
0
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5.5
5.0
4.5
4.0
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5.0
10.0
15.0
20.0
ml
pH
% buffer B
Anti-HER2
FAB-Fc 133

FIG. 4B

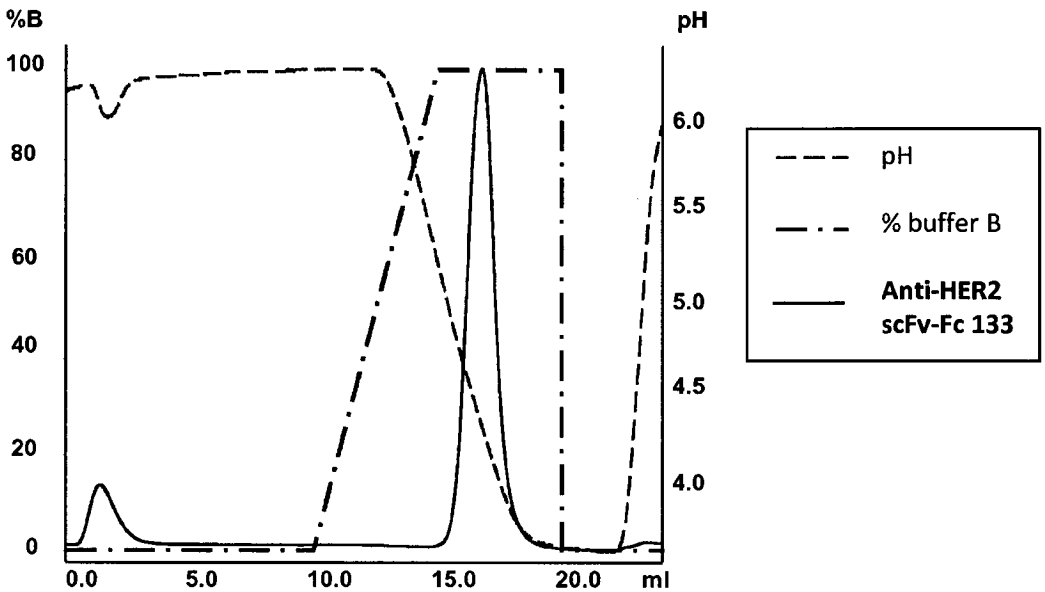

%B
pH
100
80
60
40
20
0
6.0
5.5
5.0
4.5
4.0
0.0
5.0
10.0
15.0
20.0
ml
pH
% buffer B
Anti-HER2 scFv-Fc 133

FIG. 4C

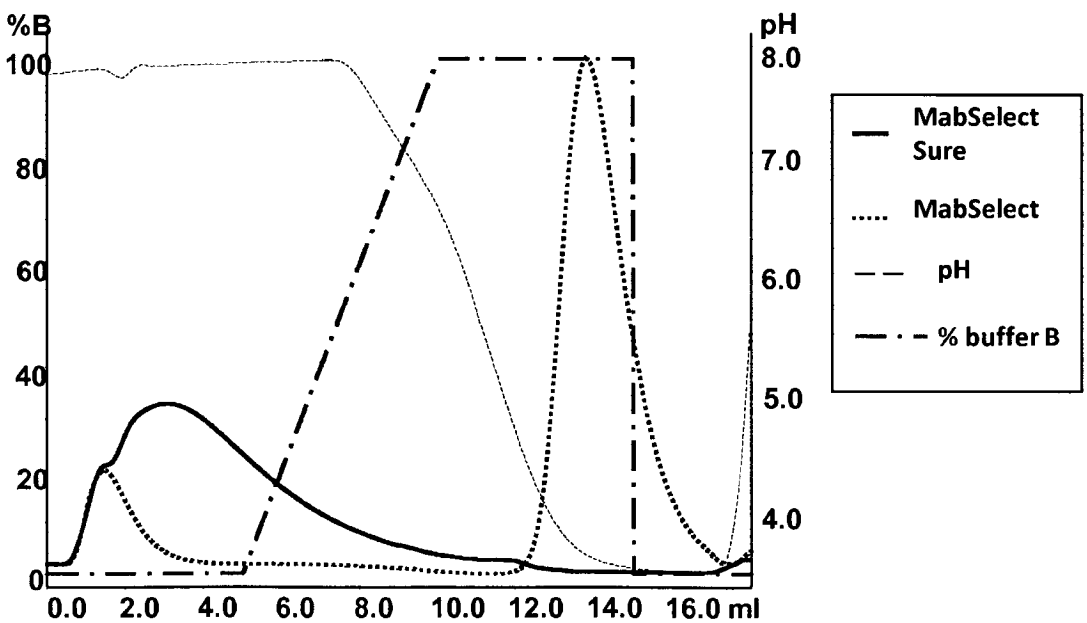

%B
pH
100
80
60
40
20
0
8.0
7.0
6.0
5.0
4.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0 ml
MabSelect Sure
MabSelect
pH
% buffer B

FIG. 5

```
                          10        20        30        40        50
                   ....|....|....|....|....|....|....|....|....|....|
Kabat numbering    12345678901234567890123456789012345AB678901234567890
Human IGHV1-3      QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAMH..WVRQAPGQRLEWMGW
Human IGHV2-26     QVTLKESGPVLVKPTETLTLTCTVSGFSLSNARMGVSWIRQPPGKALEWLAH
Human IGHV3-23     EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS..WVRQAPGKGLEWVSA
Human IGHV4-28     QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWG.WIRQPPGKGLEWIGY
Human IGHV5-51     EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIG..WVRQMPGKGLEWMGI
Human IGHV6-1      QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGR
Human IGHV7-4-1    QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMN..WVRQAPGQGLEWMGW

                              60        70        80            90
                   ....|....|....|....|....|....|....|....|....|....|
Kabat numbering    12AB3456789012345678901234567890 12ABC3456789012345
Human IGHV1-3      INA.GNGNTKYSQKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCAR.
Human IGHV2-26     IF..SNDEKSYSTSLKSRLTISKDTSKSQVVLTMTNMDPVDTATYYCARI
Human IGHV3-23     ISG.SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK.
Human IGHV4-28     IY..YSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYYCAR.
Human IGHV5-51     IYP.GDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCAR.
Human IGHV6-1      TYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCAR.
Human IGHV7-4-1    INT.NTGNPTYAQGFTGRFVFSLDTSVSTAYLQICSLKAEDTAVYYCAR.
```

FIG. 6A

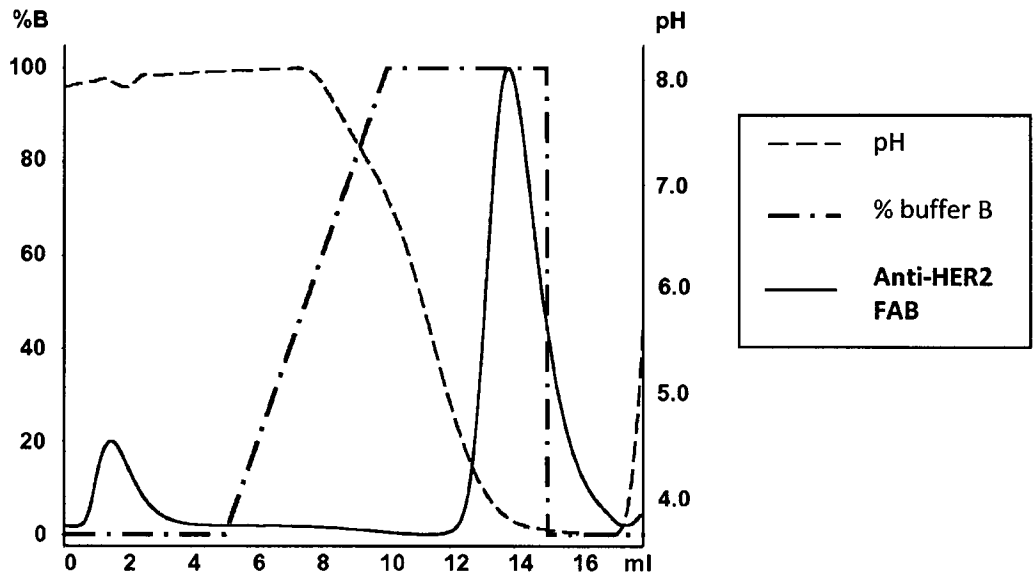

%B
pH
100
80
60
40
20
0
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% buffer B
Anti-HER2 FAB

FIG. 6B

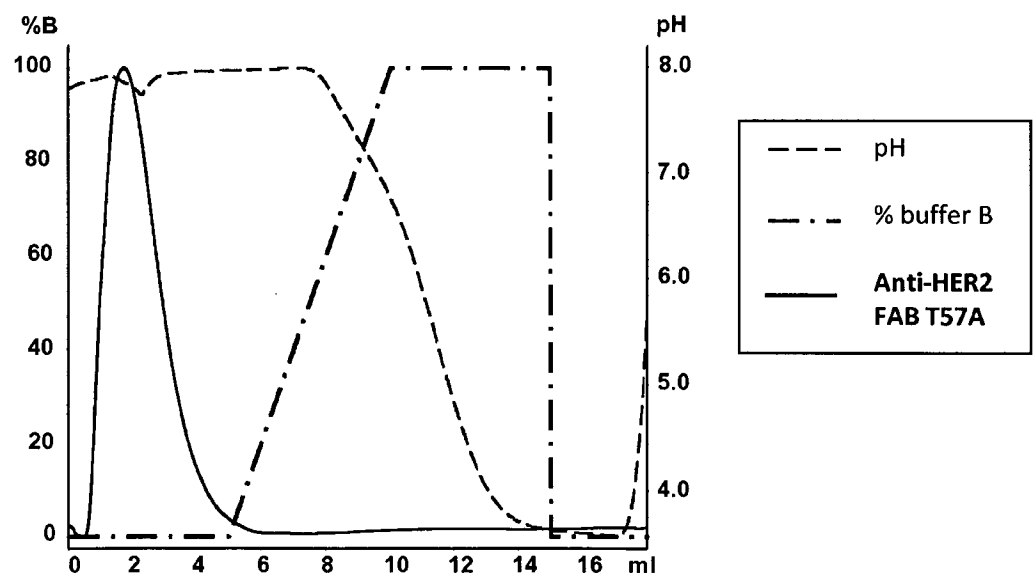

%B
pH
100
80
60
40
20
0
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% buffer B
Anti-HER2 FAB T57A

FIG. 6C

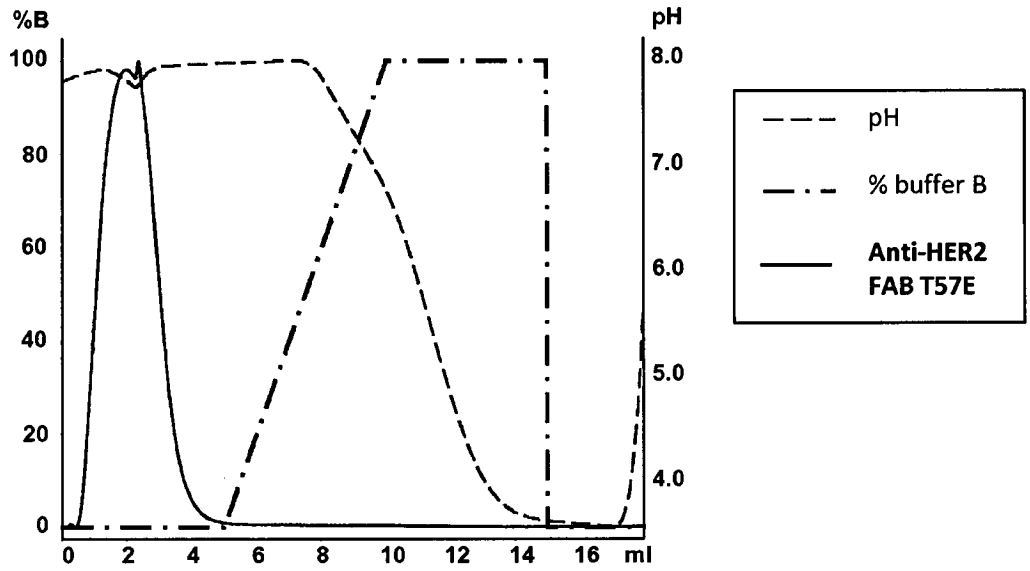

%B
100
80
60
40
20
0
pH
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% buffer B
Anti-HER2 FAB T57E

FIG. 6D

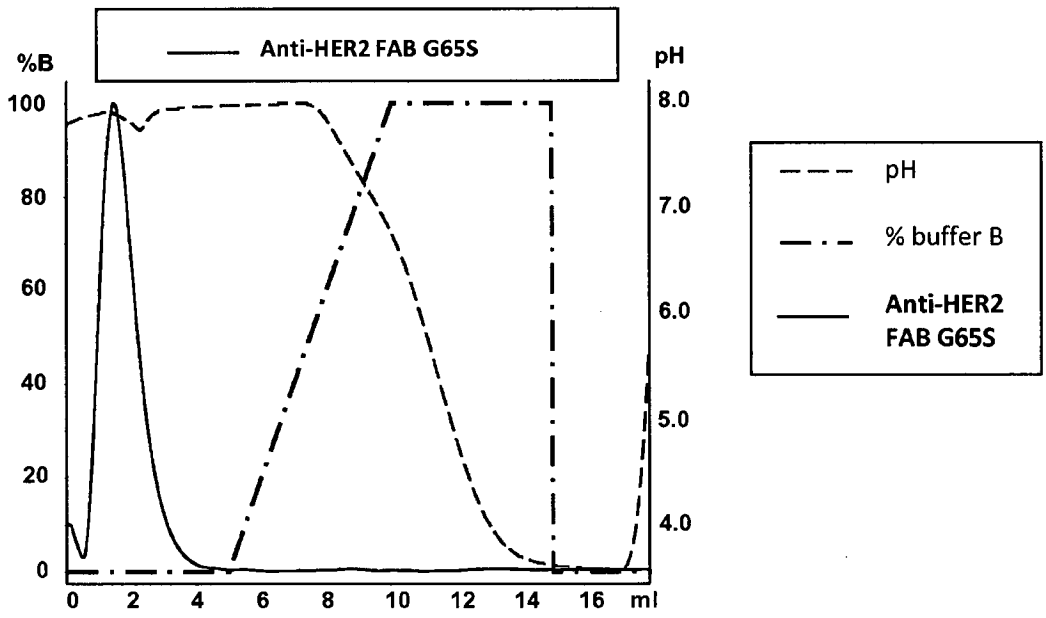

Anti-HER2 FAB G65S
%B
100
80
60
40
20
0
pH
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% buffer B
Anti-HER2 FAB G65S

FIG. 6E

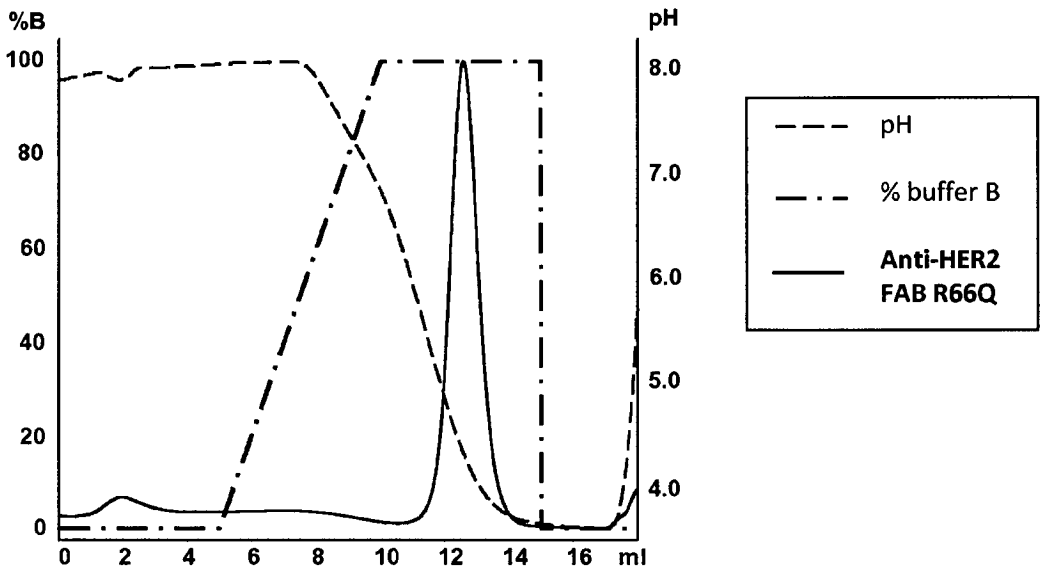

%B
100
80
60
40
20
0
pH
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% buffer B
Anti-HER2 FAB R66Q

FIG. 6F

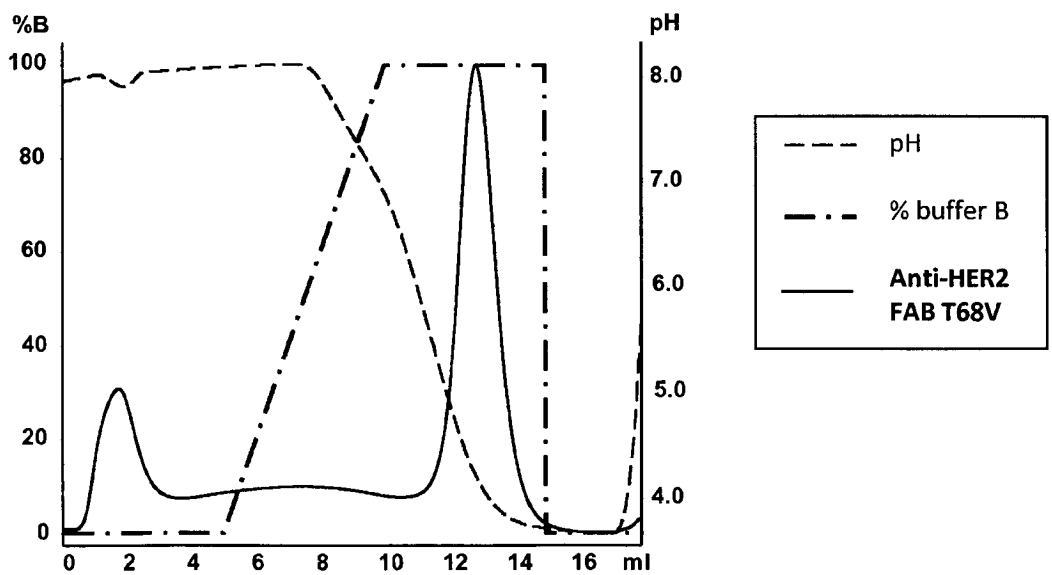

%B
100
80
60
40
20
0
pH
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% buffer B
Anti-HER2 FAB T68V

FIG. 6G

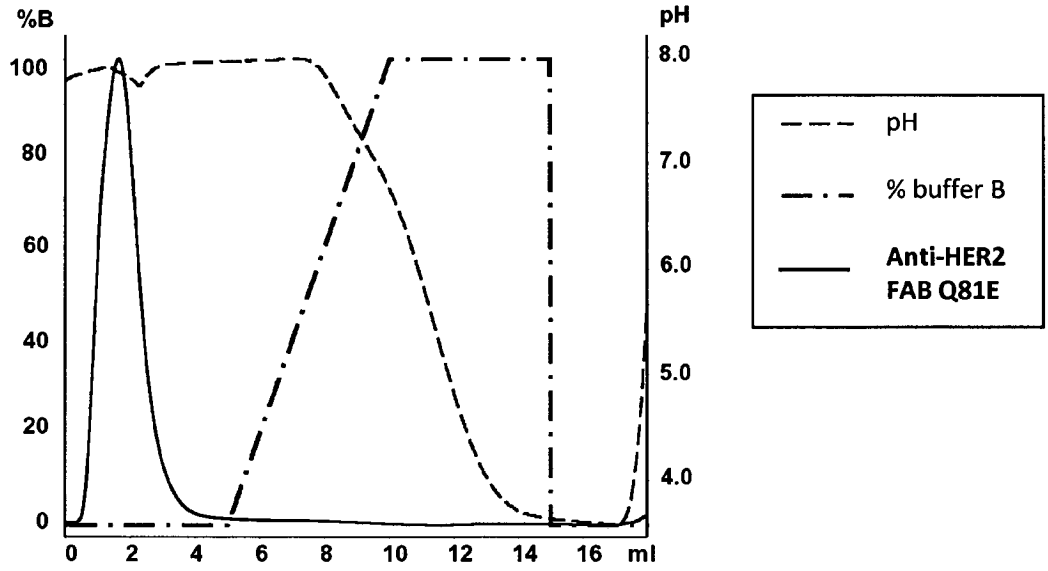

%B
pH
100
80
60
40
20
0
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% buffer B
Anti-HER2 FAB Q81E

FIG. 6H

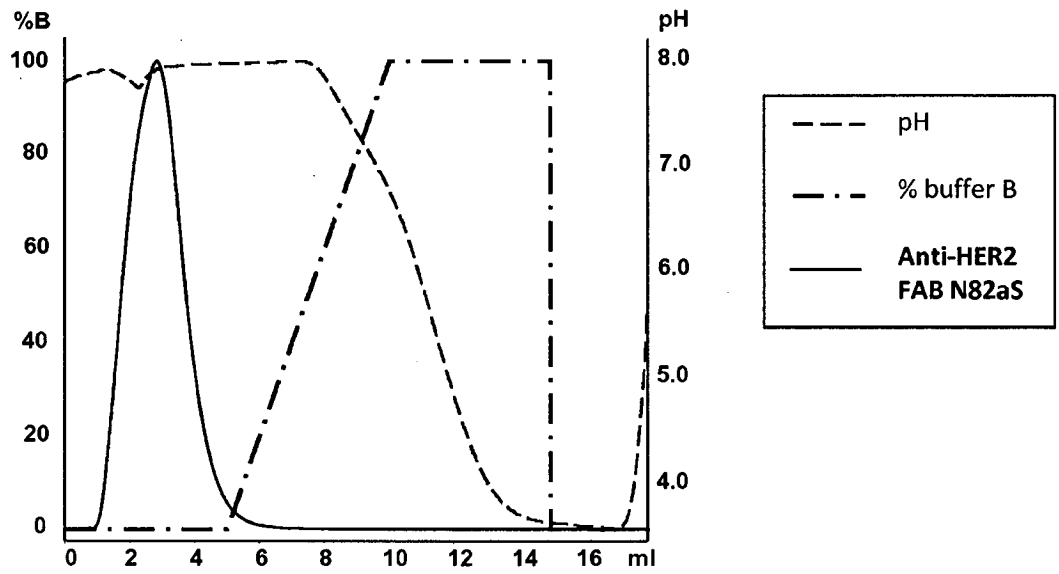

%B
pH
100
80
60
40
20
0
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% buffer B
Anti-HER2 FAB N82aS

FIG. 6I

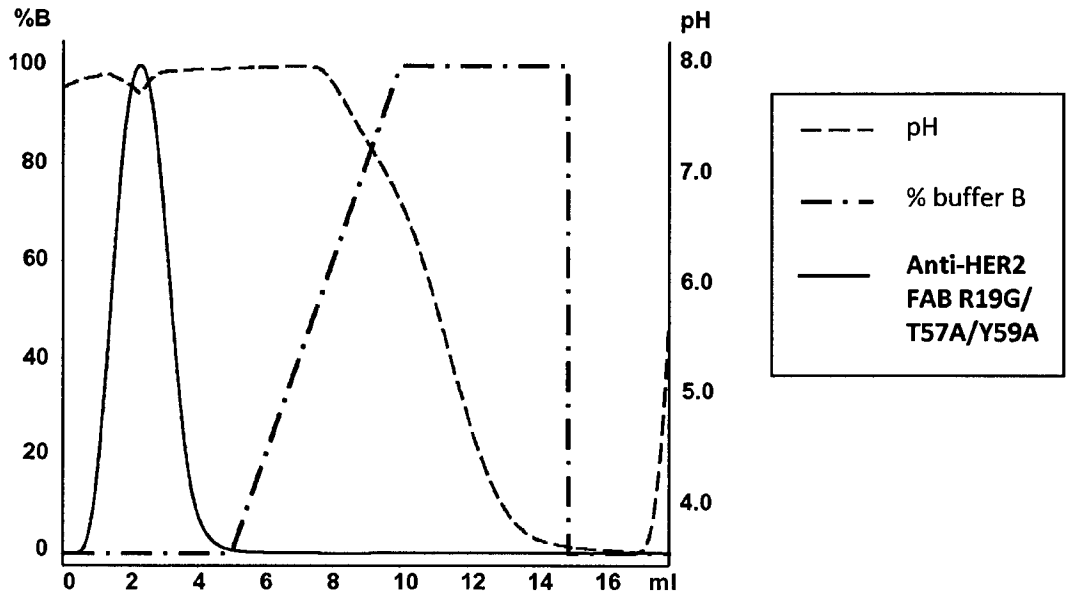


FIG. 7

	KD (pM)
Anti-HER2 FAB	153 ± 9
Anti-HER2 FAB N82aS	162 ± 19
Anti-HER2 FAB G65S	142 ± 30

FIG. 8A

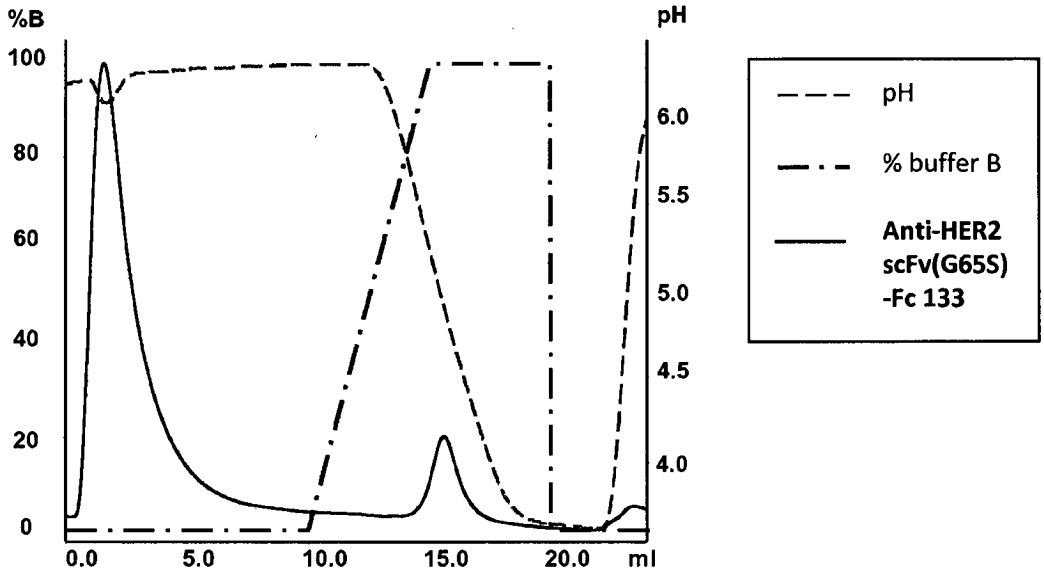

%B
100
80
60
40
20
0
pH
6.0
5.5
5.0
4.5
4.0
0.0
5.0
10.0
15.0
20.0
ml
pH
% buffer B
Anti-HER2 scFv(G65S) -Fc 133

FIG. 8B

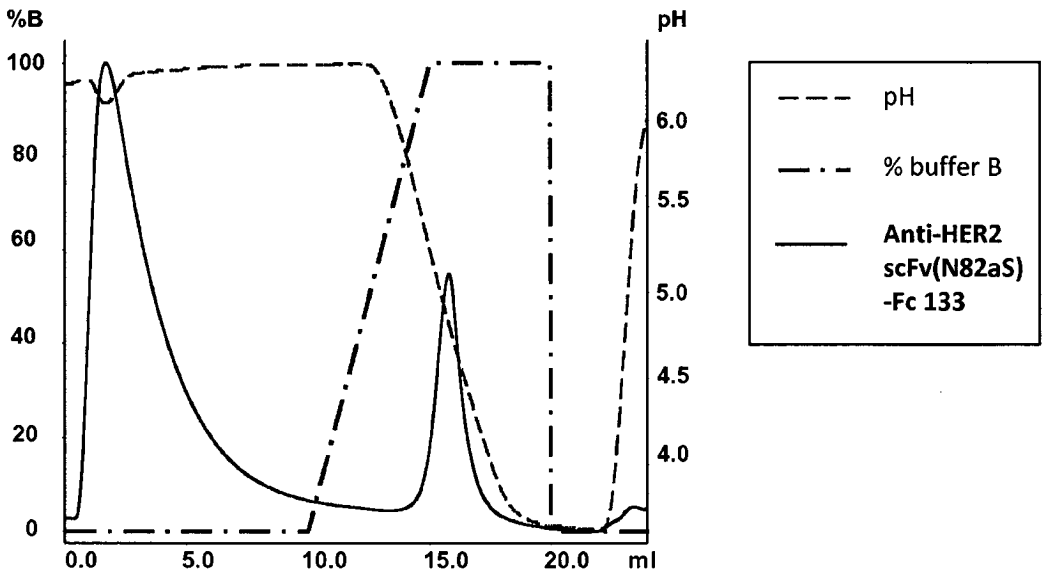

%B
100
80
60
40
20
0
pH
6.0
5.5
5.0
4.5
4.0
0.0
5.0
10.0
15.0
20.0
ml
pH
% buffer B
Anti-HER2 scFv(N82aS) -Fc 133

FIG. 8C

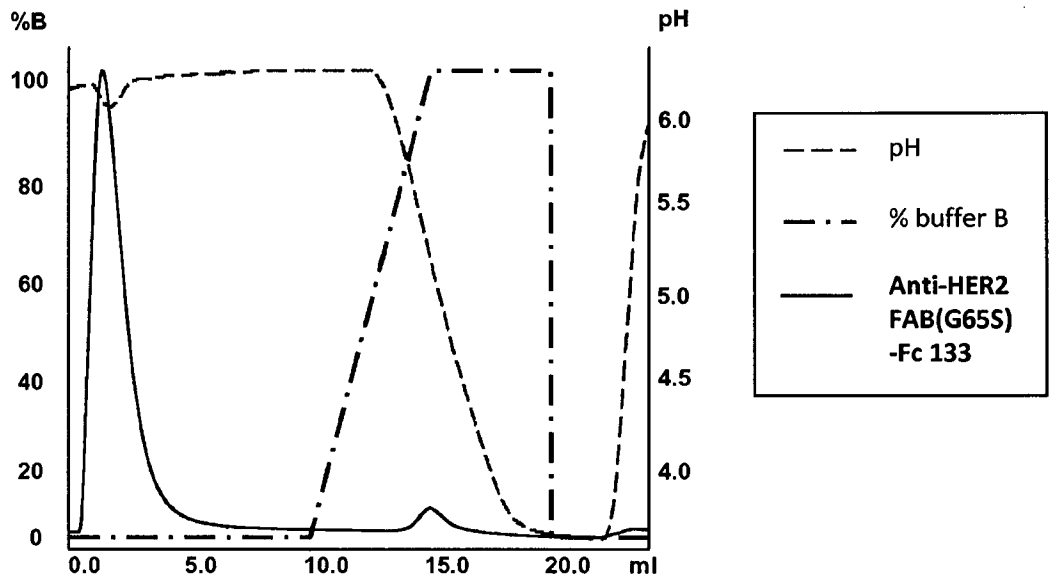

%B
pH
100
80
60
40
20
0
6.0
5.5
5.0
4.5
4.0
0.0
5.0
10.0
15.0
20.0
ml
pH
% buffer B
Anti-HER2 FAB(G65S) -Fc 133

FIG. 8D

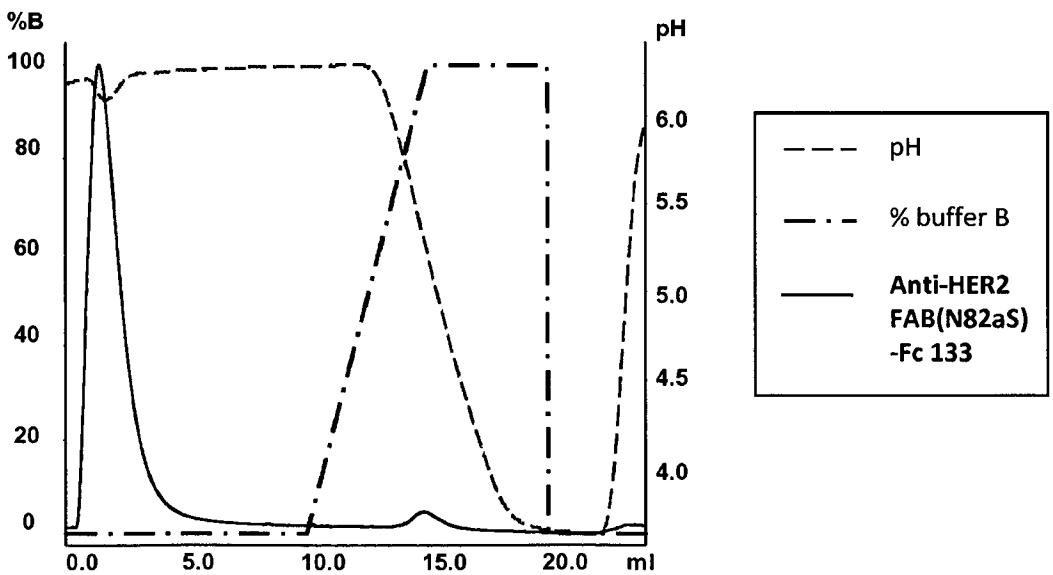

%B
pH
100
80
60
40
20
0
6.0
5.5
5.0
4.5
4.0
0.0
5.0
10.0
15.0
20.0
ml
pH
% buffer B
Anti-HER2 FAB(N82aS) -Fc 133

FIG. 9A

Antibody	Back-mutations VH / VL	SEQ ID NO H / L chains	Transient expression (mg/L)	FAB Tm (°C)	HPB-ALL staining relative to OKT3 chimera
Chimeric OKT3	N.A.	25/26	20	80.7	+++
VH/VL	- / -	27/39	50	88.1	-
VH/VL2	- / M4L	27/41	47	90.6	-
VH/VL3	- / M4L- F71Y	27/42	43	89.8	+
VH1/VL1	A49G / F71Y	28/40	22	90.1	-
VH1/VL2	A49G / M4L	28/41	42	90.5	-
VH1/VL3	A49G / M4L-F71Y	28/42	40	90.7	+
VH2/VL1	I34M-A49G / F71Y	29/40	51	89.5	-
VH2/VL2	I34M-A49G / M4L	29/41	43	90.1	-
VH2/VL3	I34M-A49G / M4L- F71Y	29/42	42.5	89.5	+
VH3/VL1	A49G-A71T / F71Y	30/40	33.5	89.7	-
VH3/VL2	A49G-A71T / M4L	30/41	42	90.4	-
VH3/VL3	A49G-A71T / M4L- F71Y	30/42	56.5	89.8	+

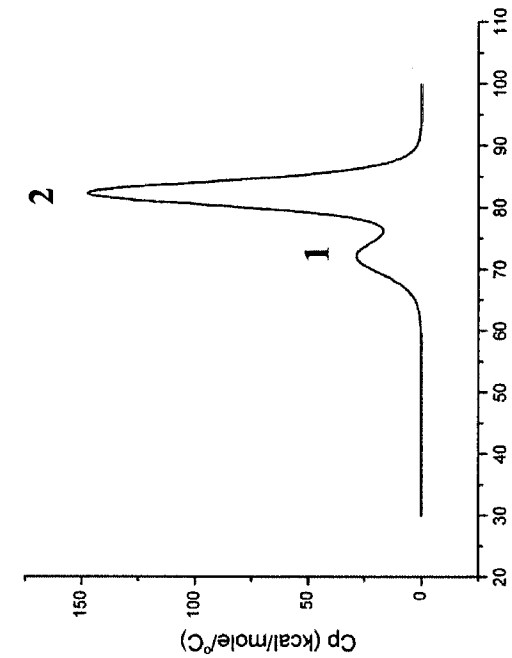
FIG. 9B

Antibody	Back-mutations	SEQ ID NO H / L chains	Transient expression (mg/l)	FAB Tm (°C)	HPB-ALL staining relative to OKT3 chimera
VH4/VL2	I34M-A49G-A71T / M4L	31/41	20	88.4	+ +
VH4/VL3	I34M-A49G-A71T / M4L-F71Y	31/42	34	88.7	+ +
VH5/VL2	I34M-A49G-I69L-A71T-T73K / M4L	32/41	40	87	+ +
VH5/VL3	I34M-A49G-I69L-A71T-T73K / M4L-F71Y	32/42	36	87.2	+ +
VH5/VL4	I34M-A49G-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	32/43	12	80.3	+++
VH5/VL6	I34M-A49G-I69L-A71T-T73K / M4L-L46R-L47W-F71Y-P96F	32/45	15.6	78.6	+++
VH5/VL7	I34M-A49G-I69L-A71T-T73K / M4L-V33M-A34N-F71Y-P96F	32/46	35.4	84.1	++
VH6/VL3	I34M-V48I-A49G-I69L-A71T-T73K / M4L-F71Y	33/42	23	88.3	++
VH6/VL4	I34M-V48I-A49G-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	33/43	14	80.8	+++
VH6/VL5	I34M-V48I-A49G-I69L-A71T-T73K / M4L-V33M-A34N-F71Y	33/44	26	86.1	++
VH6/VL6	I34M-V48I-A49G-I69L-A71T-T73K / M4L-L46R-L47W-F71Y-P96F	33/45	14.8	79.1	+++
VH6/VL7	I34M-V48I-A49G-I69L-A71T-T73K / M4L-V33M-A34N-F71Y-P96F	33/46	32	85.3	++

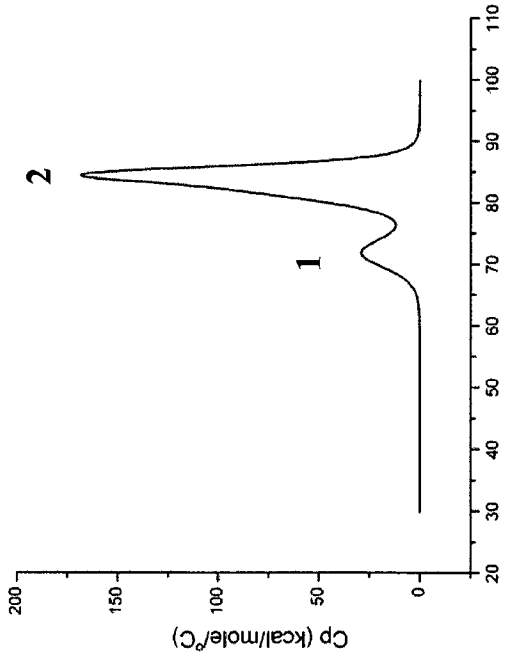
FIG. 9C

Antibody	Back-mutations	SEQ ID NO H / L chains	Transient expression (mg/l)	FAB Tm (°C)	HPB-ALL staining relative to OKT3 chimera
VH6/VL8	I34M-V48I-A49G-I69L-A71T-T73K / M4L-L46R-L47W-R66G-F71Y	33/47	7	80.6	+++
VH7/VL3	I34M-A49G-R58N-I69L-A71T-T73K / M4L-F71Y	34/42	21	86.1	++
VH7/VL4	I34M-A49G-R58N-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	34/43	25	80.5	+++
VH7/VL5	I34M-A49G-R58N-I69L-A71T-T73K / M4L-V33M-A34N-F71Y	34/44	26	84.12	++
VH8/VL4	I34M-V48I-A49G-R58Y-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	35/43	7	80.9	+++
VH8/VL8	I34M-V48I-A49G-R58Y-I69L-A71T-T73K / M4L-L46R-L47W-R66G-F71Y	35/47	23	83.5	+++
VH9/VL8	I34M-V48I-A49G-R58Y-G65S-I69L-A71T-T73K / M4L-L46R-L47W-R66G-F71Y	36/47	13	82	+++
VH10/VL4	I34M-V48I-A49G-R58Y-G65S-F67A-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	37/43	7	78.6	+++
VH10/VL8	I34M-V48I-A49G-R58Y-G65S-F67A-I69L-A71T-T73K / M4L-L46R-L47W-R66G-F71Y	37/47	10	80.4	+++
VH11/VL4	I34M-V48I-A49G-R58Y-I69L-A71T-T73K-N82aS / M4L-L46R-L47W-F71Y	38/43	8	80.3	+++
VH11/VL8	I34M-V48I-A49G-R58Y-I69L-A71T-T73K-N82aS / M4L-L46R-L47W-R66G-F71Y	38/47	15	82.3	+++

FIG. 9D



Tm(1): 72.1; Tm(2): 82.3 (FAB)



Tm(1): 71.8; Tm(2): 83.5 (FAB)

FIG. 9E

scFv-Fc fusion	SEQ ID NO	Transient expression (mg/L)	scFv Tm (°C)	HPB-ALL staining relative to OKT3 chimera
Mouse OKT3	52	0.35	-	++
VH5-VL3	53	21	71.2	+
VH6-VL4	54	29	65.3	++
VH6-VL5	55	27	71.2	++
VH8-VL4	56	28	66.4	+++
VH8-VL8	57	28	69.2	+++

FIG. 9F

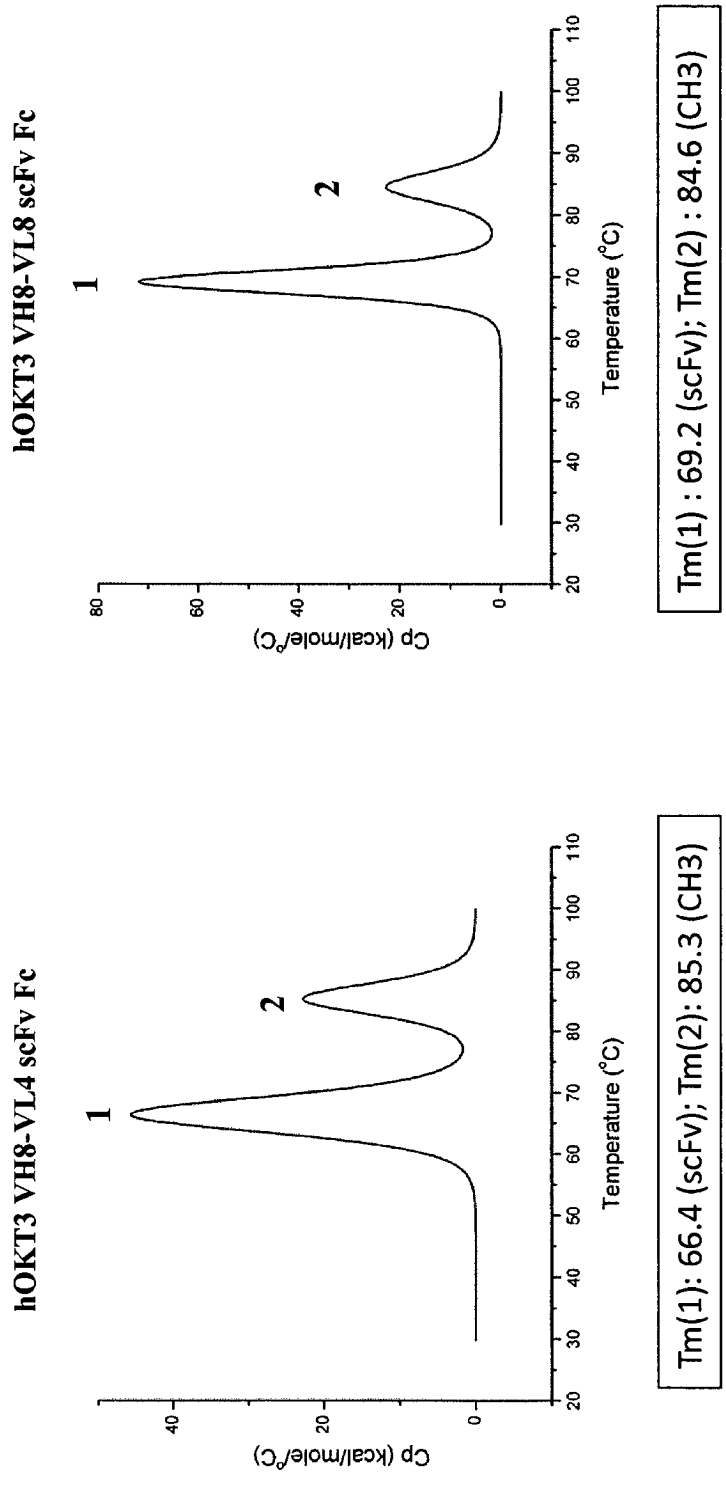

hOKT3 VH8-VL8 scFv Fc
1
2
Cp (kcal/mole/°C)
Temperature (°C)
Tm(1) : 69.2 (scFv); Tm(2) : 84.6 (CH3)
hOKT3 VH8-VL4 scFv Fc
1
2
Cp (kcal/mole/°C)
Temperature (°C)
Tm(1): 66.4 (scFv); Tm(2): 85.3 (CH3)

FIG. 10A

Antibody	Back-mutations VH/VL	SEQ ID NO H/L chains	Format	Transient expression (mg/L)	SPR-Binding to human/cynomolgus monkey CD3 epsilon 1-26_Fc proteins
Chimeric SP34	N.A.	62/63	IgG1	7	++++
VH1/VL1	-/-	64/69	IgG1	8	+
VH1/VL2	-/Q89A	64/70	IgG1	1	++++
VH1/VL3	-/deleted 8P	64/71	IgG1	0.5	-
VH1/VL4	-/deleted 8P-Q89A	64/72	IgG1	0.5	++++
VH1/VL5	-/A2I-Q89A	64/73	IgG1	2.6	++++
VH1/VL6	-/F44P-Q89A	64/74	IgG1	0.9	++++
VH1/VL7	-/A2I-F44P-Q89A	64/75	IgG1	No expression	Not tested
VH1/VL8	-/L66G-Q89A	64/76	IgG1	0.8	+
VH1/VL9	-/A2I-L66G-Q89A	64/77	IgG1	No expression	Not tested
VH1/VL10	-/F87Y-Q89A	64/78	IgG1	6	++++
VH1/VL11	-/L66G-D69T-Q89A	64/79	IgG1	0.8	+
VH1/VL12	-/D69T-Q89A	64/80	IgG1	1.5	++
VH1/VL13	-/S25A/Q89A	64/81	IgG1	1	+++
VH1/VL14	-/G46L-Q89A	64/82	IgG1	3	-
VH1/VL15	-/E38Q-Q89A	64/83	IgG1	3	++++
VH1/VL16	-/A2I-D69T-Q89A	64/84	IgG1	0.5	+
VH1/VL17	-/A2I-S25A-Q89A	64/85	IgG1	1	++
VH1/VL18	-/A2I-Q89A-Q100G	64/86	IgG1	1	++
VH1/VL19	-/A2I-D69T-F87Y-Q89A	64/87	IgG1	0.2	+
VH1/VL20	-/A2I-E38Q-D69T-F87Y-Q89A	64/88	IgG1	No expression	Not tested
VH1/VL21	-/A2I-F87Y-Q89A	64/89	IgG1	8	++++
VH1/VL22	-/A2I-E38Q-F87Y-Q89A	64/90	IgG1	2	+++

FIG. 10B

ScFv-Fc fusion	Back-mutations VH/VL	SEQ ID NO H/L chains	Format	Transient expression (mg/L)	SPR-Binding to human/cynomolgus monkey CD3 epsilon 1-26_Fc proteins
VH2/VL21	G65S /A2I -F87Y-Q89A	91	scFv-Fc	5	+++
VH3/VL23	G65S-W100eY /A2I-F87Y-Q89A-W91F	92	scFv-Fc	10	++
VH4/VL23	G65S-W100eF /A2I-F87Y-Q89A-W91F	93	scFv-Fc	5.5	++++
VH5/VL23	W100eY/A2I-F87Y-Q89A-W91F	94	scFv-Fc	15	++++
VH1/VL24	-/A2I-T27A-G27aA- F87Y-Q89A	349	scFvFc	No expression	Not tested
VH1/VL25	-/A2I-V27cA-T28A- F87Y-Q89A	350	scFvFc	4	-
VH1/VL26	-/A2I-T29A-S30A- F87Y-Q89A	351	scFvFc	12	++++
VH1/VL27	-/A2I-N31A-Y32A- F87Y-Q89A	95	scFv-Fc	2	-
VH1/VL28	-/A2I-N52A-K53A- F87Y-Q89A	96	scFv-Fc	No expression	Not tested
VH1/VL29	-/A2I-R54A-P56A- F87Y-Q89A	97	scFv-Fc	4	-
VH1/VL30	-/A2I-Y92A-S93A- F87Y-Q89A	98	scFv-Fc	2	+
VH1/VL31	-/A2I-N94A -F87Y-Q89A	99	scFv-Fc	2	++
VH5/VL32	W100eY/A2I-T29A-S30A-T51A-F87Y-Q89A-W91F	100	scFv-Fc	25	++++

FIG. 11A

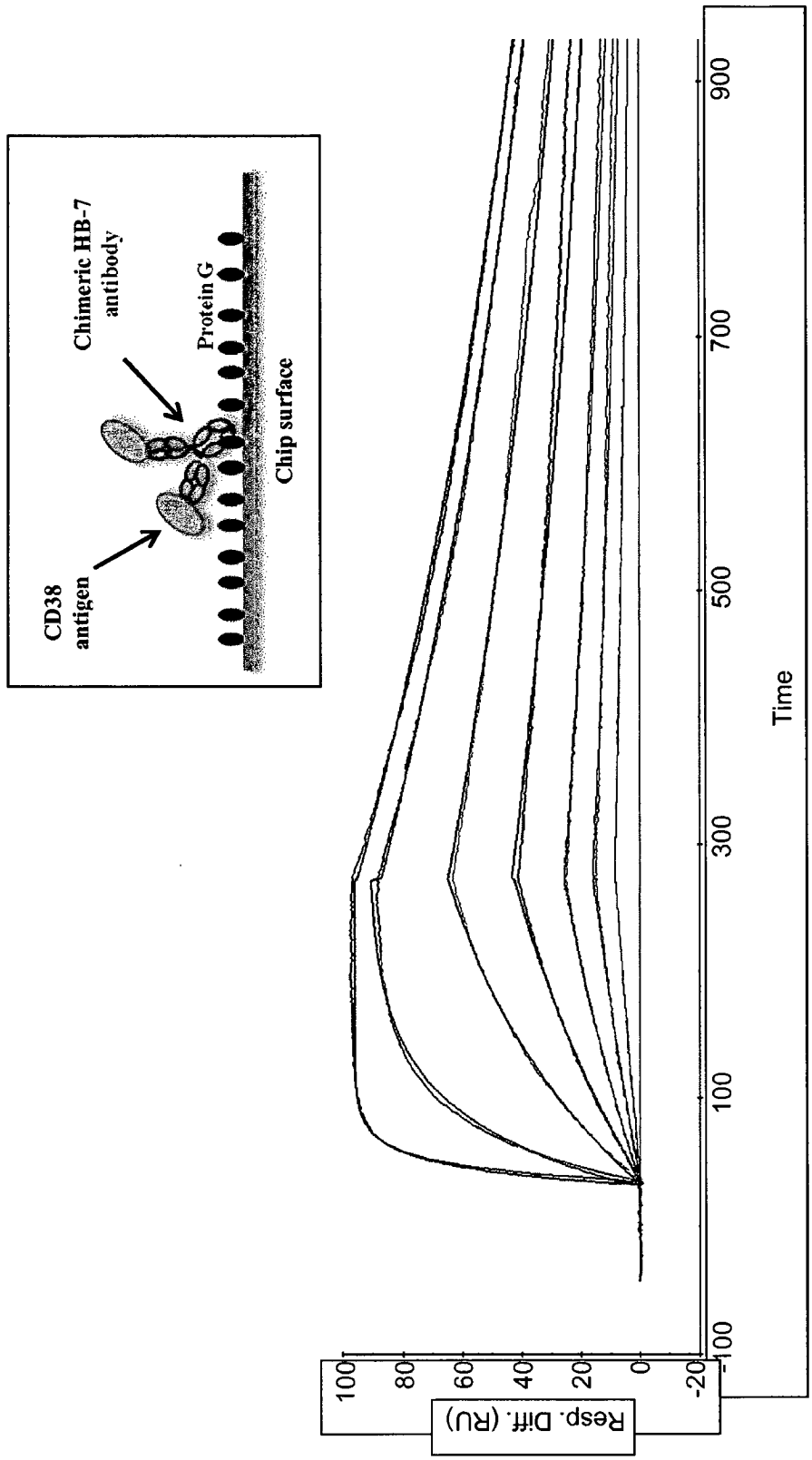

CD38 antigen
Chimeric HB-7 antibody
Protein G
Chip surface
Resp. Diff. (RU)
100
80
60
40
20
0
-20
-100
100
300
500
700
900
Time

FIG. 11B

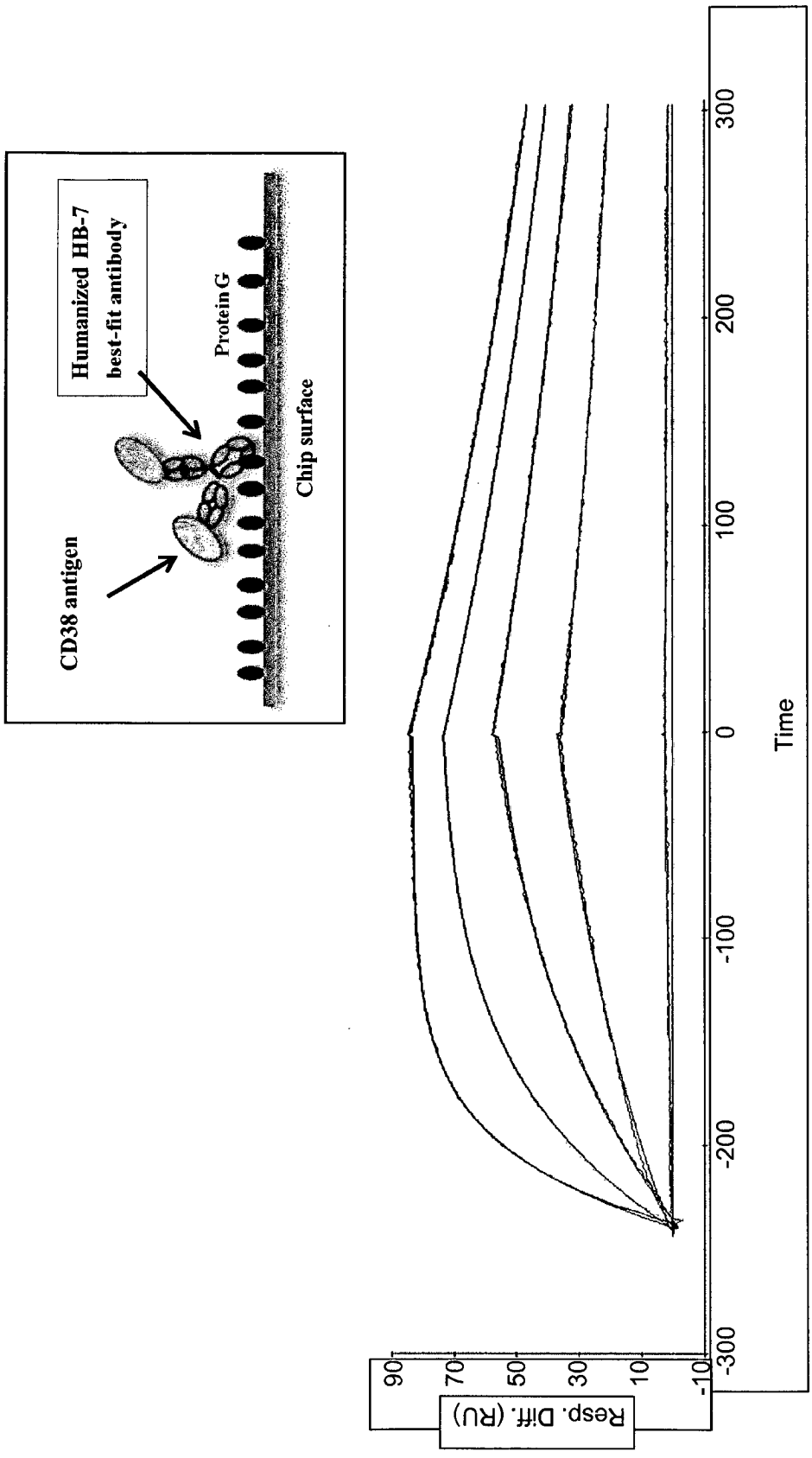

CD38 antigen
Humanized HB-7 best-fit antibody
Protein G
Chip surface
Resp. Diff. (RU)
90
70
50
30
10
-10
-300
-200
-100
0
100
200
300
Time

FIG. 11C

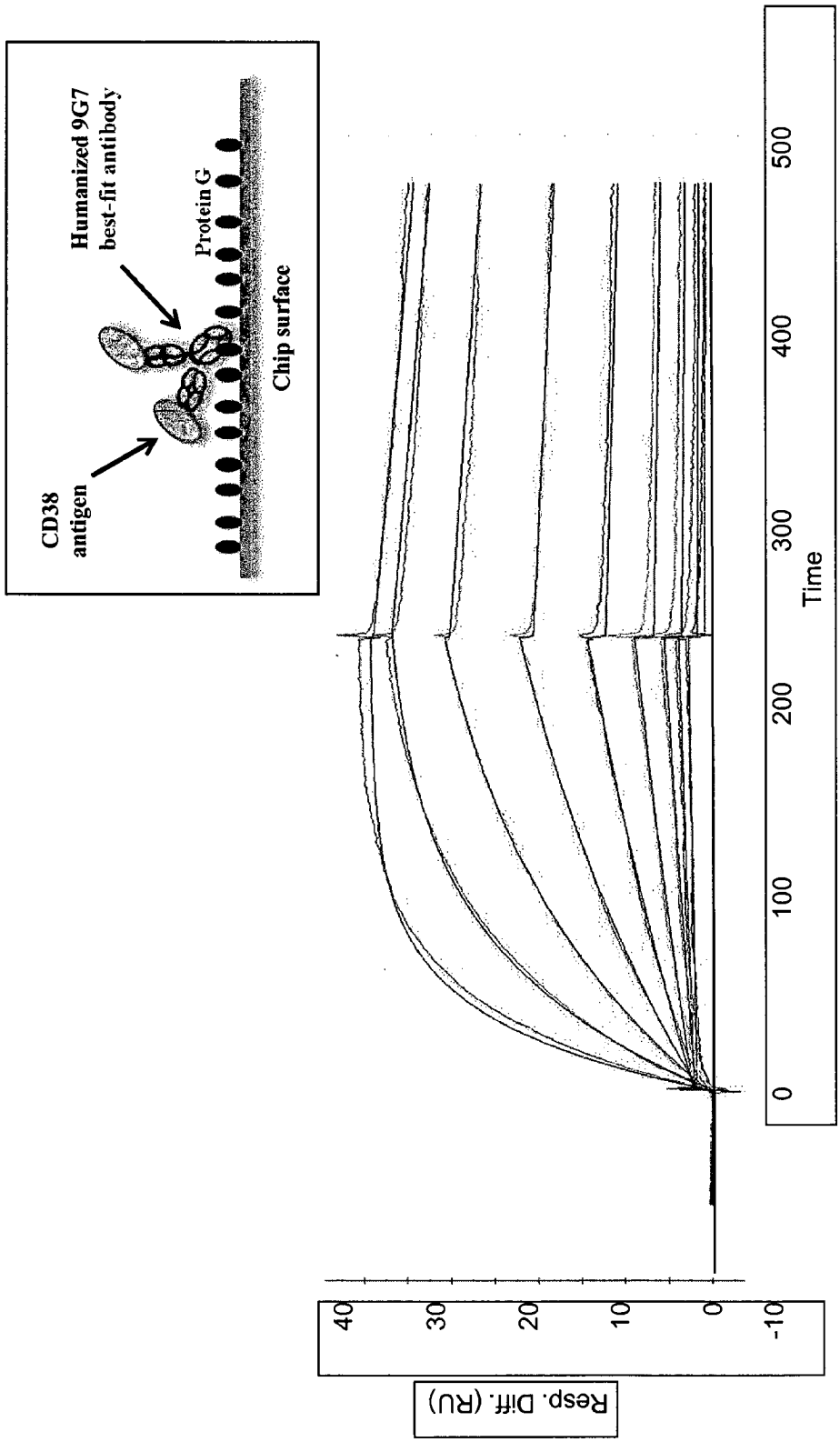

Humanized 9G7
best-fit antibody
Protein G
Chip surface
CD38
antigen
Resp. Diff. (RU)
40
30
20
10
0
-10
0
100
200
300
400
500
Time

FIG. 11D

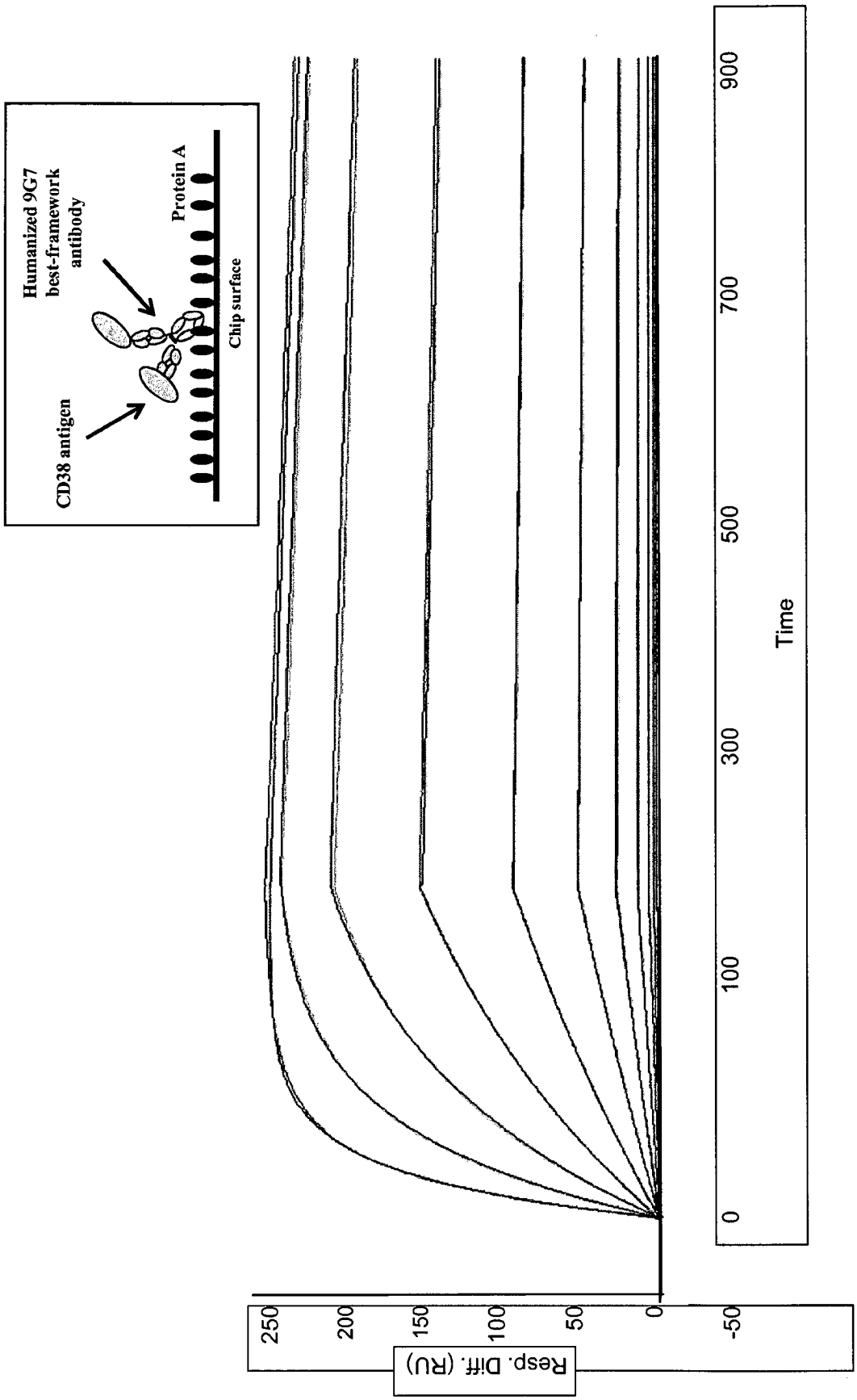
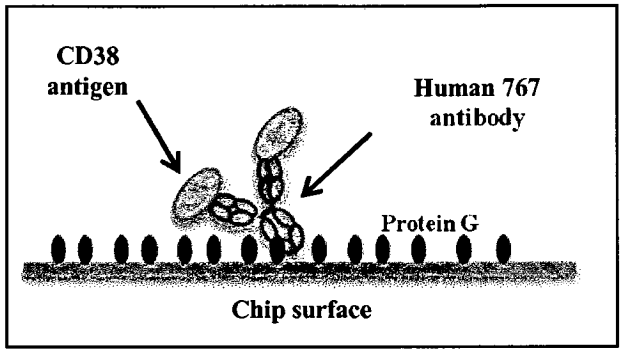

Humanized 9G7
best-framework
antibody
Protein A
CD38 antigen
Chip surface
Resp. Diff. (RU)
250
200
150
100
50
0
-50
0
100
300
500
700
900
Time

FIG. 11E


CD38
antigen
Human 767
antibody
Protein G
Chip surface

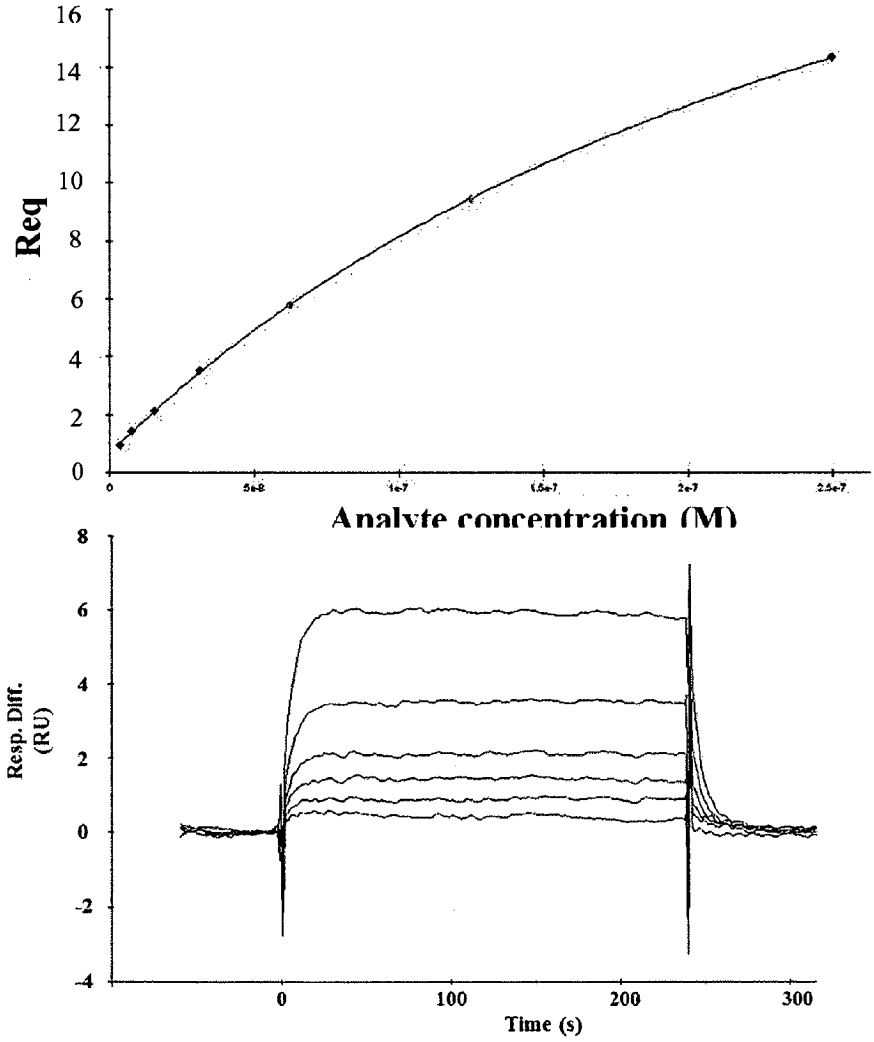

Req
Analyte concentration (M)
Resp. Diff.
(RU)
Time (s)

FIG. 11F

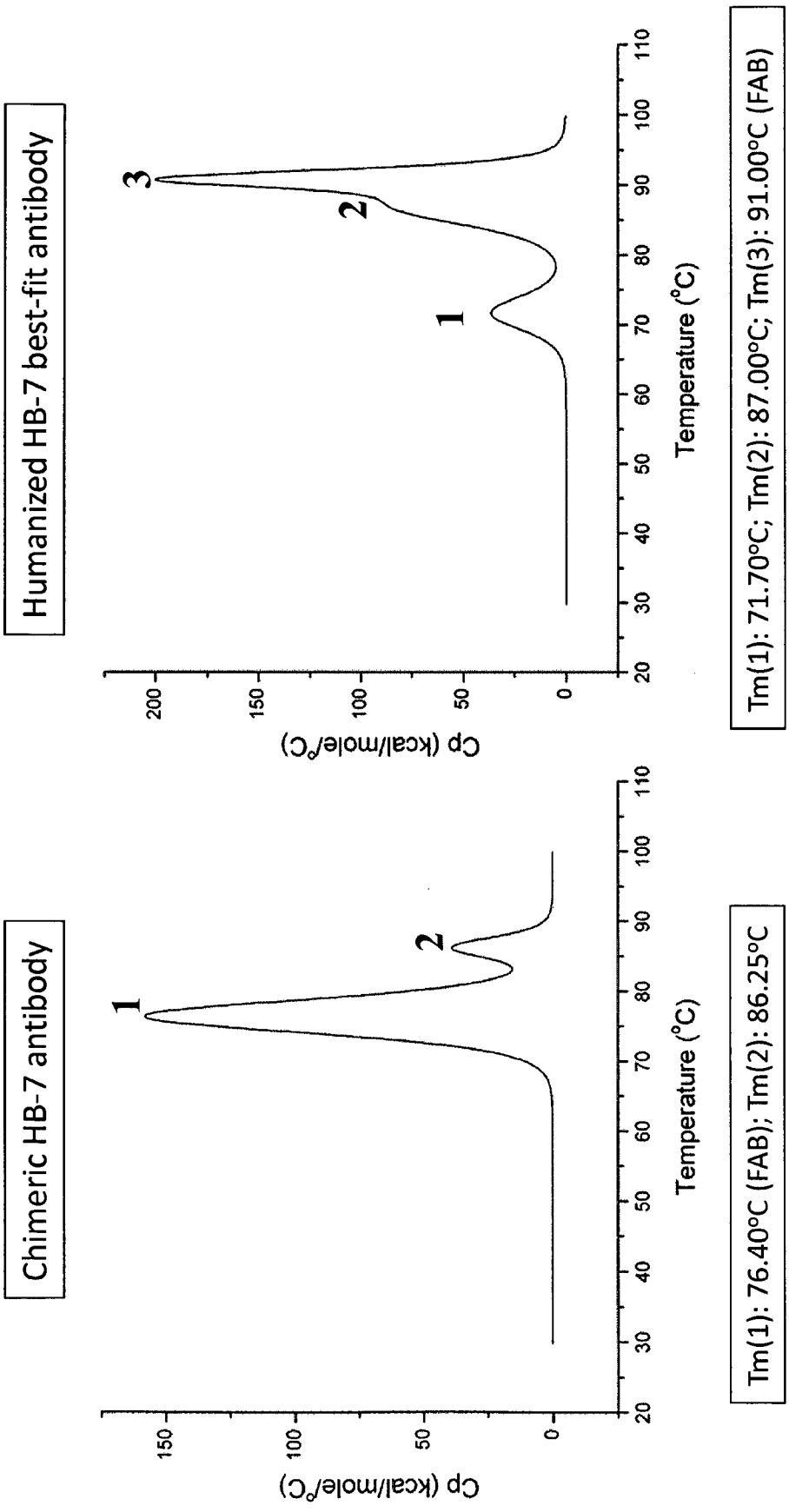

Humanized HB-7 best-fit antibody
Cp (kcal/mole/°C)
Temperature (°C)
1
2
3
Tm(1): 71.70°C; Tm(2): 87.00°C; Tm(3): 91.00°C (FAB)
Chimeric HB-7 antibody
Cp (kcal/mole/°C)
Temperature (°C)
1
2
Tm(1): 76.40°C (FAB); Tm(2): 86.25°C

FIG. 11G

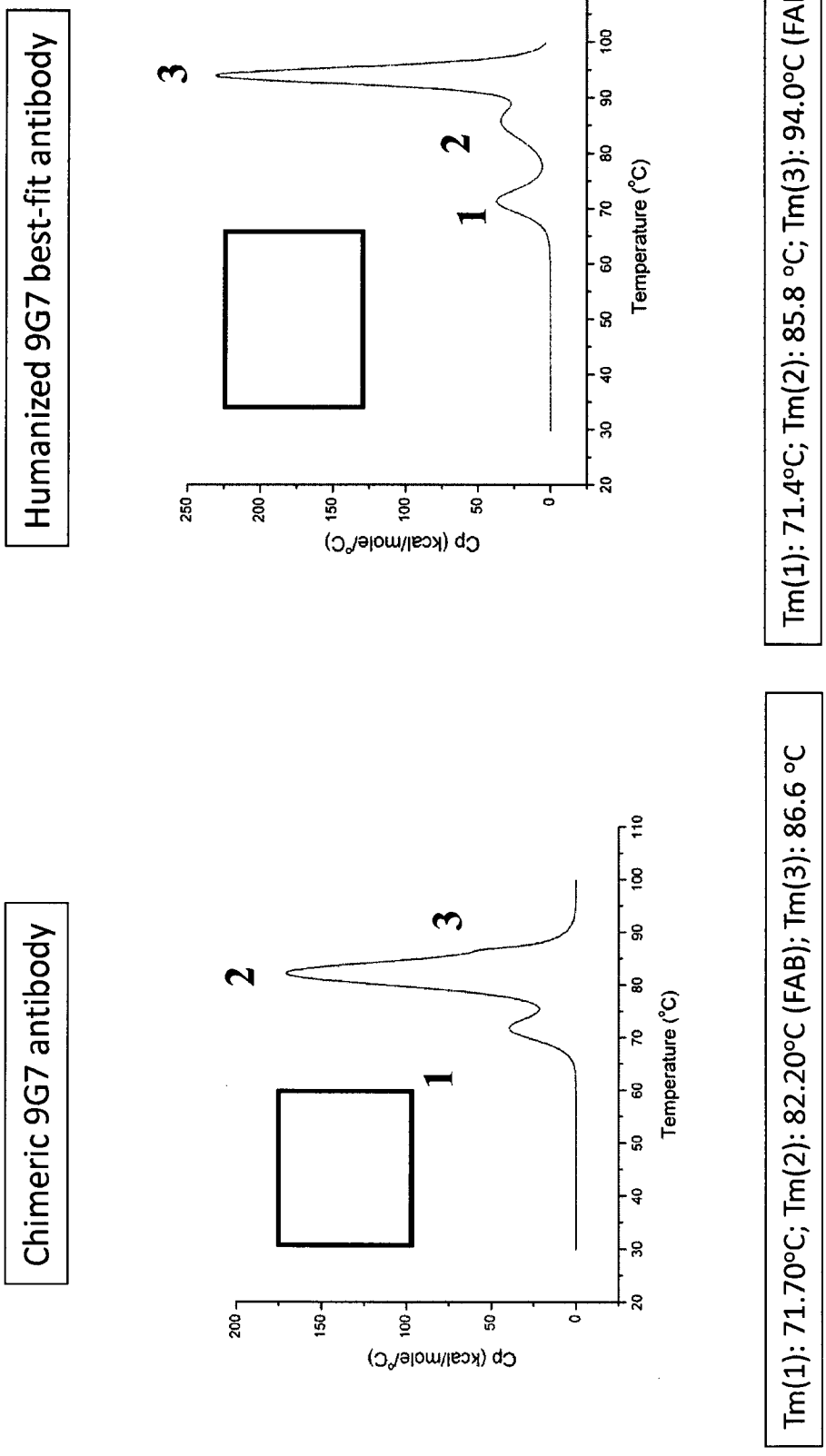
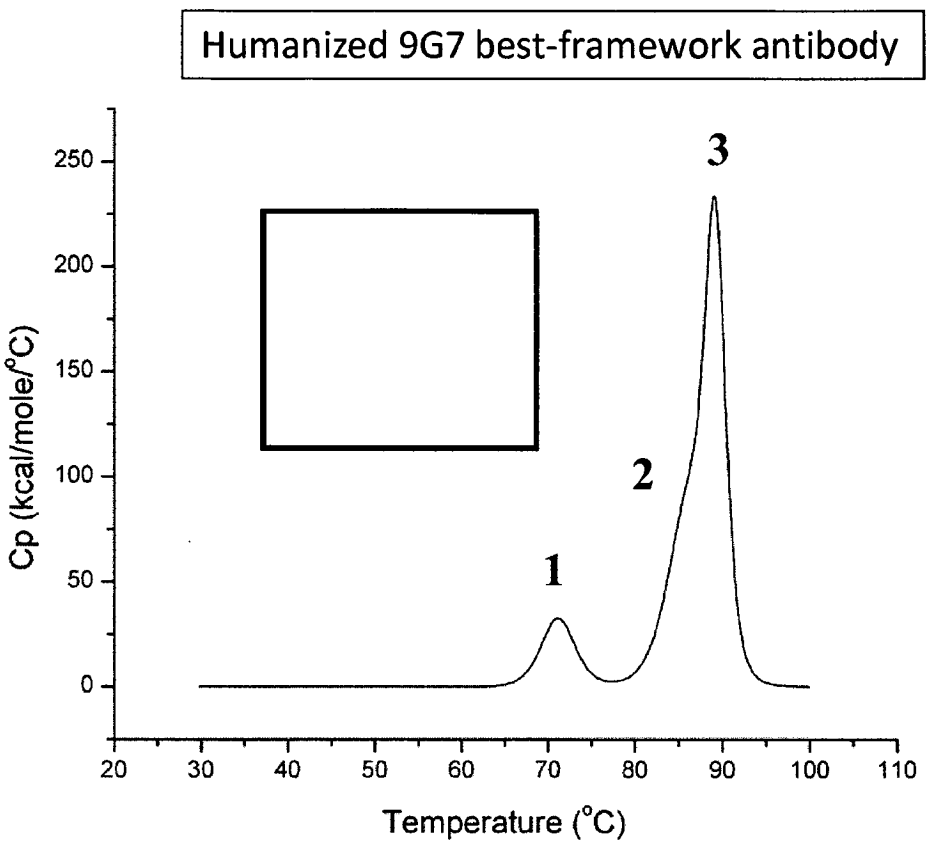

Humanized 9G7 best-fit antibody
Chimeric 9G7 antibody
Cp (kcal/mole/°C)
Temperature (°C)
1
2
3
Tm(1): 71.4°C; Tm(2): 85.8 °C; Tm(3): 94.0°C (FAB)
Tm(1): 71.70°C; Tm(2): 82.20°C (FAB); Tm(3): 86.6 °C

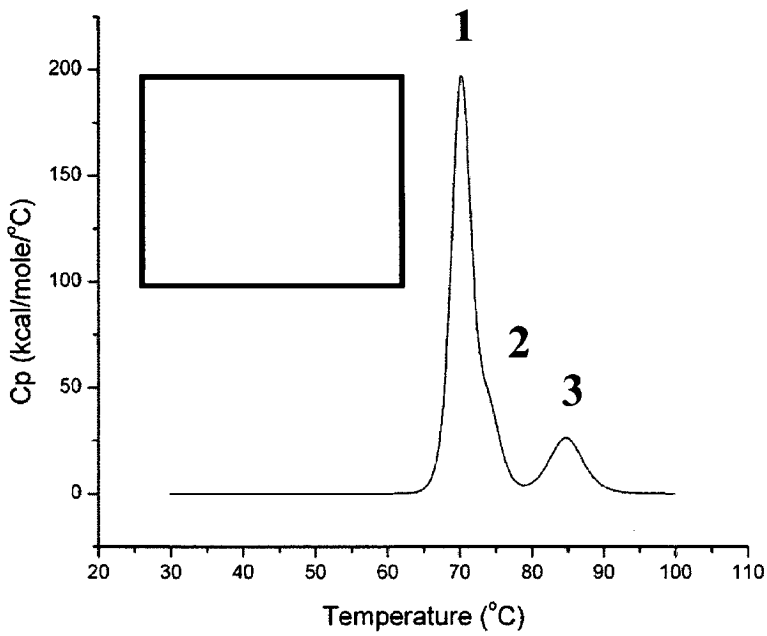
FIG. 11H



Tm(1): 71.2°C; Tm(2):86.2 °C; Tm(3):89.2 °C (FAB)

FIG. 11I

Human 767 antibody



Tm(1): 70.2°C (FAB); Tm(2):74.1 °C; Tm(3):84.7 °C

FIG. 11J

	Chimeric 9G7 antibody	Humanized 9G7 best-fit antibody	Humanized 9G7 best-framework antibody
HC/LC SEQ ID NO	126/127	124/128	131/132
Transient expression levels (mg/l)	20	11	17
KD human CD38 (nM)	0.4	0.5	0.4
KD cynomolgus monkey CD38 (nM)	1	3.2	1
FAB Tm (°C)	82.2	94	89.2

FIG. 12A

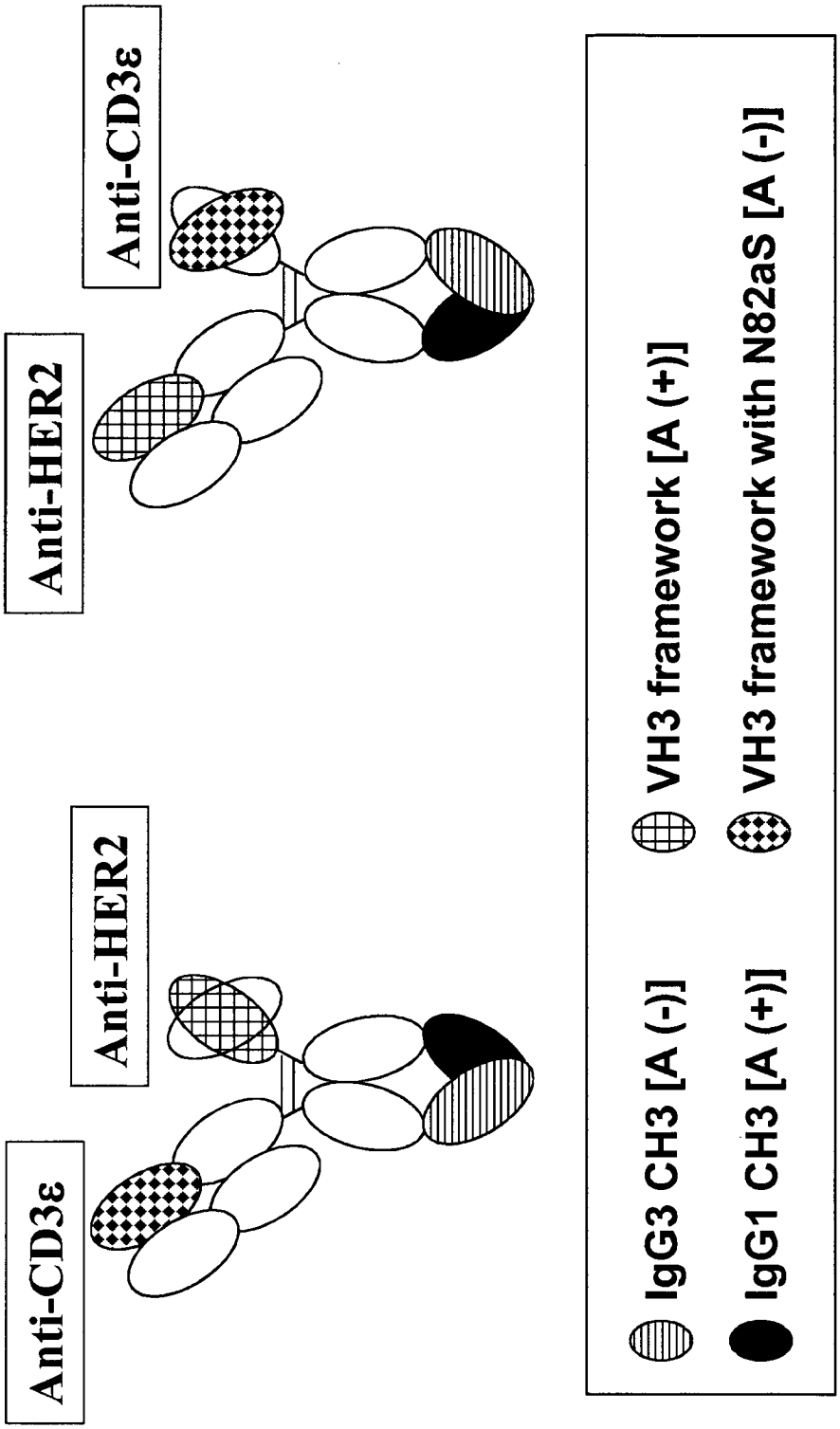

A
Anti-CD3ε
Anti-HER2
B
Anti-HER2
Anti-CD3ε
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 framework [A (+)]
VH3 framework with N82aS [A (-)]

FIG. 12B

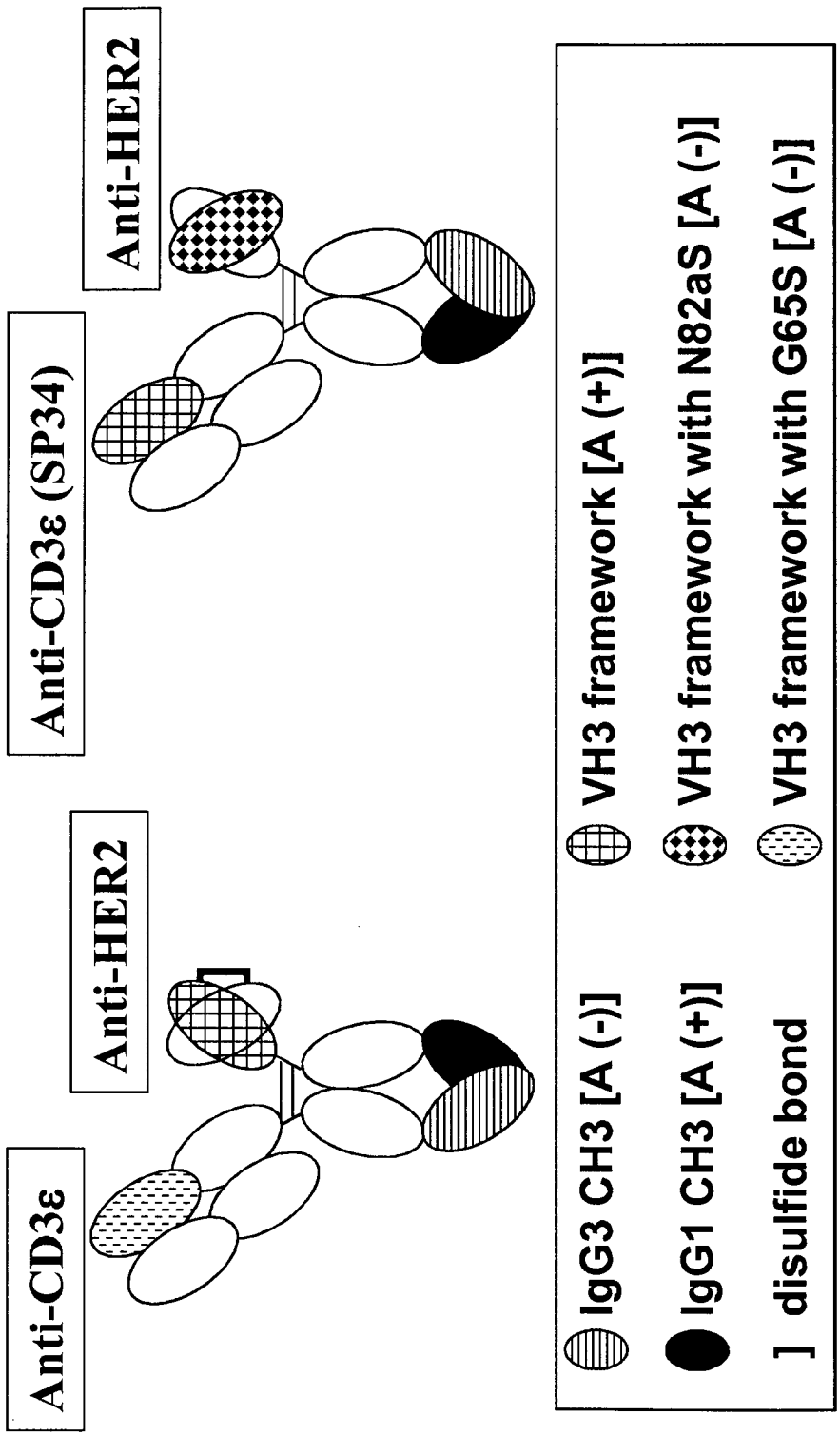

C
Anti-CD3ε
Anti-HER2
D
Anti-CD3ε (SP34)
Anti-HER2
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
] disulfide bond
VH3 framework [A (+)]
VH3 framework with N82aS [A (-)]
VH3 framework with G65S [A (-)]

FIG. 12C

E

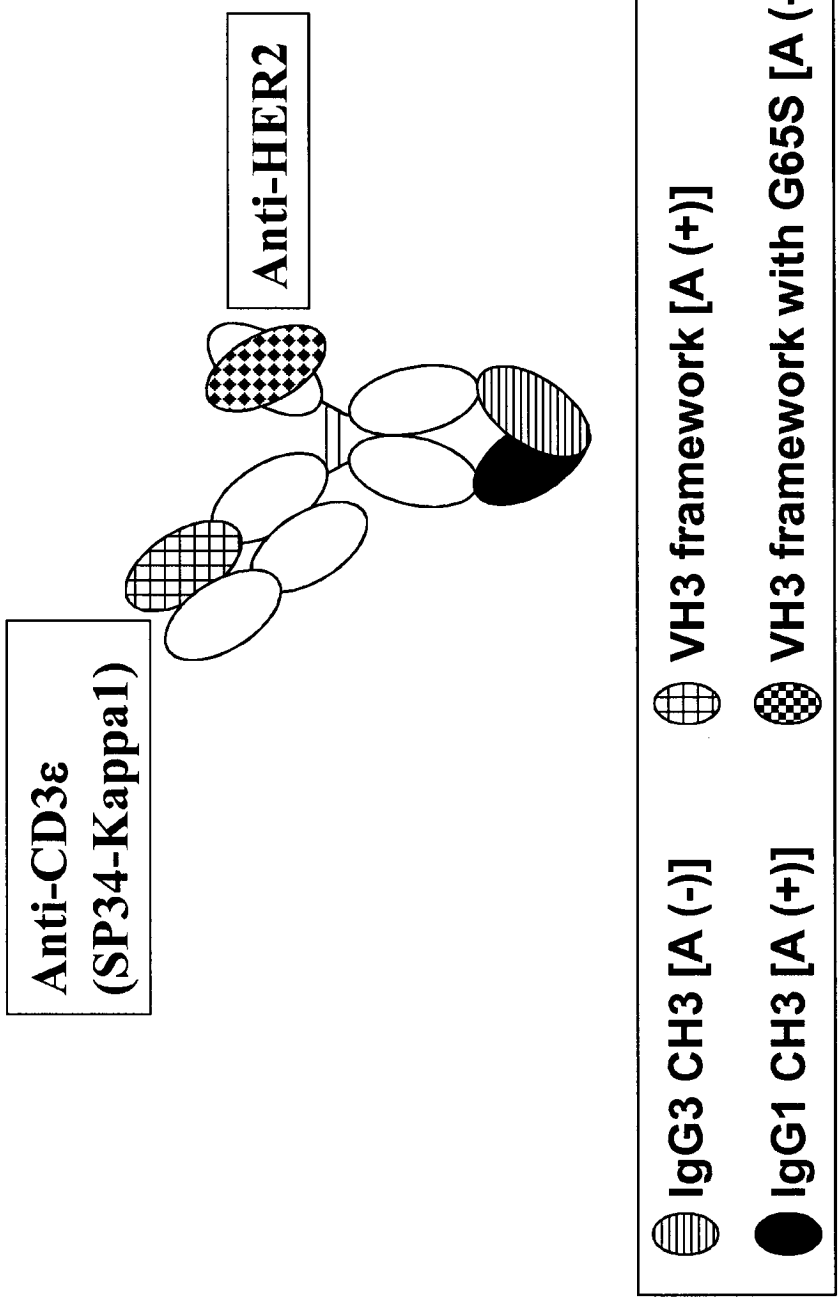

Anti-CD3ε
(SP34-Kappa1)
Anti-HER2
IgG3 CH3 [A (-)]
VH3 framework [A (+)]
IgG1 CH3 [A (+)]
VH3 framework with G65S [A (-)]

FIG. 13

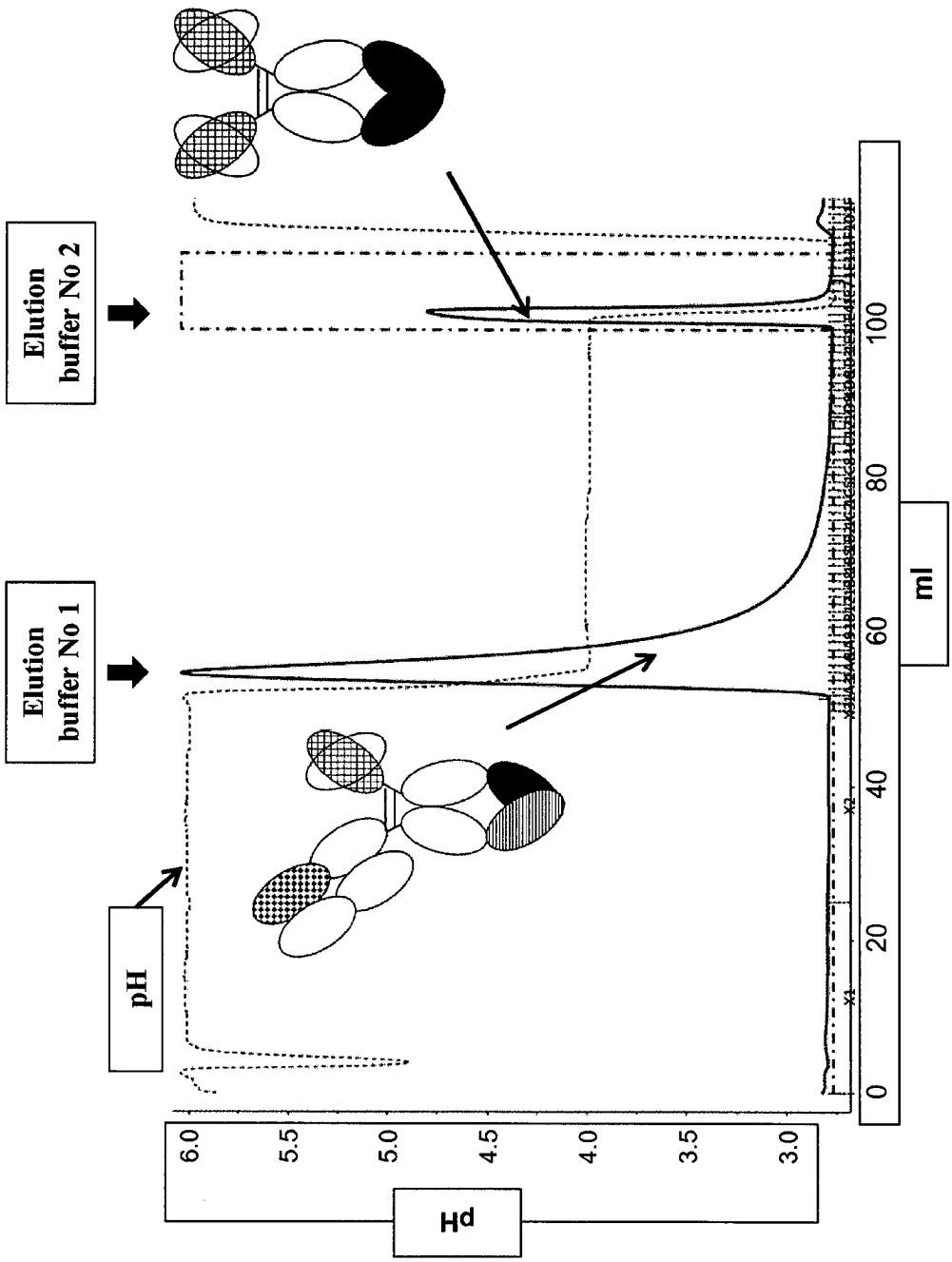
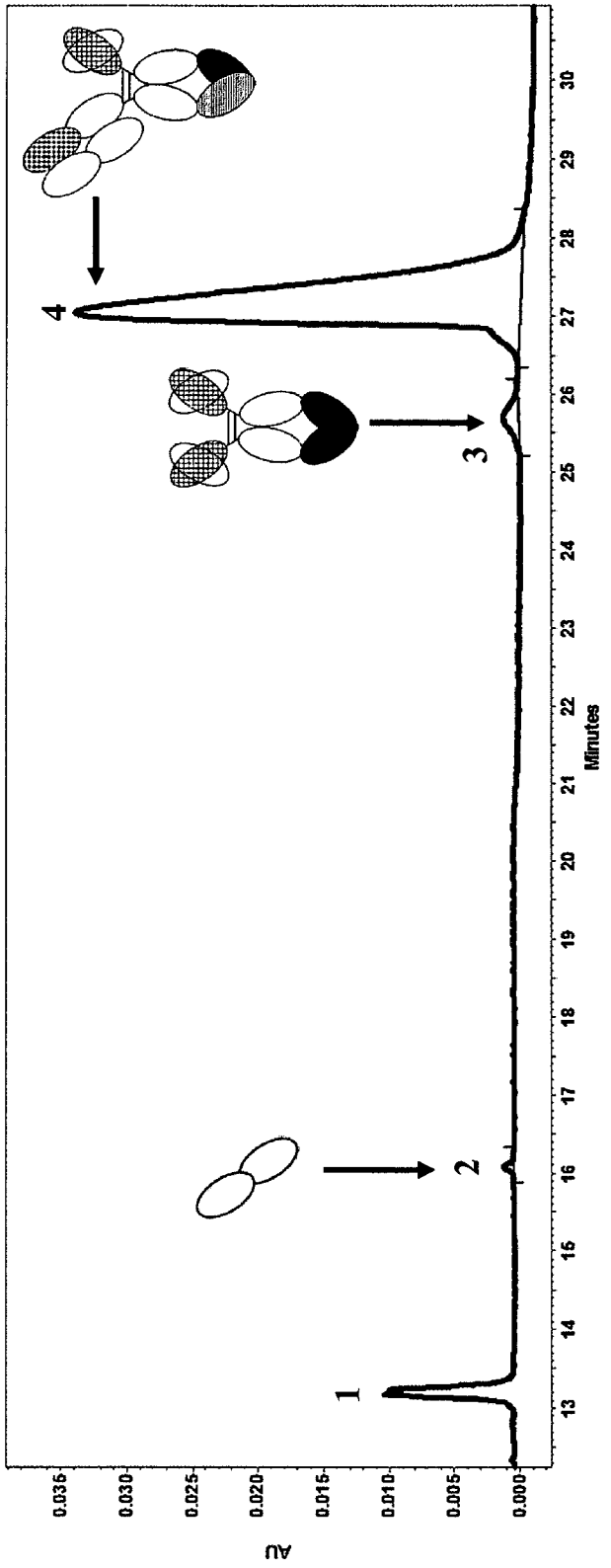

Elution buffer No 2
Elution buffer No 1
pH
6.0
5.5
5.0
4.5
4.0
3.5
3.0
pH
0
20
40
60
80
100
ml

FIG. 14



Peak	Species	% of total AUC
1	Internal standard	N.A.
2	Light chain (25 kDa)	0.5
3	Homodimer (100 kDa)	2.5
4	Heterodimer (125 kDa)	97

IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 framework [A (+)]
VH3 framework with N82aS [A (-)]

FIG. 15A

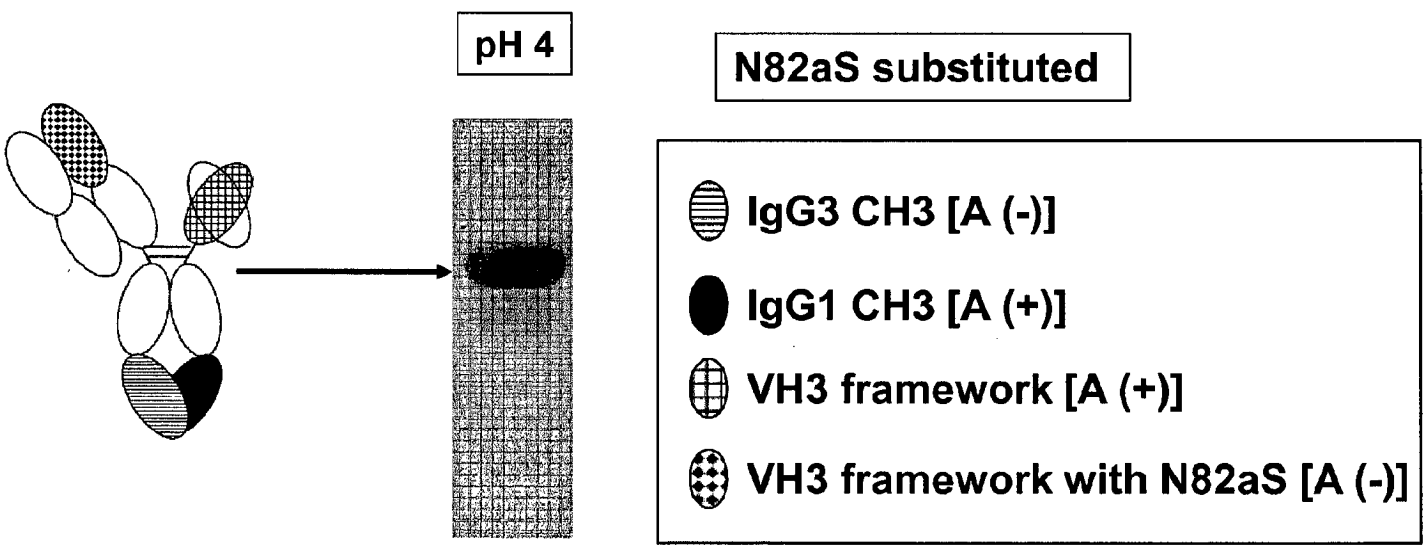

pH 4
N82aS substituted
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 framework [A (+)]
VH3 framework with N82aS [A (-)]

FIG. 15B

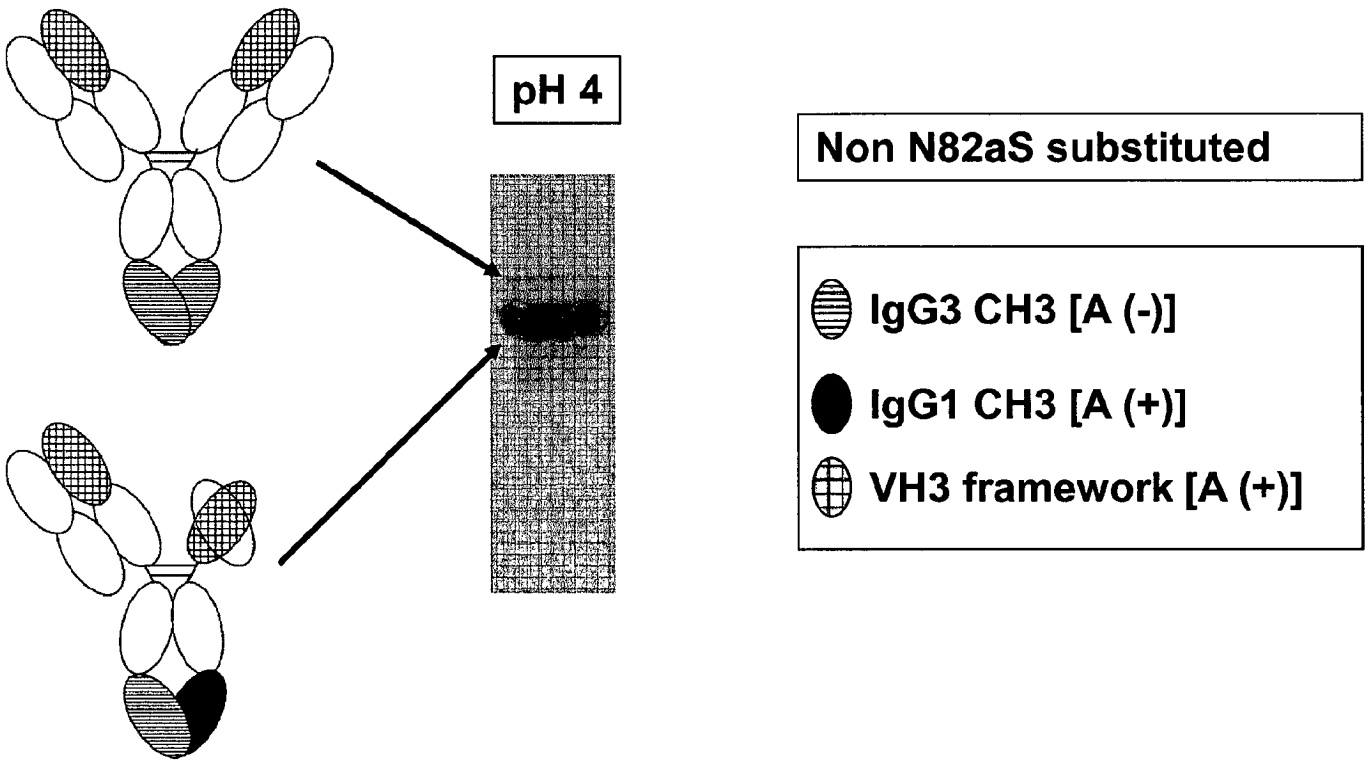
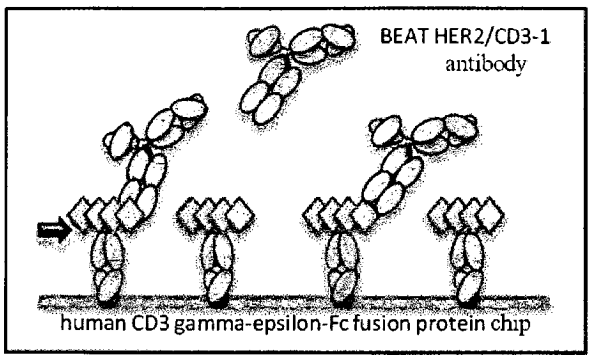
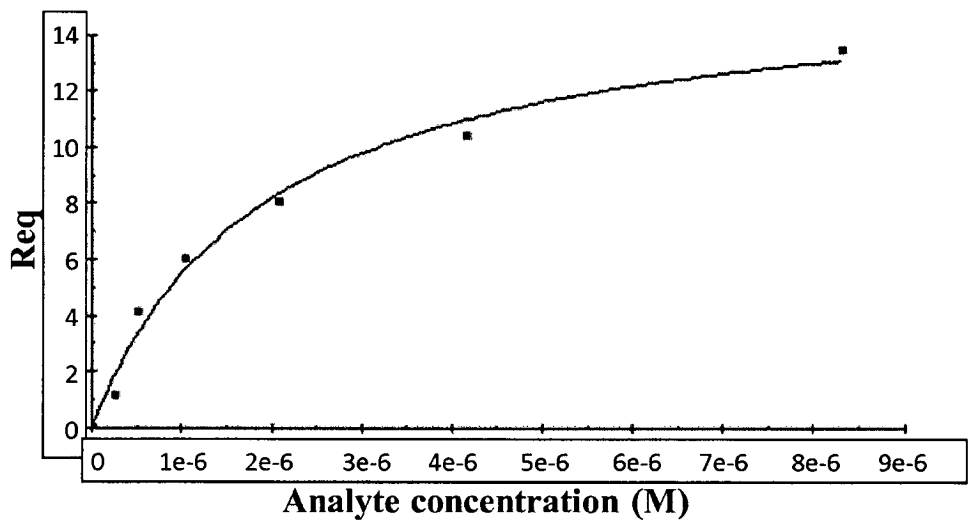

pH 4
Non N82aS substituted
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 framework [A (+)]

FIG. 16A


BEAT HER2/CD3-1
antibody
human CD3 gamma-epsilon-Fc fusion protein chip


Req
14
12
10
8
6
4
2
0
0
1e-6
2e-6
3e-6
4e-6
5e-6
6e-6
7e-6
8e-6
9e-6
Analyte concentration (M)

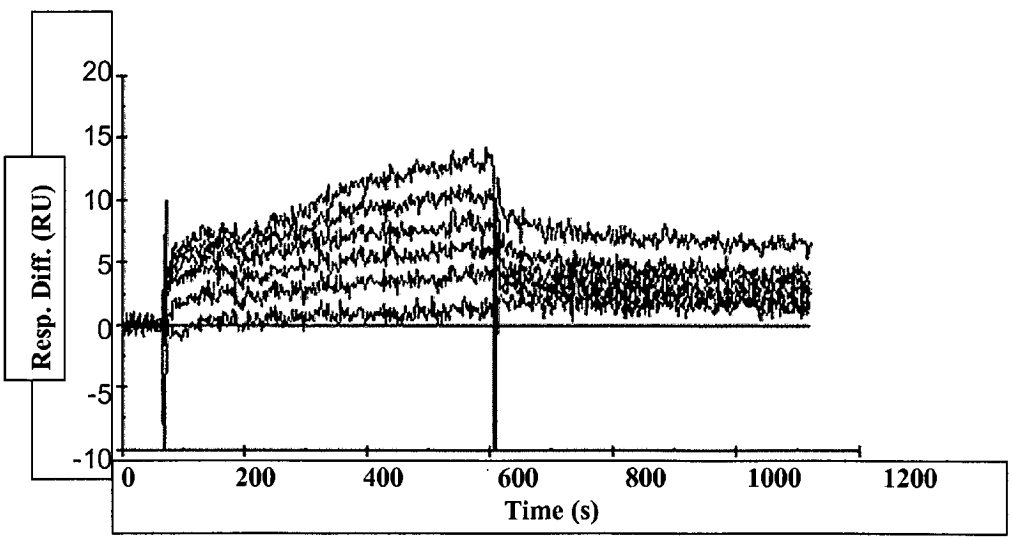

Resp. Diff. (RU)
20
15
10
5
0
-5
-10
0
200
400
600
800
1000
1200
Time (s)

FIG. 16B

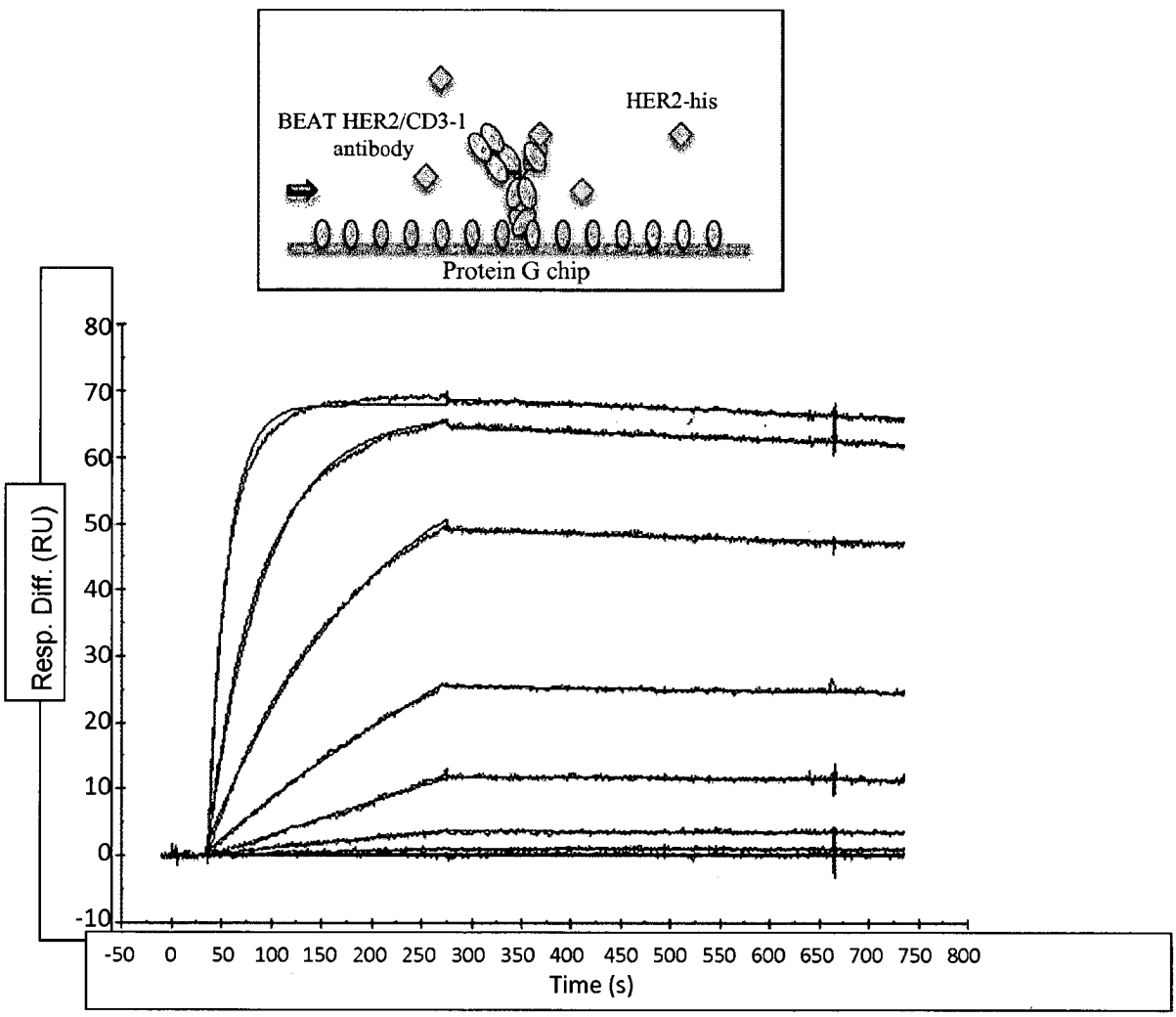

BEAT HER2/CD3-1
antibody
HER2-his
Protein G chip
Resp. Diff. (RU)
80
70
60
50
40
30
20
10
0
-10
-50 0 50 100 150 200 250 300 350 400 450 500 550 600 650 700 750 800
Time (s)

FIG. 16C

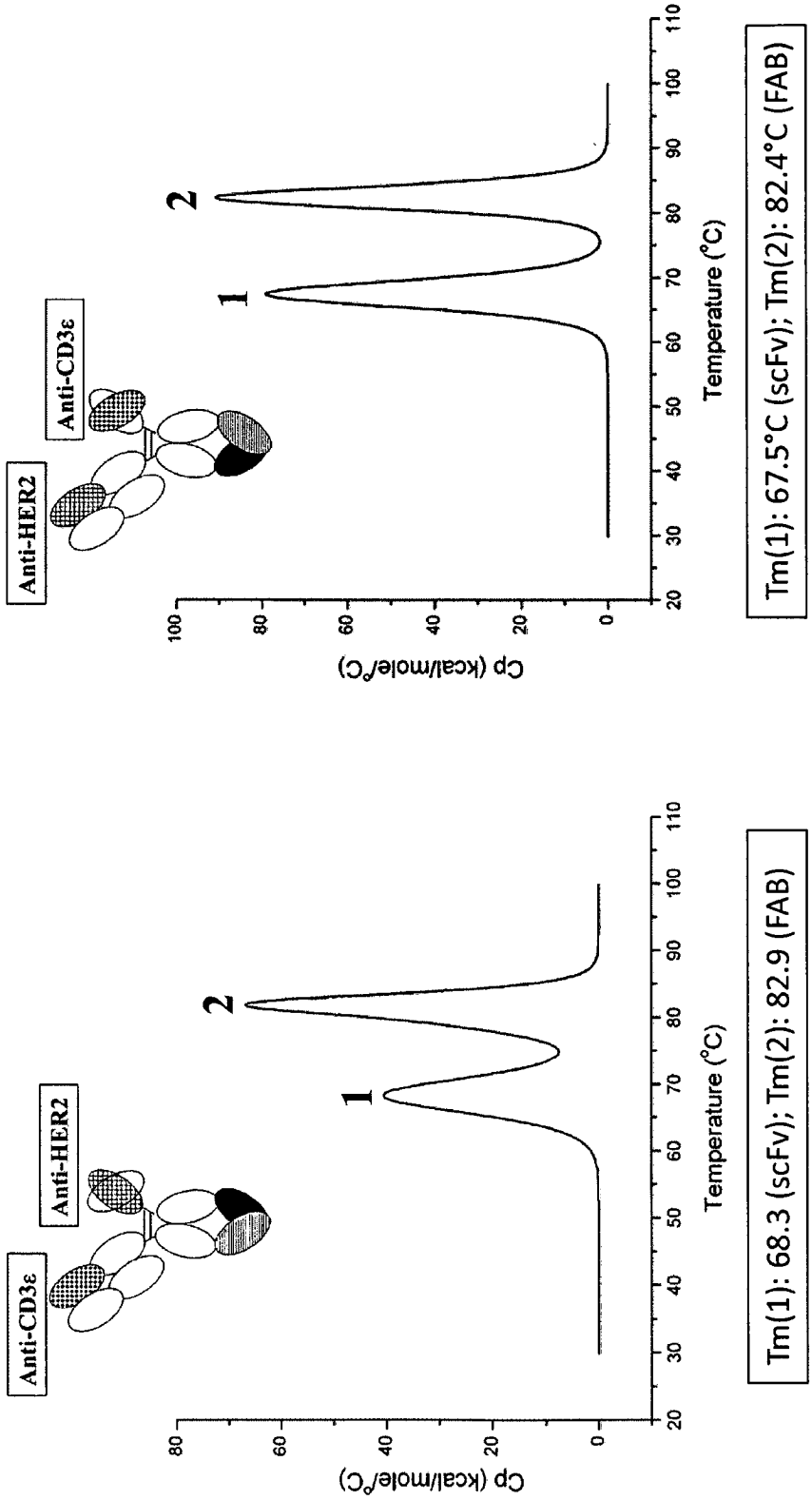

A
Anti-CD3ε
Anti-HER2
1
2
Cp (kcal/mole/°C)
Temperature (°C)
Tm(1): 68.3 (scFv); Tm(2): 82.9 (FAB)
B
Anti-HER2
Anti-CD3ε
1
2
Cp (kcal/mole/°C)
Temperature (°C)
Tm(1): 67.5°C (scFv); Tm(2): 82.4°C (FAB)

FIG. 17A

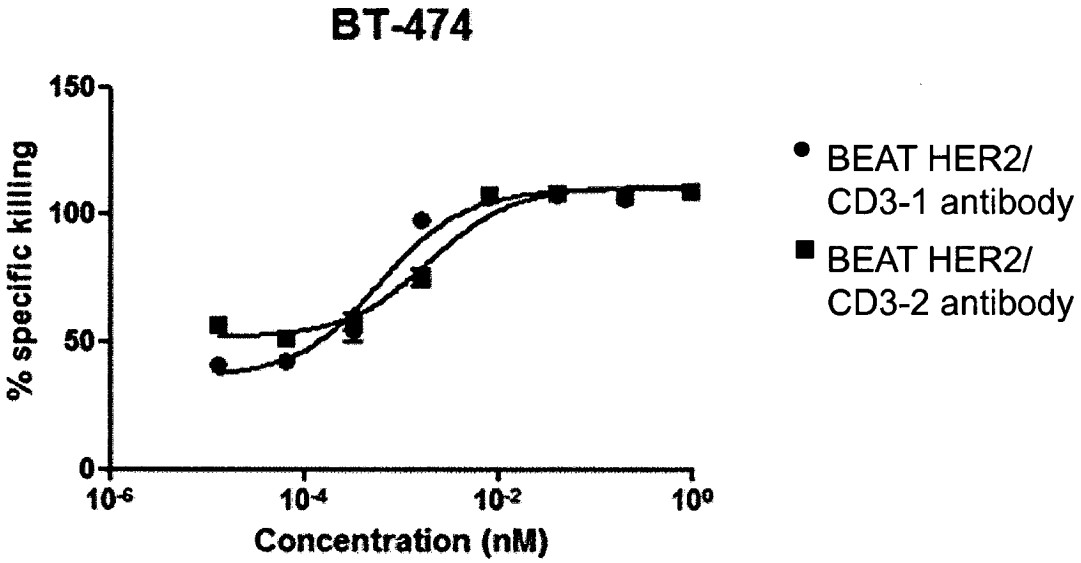


FIG. 17B

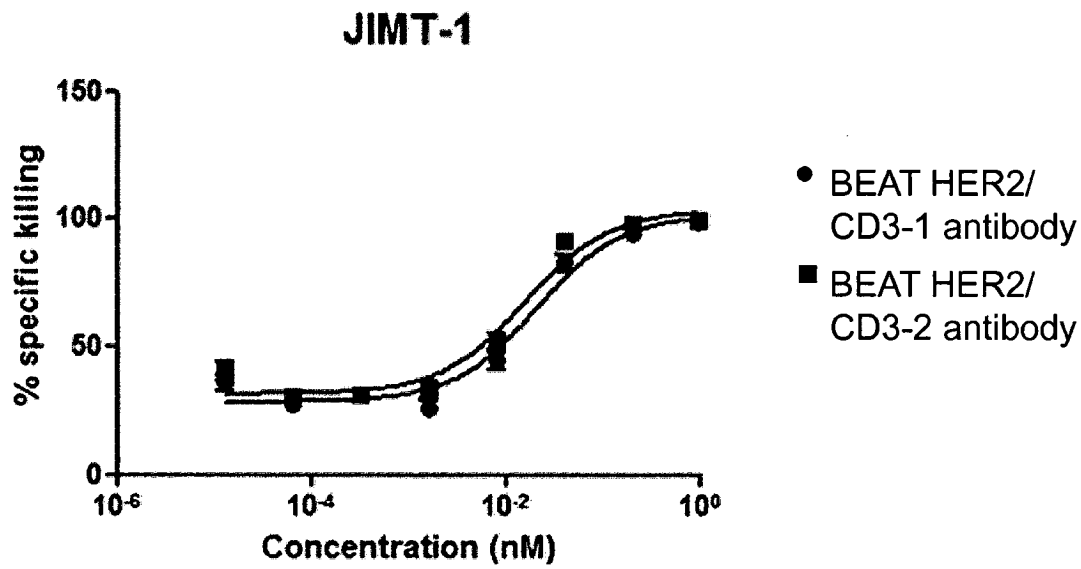


FIG. 17C

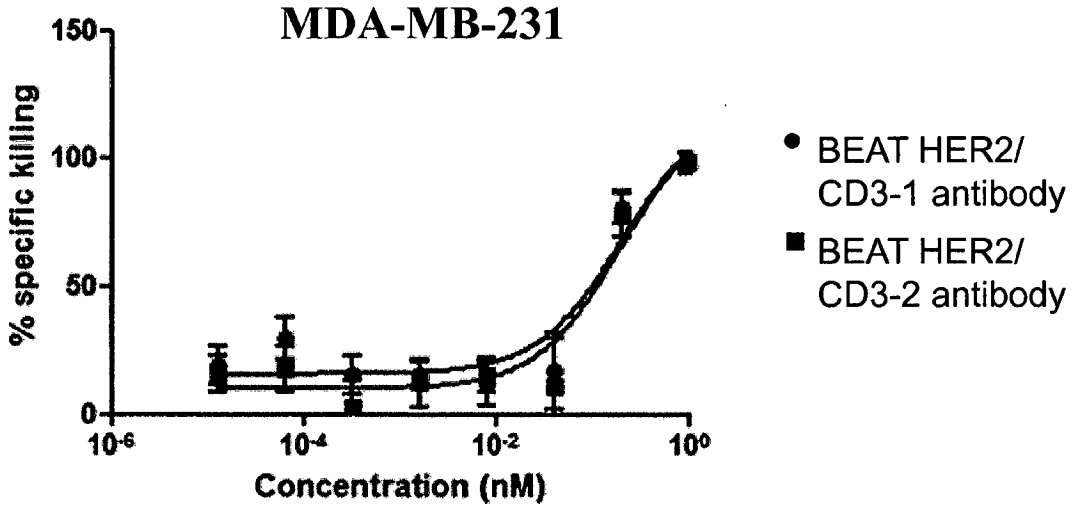


FIG. 17D

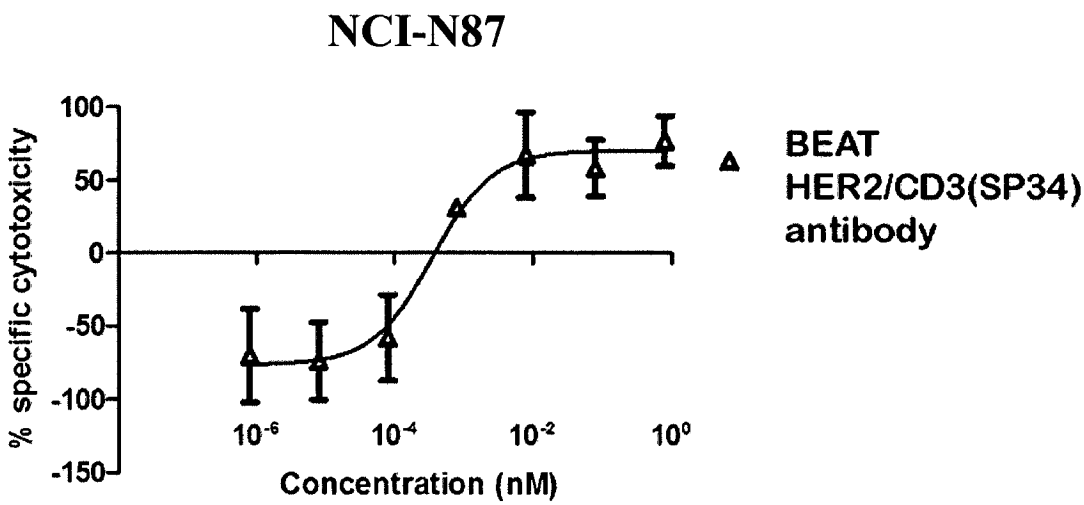


FIG. 17E

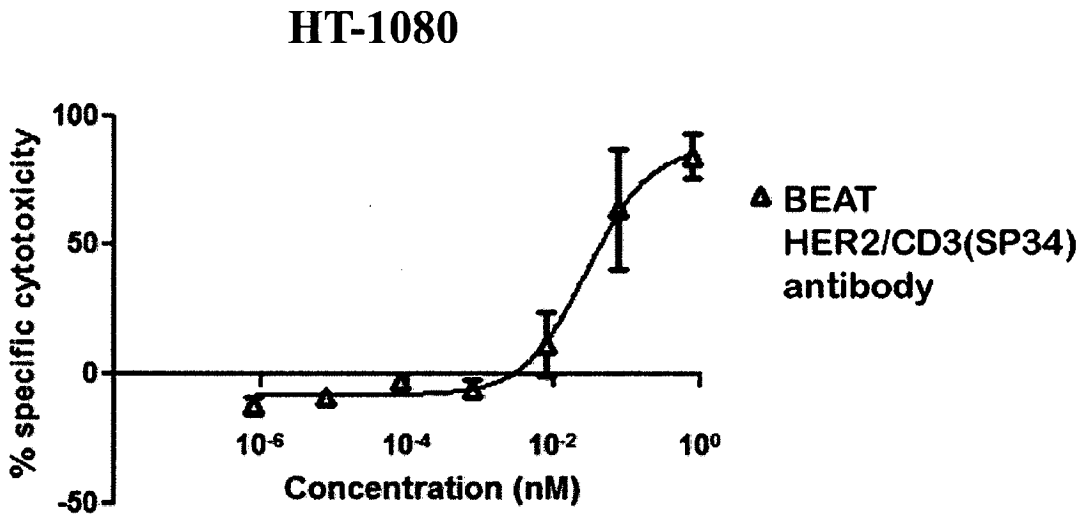


FIG. 17F

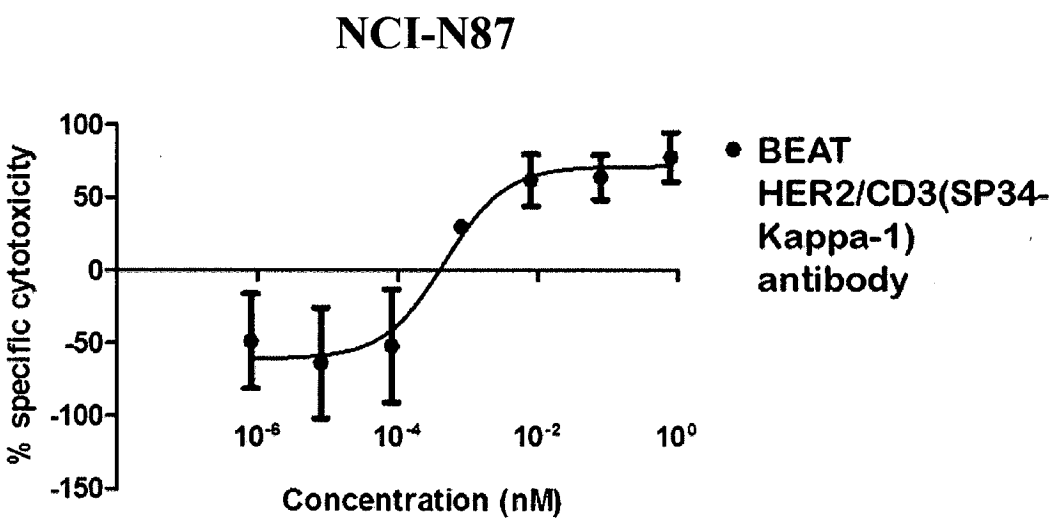


FIG. 17G

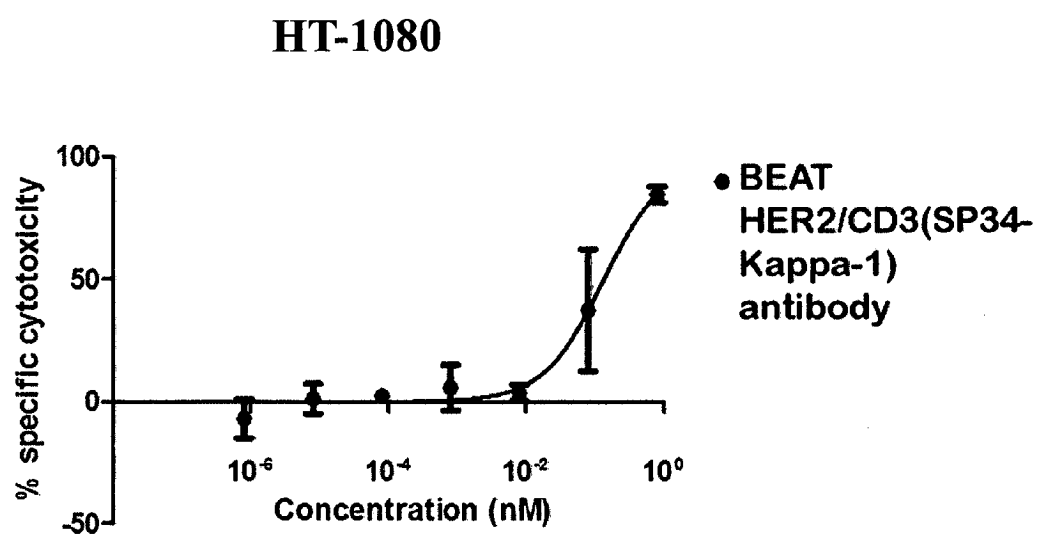


FIG. 18A

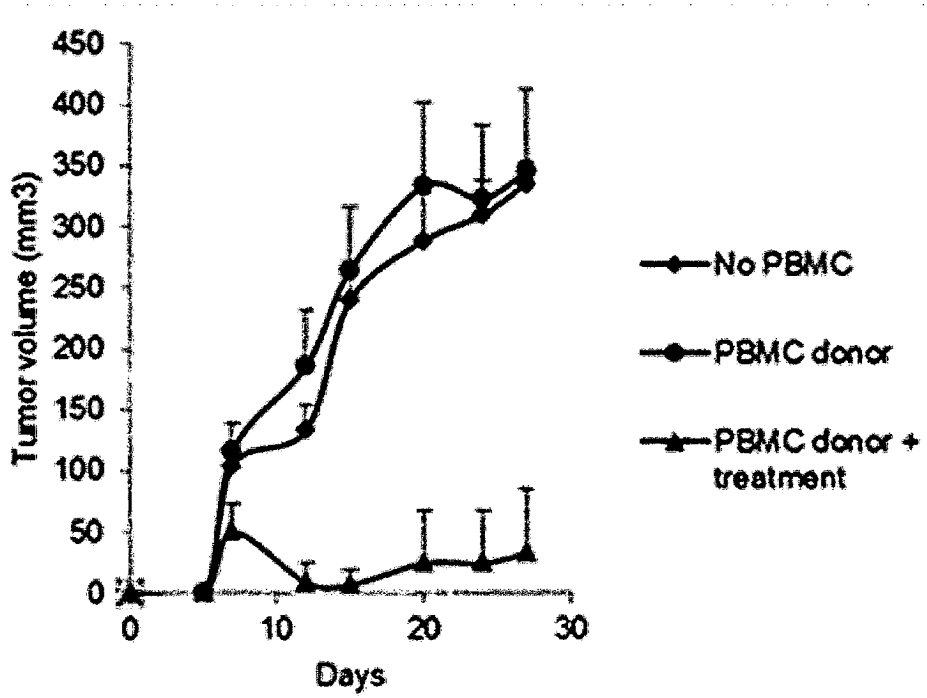


FIG. 18B

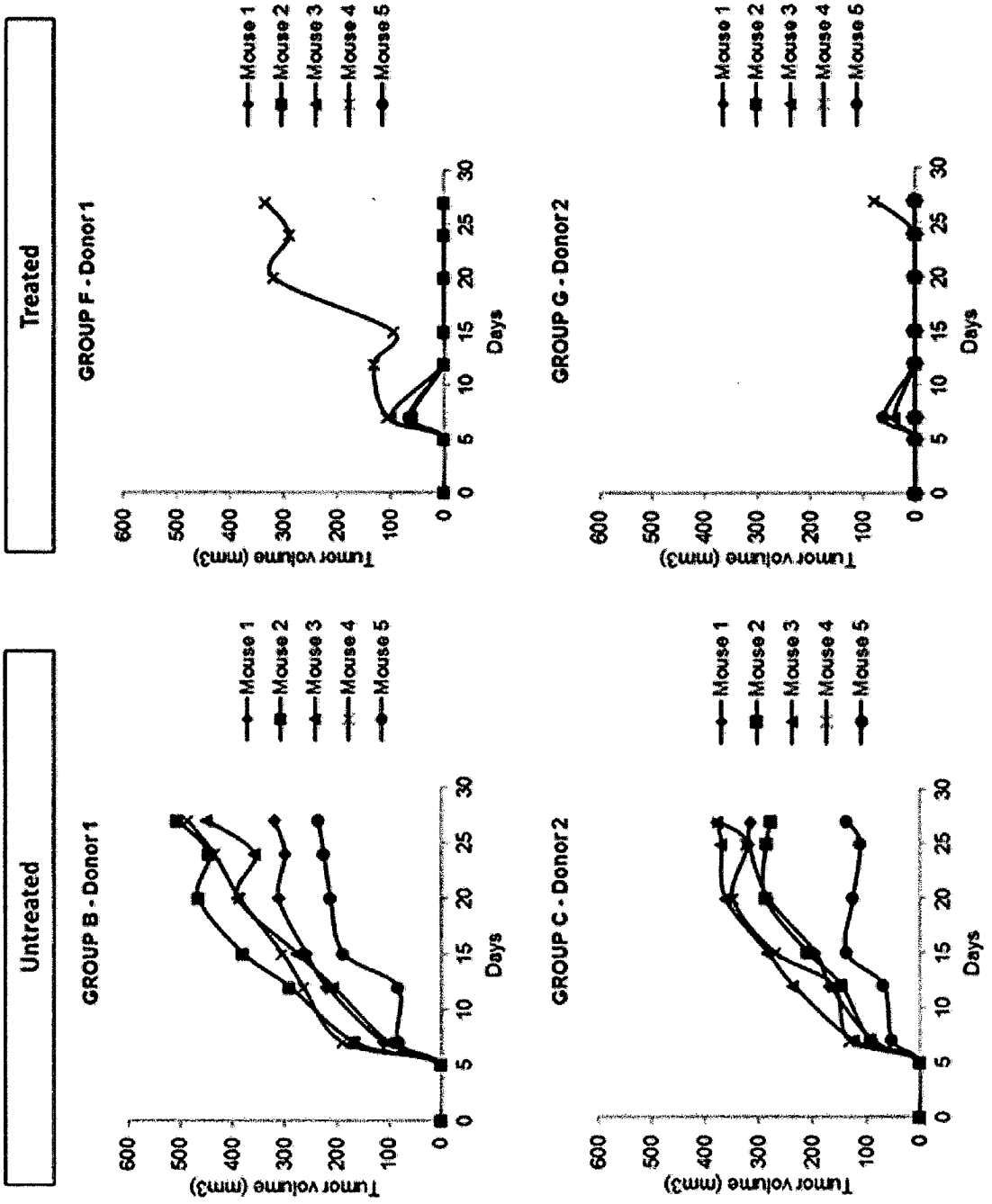

Untreated
Treated
GROUP B - Donor 1
GROUP C - Donor 2
GROUP F - Donor 1
GROUP G - Donor 2
Tumor volume (mm3)
Days
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
0
100
200
300
400
500
600
0
5
10
15
20
25
30

FIG. 18C

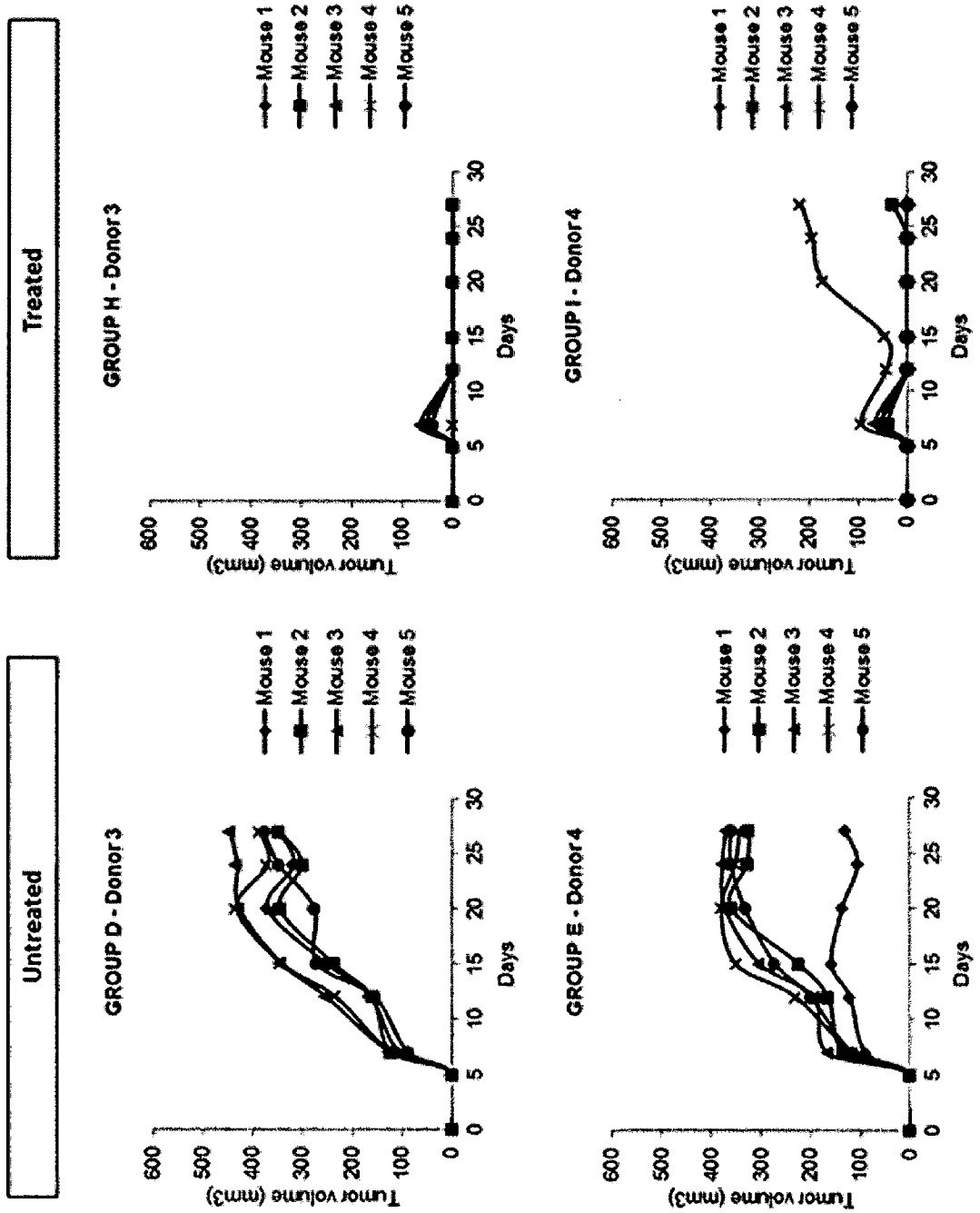

Untreated
Treated
GROUP D - Donor3
GROUP E - Donor4
GROUP H - Donor3
GROUP I - Donor4
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Tumor volume (mm3)
Days
0
100
200
300
400
500
600
0
5
10
15
20
25
30

FIG. 19

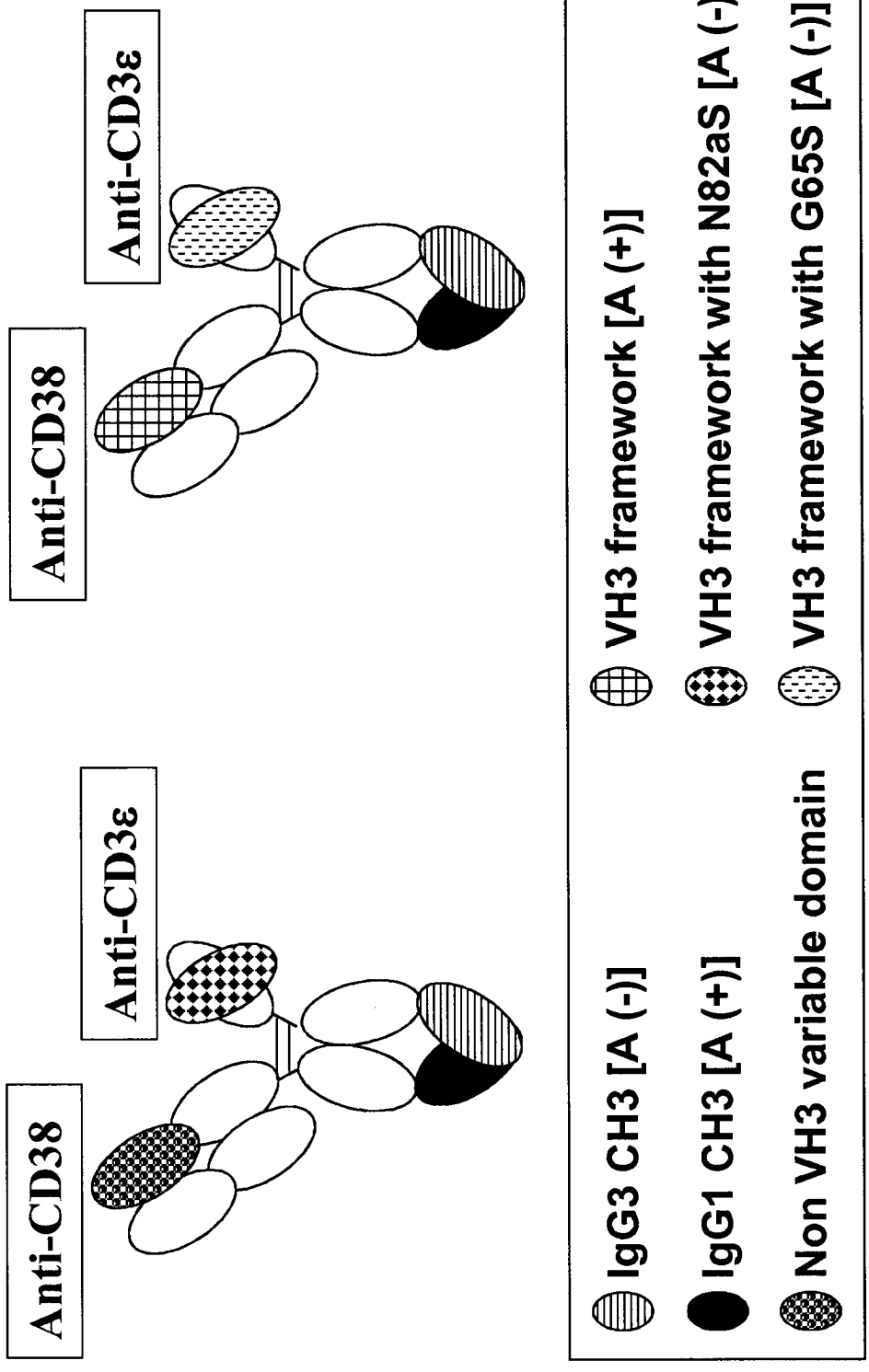

A
Anti-CD38
Anti-CD3ε
B
Anti-CD38
Anti-CD3ε
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
Non VH3 variable domain
VH3 framework [A (+)]
VH3 framework with N82aS [A (-)]
VH3 framework with G65S [A (-)]

FIG. 20A

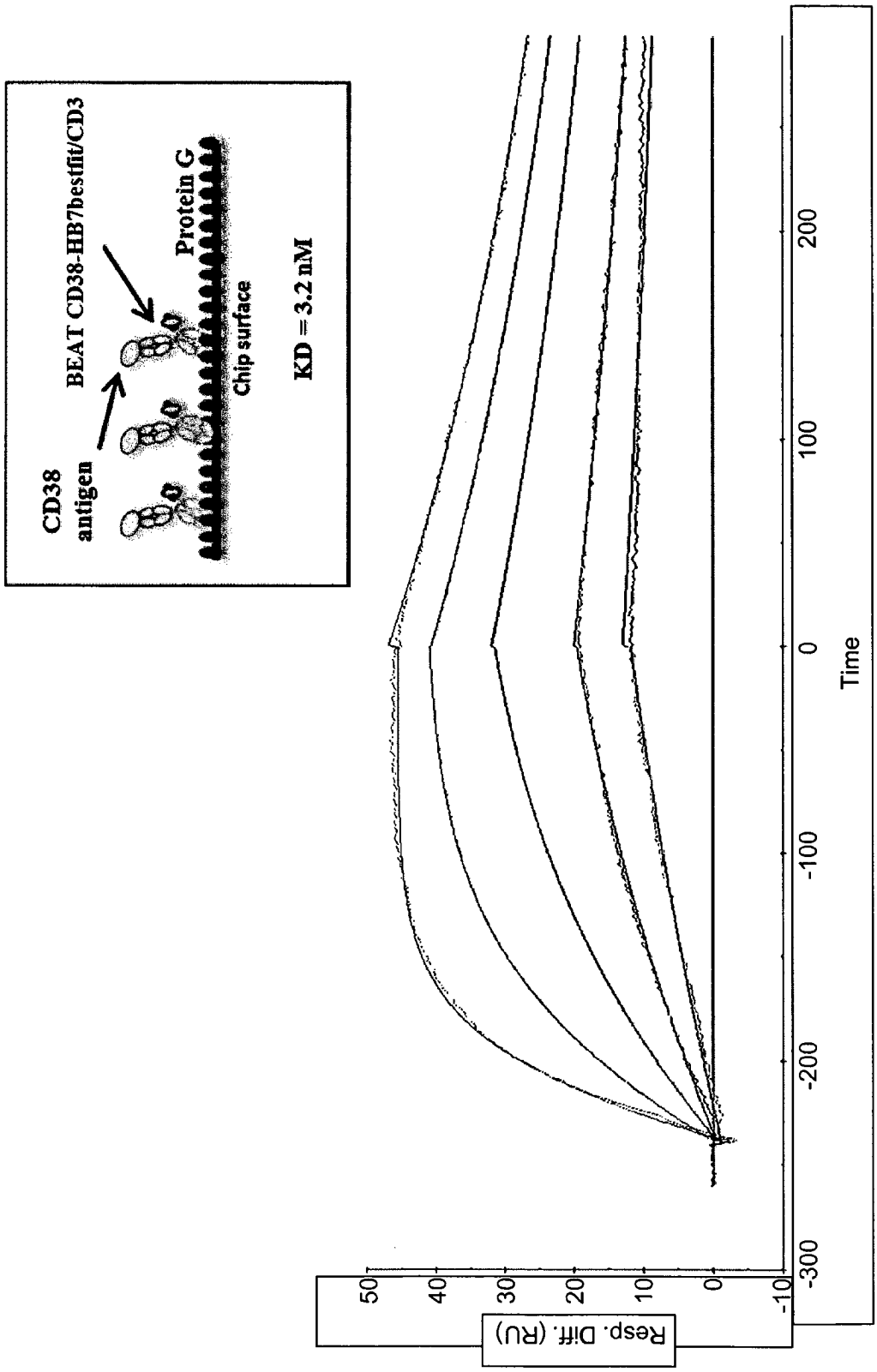
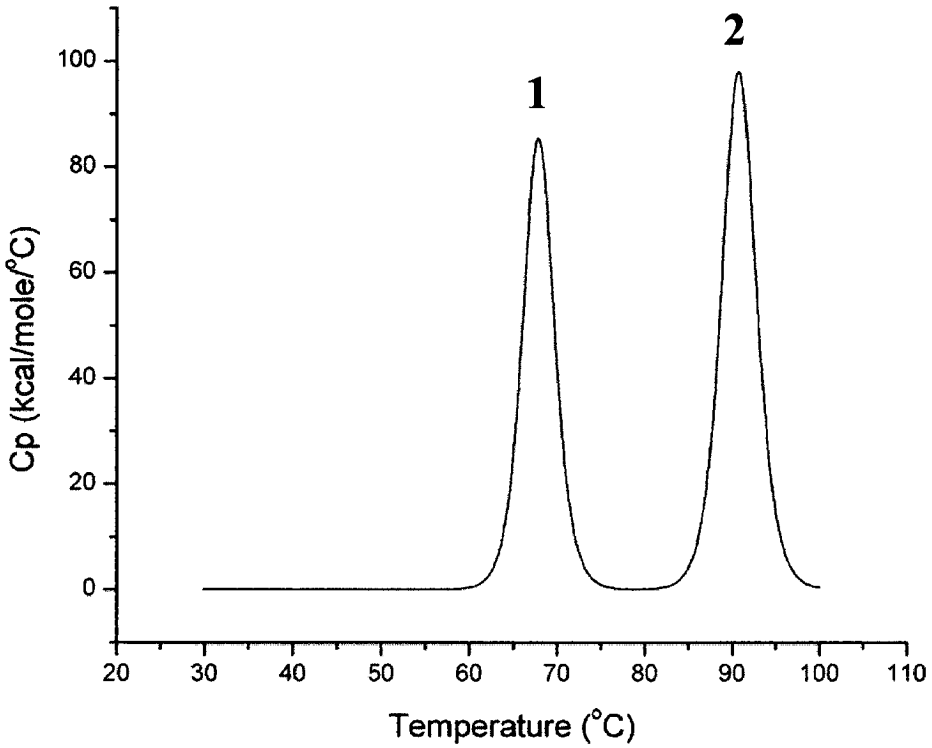

CD38 antigen
BEAT CD38-HB7bestfit/CD3
Protein G
Chip surface
KD = 3.2 nM
Resp. Diff. (RU)
50
40
30
20
10
0
-10
-300
-200
-100
0
100
200
Time

FIG. 20B



Tm(1): 67.9°C (scFv); Tm(2): 90.8°C (FAB)

FIG. 21

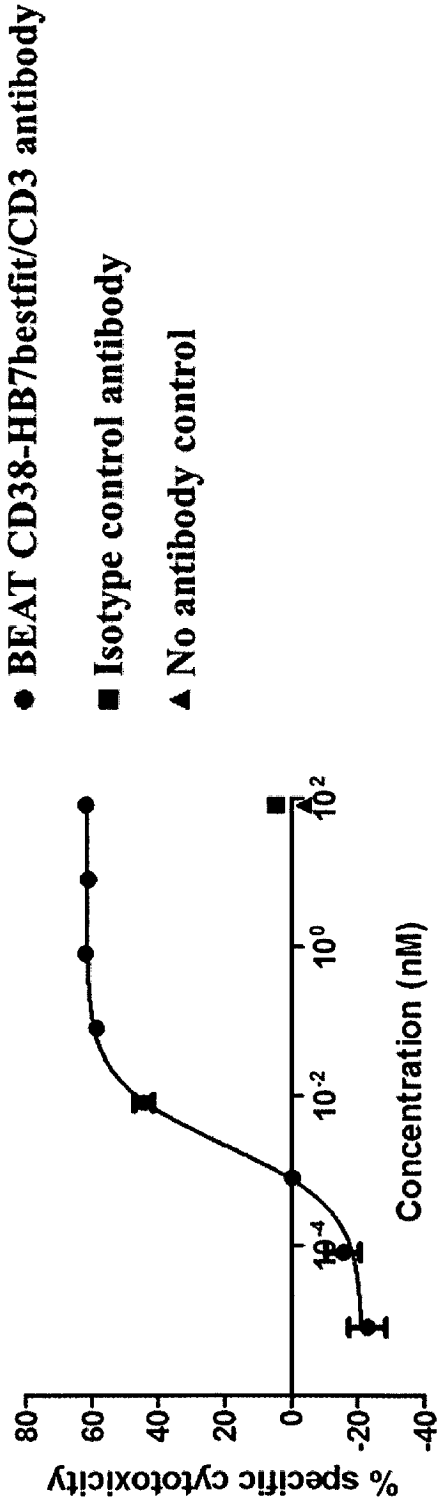

RPMI 8226
● BEAT CD38-HB7bestfit/CD3 antibody
■ Isotype control antibody
▲ No antibody control
% specific cytotoxicity
80
60
40
20
0
-20
-40
10^-4
10^-2
10^0
10^2
Concentration (nM)

FIG. 22

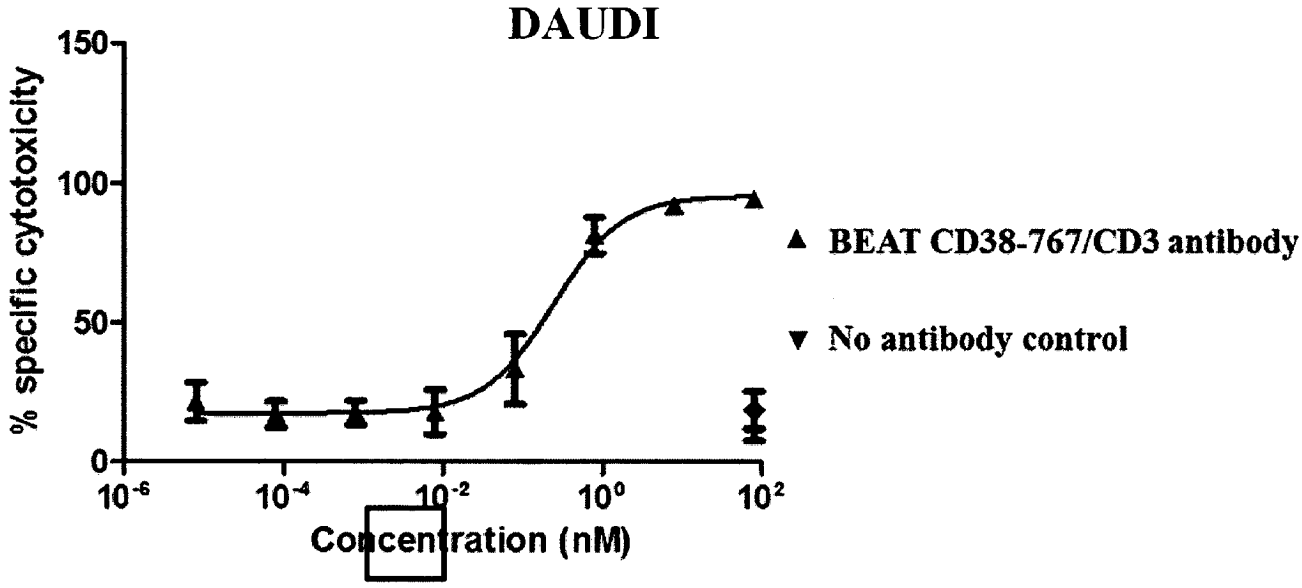

DAUDI
% specific cytotoxicity
150
100
50
0
10-6
10-4
10-2
100
102
Concentration (nM)
BEAT CD38-767/CD3 antibody
No antibody control

FIG. 23

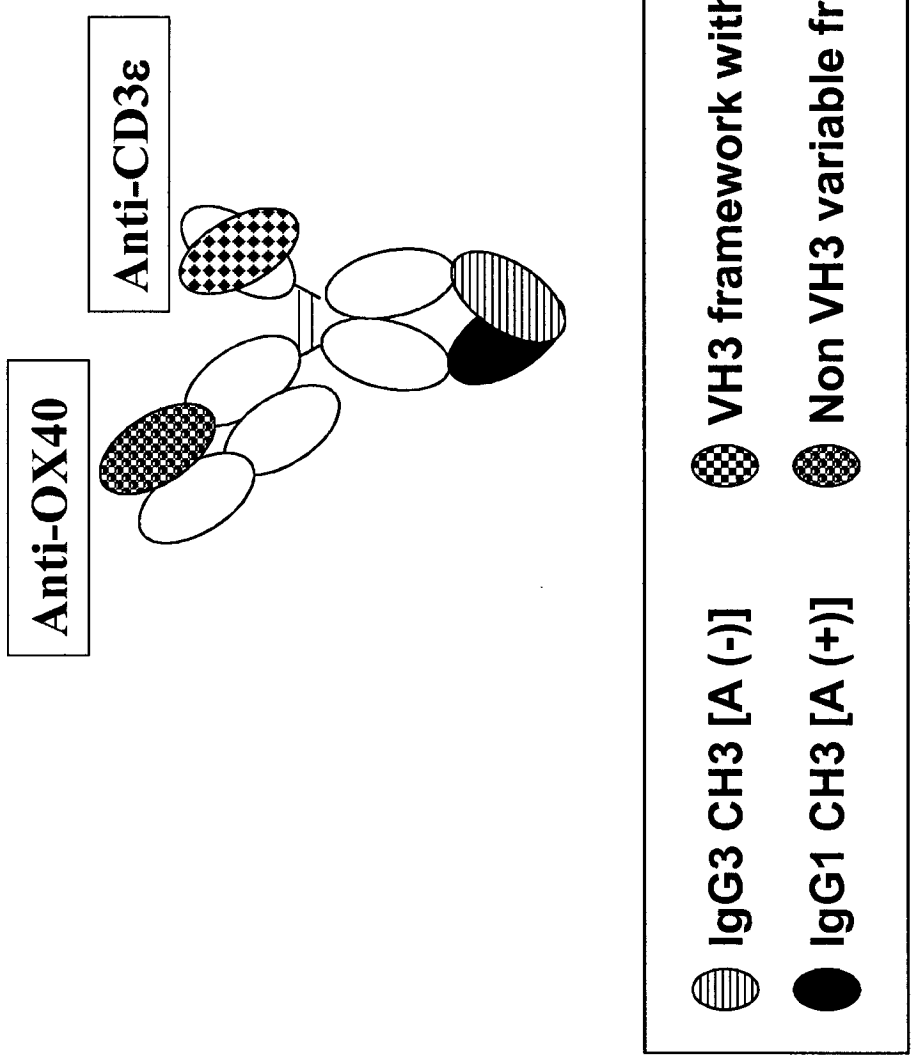

Anti-OX40
Anti-CD3ε
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 framework with N82aS [A (-)]
Non VH3 variable framework

FIG. 24

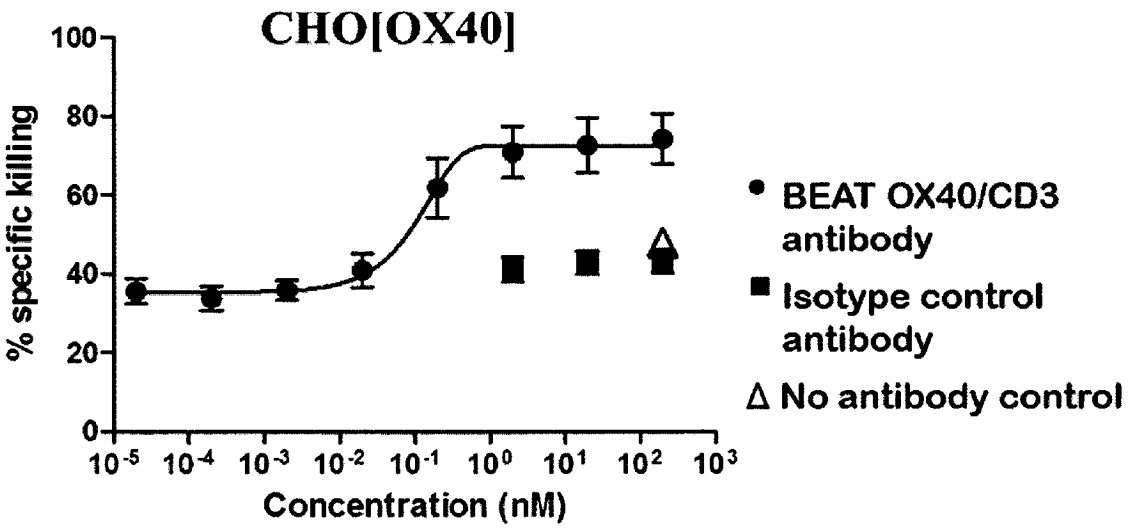

CHO[OX40]
% specific killing
100
80
60
40
20
0
10-5 10-4 10-3 10-2 10-1 100 101 102 103
Concentration (nM)
BEAT OX40/CD3 antibody
Isotype control antibody
No antibody control

FIG. 25

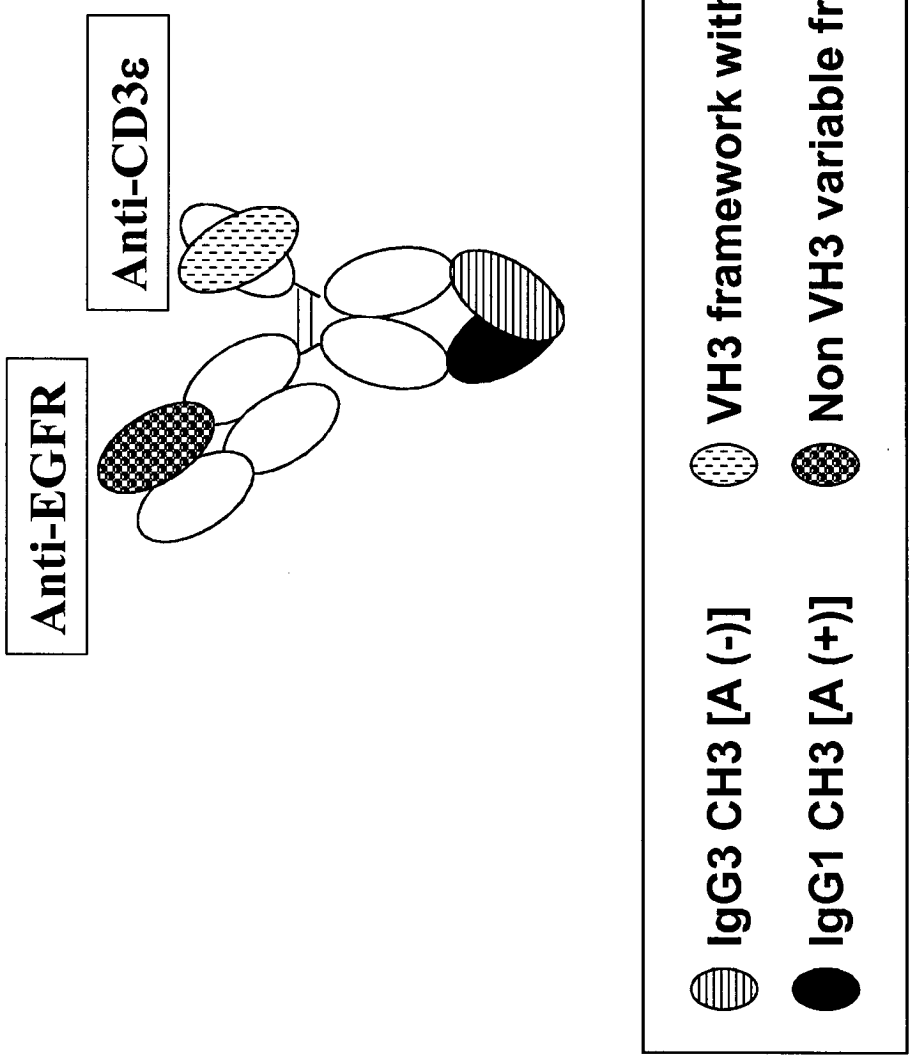

Anti-EGFR
Anti-CD3ε
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 framework with G65S [A (-)]
Non VH3 variable framework

FIG. 26

HT-29

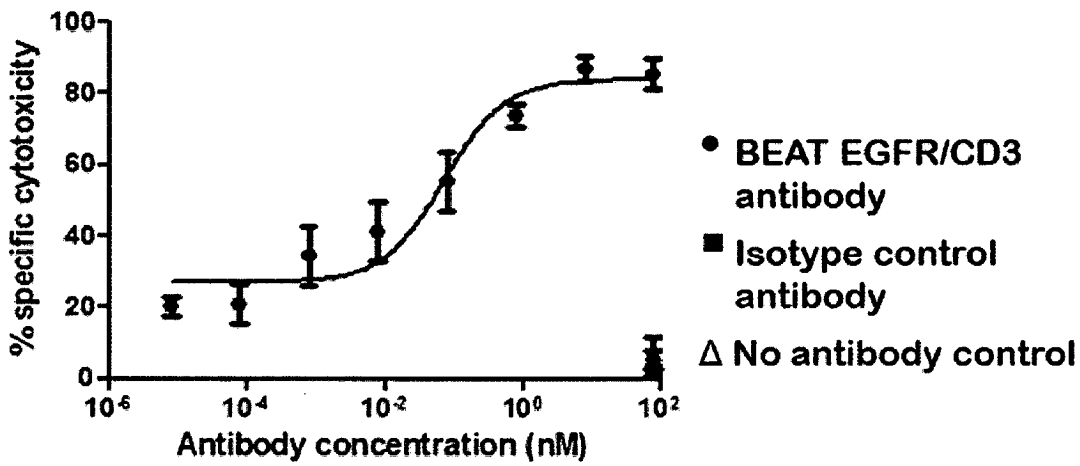

% specific cytotoxicity
100
80
60
40
20
0
10^{-6}
10^{-4}
10^{-2}
10^{0}
10^{2}
Antibody concentration (nM)
BEAT EGFR/CD3 antibody
Isotype control antibody
Δ No antibody control

FIG. 27

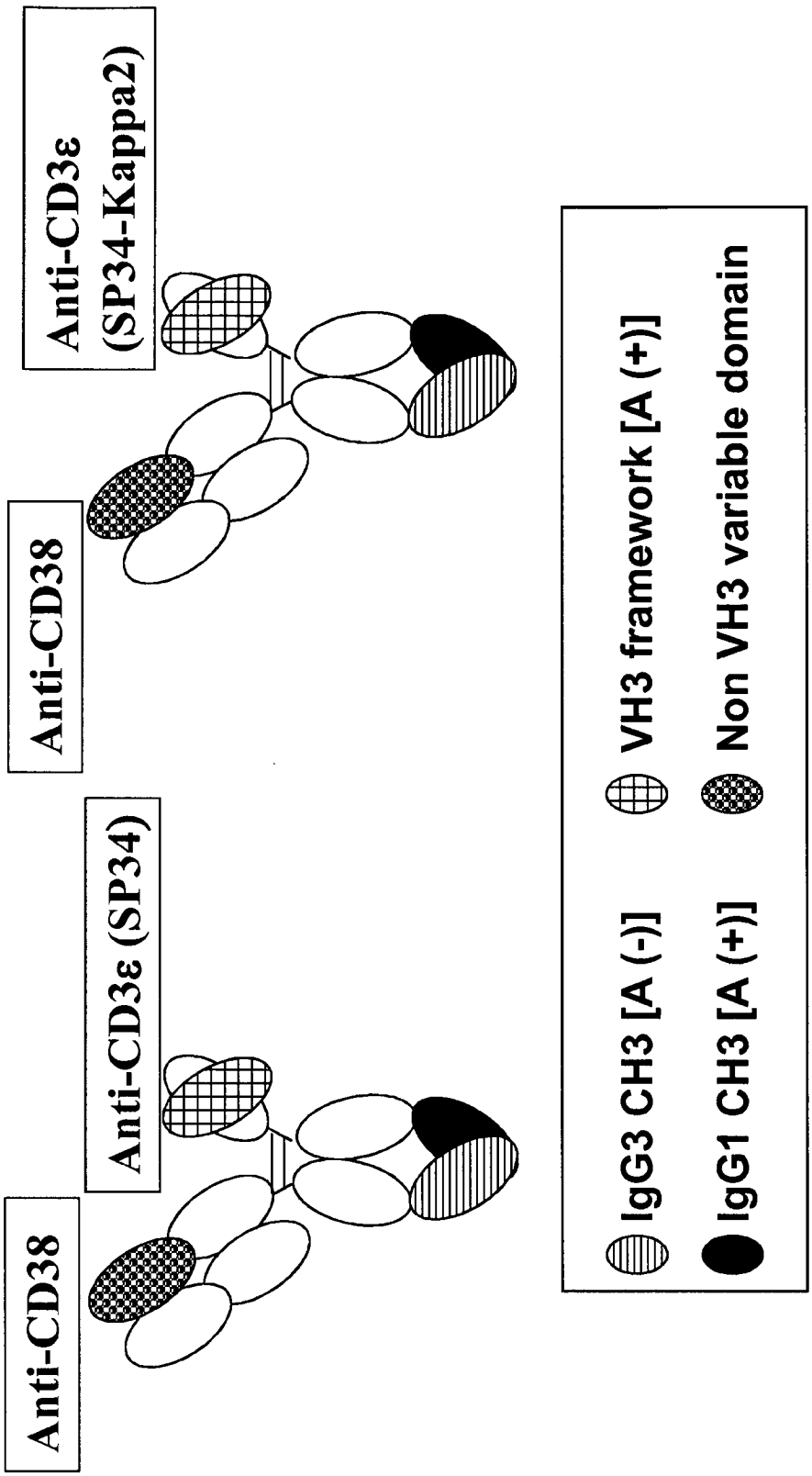

A
Anti-CD38
Anti-CD3ε (SP34)
B
Anti-CD38
Anti-CD3ε (SP34-Kappa2)
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 framework [A (+)]
Non VH3 variable domain

FIG. 28

DAUDI

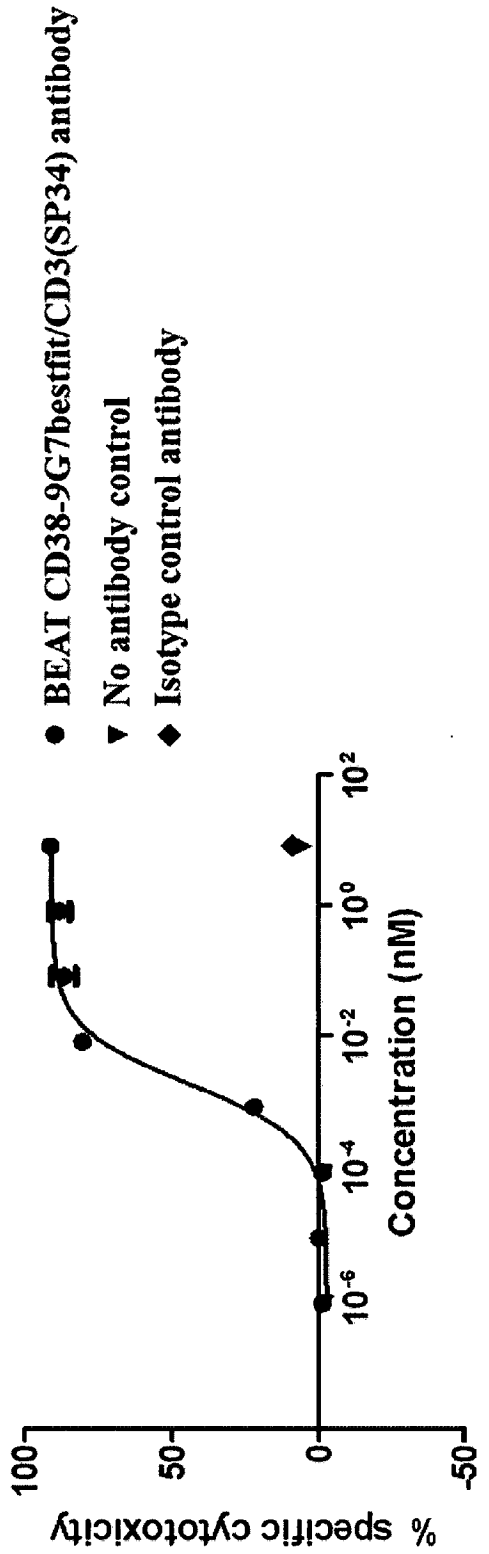

BEAT CD38-9G7bestfit/CD3(SP34) antibody
No antibody control
Isotype control antibody
% specific cytotoxicity
100
50
0
-50
10^{-6}
10^{-4}
10^{-2}
10^{0}
10^{2}
Concentration (nM)

FIG. 29

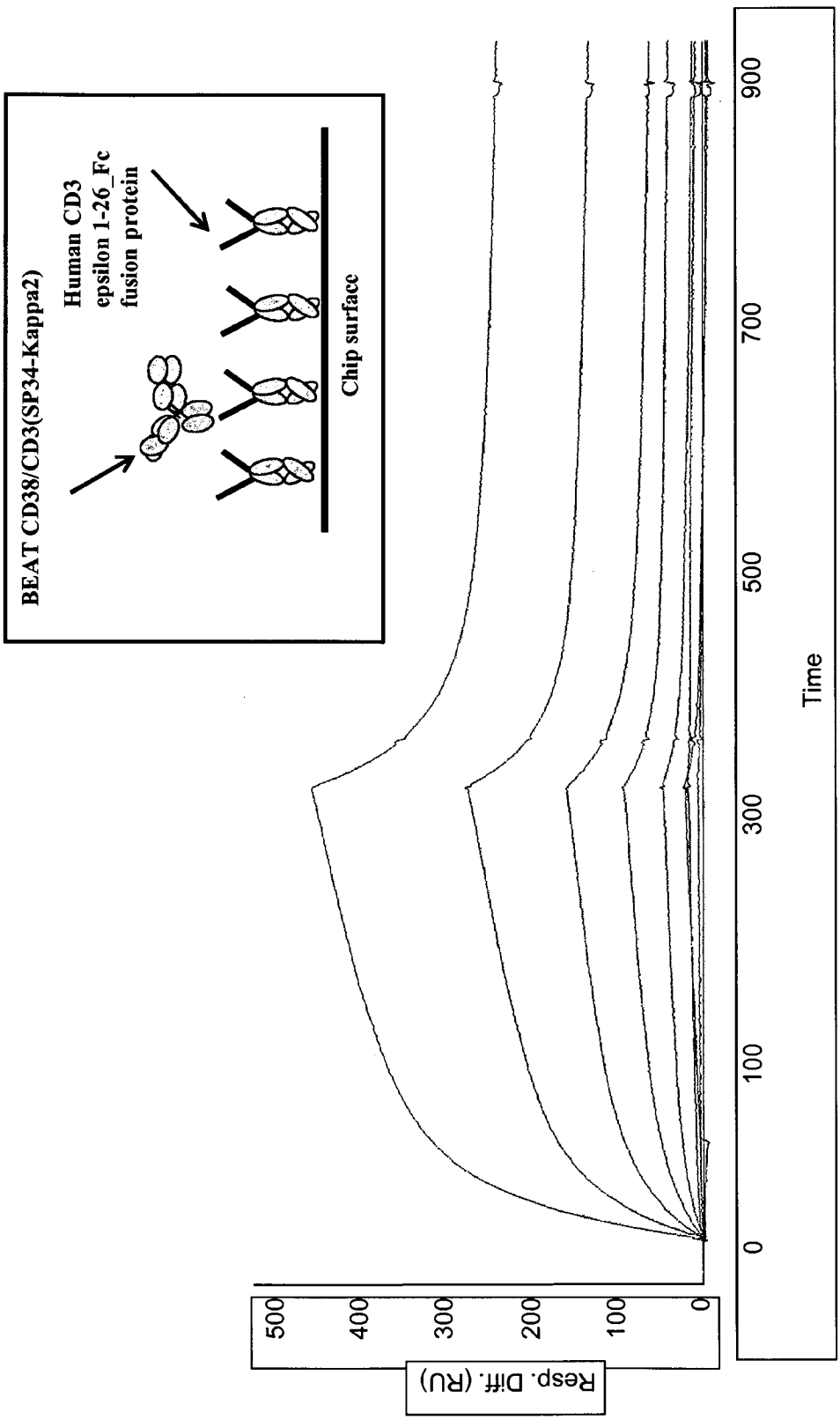

BEAT CD38/CD3(SP34-Kappa2)
Human CD3 epsilon 1-26_Fc fusion protein
Chip surface
Resp. Diff. (RU)
500
400
300
200
100
0
0
100
300
500
700
900
Time

FIG. 30

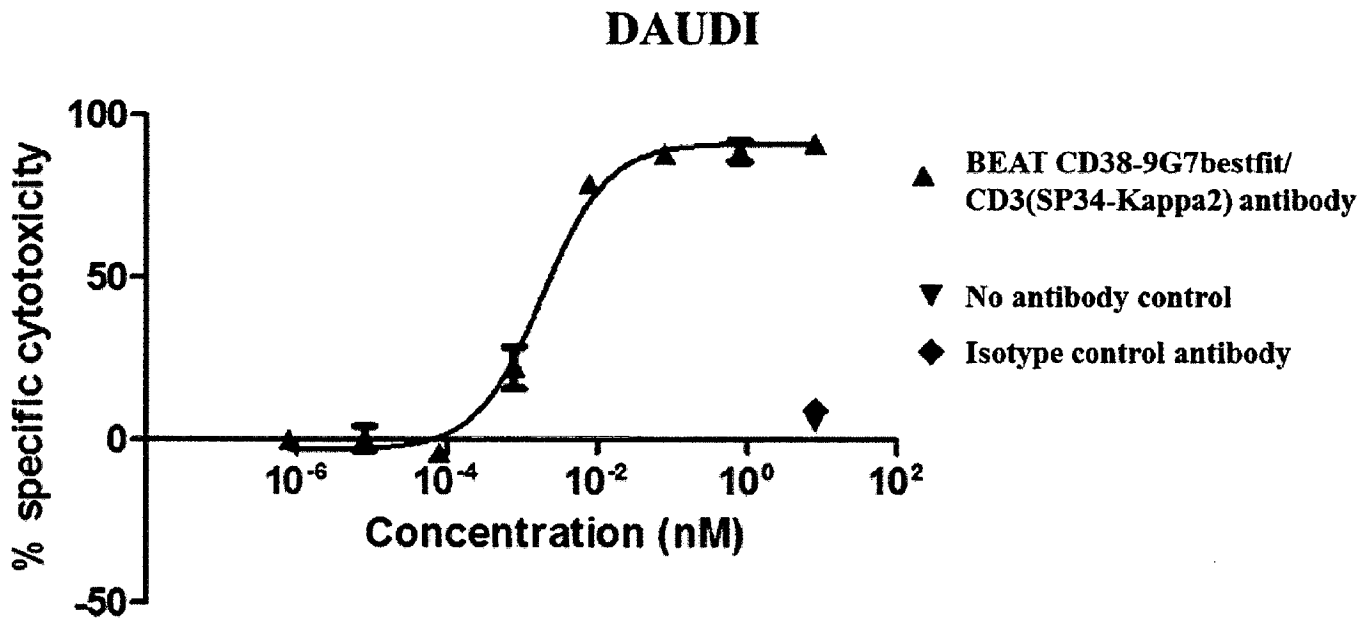

DAUDI
% specific cytotoxicity
100
50
0
-50
10^{-6}
10^{-4}
10^{-2}
10^{0}
10^{2}
Concentration (nM)
BEAT CD38-9G7bestfit/ CD3(SP34-Kappa2) antibody
No antibody control
Isotype control antibody

FIG. 31

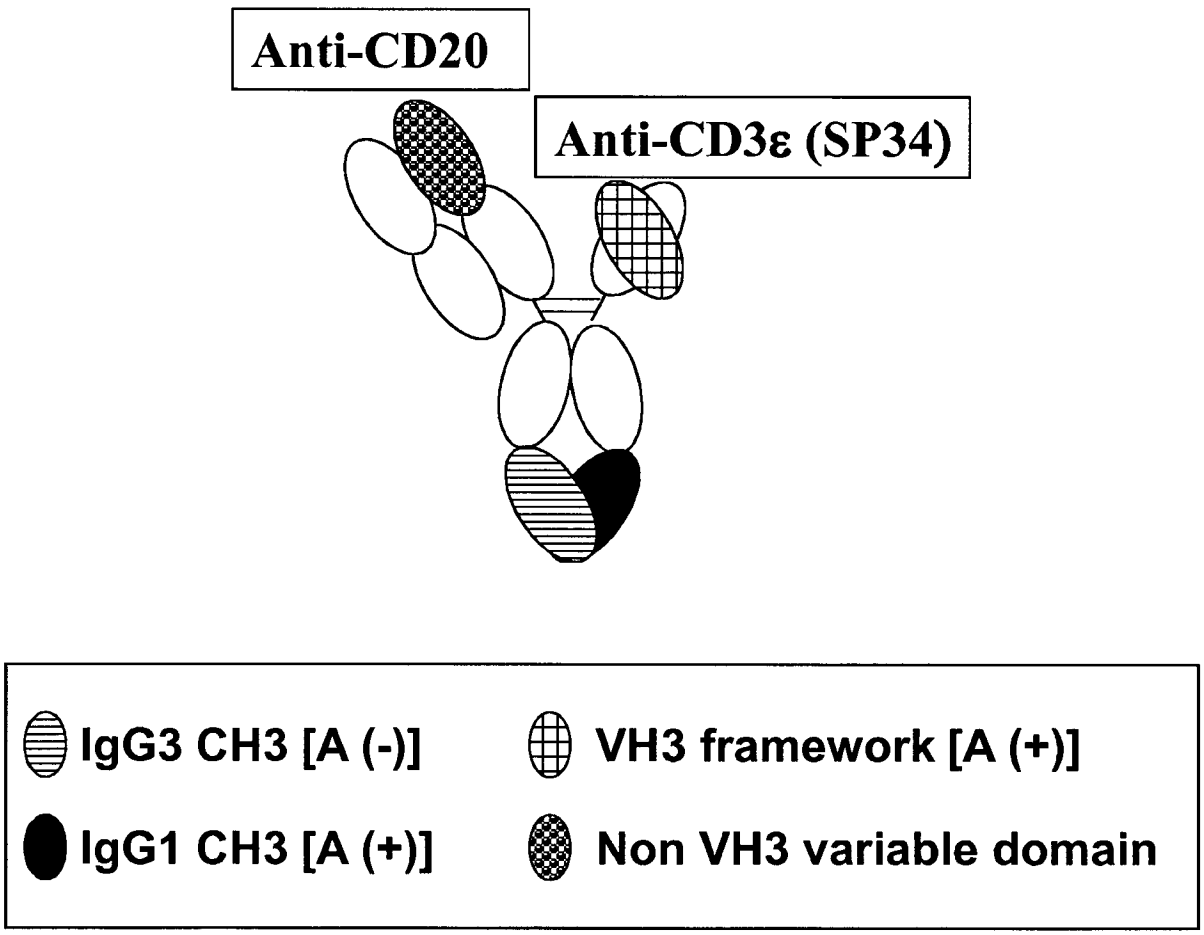

Anti-CD20
Anti-CD3ε (SP34)
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 framework [A (+)]
Non VH3 variable domain

FIG. 32

DAUDI

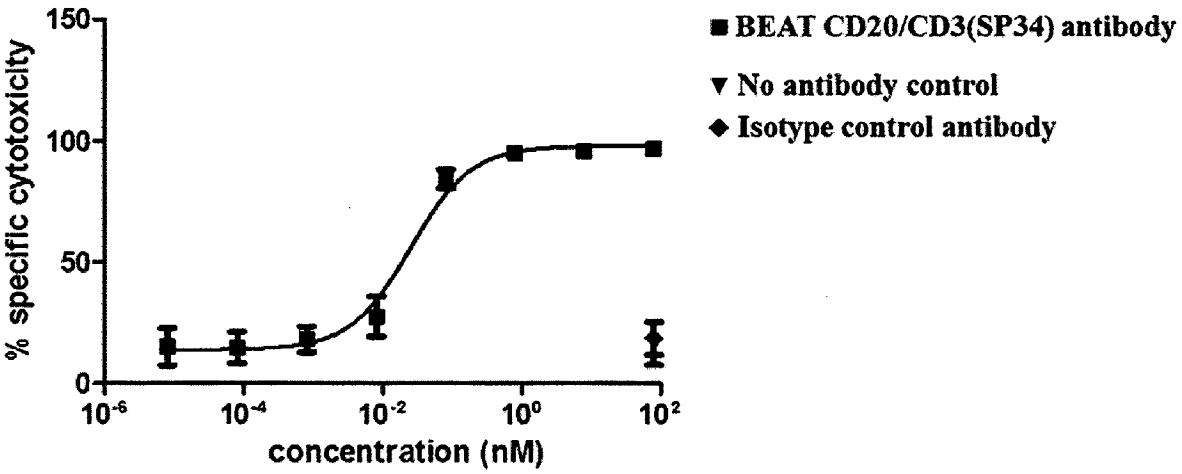

150
100
50
0
% specific cytotoxicity
10-6
10-4
10-2
100
102
concentration (nM)
BEAT CD20/CD3(SP34) antibody
No antibody control
Isotype control antibody

FIG. 33

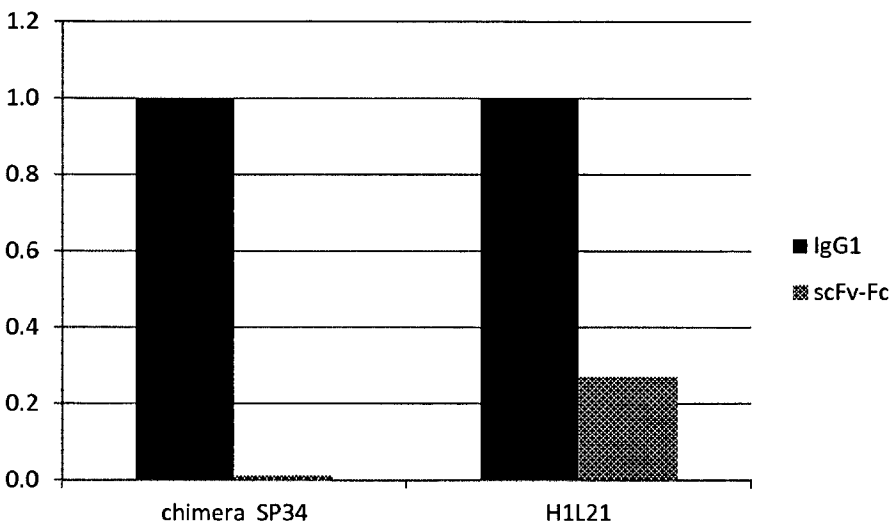


FIG. 34

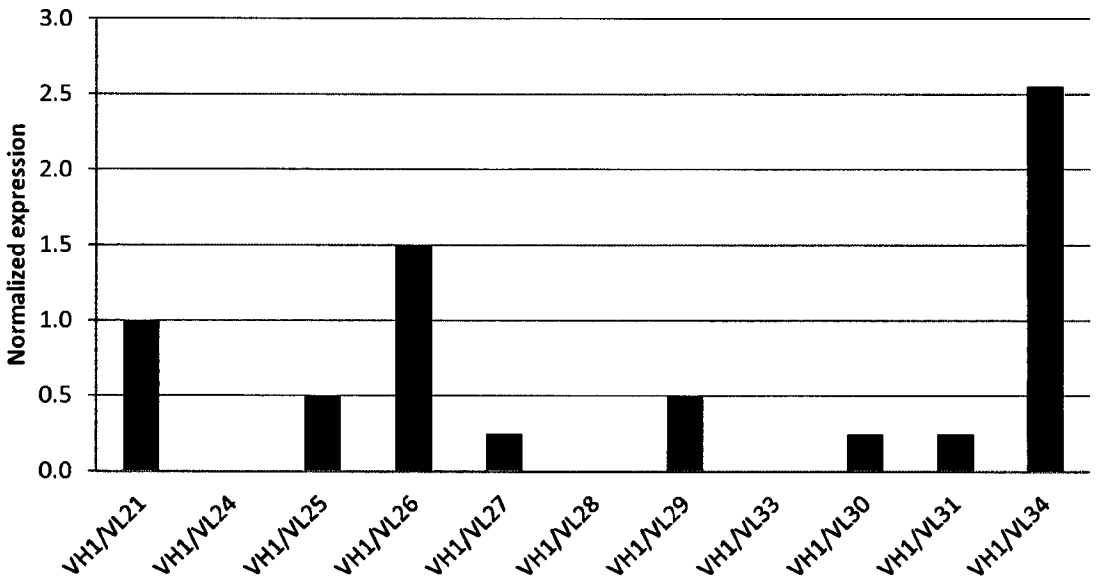
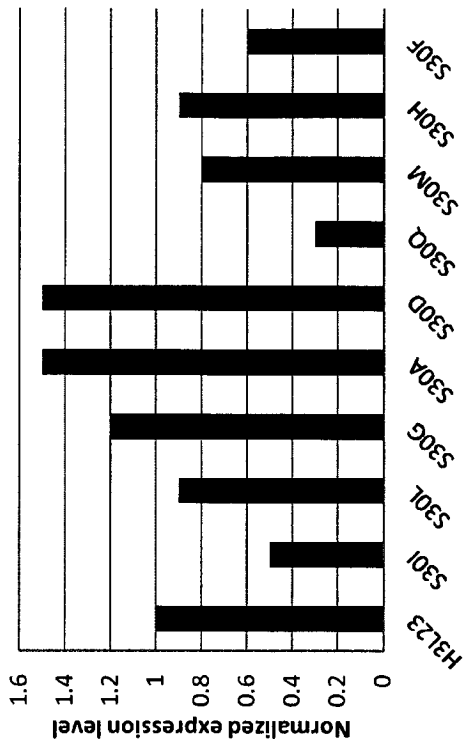
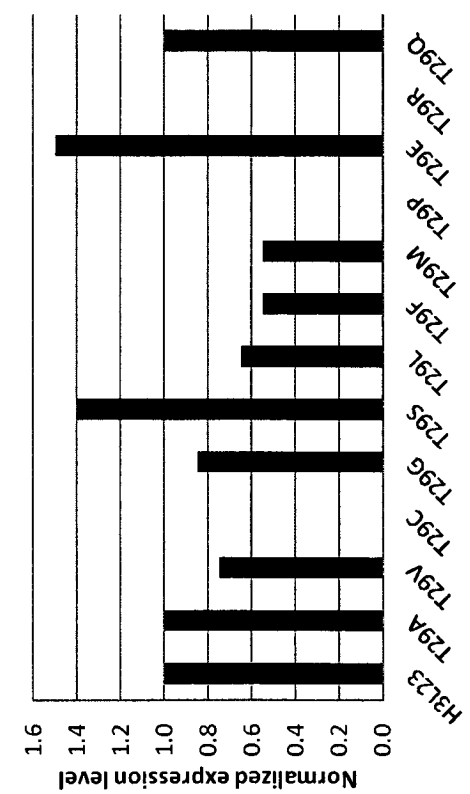

3.0
2.5
2.0
1.5
1.0
0.5
0.0
Normalized expression
VH1/VL21
VH1/VL24
VH1/VL25
VH1/VL26
VH1/VL27
VH1/VL28
VH1/VL29
VH1/VL33
VH1/VL30
VH1/VL31
VH1/VL34

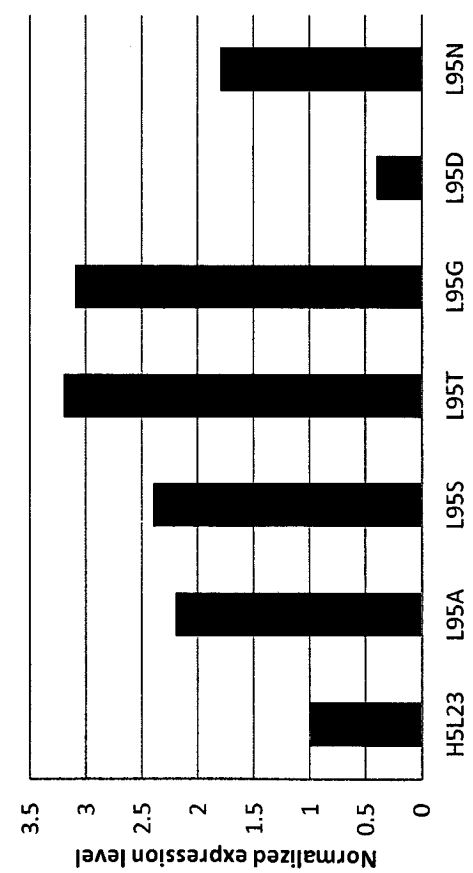
FIG. 35


Normalized expression level
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
H3L23
S30I
S30L
S30G
S30A
S30D
S30Q
S30M
S30H
S30F

b


Normalized expression level
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
H3L23
T29A
T29V
T29C
T29G
T29S
T29L
T29F
T29M
T29P
T29E
T29R
T29Q

a


Normalized expression level
3.5
3
2.5
2
1.5
1
0.5
0
H5L23
L95A
L95S
L95T
L95G
L95D
L95N

c

FIG. 36

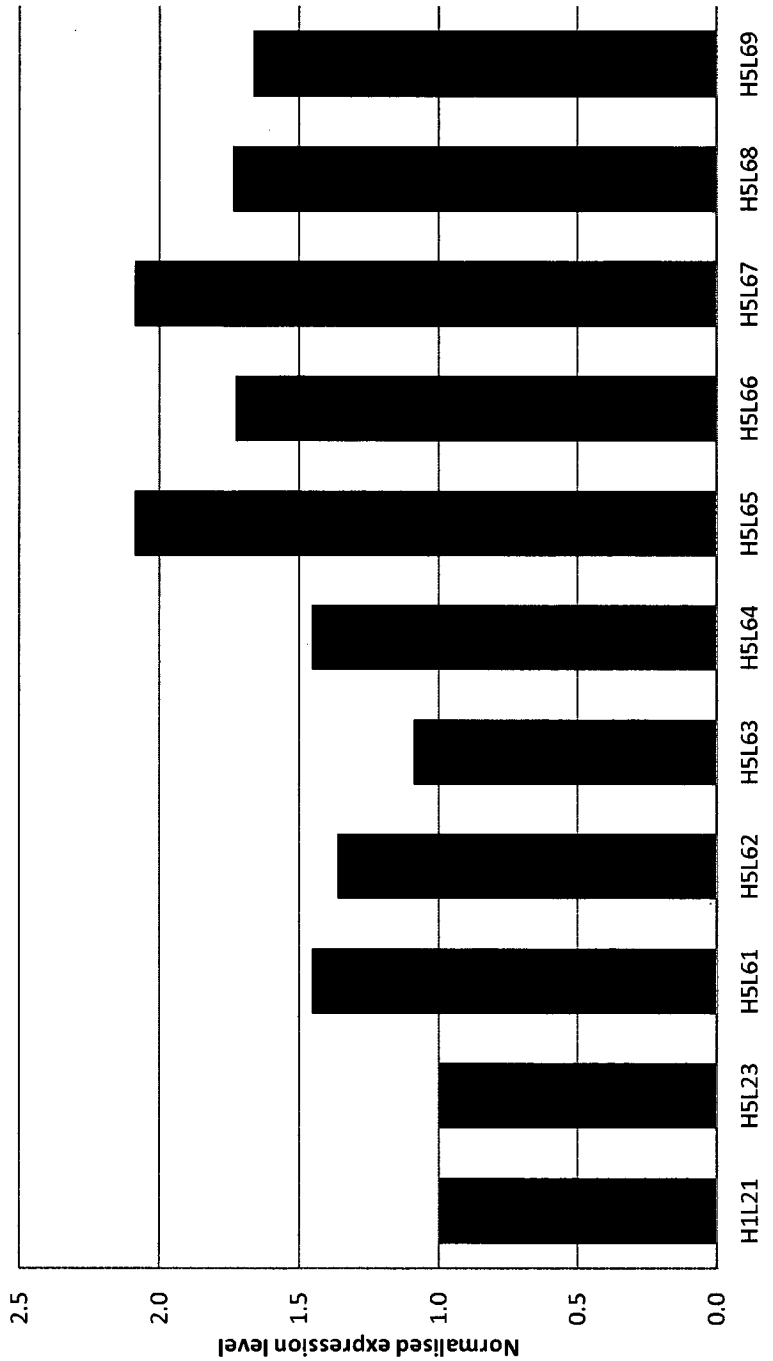
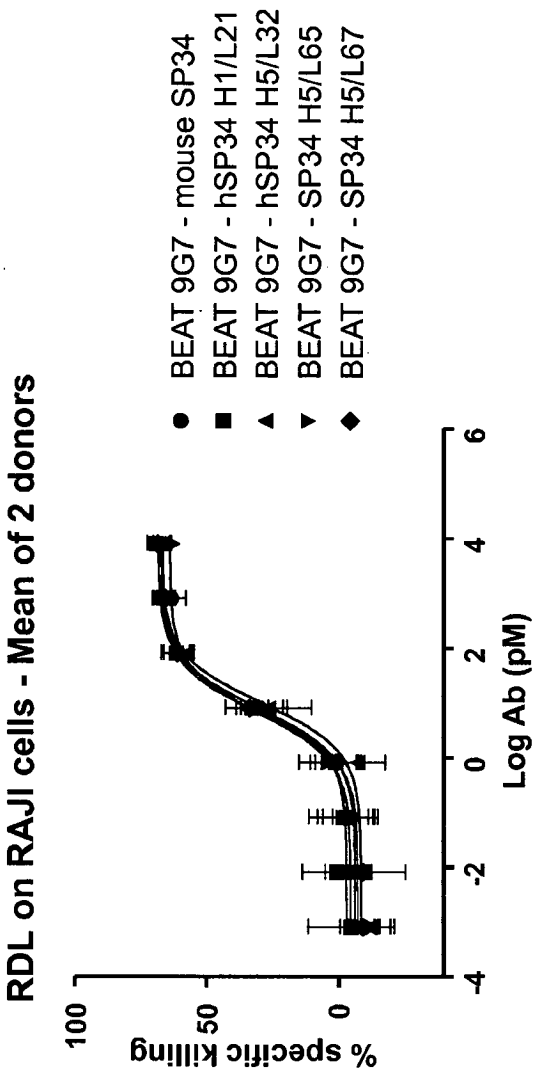
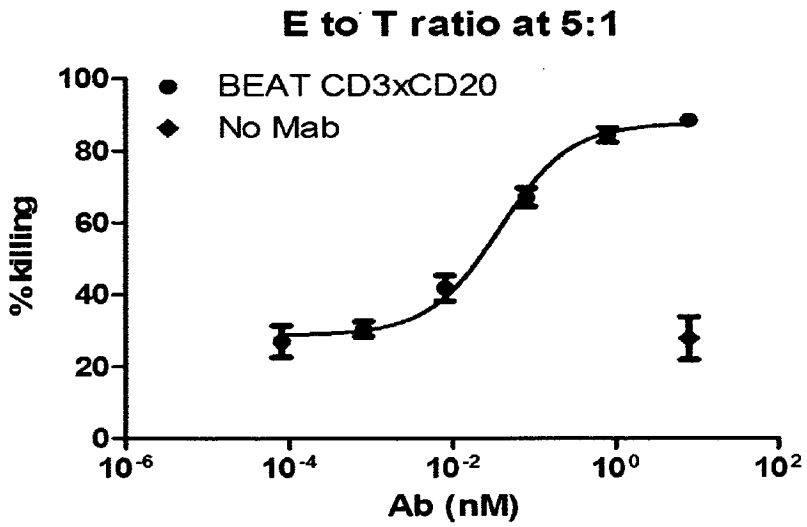

Normalised expression level
0.0
0.5
1.0
1.5
2.0
2.5
H1L21
H5L23
H5L61
H5L62
H5L63
H5L64
H5L65
H5L66
H5L67
H5L68
H5L69

FIG. 37



	EC50 BEAT 9G7 - mouse SP34	EC50 BEAT 9G7 - hSP34 H1L21	EC50 BEAT 9G7 - hSP34 H5L32	EC50 BEAT 9G7 - hSP34 H5L65	EC50 BEAT 9G7 - hSP34 H5L67
Mean	7.883	8.807	10.09	6.170	6.493
Std. Deviation	2.879	2.070	5.098	1.185	1.526

FIG. 38



BEAT CD3xCD20	EC50 (pM)
E to T ratio 5:1	37.56

FIG. 39

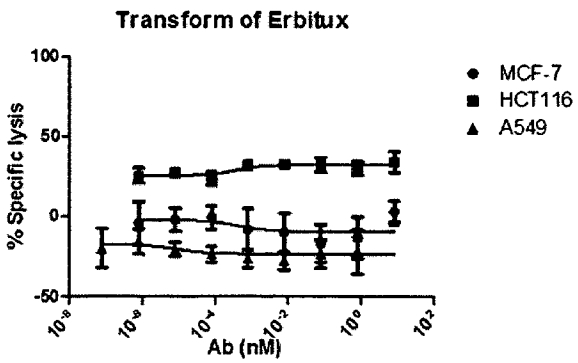

Transform of Erbitux
100
50
0
-50
% Specific lysis
MCF-7
HCT116
A549
Ab (nM)

FIG. 40

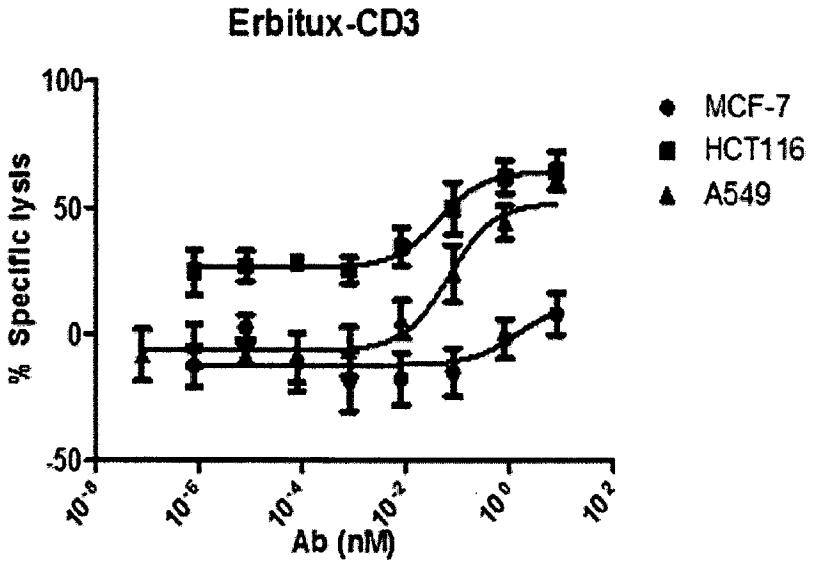

Erbitux-CD3
% Specific lysis
100
50
0
-50
10^{-8}
10^{-6}
10^{-4}
10^{-2}
10^{0}
10^{2}
Ab (nM)
MCF-7
HCT116
A549

FIG. 41

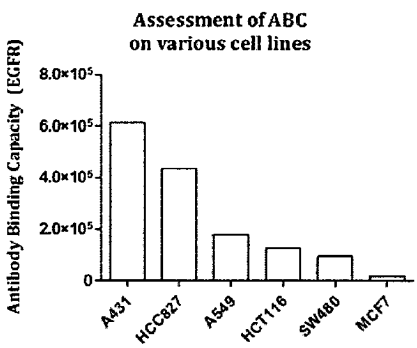

Assessment of ABC
on various cell lines
Antibody Binding Capacity (EGFR)
8.0×10⁵
6.0×10⁵
4.0×10⁵
2.0×10⁵
0
A431
HCC827
A549
HCT116
SW480
MCF7

FIG. 42

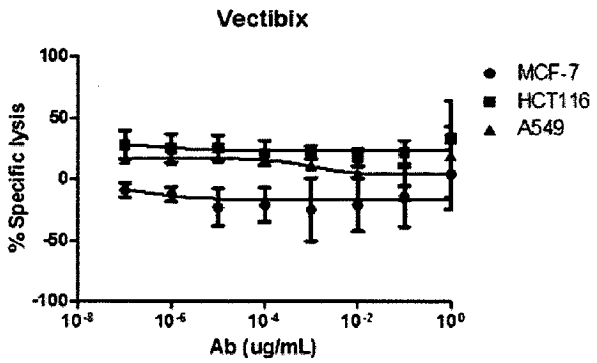
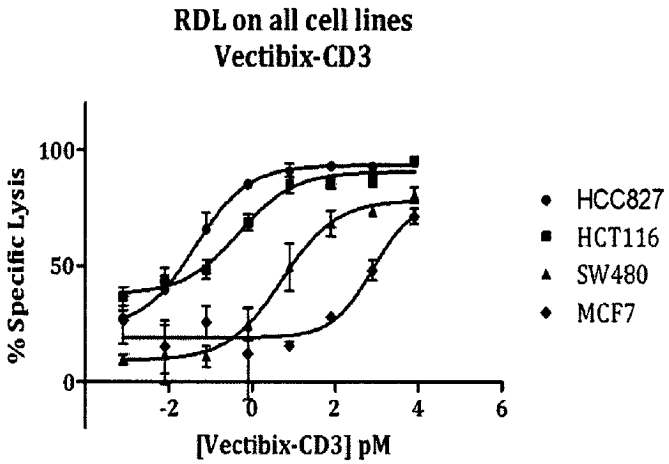

Vectibix
100
50
0
-50
-100
% Specific lysis
10-8
10-6
10-4
10-2
100
Ab (ug/mL)
MCF-7
HCT116
A549

FIG. 43



	HCC827	HCT116	SW480	MCF7
EC50	0.04302	0.4863	5.260	795.8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/060003

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/46 B01D15/38 C07K16/28 C07K16/40
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 2 522 724 A1 (CHUGAI PHARMACEUTICAL CO LTD [JP]) 14 November 2012 (2012-11-14) paragraph [0012] paragraph [0014] paragraph [0075] paragraph [0015] paragraph [0016] paragraphs [0073] - [0074] figure 4; example 8	1-10
A	WO 2012/131555 A2 (GLENMARK PHARMACEUTICALS SA [CH]; BLEIN STANISLAS [CH]; SKEGRO DARKO []) 4 October 2012 (2012-10-04) page 6, line 15 - page 11, line 16	1-10

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 July 2015	10/07/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Irion, Andrea

3

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2010/075548 A2 (GENENTECH INC [US]; YEUNG YIK [US]; LOWMAN HENRY B [US]) 1 July 2010 (2010-07-01) page 2, lines 4-15 page 49, line 29 - page 50, line 22; example 2 page 28, line 25 - page 30, line 31	1-10
A	ROBEN P W ET AL: "VH3 family antibodies bind domain D of staphylococcal protein A", THE JOURNAL OF IMMUNOLOGY, THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 154, no. 12, 15 June 1995 (1995-06-15), pages 6437-6445, XP002508063, ISSN: 0022-1767 abstract	1-10
A	POTTER K N ET AL: "Staphylococcal protein A binding to VH3 encoded immunoglobulins", INTERNATIONAL REVIEWS OF IMMUNOLOGY, HARWOOD ACADEMIC PUBLISHERS, LONDON, GB, vol. 14, no. 4, 1 January 1997 (1997-01-01), pages 291-308, XP008116757, ISSN: 0883-0185 abstract	1-10
A	GRAILLE M ET AL: "Crystal structure of a Staphylococcus aureus protein A domain complexed with the Fab fragment of a human IgM antibody: Structural basis for recognition of B-cell receptors and superantigen activity", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, US, vol. 97, no. 10, 9 May 2000 (2000-05-09), pages 5399-5404, XP002284947, ISSN: 0027-8424, DOI: 10.1073/PNAS.97.10.5399 abstract figure 1; table 1	1-10
Y	ALMAGRO JUAN C ET AL: "Humanization of antibodies", FRONTIERS IN BIOSCIENCE, FRONTIERS IN BIOSCIENCE, ALBERTSON, NY, US, vol. 13, 1 January 2008 (2008-01-01), pages 1619-1633, XP009126790, ISSN: 1093-9946 p. 1624 right-hand col. 3rd paragraph - p. 1625 right-hand col. 2nd paragraph	1-10

-/--

3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/060003

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2010/151792 A1 (REGENERON PHARMA [US]; DAVIS SAMUEL [US]; SMITH ERIC [US]; MACDONALD D) 29 December 2010 (2010-12-29) paragraphs [0024] - [0028] paragraph [0165]; example 7	1-10
Y	WO 2014/049003 A1 (GLENMARK PHARMACEUTICALS SA [CH]) 3 April 2014 (2014-04-03) p. 9 l. 1-13, p. 11 l. 27 - p. 12 l. 29, p. 66 l. 15 - p. 69 l. 33, claims 55-63, 67-69	1-10
Y	MELANIE L. CONRAD ET AL: "TCR and CD3 antibody cross-reactivity in 44 species", CYTOMETRY PART A, vol. 71A, no. 11, 1 January 2007 (2007-01-01), pages 925-933, XP55151407, ISSN: 1552-4922, DOI: 10.1002/cyto.a.20435 the whole document abstract table 1	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/060003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2522724 A1	14-11-2012	AU 2010336277 A1	16-08-2012
		CA 2785414 A1	30-06-2011
		CN 102770537 A	07-11-2012
		EP 2522724 A1	14-11-2012
		KR 20120123055 A	07-11-2012
		RU 2012131671 A	27-01-2014
		US 2013018174 A1	17-01-2013
		WO 2011078332 A1	30-06-2011
WO 2012131555 A2	04-10-2012	AU 2012235758 A1	18-04-2013
		CA 2812739 A1	04-10-2012
		CN 103608357 A	26-02-2014
		EA 201391175 A1	30-07-2014
		EP 2688909 A2	29-01-2014
		JP 2014511836 A	19-05-2014
		KR 20130140112 A	23-12-2013
		SG 188638 A1	31-05-2013
		US 2013178605 A1	11-07-2013
		US 2014187753 A1	03-07-2014
		WO 2012131555 A2	04-10-2012
WO 2010075548 A2	01-07-2010	AU 2009329866 A1	07-07-2011
		CA 2746330 A1	01-07-2010
		CN 102264759 A	30-11-2011
		EP 2382234 A2	02-11-2011
		JP 2012513414 A	14-06-2012
		KR 20110103969 A	21-09-2011
		US 2012009182 A1	12-01-2012
		WO 2010075548 A2	01-07-2010
WO 2010151792 A1	29-12-2010	AU 2010265933 A1	19-01-2012
		CA 2766220 A1	29-12-2010
		CN 102471378 A	23-05-2012
		CN 103833855 A	04-06-2014
		EP 2445936 A1	02-05-2012
		JP 2012531439 A	10-12-2012
		KR 20120027055 A	20-03-2012
		KR 20140112569 A	23-09-2014
		MY 152963 A	15-12-2014
		RU 2012102602 A	10-08-2013
		SG 176868 A1	28-02-2012
		TW 201116625 A	16-05-2011
		US 2010331527 A1	30-12-2010
		US 2014248664 A1	04-09-2014
		WO 2010151792 A1	29-12-2010
WO 2014049003 A1	03-04-2014	AU 2013322710 A1	16-04-2015
		CA 2886036 A1	03-04-2014
		WO 2014049003 A1	03-04-2014

(19)中华人民共和国国家知识产权局

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14/532,923 2014.11.04 US
(85)PCT国际申请进入国家阶段日
2017.07.03
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PCT/EP2015/060003 2015.05.06
(87)PCT国际申请的公布数据
WO2016/071004 EN 2016.05.12
(71)申请人 格兰马克药品股份有限公司
地址 瑞士拉绍德封
(72)发明人 R·奥利耶
(74)专利代理机构 北京市中咨律师事务所 11247
代理人 张莉 黄革生
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(54)发明名称

T细胞重靶向性异源二聚体免疫球蛋白

(57)摘要

本发明描述结合CD3和疾病相关抗原的新异源二聚体免疫球蛋白或其片段。这些异源二聚体免疫球蛋白已经被工程化改造，以利于异源二聚体在表达过程中形成并且可以使用蛋白A差异纯化技术纯化至高纯度。

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1.一种结合人CD3的表位结合蛋白或其片段，其至少包含选自SEQ ID NO:101、SEQ ID NO:102、SEQ ID NO:103和SEQ ID NO:104的重链可变结构域和选自SEQ ID NO:105、SEQ ID NO:106、SEQ ID NO:401和SEQ ID NO:402的轻链可变结构域。

2.根据权利要求1所述的表位结合蛋白或其片段，其包含选自SEQ ID NO:101和SEQ ID NO:105、SEQ ID NO:104和SEQ ID NO:106、SEQ ID NO:104和SEQ ID NO:401、SEQ ID NO:104和SEQ ID NO:402的重链可变结构域和轻链可变结构域对。

3.根据权利要求1所述的表位结合蛋白或其片段，其包含选自SEQ ID NO:359和SEQ ID NO:399、SEQ ID NO:359和SEQ ID NO:400的重链和轻链对。

4.根据权利要求1、2或3所述的表位结合蛋白或其片段，其中所述抗原结合蛋白或片段是异源二聚体免疫球蛋白或其片段。

5.根据权利要求4所述的异源二聚体免疫球蛋白或其片段，其中所述异源二聚体免疫球蛋白或片段与第二表位结合。

6.根据权利要求4或5所述的异源二聚体免疫球蛋白或其片段，其中第一多肽的表位结合区是FAB，第二多肽的表位结合区是scFv，或者其中第一多肽的表位结合区是scFv，第二多肽的表位结合区是FAB。

7.根据权利要求6所述的异源二聚体免疫球蛋白或其片段，其中scFv与人CD3结合并且包含选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的序列。

8.根据权利要求6或7所述的异源二聚体免疫球蛋白或其片段，其中与包含SEQ ID NO:60和61编码的重链和轻链可变区的scFv-Fc形式的SP34嵌合体相比，所述scFv在表达方面具有至少两倍的提高。

9.根据权利要求6至8中任一项所述的异源二聚体免疫球蛋白或其片段，其中，所述scFv在包含FAB臂的双特异性抗体中表达时，与包含SEQ ID NO:60和61编码的重链和轻链可变区的scFv形式的SP34嵌合体在包含FAB臂的双特异性抗体中表达时相比，具有至少两倍的表达改善。

10.根据权利要求1至9中任一项所述的表位结合蛋白或其片段或异源二聚体免疫球蛋白或其片段，其中所述表位结合蛋白或其片段或异源二聚体免疫球蛋白或其片段，与包含SEQ ID NO:60和61编码的重链和轻链可变区的表位结合蛋白或其片段或异源二聚体免疫球蛋白或其片段相比，具有更大的热稳定性。

T细胞重靶向性异源二聚体免疫球蛋白

技术领域

[0001] 本发明涉及靶向人CD3抗原组分和疾病相关抗原的异源二聚体免疫球蛋白及其制备方法。

背景技术

[0002] T细胞重定向杀伤是许多治疗领域中期望的作用模式。在临床前和临床研究中已经证实多种双特异性抗体形式可以介导T细胞重定向(May C等,(2012)Biochem Pharmacol,84(9):1105-12;Frankel SR&Baeuerle PA,(2013)Curr Opin Chem Biol,17(3):385-92)。所有的T细胞重靶向性双特异性抗体或其片段经工程化而具有至少两个抗原结合位点,其中一个位点结合靶细胞上表面抗原,而另一位点结合T细胞表面抗原。在T细胞表面抗原中,TCR蛋白复合物中的人CD3ε亚基是最常被靶向以实现T细胞杀伤重定向的抗原。

[0003] 许多双特异性抗体形式已经用于重定向T细胞杀伤,主要包括串联的scFv片段和基于双抗体(diabody)的形式,仅有少数基于Fc的双特异性抗体形式的例子被报道(Moore PA等,(2011)Blood,117(17):4542-51;May C等,(2012)同上引文;Frankel SR&Baeuerle PA,(2013)同上引文)。包含人Fc区的双特异性形式将具有更长的循环半衰期,这可以导致增强的功效和/或更低频率的给药方案。在可能的基于Fc的双特异性形式中,用于重定向T细胞杀伤的一个优选形式是所谓的重链异源二聚体形式。该形式是尤其有意义的,因为它不允许多个拷贝的人CD3分子聚集在T细胞表面,从而防止了任何的T细胞失活(Klein C et al.,(2012)MAbs,4(6):653-63)。

[0004] 第一个描述的用于工程化构建重链异源二聚体的方法是称作"杵臼"(knob-into-hole)法的方法(PCT公布号WO199627011;Merchant AM等(1998)Nat Biotechnol,16(7):677-81)。近来报道了一种称作FAB-臂交换法的化学方法,其中两个抗体通过还原和半免疫球蛋白的体外改组而组合成一个双特异性抗体(PCT公布号WO2008119353(Schuurman J等)和WO2013060867(Gramer M等);Labrijn AF等,(2013)Proc Natl Acad Sci USA,110(13):5145-50)。

[0005] 目前的方法及其衍生方案不足以在哺乳动物细胞宿主中产生基于Fc的双特异性抗体形式。当在哺乳动物细胞宿主中表达"杵臼"重链异源二聚体时,同源二聚体的存在对双特异性抗体的回收造成不利影响(Jackman J等人,(2010)J Biol Chem,285(27):20850-9;Klein C等人,同上)。FAB-臂交换法及其衍生方案也存在相同的缺点,此外还存在必须首先分开产生该两种"单特异性"抗体的另一问题。

[0006] 当开发通过啮合CD3亚基来重定向T细胞杀伤的双特异性抗体时,重要的是在药物终产品中不存在特异于CD3亚基的同源二聚体。在靶向CD3ε亚基的情况下,痕量的抗人CD3ε抗体(针对人CD3ε抗原的单特异性和二价抗体)就可能在导致T细胞凋亡前触发瞬时T细胞活化和细胞因子释放,从而干扰受控的和特异性的T细胞活化的目的。生产有效地重定向T细胞杀伤的稳定且安全的基于Fc的双特异性抗体,对制药工业而言在纯度和产率方面仍然

具有挑战性。

[0007] 因此，仍然需要一种技术来有效地生产不含抗人CD3同源二聚体的基于抗人CD3的重链异源二聚体，其中分泌的双特异性抗体产物可以容易地从重组哺乳动物宿主细胞系的细胞培养物上清液中分离。

[0008] 已经描述了基于对试剂的不同亲和力而使重链异源二聚体优于同源二聚体获得纯化的技术。已知的差异亲和纯化技术的第一个实例涉及使用来自两种不同动物物种的两个不同重链，其中一个重链不结合亲和试剂蛋白A(Lindhofer H等人，(1995)J Immunol，155(1)：219-225)。该作者还描述了使用源自两种不同人免疫球蛋白同种型(IGHG1和IGHG3)的两个不同重链，其中一个重链不结合亲和试剂蛋白A(IGHG3；见US6,551,592 Lindhofer H等人)。最近，Davis S等人报道了该技术的一种变型方式(PCT公开号：WO2010151792)，在异源二聚体重链之一中利用Jendeberg(1997)(Jendeberg等人(1997)J Immunol Methods,201(1)：25-34)描述的两个氨基酸取代H435R和Y436F来消除对试剂蛋白A的亲和力。

[0009] 本发明中优选的已知差异蛋A白亲和纯化技术对应于这样一种技术，其中所有三个物质，即，两个同源二聚体和一个目的异源二聚体，在蛋白A结合位点总数上相差至少一个位点，并且其中两个同源二聚体之一没有蛋白A结合位点，因此不结合蛋白A(如图1所示)。

[0010] 药物稳定性是药物开发成功的重要方面，而基于VH3的免疫球蛋白或其片段对生物药物行业至关重要。基于VH3亚类的治疗性抗体，由于框架结合蛋白A并有助于在抗体片段形成免疫球蛋白之前测试抗体片段，因此已经被广泛开发；例如，用于发现抗体的许多合成抗体噬菌体展示文库是基于VH3亚类的。此外，基于VH3的抗体常因其良好的表达和稳定性而优于其他已知的重链可变结构域亚类被选择。

[0011] 与具有两个亲和力较强的位点的Fc区比较时，VH3结构域仅具有一个亲和力较弱的蛋白A结合位点(Roben PW等人，(1995)J Immunol,154(12)：6437-45)，但是亲和力足以干扰已知的差异蛋白A亲和纯化技术。在处理重链异源二聚体的纯化时，对于在Fc区中经工程化而不具有蛋白A结合的重链，当其包含一个基于VH3的抗原结合位点时，蛋白A结合可以通过VH3结构域而得以恢复，如图1和上述的优选技术不再有用(图2A)。在这种情况下，在基于VH3的抗原结合位点中消除蛋白A结合可以提供直接的解决方案，其允许保持期望异源二聚体的初始构造(图2B)。或者，可以对重链异源二聚体进行重新工程化，以使基于VH3的抗原结合位点位于在其Fc区中结合蛋白A的重链上(图2C；注意，VH3结构域与Fc单体相比对蛋白A具有较弱的亲和力，因此，相对于其他同源二聚体物质，目的异源二聚体仍可以在分开的不同pH值洗脱(通常在pH 4)，而结合蛋白A的同源二聚体物质现包含两个另外的蛋白A结合位点，在pH值≤3时洗脱)。

[0012] 更重要的是，在纯化重链异源二聚体时，如果两条重链都包含基于VH3的抗原结合位点，则上述的重定位策略可能仅部分有用(图2D和图15B)。只有在至少一个(图2E)或两个(图2F)基于VH3的抗原结合位点中的蛋白A结合被消除时，基于蛋白A的差异纯化才是可行的。

[0013] 因此，在生产包含VH3可变结构域亚类的重链异源二聚体时，仍然需要消除VH3结构域中的蛋白A结合。

[0014] 发明概述

[0015] 本发明提供新的抗人CD3双特异性抗体，其包含能识别和结合疾病相关抗原的第二结合臂。

[0016] 在本发明的上下文中，疾病相关抗原是指与病理状态相关的任何抗原或表位，例如致癌标志物或一些其他代谢或免疫功能障碍的标志物。另外，疾病标志物也可以涉及感染性疾病如致病性病毒或细菌。

[0017] 根据本发明，抗人CD3结合臂的靶结合部分包含SP34的单链可变片段(scFv)。特别地，与包含SEQ ID NO:60和61编码的重链和轻链可变区的scFv-Fc相比，该scFv具有更好的表达谱，同时保持其CD3结合特性。

[0018] 特别是当表达为scFv-Fc时，与包含SEQ ID NO:60和61编码的重链和轻链可变区的scFv-Fc形式的SP34嵌合体相比，其具有至少两倍的表达改善。优选地，该改良的SP34 scFv，与包含SEQ ID NO:60和61编码的重链和轻链可变区的scFv形式的SP34嵌合体相比，具有至少六倍的表达改善，最优选地，与包含SEQ ID NO:60和61编码的重链和轻链可变区的scFv形式的SP34嵌合体相比，具有至少十二倍的表达改善。

[0019] 根据本发明的另一方面，当在BEAT形式的包含FAB臂的双特异性抗体中表达为scFv时，其与包含SEQ ID NO:60和61编码的重链和轻链可变区的scFv形式的SP34嵌合体相比，具有至少二倍的表达改善。优选地，该改良SP34 scFv作为BEAT双特异性抗体的组分，与包含SEQ ID NO:60和61编码的重链和轻链可变区的scFv形式的SP34嵌合体作为BEAT双特异性抗体的组分相比，具有至少五倍的表达改善。

[0020] 根据本发明，抗人CD3双特异性抗体的两个结合臂各自包含免疫球蛋白恒定区，并且其中第一臂或多肽结合蛋白A，第二臂或多肽不结合蛋白A。

[0021] 根据本发明，第一多肽与蛋白A结合而第二多肽与蛋白A不结合，并不意味着第二多肽不可以具有一些残余的蛋白A结合，而是指与第一臂相比，第二多肽与蛋白A的结合较差。

[0022] 根据本发明，异源二聚体免疫球蛋白或其片段的第一和第二多肽，包含具有修饰的CH3区的工程化免疫球蛋白恒定区，所述修饰的CH3区具有有利于异源二聚体形成(相比于同源二聚体形成)的蛋白-蛋白界面。在优选的实施方案中，本发明提供异源二聚体免疫球蛋白或其片段，其中第一和第二多肽包含具有修饰的CH3结构域的工程化免疫球蛋白恒定区，所述修饰的CH3结构域具有蛋白-蛋白界面，其中第一多肽的蛋白-蛋白界面包含在选自以下位置的氨基酸取代：3、5、7、20、22、26、27、79、81、84、84.2、85.1、86、88和90(**IMGT®**编号)，并且其中第二多肽的蛋白-蛋白界面包含在选自以下位置的氨基酸取代：3、5、7、20、22、26、27、79、81、84、84.2、84.4、85.1、86、88和90(**IMGT®**编号)。

[0023] 优选地，其中第二多肽的蛋白-蛋白界面包含位置84.4的氨基酸取代和在选自以下位置的至少一个另外的取代：3、5、7、20、22、26、27、79、81、84、84.2、85.1、86、88和90(**IMGT®**编号)。

[0024] 在另一个实施方案中，本发明提供了异源二聚体免疫球蛋白或其片段，其中第一和第二多肽包含具有修饰的CH3结构域的工程化免疫球蛋白恒定区，所述修饰的CH3结构域具有蛋白-蛋白界面，其中第一多肽的蛋白-蛋白界面包含在位置88和在选自位置3、5、7、20、22、26、27、79、81、84、84.2、85.1、86和90的位置上的氨基酸取代(**IMGT®**编号)，并且

其中第二多肽的蛋白-蛋白界面包含在位置85.1和/或86以及在选自位置3、5、7、20、22、26、27、79、81、84、84.2、84.4、88和90的位置上的氨基酸取代(IMGT®编号)。

[0025] 根据本发明的另一方面，第一多肽的表位结合区结合CD3蛋白复合物和第二多肽的表位结合区结合疾病相关抗原，或其中第一多肽的表位结合区结合疾病相关抗原和第二多肽的表位结合区结合CD3蛋白复合物；并且其中结合CD3蛋白复合物的表位结合区包含：包含氨基酸序列SEQ ID NO:200的重链CDR1、包含氨基酸序列SEQ ID NO:201的的重链CDR2和包含氨基酸序列SEQ ID NO:202的重链CDR3；和包含氨基酸序列SEQ ID NO:203的轻链CDR1、包含氨基酸序列SEQ ID NO:204的轻链CDR2和包含氨基酸序列SEQ ID NO:205的轻链CDR3；或者

[0026] 其中结合CD3蛋白复合物的表位结合区包含：包含氨基酸序列SEQ ID NO:352的重链CDR1、包含氨基酸序列SEQ ID NO:353的重链CDR2和包含氨基酸序列SEQ ID NO:354的重链CDR3；和包含氨基酸序列SEQ ID NO:355的轻链CDR1、包含氨基酸序列SEQ ID NO:356的轻链CDR2和包含氨基酸序列SEQ ID NO:357的轻链CDR3。

[0027] 这些新的抗人CD3双特异性抗体的用途不限于但包括治疗各种人癌症和自身免疫性和炎性疾病。相对健康细胞和组织，对癌细胞的特异性破坏是肿瘤学的主要目标。可以安全地将T细胞杀伤重定向于肿瘤相关细胞表面抗原的治疗剂，可提供改善的临床疗效。肿瘤学中尚未满足临床需求的已知领域包括但不限于乳癌、转移性乳癌、卵巢癌、胰腺癌、肺癌、淋巴瘤和多发性骨髓瘤。在自身免疫性和炎性疾病如银屑病、多发性硬化和糖尿病的治疗中，消除引起疾病的T细胞可能比抑制T细胞分化更有利。

[0028] 一组优选的疾病相关抗原来自基因产物CCR3、CCR6、CRTH2、PDL1、BLUT1、PirB、CD33、TROP2、CD105、GD2、GD3、CEA、VEGFR1、VEGFR2、NCAM、CD133、CD123、ADAM17、MCSP、PSCA、FOLR1、CD19、CD20、CD38、EpCAM、HER2、EGFR、PSMA、IgE、整合素a4b1、CCR5、LewisY、FAP、MUC-1、Wue-1、MSP、EGFRvIII、P糖蛋白、AFP、ALK、BAGE蛋白、CD30、CD40、CTLA4、ErbB3、ErbB4、间皮素(Mesothelin)、OX40、CA125、CAIX、CD66e、cMet、EphA2、HGF/SF、MUC1、磷脂酰丝氨酸、TAG-72、TPBG、β-联蛋白(β-catenin)、brc-abl、BRCA1、BORIS、CA9、胱天蛋白酶-8、CDK4、细胞周期蛋白-B1、CYP1B1、ETV6-AML、Fra-1、FOLR1、GAGE-1、GAGE-2、GloboH、磷脂酰肌醇聚糖-3、GM3、gp100、HLA/B-raf、HLA/k-ras、HLA/MAGE-A3、hTERT、LMP2、MAGE1、MAGE2、MAGE3、MAGE4、MAGE6、MAGE12、MART-1、ML-IAP、Muc2、Muc3、Muc4、Muc5、Muc16、MUM1、NA17、NY-BR1、NY-BR62、NY-BR-85、NY-ESO1、p15、p53、PAP、PAX3PAX5、PCTA-1、PLAC1、PRLR、PRAME、RAGE蛋白、Ras、RGS5、Rho、SART-1、SART-3、Steap-1、Steap-2、生存素(survivin)、TAG-72、TGF-β、TMPRSS2、Tn、TRP-1、TRP-2、酪氨酸酶、uroplakin-3。

[0029] 根据本发明的异源二聚体免疫球蛋白或其片段，其中结合疾病相关抗原的表位结合区包含重链CDR1、CDR2和CDR3氨基酸序列和轻链CDR1、CDR2和CDR3氨基酸序列，分别选自：

[0030] i)SEQ ID NOs:206-211；

[0031] ii)SEQ ID NOs:212-217；

[0032] iii)SEQ ID NOs:218-223；

[0033] iv)SEQ ID NOs:224-229；

[0034] v)SEQ ID NOs:230-235；

[0035] vi) SEQ ID NOs:236-241;

[0036] vii) SEQ ID NOs:242-247;

[0037] viii) SEQ ID NOs:248-253;

[0038] ix) SEQ ID NOs:254-259;

[0039] x) SEQ ID NOs:260-265;

[0040] xi) SEQ ID NOs:266-271;和

[0041] xii) SEQ ID NOs:272-277。

[0042] 根据本发明的再一方面，异源二聚体免疫球蛋白或其片段的第二多肽的恒定区，包含IgG3 CH3区。

[0043] 根据本发明的再一方面，异源二聚体免疫球蛋白或其片段的第二多肽的恒定区，包含非来自IgG的CH3区，并且该非IgG3 CH3区包含至少一个取代以降低/消除蛋白A结合。

[0044] 根据本发明的再一方面，异源二聚体免疫球蛋白或其片段的第二多肽的表位结合区包含VH3区，该VH3区包含至少一个降低蛋白A结合的修饰。

[0045] 本发明人已经证明，基于VH3的抗原结合位点可以容易地在单个蛋白A色谱步骤中以高纯度产生和纯化。除了其易于生产之外，这些抗体可表现出比现有疗法更高的功效。

[0046] 本发明还提供，从重组哺乳动物宿主细胞系生产抗人CD3双特异性重链异源二聚体的方法，所述抗人CD3双特异性重链异源二聚体具有至少一个基于VH3的抗原结合位点，其中该双特异性抗体产物可以容易地在单个蛋白A色谱步骤之后以高纯度分离。

[0047] 特别地，该修饰的VH3区包含选自以下的氨基酸取代:57,65,81,82a和组合19/57/59(Kabat编号)；甚至更优选地，该修饰的VH3区包含选自以下的氨基酸取代:57A,57E,65S,81E,82aS和组合19G/57A/59A(Kabat编号)。

[0048] 根据本发明的再一方面，异源二聚体免疫球蛋白或其片段，可以包含其它取代，其中重链可变框架区包含选自以下的氨基酸取代:I34M,V48I,A49G,R58N/Y,I69L,A71T和T73K(Kabat编号)，且轻链可变框架区包含选自以下的氨基酸取代:M4L,V33M,A34N,L46R,L47W,T51A,R66G,F71Y和P96F(Kabat编号)；或者其中重链可变框架区包含氨基酸取代I34M,A49G和A71T(Kabat编号)，且轻链可变框架区包含氨基酸取代M4L,L46R,L47W和F71Y(Kabat编号)。

[0049] 在另一个实施方案中，结合CD3蛋白复合物的表位结合区包含重链可变框架区，其是人VH3亚类的产物或衍生自人VH3亚类。优选地，重链可变框架区是人IGHV3-23的产物或衍生自人IGHV3-23。更优选地，重链可变框架区是人IGHV3-23*04(SEQ ID NO:22)的产物或衍生自人IGHV3-23*04(SEQ ID NO:22)。该重链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:18或SEQ ID NO:60的相应鼠抗体的重链可变区的相应框架区的氨基酸修饰。

[0050] 在一个优选的实施方案中，结合CD3蛋白复合物的第一多肽的表位结合区包含轻链可变框架区，其是人VK1亚类或人VK3亚类的产物或衍生自人VK1亚类或人VK3亚类。优选地，轻链可变框架区是人VK1-39或VK3-20的产物或衍生自人VK1-39或VK3-20。更优选，轻链可变框架区是人IGKV1-39*01(SEQ ID NO:23)或IGKV3-20*01(SEQ ID NO:24)的产物或衍生自人IGKV1-39*01或IGKV3-20*01(SEQ ID NO:24)。该轻链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:19或SEQ ID NO:61的相应鼠抗体的轻链可变区的相应框

架区的氨基酸修饰。

[0051] 在一个优选实施方案中，结合CD3蛋白复合物的表位结合区包含具有选自以下的回复突变的人源化重链可变结构域：I34M，V48I，A49G，R58N/Y，I69L，A71T和T73K(Kabat编号)，和具有选自以下的回复突变的人源化轻链可变结构域：M4L，V33M，A34N，L46R，L47W，R66G，F71Y和P96F(Kabat编号)。更优选地，结合CD3蛋白复合物的表位结合区包含具有回复突变I34M，A49G和A71T(Kabat编号)的人源化重链可变结构域，和具有回复突变M4L，L46R，L47W和F71Y(Kabat编号)的人源化轻链可变结构域。

[0052] 根据本发明的另一方面，异源二聚体免疫球蛋白或其片段中结合CD3蛋白复合物的表位结合区，

[0053] 其中结合CD3蛋白复合物的表位结合区包含：包含氨基酸序列SEQ ID NO:101的重链可变区和包含氨基酸序列SEQ ID NO:105的轻链可变区；或者

[0054] 其中结合CD3蛋白复合物的表位结合区包含：包含氨基酸序列SEQ ID NO:103的重链可变区和包含氨基酸序列SEQ ID NO:106的轻链可变区；或者

[0055] 其中结合CD3蛋白复合物的表位结合区包含：包含氨基酸序列SEQ ID NO:104的重链可变区和包含氨基酸序列SEQ ID NO:106的轻链可变区；或者

[0056] 其中结合CD3蛋白复合物的表位结合区包含：包含氨基酸序列SEQ ID NO:104的重链可变区和包含氨基酸序列SEQ ID NO:401的轻链可变区；或者

[0057] 其中结合CD3蛋白复合物的表位结合区包含：包含氨基酸序列SEQ ID NO:104的重链可变区和包含氨基酸序列SEQ ID NO:402的轻链可变区。

[0058] CD3蛋白复合物包含多个亚基，例如δ、ε和γ。在一个优选实施方案中，结合CD3蛋白复合物的表位结合区结合CD3的ε亚基。

[0059] 本文描述的表位结合区包括一个或多个重链可变结构域和一个或多个互补轻链可变结构域的组合，其一起形成允许异源二聚体免疫球蛋白或其片段特异性结合一个或多个表位的结合位点。在本发明的一个实施方案中，第一多肽的表位结合区包含FAB，第二多肽的表位结合区包含scFv。或者，第一多肽的表位结合区包含scFv，第二多肽的表位结合区包含FAB。

[0060] 在一个实施方案中，结合疾病相关抗原的表位结合区结合HER2。该表位结合区包含重链可变框架区和轻链可变框架区，所述重链可变框架区是人VH3亚类的产物或衍生自人VH3亚类，优选是人VH3-23的产物或衍生自人VH3-23、更优选是人IGHV3-23*04(SEQ ID NO:22)的产物或衍生自人IGHV3-23*04(SEQ ID NO:22)，所述轻链可变框架区是人VK1亚类的产物或衍生自人VK1亚类，优选是人VK1-39的产物或衍生自人VK1-39，更优选是人IGKV1-39*01(SEQ ID NO:23)的产物或衍生自人IGKV1-39*01(SEQ ID NO:23)。

[0061] 在一个优选的实施方案中，结合疾病相关抗原HER2的表位结合区包含：包含氨基酸序列SEQ ID NO:20的重链可变结构域和包含氨基酸序列SEQ ID NO:21的轻链可变结构域。在另一个优选的实施方案中，结合HER2的表位结合区可以包含重链可变结构域和轻链可变结构域，其通过G_4S接头连接形成scFv片段，所述scFv片段包含氨基酸序列SEQ ID NO:107。优选地，scFv片段的可变结构域包含消除蛋白A结合的修饰，其中该氨基酸取代为65S(Kabat编号)并且其中scFv片段包含氨基酸序列SEQ ID NO:109，或其中该氨基酸取代是82aS(Kabat编号)并且其中scFv片段包含氨基酸序列SEQ ID NO:111。

[0062] 特别地，其中所述赫赛汀(Herceptin)结合臂包含由SEQ ID NO:20编码的重链可变区和由SEQ ID NO:21编码的轻链可变区。

[0063] 在另一个实施方案中，结合疾病相关抗原的表位结合区结合CD38。该表位结合区包含重链可变框架区，所述重链可变框架区是人VH3亚类的产物或衍生自人VH3亚类，优选是人VH3-23的产物或衍生自人VH3-23，更优选是人IGHV3-23*04(SEQ ID NO:22)的产物或衍生自人IGHV3-23*04(SEQ ID NO:22)。该重链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:112或114或122的相应鼠抗体的重链可变区的相应框架区的氨基酸修饰。该表位结合区还可以包含轻链可变框架区，所述轻链可变框架区是人VK1亚类的产物或衍生自人VK1亚类，优选是人VK1-39的产物或衍生自人VK1-39，更优选是人IGKV1-39*01(SEQ ID NO:23)的产物或衍生自人IGKV1-39*01(SEQ ID NO:23)。该轻链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:113或115或123的相应鼠抗体的轻链可变区的相应框架区的氨基酸修饰。

[0064] 特别地，CD38结合多肽包含由SEQ ID NO:116/117、129/130、133/134和135/136编码的可变重链结构域和可变轻链结构域对。

[0065] 在一个实施方案中，结合疾病相关抗原的表位结合区结合OX40。该表位结合区包含重链可变框架区，所述重链可变框架区是人VH3亚类的产物或衍生自人VH3亚类，优选是人VH3-23的产物或衍生自人VH3-23，更优选是人IGHV3-23*04(SEQ ID NO:22)的产物或衍生自人IGHV3-23*04(SEQ ID NO:22)。该重链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:139的相应鼠抗体的重链可变区的相应框架区的氨基酸修饰。该表位结合区还可以包含轻链可变框架区，所述轻链可变框架区是人VK1亚类的产物或衍生自人VK1亚类，优选地是人VK1-39的产物或衍生自人VK1-39，更优选是人IGKV1-39*01(SEQ ID NO:23)的产物或衍生自人IGKV1-39*01(SEQ ID NO:23)。该轻链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:140的相应鼠抗体的轻链可变区的相应框架区的氨基酸修饰。

[0066] 最优选地，人源化重链可变结构域包含消除蛋白A结合的修饰，所述修饰包含取代G65S或取代N82aS(Kabat编号)。

[0067] 特别地，OX40结合多肽包含由SEQ ID NO:141/142、278/280和279/281编码的可变重链结构域和可变轻链结构域对。

[0068] 在一个实施方案中，结合疾病相关抗原的表位结合区结合CD19。该表位结合区包含重链可变框架区，所述重链可变框架区是人VH3亚类的产物或衍生自人VH3亚类，优选地是人VH3-23的产物或衍生自人VH3-23，更优选地是人IGHV3-23*04(SEQ ID NO:22)的产物或衍生自人IGHV3-23*04(SEQ ID NO:22)，最优选包含氨基酸序列SEQ ID NO:296。表位结合区还可以包含轻链可变框架区，所述轻链可变框架区是人VK1亚类的产物或衍生自人VK1亚类，优选地是人VK1-39的产物或衍生自人VK1-39，更优选地是人IGKV1-39*01(SEQ ID NO:23)的产物或衍生自人IGKV1-39*01(SEQ ID NO:23)，最优选包含氨基酸序列SEQ ID NO:297。在一个优选的实施方案中，重链可变结构域包含消除蛋白A结合的修饰，所述修饰包含取代G65S或取代N82aS(Kabat编号)。

[0069] 特别地，CD19结合多肽包含由SEQ ID NO:296/297编码的可变重链结构域和可变轻链结构域对。

[0070] 在一个实施方案中，结合疾病相关抗原的表位结合区结合CD20。该表位结合区包含重链可变框架区，所述重链可变框架区是人VH3亚类的产物或衍生自人VH3亚类，优选地是人VH3-23的产物或衍生自人VH3-23，更优选是人IGHV3-23*04(SEQ ID NO:22)的产物或衍生自人IGHV3-23*04(SEQ ID NO:22)。该重链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:143的相应鼠抗体的重链可变区的相应框架区的氨基酸修饰。表位结合区还可以包含轻链可变框架区，所述轻链可变框架区是人VK1亚类的产物或衍生自人VK1亚类，优选是人VK1-39的产物或衍生自人VK1-39，更优选是人IGKV1-39*01(SEQ ID NO:23)的产物或衍生自人IGKV1-39*01(SEQ ID NO:23)。轻链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:144的相应鼠抗体的轻链可变区的相应框架区的氨基酸修饰。

[0071] 最优选地，人源化重链可变结构域包含消除蛋白A结合的修饰，所述修饰包含取代G65S或取代N82aS(Kabat编号)。

[0072] 特别地，CD20结合多肽包含由SEQ ID NO:143/144、282/284、283/285编码的可变重链结构域和可变轻链结构域对。

[0073] 在一个实施方案中，结合疾病相关抗原的表位结合区结合EGFR。该表位结合区包含重链可变框架区，所述重链可变框架区是人VH3亚类的产物或衍生自人VH3亚类，优选地是人VH3-23的产物或衍生自人VH3-23，更优选是人IGHV3-23*04(SEQ ID NO:22)的产物或衍生自人IGHV3-23*04(SEQ ID NO:22)。重链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:145的相应鼠抗体的重链可变区的相应框架区的氨基酸修饰。表位结合区还可以包含轻链可变框架区，所述轻链可变框架区是人VK1亚类的产物或衍生自人VK1亚类，优选地是人VK1-39的产物或衍生自人VK1-39，更优选是人IGKV1-39*01(SEQ ID NO:23)的产物或衍生自人IGKV1-39*01(SEQ ID NO:23)。轻链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:146的相应鼠抗体的轻链可变区的相应框架区的氨基酸修饰。

[0074] 最优选地，人源化重链可变结构域包含消除蛋白A结合的修饰，所述修饰包含取代G65S或取代N82aS(Kabat编号)。

[0075] 特别地，EGFR结合多肽包含由SEQ ID NO:145/146、286/288、287/289、290/291、292/294编码的可变重链结构域和可变轻链结构域对。

[0076] 在一个实施方案中，结合疾病相关抗原的表位结合区结合IgE。该表位结合区包含重链可变框架区，所述重链可变框架区是人VH3亚类的产物或衍生自人VH3亚类，优选地是人VH3-23产物或衍生自人VH3-23，更优选是人IGHV3-23*04(SEQ ID NO:22)的产物或衍生自人IGHV3-23*04(SEQ ID NO:22)。重链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:298的相应人源化抗体或包含氨基酸序列SEQ ID NO:304的相应鼠抗体的重链可变区的相应框架区的氨基酸修饰。表位结合区还可以包含轻链可变框架区，所述轻链可变框架区是人VK1亚类的产物或衍生自人VK1亚类，优选地是人VK1-39的产物或衍生自人VK1-39，更优选是人IGKV1-39*01(SEQ ID NO:23)的产物或衍生自人IGKV1-39*01(SEQ ID NO:23)。轻链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:299的相应人源化抗体或包含氨基酸序列SEQ ID NO:305的相应鼠抗体的轻链可变区的相应框架区的氨基酸修饰。

[0077] 最优选地，重链可变结构域包含消除蛋白A结合的修饰，所述修饰包含取代G65S或取代N82aS(Kabat编号)。

[0078] 特别地，IgE结合多肽包含由SEQ ID NO:298/299、300/302、301/303、304/305、306/308、307/309编码的可变重链结构域和可变轻链结构域对。

[0079] 已经发现，抗CD3抗体可以通过直接和间接制剂触发毒性。间接机制由CD3抗体的Fc区介导，其与表达Fc受体的免疫细胞作用，导致瞬时T细胞活化和细胞因子释放。因此，为了改善本文所述异源二聚体免疫球蛋白或其片段的安全性，第一和/或第二多肽的免疫球蛋白恒定区对效应免疫细胞和/或补体C1q具有降低的结合或没有结合。优选地，工程化免疫球蛋白恒定区，以消除下铰链区(lower hinge region)中的Fc受体结合。更优选地，第一和/或第二多肽的免疫球蛋白恒定区包含取代L234A和/或L235A(EU编号)。最优选地，第一和/或第二多肽的免疫球蛋白恒定区包含取代L234A和L235A(EU编号)。

[0080] 在另一方面，本发明的公开还描述了异源二聚体免疫球蛋白或其片段，其中表位结合区结合CD3蛋白复合物的CD3ε亚基并且包含FAB，如通过差示扫描量热法(DSC)所测量(如表1)，该FAB具有的FAB热稳定性优于SP34嵌合体的FAB热稳定性，其中所述SP34嵌合体包含氨基酸序列SEQ ID NO:60的重链可变结构域和氨基酸序列SEQ ID NO:61的轻链可变结构域。这种增加的热稳定性意味着，这些改进的SP34结合臂将具有增加的体内和体外稳定性，意味着作为治疗剂以及在稳定性/储存期限/保存期限方面更好的性能。

[0081] 根据本发明的另一方面，提供了改善包含SP34的可变重链和轻链结构域序列SEQ ID NO:60和61的scFv的表达的方法，包括以下步骤：

[0082] a)修饰由SEQ ID NO:60和61编码的重链或轻链可变结构域中的至少一个残基；

[0083] b)表达来自步骤a)的修饰的构建体；

[0084] c)将修饰构建体的表达水平与包含SEQ ID NO:60和SEQ ID NO:61或SEQ ID NO:62和SEQ ID NO:63或SEQ ID NO:64和SEQ ID NO:69编码的重/轻链可变结构域的scFv进行比较；

[0085] d)如果修饰构建体比包含SEQ ID NO:60和SEQ ID NO:61或SEQ ID NO:62和SEQ ID NO:63或SEQ ID NO:64和SEQ ID NO:69编码的重/轻链可变结构域的scFv更多地表达，则选择该修饰的构建体。

[0086] 根据本发明，至少在位置100e修饰重链可变结构域。

[0087] 特别地，将残基100e取代为具有疏水侧链的氨基酸，特别是苯丙氨酸或酪氨酸残基。

[0088] 根据本发明，至少在位置29、30、91、95之一上修饰轻链可变结构域。

[0089] 根据本发明，残基被随机突变。

[0090] 根据本发明，通过定点诱变，将残基突变为所有标准氨基酸和/或非标准氨基酸或其子集。

[0091] 根据本发明的一个优选方面，通过定点诱变，将残基突变为在可变重链和轻链结构域的其他已表征过的版本中位于相同位置上的其他残基。

[0092] 特别地，将残基29取代为丙氨酸、谷氨酸或丝氨酸残基；将残基30取代为丙氨酸或天冬氨酸残基；将残基91取代为苯丙氨酸残基，将残基95取代为甘氨酸或苏氨酸残基。

[0093] 在另一方面，本发明提供了如本文所述的异源二聚体免疫球蛋白或其片段，其中一个表位结合区结合CD3蛋白复合物的CD3ε亚基，结合疾病相关抗原的另一表位结合区结合HER2。这种异源二聚体免疫球蛋白或其片段重定向T细胞杀伤的效力可以在体外测定法

中使用流式细胞术(RDL-FACS)或基于比色的方法(RDL-MTS)在表达HER2的细胞系如JIMT-1、BT-474和MDA-MB-231上测量，如实施例中所述。

[0094] 在一个优选的实施方案中，本发明提供了异源二聚体免疫球蛋白或其片段，其结合：

[0095] i)CD3蛋白复合物和HER2，其中第一多肽具有选自SEQ ID NO:359的氨基酸序列、并与氨基酸序列SEQ ID NO:399或400的轻链组装并结合CD3ε，并且其中第二多肽具有SEQ ID NO:167的氨基酸序列并结合HER2；

[0096] ii)CD3蛋白复合物和HER2，其中第一多肽具有选自SEQ ID NO:359的氨基酸序列、并与氨基酸序列SEQ ID NO:399或400的轻链组装并结合CD3ε，并且其中第二多肽具有SEQ ID NO:167的氨基酸序列并结合HER2；

[0097] iii)CD3蛋白复合物和CD38，其中第一多肽具有选自SEQ ID NO:359的氨基酸序列、并与氨基酸序列SEQ ID NO:399或400的关连轻链组装并结合CD38，并且其中第二多肽具有SEQ ID NO:162的氨基酸序列、并结合CD3ε；

[0098] iv)CD3蛋白复合物和CD38，其中第一多肽具有选自SEQ ID NO:170的氨基酸序列、并与氨基酸序列SEQ ID NO:138的关连轻链组装并结合CD38，并且其中第二多肽具有SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0099] v)CD3蛋白复合物和CD38，其中第一多肽具有SEQ ID NO:176的氨基酸序列，并与氨基酸序列SEQ ID NO:119的关连轻链组装并结合CD38，并且其中第二多肽具有SEQ ID NO:SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0100] vi)CD3蛋白复合物和CD38，其中第一多肽具有SEQ ID NO:178的氨基酸序列，并与氨基酸序列SEQ ID NO:128的关连轻链组装并结合CD38，并且其中第二多肽具有SEQ ID NO:SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0101] vii)CD3蛋白复合物和OX40，其中第一多肽具有选自SEQ ID NO:359的氨基酸序列，并与氨基酸序列SEQ ID NO:399或400的关连轻链组装并结合OX40，并且其中第二多肽具有SEQ ID NO:162的氨基酸序列并结合CD3ε；

[0102] viii)CD3蛋白复合物和EGFR，其中第一多肽具有SEQ ID NO:174的氨基酸序列，并且与氨基酸序列SEQ ID NO:175的关连轻链组装并结合EGFR，并且其中第二多肽具有SEQ ID NO:SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0103] ix)CD3蛋白复合物和CD20，其中第一多肽具有SEQ ID NO:180的氨基酸序列，并与氨基酸序列SEQ ID NO:181的关连轻链组装并结合CD20，并且其中第二个多肽具有SEQ ID NO:SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε。

[0104] 在另一个实施方案中，本发明提供了异源二聚体免疫球蛋白或其片段，其结合：

[0105] CD3蛋白复合物和HER2，其中第一多肽具有SEQ ID NO:310的氨基酸序列，并与氨基酸序列SEQ ID NO:3的轻链组装并结合HER2，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0106] CD3蛋白复合物和CD38，其中第一多肽具有SEQ ID NO:312或404的氨基酸序列，并与氨基酸序列SEQ ID NO:132的轻链组装并结合CD38，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0107] CD3蛋白复合物和CD38，其中第一多肽具有SEQ ID NO:313的氨基酸序列，并与氨基酸序列SEQ ID NO:138的轻链组装并结合CD38，并且其中第二多肽具有其选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0108] CD3蛋白复合物和OX40，其中第一多肽具有SEQ ID NO:314的氨基酸序列，并与氨基酸序列SEQ ID NO:315的轻链组装并结合OX40，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0109] CD3蛋白复合物和OX40，其中第一多肽具有SEQ ID NO:316的氨基酸序列，并与氨基酸序列SEQ ID NO:317的轻链组装并结合OX40，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0110] CD3蛋白复合物和CD20，其中第一多肽具有SEQ ID NO:318的氨基酸序列，并与氨基酸序列SEQ ID NO:319的轻链组装并结合CD20，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0111] CD3蛋白复合物和CD20，其中第一多肽具有SEQ ID NO:320的氨基酸序列，并与氨基酸序列SEQ ID NO:321的轻链组装并结合CD20，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0112] CD3蛋白复合物和EGFR，其中第一多肽具有SEQ ID NO:322的氨基酸序列，并与氨基酸序列SEQ ID NO:323的轻链组装并结合EGFR，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0113] CD3蛋白复合物和EGFR，其中第一多肽具有SEQ ID NO:324的氨基酸序列，并与氨基酸序列SEQ ID NO:325的轻链组装并结合EGFR，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0114] CD3蛋白复合物和EGFR，其中第一多肽具有SEQ ID NO:326的氨基酸序列，并与氨基酸序列SEQ ID NO:327的轻链组装并结合EGFR，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0115] CD3蛋白复合物和EGFR，其中第一多肽具有SEQ ID NO:328的氨基酸序列，并与氨基酸序列SEQ ID NO:329的轻链组装并结合EGFR，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0116] CD3蛋白复合物和CD19，其中第一多肽具有SEQ ID NO:330的氨基酸序列，并与氨基酸序列SEQ ID NO:331的轻链组装并结合CD19，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0117] CD3蛋白复合物和IgE，其中第一多肽具有SEQ ID NO:332的氨基酸序列，并与氨基酸序列SEQ ID NO:333的轻链组装并结合IgE，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0118] CD3蛋白复合物和IgE，其中第一多肽具有SEQ ID NO:334的氨基酸序列，并与氨基酸序列SEQ ID NO:335的轻链组装并结合IgE，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0119] CD3蛋白复合物和IgE，其中第一多肽具有SEQ ID NO:336的氨基酸序列，并与氨基酸序列SEQ ID NO:337的轻链组装并结合IgE，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0120] CD3蛋白复合物和IgE，其中第一多肽具有SEQ ID NO:338的氨基酸序列并与氨基酸序列SEQ ID NO:339的轻链组装并结合IgE，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0121] CD3蛋白复合物和OX40，其中第一多肽具有SEQ ID NO:340的氨基酸序列，并与氨基酸序列SEQ ID NO:173的轻链组装并结合OX40，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0122] CD3蛋白复合物和CD20，其中第一多肽具有SEQ ID NO:341的氨基酸序列，并与氨基酸序列SEQ ID NO:181的轻链组装并结合CD20，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0123] CD3蛋白复合物和EGFR，其中第一多肽具有SEQ ID NO:342的氨基酸序列，并与氨基酸序列SEQ ID NO:175的轻链组装并结合EGFR，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0124] CD3蛋白复合物和EGFR，其中第一多肽具有SEQ ID NO:343的氨基酸序列，并与氨基酸序列SEQ ID NO:344的轻链组装并结合EGFR，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0125] CD3蛋白复合物和IgE，其中第一多肽具有SEQ ID NO:345的氨基酸序列，并与氨基酸序列SEQ ID NO:346的轻链组装并结合IgE，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε；

[0126] CD3蛋白复合物和IgE，其中第一多肽具有SEQ ID NO:347的氨基酸序列，并与氨基酸序列SEQ ID NO:348的轻链组装并结合IgE，并且其中第二多肽具有选自SEQ ID NO:361、SEQ ID NO:311、SEQ ID NO:394和SEQ ID NO:396的氨基酸序列并结合CD3ε。

[0127] 根据本发明的另一方面，异源二聚体免疫球蛋白或其片段，其中所述CD3结合多肽包含选自以下的重链和轻链可变区中的至少一个或其组合：SEQ ID NOs:101/105、103/106、104/106、104/401、104/402。

[0128] 如上文针对双特异性抗体产生所讨论的，需要有效地产生不含抗人CD3同源二聚体的基于抗人CD3的重链异源二聚体，其中分泌的双特异性抗体产物可以容易从重组哺乳动物宿主细胞系的细胞培养物上清液中分离出来。为此，基于蛋白A的差异纯化技术可用于分离包含VH3可变结构域亚类的异源二聚体免疫球蛋白或其片段，其中消除至少一个但优选两个基于VH3的表位结合区中的蛋白A结合位点。因此，在另一方面，本发明提供用于产生如本文所述的异源二聚体免疫球蛋白或其片段的体外方法，包括以下步骤：

[0129] ia)制备编码第一多肽的重链的DNA载体和编码第二多肽的重链的DNA载体，其中一个或两个DNA载体或第三DNA载体任选地编码共同的轻链或与第一或第二多肽的重链组装的轻链；或者

[0130] ib)制备编码第一和第二多肽的重链的一个DNA载体，其中该DNA载体任选地编码共同的轻链或与第一或第二多肽的重链组装的轻链；和

[0131] 其中所述DNA载体适于在哺乳动物宿主细胞中瞬时或稳定表达；

[0132] ii)在哺乳动物宿主细胞系中转染或共转染来自(i)的DNA载体;

[0133] iii)培养转染的细胞系或从其稳定选择的克隆,并收获细胞培养物上清液;

[0134] iv)使细胞培养物上清液与蛋白A亲和色谱树脂接触;

[0135] v)洗脱和收集目的异源二聚体免疫球蛋白。

[0136] 优选,在来自步骤(v)的纯化物质中异源二聚体免疫球蛋白或其片段为至少95%纯。更优选地,在步骤(v)的纯化物质中发现的异源二聚体免疫球蛋白或其片段至少为96%纯。甚至更优选地在步骤(v)的纯化物质中发现的异源二聚体免疫球蛋白或其片段至少为97%纯。在纯化物质中异源二聚体免疫球蛋白或其片段的纯度可以通过毛细管电泳测定。

[0137] 附图简述

[0138] 图1:使用蛋白A的优选差异亲和纯化技术的示意图。重链都不包含基于VH3的抗原结合位点。图例:[(A+)]表示功能性蛋白A结合位点,[(A-)]表示非功能性蛋白A结合位点。示出pH洗脱。

[0139] 图2A-F:示意图,举例说明使用差异蛋白A色谱法纯化包含一个或多个VH3结构域的重链异源二聚体时所面临的问题。示出了基于突变异源二聚体的至少一个VH3结构域内的蛋白A结合位点的解决方案实例。图2A:当重链异源二聚体在Fc区不结合蛋白A的重链中包含VH3结构域时所面临的问题。图2B:图2A中描述的纯化问题的解决方案,Fc区不结合蛋白A的异源二聚体的重链包含已被突变消除了其蛋白A结合位点的VH3结构域。图2C:图2A中描述的问题的替代解决方案,异源二聚体仅包含一个VH3结构域,并且对异源二聚体进行工程化以使其VH3结构域位于在Fc区中结合蛋白A的重链上(VH3域再定位策略作为解决方案)。图2D:当异源二聚体的两个重链都包含VH3结构域时面临的问题。图2E:图2D中描述的纯化问题的解决方案,Fc区不结合蛋白A的异源二聚体的重链包含已被突变消除了其蛋白A结合位点的VH3结构域。图2F:图2D中描述的纯化问题的替代解决方案,每个VH3结构域的蛋白A结合位点都被消除。加框的物质表明这些物质在差异蛋白A色谱过程中共洗脱。pH值A和B差异约一个pH单位,允许有效分离结合蛋白A的物质。通常,pH A和pH B的pH值分别为4和3。所有图的图例:[(A+)]表示功能性蛋白A结合位点,[(A-)]表示非功能性蛋白A结合位点。

[0140] 图3:Fc133的蛋白A梯度模式色谱曲线(HiTrap™ MabSelect SuRe™蛋白A柱)。实线表示280nm吸光度vs.流动相总体积的曲线。虚线和点划线分别表示流动相pH的曲线和洗脱缓冲液(B)在流动相中的存在百分比的曲线。

[0141] 图4A-C:蛋白A梯度模式色谱曲线。实线表示280nm吸光度vs.流动相总体积的曲线。虚线和点划线分别表示流动相pH的曲线和洗脱缓冲液(B)在流动相中的存在百分比的曲线。图4A:抗HER2 FAB-Fc 133(HiTrap™ MabSelect SuRe™蛋白A柱)。图4B:抗HER2 scFv-Fc 133(HiTrap™ MabSelect SuRe™蛋白A柱)。图4C:抗HER2 FAB(HiTrap™ MabSelect SuRe™蛋白A柱和HiTrap™ MabSelect™蛋白A柱)。

[0142] 图5:七种已知人VH框架亚类的各自代表性氨基酸序列。根据Kabat编号对序列进行比对。与蛋白A结构域D相互作用的人VH3-23框架亚类中的位置以粗体显示。

[0143] 图6A-I:蛋白A梯度模式色谱曲线(HiTrap™ MabSelect™蛋白A柱)。实线表示280nm吸光度vs.流动相总体积的曲线。虚线和点划线分别表示流动相pH的曲线和洗脱缓冲液(B)在流动相中的存在百分比的曲线。图6A:抗HER2 FAB。图6B:抗HER2 FAB T57A。图6C:抗HER2 FAB T57E。图6D:抗HER2 FAB G65S。图6E:抗HER2 FAB R66Q。图6F:抗HER2 FAB

T68V。图6G:抗HER2 FAB Q81E。图6H:抗HER2 FAB N82aS。图6I:抗HER2 FAB R19G/T57A/Y59A。

[0144] 图7:选择的抗HER2 FAB变体对HER2抗原的平衡解离常数(KD)。

[0145] 图8A-D:蛋白A梯度模式色谱曲线(HiTrap™MabSelect SuRe™蛋白A柱)。实线表示280nm吸光度vs.流动相总体积的曲线。虚线和点划线分别表示流动相pH的曲线和洗脱缓冲液(B)在流动相中的存在百分比的曲线。图8A:抗HER2 scFv(G65S)-Fc 133。图8B:抗HER2 scFv(N82aS)-Fc 133。图8C:抗HER2 FAB(G65S)-Fc 133。图8D:抗HER2 FAB(N82aS)-Fc 133。

[0146] 图9A-F:这些图都与在稳定的人框架上的OKT3人源化有关。图9A-C:人IgG1抗体形式的人源化候选物的总结。相对于嵌合OKT3抗体的HPB-ALL染色:(-)表示没有结合,(+)表示较弱的结合,(++)表示中度结合,(+++)表示相似的结合。图9D:选择的候选抗体的DSC谱。图9E:scFv-Fc融合形式的人源化候选物的总结。相对于嵌合OKT3抗体的HPB-ALL染色:(-)表示没有结合,(+)表示较弱的结合,(++)表示中度结合,(+++)表示相似的结合。图9F:选择的scFv-Fc候选物的DSC谱。

[0147] 图10A-B:这些图都与在稳定的人框架上的SP34人源化有关。图10A:人IgG1抗体形式的人源化候选物的总结。图10B:scFv-Fc融合蛋白形式的人源化候选物的总结(人IgG1同种型的Fc)。对于人和猕猴CD3ε1-26_Fc融合蛋白,相对于嵌合SP34抗体的SPR数据:(-)表示没有结合,(+)表示较弱的结合,(++)表示中度结合,(+++)表示强但不相似的结合,(++++)表示相似的结合。

[0148] 图11A-J:这些图都与抗人CD38抗体有关。

[0149] 图11A:嵌合HB-7抗体和人CD38抗原之间通过SPR测量的抗体-抗原相互作用。CM5传感器芯片与蛋白G共价偶联,捕获200RU的嵌合HB-7抗体。以125、31、7.8、3.9、1.9、1和0.5nM、以30μl/min的流速在HBS-P中注射人CD38蛋白(具有多组氨酸标签的人CD38细胞外结构域)。图11B:人源化HB-7最适配(best-fit)抗体与人CD38抗原之间通过SPR测量的抗体-抗原相互作用。CM5传感器芯片与蛋白G共价偶联,捕获200RU的人源化HB-7最适配抗体。在HBS-P中以50、25、12.5、6.25和0.39nM、以30μl/min的流速注射人CD38蛋白(具有多组氨酸标签的人CD38细胞外结构域)。图11C:人源化9G7最适配抗体与人CD38抗原之间通过SPR测量的抗体-抗原相互作用。CM5传感器芯片与蛋白G共价偶联,捕获200RU的人源化9G7最适配抗体。在HBS-P中以25、12.5、6.25、3.12、1.56、0.78、0.39、0.19和0.1nM、以30μl/min的流速注射人CD38蛋白(具有多组氨酸标签的人CD38细胞外结构域)。图11D:人源化9G7最佳框架抗体和人CD38抗原之间通过SPR测量的抗体-抗原相互作用。CM5传感器芯片与蛋白G共价偶联,捕获了200RU的人源化9G7最佳框架抗体。在HBS-P中以50、25、12.5、6.25、3.12、1.56、0.78、0.39、0.19,和0.1nM、以30μL/min的流速注射人CD38蛋白(具有多组氨酸标签的人CD38胞外结构域)。图11E:人767抗体和人CD38抗原之间通过SPR测量的抗体-抗原相互作用。CM5传感器芯片与蛋白G共价偶联,捕获200RU的人767抗体。在HBS-P中以500、250、125、62.5、31.25和15.6nM、30μl/min的流速注射人CD38蛋白(具有多组氨酸标签的人CD38细胞外结构域)。根据以下公式从平衡反应(Req)vs.分析物浓度(C)的图获得亲和力:Req=KA*C*Rmax/(KA*C*n+1),KD为50%饱和的浓度。所有SPR数据表示为响应单位数(缩写为RU;Y轴)vs.时间(X轴)。图11F:嵌合HB-7和人源化HB-7最适配抗体的DSC谱。图11G:嵌合9G7和人

源化9G7最适配抗体的DSC谱。图11H:人源化9G7最佳框架抗体的DSC谱。图11I:人克隆767抗体的DSC谱。图11J:9G7人源化抗体的总结表。

[0150] 图12A-C:替代形式的BEAT HER2/CD3抗体的示意图。图12A:BEAT HER2/CD3-1(形式A)和BEAT HER2/CD3-2(形式B)抗体。图12B:BEAT HER2/CD3-3(形式C)和BEAT HER2/CD3(SP34)(形式D)抗体。图12C:BEAT HER2/CD3(SP34-κ1)(形式E)抗体。图例:[(A+)]表示功能性蛋白A结合位点。[(A-)]表示非功能性蛋白A结合位点。

[0151] 图13:BEAT HER2/CD3-1抗体的蛋白A纯化图谱(280nm吸光度曲线)。柱:1ml MabSelect SuRe。流速:1ml/min。运行缓冲液:0.2M NaH_2PO_4 pH 6。洗脱缓冲液No 1:20mM醋酸钠pH4(20ml)。洗脱缓冲液No 2:0.1M甘氨酸pH 3(20ml)。中和:1/10体积的1M Tris pH8。

[0152] 图14:BEAT HER2/CD3-1抗体制剂的毛细管电泳图谱。

[0153] 图15A:N82aS取代的BEAT HER2/CD3-1抗体的SDS-PAGE分析。图15B:N82aS非取代的BEAT HER2/CD3-1抗体变体的SDS-PAGE分析。图例:[(A+)]表示功能性蛋白A结合位点,[(A-)]表示非功能性蛋白A结合位点。示出洗脱的pH。

[0154] 图16A:BEAT HER2/CD3-1抗体与人CD3ε抗原之间通过SPR测量的抗体-抗原相互作用。CM5传感器芯片与7400Ru的人CD3γ-ε-Fc融合蛋白共价偶联。在HBS-P中以5000、2500、1250、625、312.5和156.25nM、以10μl/min的流速注射BEAT HER2/CD3-1抗体。数据表示为响应单位数(缩写为RU;Y轴)vs.时间(X轴)。根据以下公式从平衡反应(Req)vs.分析物浓度(C)图获得亲和力:Req=KA*C*Rmax/(KA*C*n+1),KD为50%饱和的浓度。图16B:BEAT HER2/CD3-1抗体与人HER2抗原之间通过SPR测量的抗体-抗原相互作用。CM5传感器芯片共价偶联蛋白G,捕获150RU的BEAT HER2/CD3-1抗体。在HBS-P中以1000、333、111、37、12、4.1、1.4、0.5和0.15nM、以30μl/min的流速注射HER2-his。数据表示为响应单位数(缩写为RU;Y轴)vs.时间(X轴)。图16C:在图A和B中分别显示的BEAT HER2/CD3-1和-2抗体的DSC谱。

[0155] 图17A-G:通过BEAT HER2/CD3抗体的T细胞重定向杀伤的实例。读取:RDL-MTS方法。效应细胞:人PBMC。效应细胞与靶细胞的比为10:1。三个供体的平均值,48小时孵育。抗体浓度以nM表示。图17A:BEAT HER2/CD3-1和BEAT HER2/CD3-2抗体,靶细胞:BT-474。图17B:BEAT HER2/CD3-1和BEAT HER2/CD3-2抗体,靶细胞:JIMT-1。图17C:BEAT HER2/CD3-1和BEAT HER2/CD3-2抗体,靶细胞:MDA-MB-231。图17D:BEAT HER2/CD3(SP34)抗体,靶细胞:NCI-N87。图17E:BEAT HER2/CD3(SP34)抗体,靶细胞:HT-1080。图17F:BEAT HER2/CD3(SP34-κ1)抗体,靶细胞:NCI-N87。图17G:BEAT HER2/CD3(SP34-κ1)抗体,靶细胞:HT-1080。

[0156] 图18A-C:JIMT-1异种移植物以及人PBMC补充。图18A:人PBMC不干扰肿瘤生长。图18B-C:BEAT HER2/CD3-1处理和未处理的小鼠的肿瘤体积(mm^3),四个人PBMC供体,五只小鼠的组。

[0157] 图19:BEAT CD38-HB7最适配/CD3(形式A)和BEAT CD38-767/CD3(形式B)抗体的示意图。[(A+)]表示功能性蛋白A结合位点。[(A-)]表示非功能性蛋白A结合位点。

[0158] 图20A:BEAT CD38-HB7最适配/CD3抗体和人CD38抗原之间通过SPR测量的抗体-抗原相互作用。CM5传感器芯片共价偶联蛋白G,捕获200U的BEAT CD38-HB7最适配/CD3抗体。在HBS-P中以50、25、12.5、6.25和0.39nM、以30μl/min的流速注射人CD38蛋白(多组氨酸标记的蛋白)。数据表示为响应单位数(缩写为RU;Y轴)vs.时间(X轴)。图20B:BEAT CD38-HB7最适配/CD3抗体DSC谱。

[0159] 图21:通过BEAT CD38-HB7最适配/CD3抗体的T细胞重定向杀伤的实例。读取:RDL-FACS方法。效应细胞:纯化的人T细胞。效应细胞与靶细胞的比为10:1。两个供体的平均值,48h孵育。靶细胞:RPMI 8226。抗体浓度以nM表示。

[0160] 图22:通过BEAT CD38-767/CD3(SP34)抗体的T细胞重定向杀伤的实例。读取:RDL-FACS方法。效应细胞:人PBMC。效应细胞与靶细胞的比为10:1。三个供体的平均值,24小时孵育。靶细胞:Daudi。抗体浓度以nM显示。

[0161] 图23:BEAT OX40/CD3抗体的示意图。图例:[(A+)]表示功能性蛋白A结合位点。[(A-)]表示非功能性蛋白A结合位点。

[0162] 图24:BEAT OX40/CD3抗体的T细胞重定向杀伤的实例。读取:RDL-MTS方法。效应细胞:人PBMC。效应细胞与靶细胞的比为20:1。三个供体的平均值,48小时孵育。靶细胞:重组稳定的CHO[OX40]细胞。抗体浓度以nM显示。

[0163] 图25:BEAT EGFR/CD3抗体的示意图。图例:[(A+)]表示功能性蛋白A结合位点。[(A-)]表示非功能性蛋白A结合位点。

[0164] 图26:通过BEAT EGFR/CD3抗体的T细胞重定向杀伤的实例。读取:RDL-MTS方法。效应细胞:人PBMC。效应细胞与靶细胞的比为10:1。四个供体的平均值,48小时孵育。靶细胞:HT-29细胞。抗体浓度以nM显示。

[0165] 图27:BEAT CD38-HB7最适配/CD3(SP34)(形式A)和BEAT CD38-9G7最适配/CD3(SP34-κ2)(形式B)抗体的示意图。[(A+)]表示功能性蛋白A结合位点。

[0166] 图28:通过BEAT CD38-HB7最适配/CD3(SP34)抗体的T细胞重定向杀伤的实例。读取:RDL-FACS方法。效应细胞:人PBMC。效应细胞与靶细胞的比为10:1。三个供体的平均,24小时孵育。靶细胞:Daudi细胞。抗体浓度以nM表示。

[0167] 图29:BEAT CD38-9G7最适配/CD3(SP34-κ2)抗体与人CD3ε1-26_Fc融合蛋白之间通过SPR测量的抗体-抗原相互作用。CM5传感器芯片共价偶联500RU的人CD3ε1-26_Fc融合蛋白。在HBS-P中以50、25、12.5、6.2、3.1、0.8和0.4nM、以30μl/min的流速注射BEAT CD38-9G7最适配/CD3(SP34-κ2)抗体。数据表示为响应单位数(缩写为RU;Y轴)vs.时间(X轴)。

[0168] 图30:通过BEAT CD38/CD3(SP34-κ2)抗体的T细胞重定向杀伤的实例。读取:RDL-FACS方法。效应细胞:人PBMC。效应细胞与靶细胞的比为10:1。三个供体的平均,24小时孵育。靶细胞:Daudi细胞。抗体浓度以nM显示。

[0169] 图31:BEAT CD20/CD3(SP34)抗体的示意图。[(A+)]表示功能性蛋白A结合位点。

[0170] 图32:通过BEAT CD20/CD3(SP34)抗体的T细胞重定向杀伤的实例。读取:RDL-FACS方法。效应细胞:人PBMC。效应细胞与靶细胞的比为10:1。三个供体的平均,24小时孵育。靶细胞:Daudi细胞。抗体浓度以nM显示。

[0171] 图33显示,对于SP34嵌合体以及Sp34H1L21,从IgG1形式重新格式化为scFv-Fc形式后的相对表达水平,其中观察到显著的表达损失。

[0172] 图34显示在位置:T27、G27a、V27c、T28、T29、S30、N31、Y32、N52、K53、R54、P56、L90、Y92、S93、N94和L95上丙氨酸扫描对SP34H1L21ScFv-Fc表达水平的影响。

[0173] 图35a显示SP34H3L23ScFv-Fc的位置29上的随机突变对表达水平的影响;b显示SP34H3L23ScFv-Fc的位置30上的随机突变对表达水平的影响;c显示SP34H5L23ScFv-Fc的位置95上的随机突变对表达水平的影响。

[0174] 图36显示了几种人源化SP34的归一化的表达水平。

[0175] 图37显示了RDL测定法中几种CD38/CD3双特异性抗体的性能，其中CD3结合臂包含：重新格式化为scFv(SEQ ID NO:403)的原始小鼠SP34、或包含重链/轻链组合H1/L21(SEQ ID NO:361)、H5/L32(SEQ ID NO:311)、H5/L65(SEQ ID NO:394)和H5/L67(SEQ ID NO:396)的修饰的人源化SP34 scFv。

[0176] 图38显示了CD3-利妥昔单抗(Rutiximab)BEAT双特异性对RAJI细胞群的作用。

[0177] 图39显示了在RDL测定法中爱必妥(Erbitux)对MCF-7、HCT116和A549细胞群的作用。

[0178] 图40显示了在RDL测定法中CD3-爱必妥BEAT双特异性抗体对MCF-7、HCT116和A549细胞群的作用。

[0179] 图41显示使用EGFR PharmDx免疫组织化学试剂盒(Dako，Cambridge，UK)测定的细胞系的EGFR状态。

[0180] 图42显示了在RDL测定法中维克替比(Vectibix)对MCF-7、HCT116和A549细胞群的作用。

[0181] 图43显示了在RDL测定法中CD3-维克替比BEAT双特异性抗体对MCF-7、HCT116和A549细胞群的作用。

[0182] 发明详述

[0183] 本发明一般涉及结合CD3蛋白复合物和疾病相关抗原的新型异源二聚体免疫球蛋白。而且，这些异源二聚体免疫球蛋白具有降低的或消除的蛋白A结合，并因此可以使用亲和色谱纯化至非常高的纯度。

[0184] 为解释本说明书的目的，应用以下定义，并且在适用时，以单数使用的术语也涵盖其复数形式且反之亦然。应当理解，本文所用术语仅用于描述特定实施方案，而不旨在构成限制。

[0185] 术语“多肽”和“蛋白”是指，氨基酸残基聚合物，其中氨基酸通过肽键组合形成氨基酸链，在该链中氨基酸已经通过脱水合成而连接在一起。多肽和蛋白可以通过化学合成或重组表达方式合成，并且没有最小氨基酸长度的限制。

[0186] 根据本发明，当蛋白质由单个多肽链组成时，多肽涵盖“蛋白质”。多肽还可以形成多聚体，例如二聚体、三聚体和更高级的寡聚体，即，由一个以上多肽分子组成。形成二聚体、三聚体等的多肽分子可以相同或不同。相应地，这些多聚体的对应高级结构被称为同源-或异源-二聚体、同源-或异源-三聚体等等。异源多聚体的一个例子是抗体分子，其天然形式由两个相同的轻链多肽和两个相同的重链多肽组成。术语“多肽”和“蛋白”也指天然修饰的多肽/蛋白，其中所述修饰可以通过例如翻译后修饰，例如糖基化、乙酰化、磷酸化等实现。这些修饰是本领域已知的。此外，出于本发明目的，“多肽”可以指相对于天然序列包括修饰的蛋白质，例如缺失、添加和取代(在性质上可以是保守的)。这些修饰可以是有意的，例如通过定点诱变引起，或可以是偶然的，例如由于产生蛋白的宿主的突变或由于PCR扩增错误引起。

[0187] 本文中，术语“CD3复合物”指称作CD3(分化簇3)T细胞共受体的蛋白质复合物(Wucherpfennig KW等，(2010)Cold Spring Harb Perspect Biol，2(4):a005140)。CD3蛋白复合物由四条分开的链组成。在哺乳动物中，该复合物含有CD3γ链、CD3δ链和两个CD3ε

链。这些链与称作T细胞受体(TCR)的分子及ζ-链缔合,在T淋巴细胞中产生活化信号(van der Merwe PA&Dushek O(2011)Nat Rev Immunol,11(1):47-55)。TCR、ζ-链和CD3分子一起组成TCR复合物。CD3γ,CD3δ,和CD3ε链是免疫球蛋白超家族中高度相关的细胞表面蛋白,含有单个细胞外免疫球蛋白结构域。CD3分子的胞内尾含有一个保守基序,称作免疫受体酪氨酸激活基序或缩写为ITAM,其是TCR的信号传导能力所必需的。由于CD3是T细胞活化所必需的,故靶向CD3的药物(常常是单克隆抗体)一直以来以及目前都被作为免疫抑制剂疗法进行研究。

[0188] 本文所用的术语"疾病相关抗原"是指参与疾病过程的分子。疾病相关抗原的实例存在于广泛的治疗领域中,如炎症、癌症和自身免疫性疾病。在肿瘤学中,疾病相关抗原是可广泛用于患者群体中筛选和/或监测和/或治疗性靶向癌症的分子,例如前列腺癌中的EpCAM抗原。肿瘤抗原可以由肿瘤直接产生或可以作为对肿瘤存在的应答由非肿瘤细胞产生,优选的肿瘤抗原是细胞表面分子。炎性疾病相关抗原是已知的,其包括但不限于,促炎细胞因子如TNF-α和IL-1。自身免疫性疾病相关抗原也是已知的,其实例包括但不限于系统性红斑狼疮中抗双链DNA的抗体和阿尔茨海默病中的淀粉样β肽。

[0189] 本文中使用的术语"免疫球蛋白"可以与"抗体"互换使用。免疫球蛋白包括全长抗体、及其任何抗原结合片段或单链。免疫球蛋白可以是同源二聚体或异源二聚体。免疫球蛋白,特别是天然存在的抗体,是以一或多拷贝的Y形单元存在的糖蛋白,所述单元由四条多肽链组成。每个"Y"形含有2个相同的重链(H)拷贝和2个相同的轻链(L)拷贝,重链和轻链因其相对分子量而得名。每条轻链与重链配对,而每条重链与另一条重链配对。共价的链间二硫键和非共价相互作用将这些链连接在一起。免疫球蛋白,特别是天然存在的抗体,含有可变区,其是两个拷贝的抗原结合位点。木瓜蛋白酶(一种蛋白水解酶)将"Y"形切割成3个独立的分子,2个被称为"Fab"或"FAB"片段(Fab=抗原结合片段),一个被称为"Fc"片段或"Fc区"(Fc=可结晶的片段)。Fab片段由完整的轻链和部分重链组成。重链含有1个可变区(VH)和3或4个恒定结构域(CH1、CH2、CH3和CH4,取决于抗体类型或同种型)。在CH1和CH2区之间的区域被称为铰链区,其使得Y形抗体分子的2个Fab臂之间具有柔性,允许其打开和闭合以适应于结合相隔固定距离的2个抗原决定簇。本文中提及的"铰链区"是长度为6-62个氨基酸的序列区,仅存在于IgA、IgD和IgG中,涵盖了桥接2条重链的半胱氨酸残基。IgA、IgD和IgG的重链都具有4个区,即,1个可变区(VH)和3个恒定区(CH1-3)。IgE和IgM的重链具有1个可变区和4个恒定区(CH1-4)。免疫球蛋白的恒定区可以介导与宿主组织或因子的结合,所述宿主组织或因子包括免疫系统的各种细胞(例如,效应细胞)和补体系统经典通路的第一成分(C1q)。每条轻链通常通过1个共价二硫键与重链连接。每条轻链含有1个可变区(VL)和1个轻链恒定区。轻链恒定区是κ轻链恒定区,在本文中称为IGKC,或者是λ轻链恒定区,在本文中称为IGLC。IGKC在本文中与Cκ或CK等价使用,具有相同的含义。IGLC在本文中与Cλ或CL等价使用,具有相同的含义。本文中使用的术语"IGLC区"指所有的λ轻链恒定区,例如,选自IGLC1、IGLC2、IGLC3、IGLC6和IGLC7的所有λ轻链恒定区。VH和VL区可以进一步细分为命名为互补决定区(CDR)的超变区,其间散布了更保守的、命名为框架区(FR或FW)的区域。每个VH和VL都包括3个CDR和4个FR,按下列顺序从氨基端至羧基端排列:FR1、CDR1、FR2、CDR2、FR3、CDR3、FR4。重链和轻链的可变区含有与抗原相互作用的表位结合区。工程化的免疫球蛋白可以包含不同的表位结合区形式,例如scFv、FAB或dAb片段。这些片段通常通过与IgG

Fc区遗传融合而组装在抗体样结构中。工程化的免疫球蛋白可以通过使用或不使用异源二聚体化增强技术而构建为同源或异源二聚体，并可以具有单或双特异性结合性质。

[0190] 本文中使用的术语“全长抗体”包含构成抗体的天然生物学形式的结构，包括可变区和恒定区。例如，在大多数哺乳动物(包括人和小鼠)中，IgG类的全长抗体是四聚体，由相同的2对组成，每对两条免疫球蛋白链，每一对具有1条轻链和1条重链，每条轻链包含免疫球蛋白区VL和轻链恒定区，每条重链包含免疫球蛋白区VH、CH1(Cγ1)、CH2(Cγ2)、CH3(Cγ3)和CH4(Cγ4，取决于抗体类型或同种型)。在一些哺乳动物中，例如骆驼和羊驼，IgG抗体可以仅由2条重链组成，每条重链包含与Fc区连接的可变区。

[0191] 由恒定区的基因决定，抗体被分类，也称为同种型。人恒定轻链分为κ(CK)和λ(Cλ)轻链。重链分为mu(μ)、delta(δ)、gamma(γ)、alpha(α)或epsilon(ε)，分别定义了抗体的同种型IgM、IgD、IgG、IgA和IgE。因此，本文中使用的“同种型”意指通过恒定区的化学和抗原特征定义的任何免疫球蛋白类别和/或亚类。已知的人免疫球蛋白同种型是IGHG1(IgG1)、IGHG2(IgG2)、IGHG3(IgG3)、IGHG4(IgG4)、IGHA1(IgA1)、IGHA2(IgA2)、IGHM(IgM)、IGHD(IgD)和IGHE(IgE)。所谓的人免疫球蛋白假-γ IGHGP基因代表了另外的人免疫球蛋白重链恒定区基因，该基因已被测序，但由于改变的转换区而不编码蛋白质(Bensmana M等人，(1988)Nucleic Acids Res.16(7):3108)。尽管具有改变的转换区，人免疫球蛋白假-γ IGHGP基因仍具有所有的重链恒定区(CH1-CH3)和铰链的可读框。其重链恒定区的所有可读框编码与具有预测的结构特征的所有人免疫球蛋白恒定区良好对齐的蛋白质结构域。该另外的假-γ同种型在本文中被称为IgGP或IGHGP。还报道了其他的假免疫球蛋白基因，如人免疫球蛋白重链恒定区ε P1和P2假基因(IGHEP1和IGHEP2)。IgG类是最常用于治疗目的的。在人中，该类别包括亚类IgG1、IgG2、IgG3和IgG4。在小鼠中，该类别包括亚类IgG1、IgG2a、IgG2b、IgG2c和IgG3。

[0192] 本文使用的术语“免疫球蛋白片段”包括但不限于(i)区域，包括例如CH1、CH2或CH3区，(ii)由VL、VH、CL或CK、和CH1区组成的Fab片段，包括Fab′和Fab′-SH，(ii)由VH和CH1区组成的Fd片段，(iii)由单可变区组成的dAb片段(Ward ES等人，(1989)Nature,341(6242):544-6)，(iv)$F(ab')_2$片段，一种包含2个相连的Fab片段的二价片段，(v)单链Fv分子(scFv)，其中VH区和VL区通过肽接头相连，所述接头允许2个区缔合形成抗原结合位点(Bird RE等人(1988)，Science、242(4877):423-6;Huston JS等人，(1988)Proc Natl Acad Sci U S A,85(16):5879-83)，(vi)“双抗体(diabody)”或“三抗体(triabodies)”，通过基因融合构建的多价或多特异性片段(Holliger P等人，(1993)Proc Natl Acad Sci U S A、90(14):6444-8;Tomlinson I和Holliger P,(2000)Methods Enzymol,326:461-79)，(vii)scFv，与Fc区融合的双抗体或区域抗体，和(viii)与相同或不同抗体融合的scFv。

[0193] 术语“可变区”是指介导抗原结合并定义特定抗体对特定抗原的特异性的区域或结构域。在天然抗体中，抗原结合位点由定义特异性的两个可变区组成：一个位于重链中，在本文中称作重链可变区(VH)，另一个位于轻链中，在本文中称作轻链可变区(VL)。在人类中，重链可变区(VH)可以分为7个亚组或亚类：VH1,VH2,VH3,VH4,VH5,VH6和VH7。一些情况下，特异性可以仅存在于两个区域的仅一个中，例如在来自驼科动物(camelids)的重链抗体的单域抗体中。这些V区通常大约110个氨基酸长度，由称作框架区(FR或“非CDR区”)的15-30个氨基酸的相对不变氨基酸序列链和称作“高变区”的7-17个氨基酸长度的极度可变

的较短区域组成，其中框架区被高变区分隔开。天然重链和轻链的可变结构域包含4个FR，这些FR大部分采取β片层构型，通过形成环的三个高变区连接。每条链中的高变区通过FR而紧靠在一起，与来自另一链的高变区一起促成抗体的抗原结合位点的形成(参见Kabat EA等，同上引文)。本文中术语"高变区"是指，负责抗原结合的抗体的氨基酸残基。高变区通常包含来自"互补决定区"或"CDR"的氨基酸残基，后者具有最大的序列变异性和/或参与抗原识别。对于所有可变区，根据Kabat(Kabat EA等，同上引文)进行编号。

[0194] 有多种CDR定义在使用，并包括在本文中。Kabat定义基于序列的变异性，是最常使用的CDR定义(Kabat EA等，同上引文)。Chothia则是指结构环的位置(Chothia&Lesk J.(1987)Mol Biol,196:901-917)。AbM定义是Kabat和Chothia定义的折中，用于Oxford Molecular的AbM抗体模建软件(Martin ACR等，(1989)Proc Natl Acad Sci USA 86:9268-9272;Martin ACR等，(1991)Methods Enzymol,203:121-153;Pedersen JT等，(1992)Immunomethods,1:126-136;Rees AR等，(1996)In Sternberg M.J.E.(ed.),Protein Structure Prediction.Oxford University Press,Oxford,141-172)。近来引入了接触定义(MacCallum RM等，(1996)J Mol Biol,262:732-745)，其基于对蛋白数据库中可得的复合物结构的分析。**IMGT®**(国际ImMunoGeneTics**信息系统®**)的CDR定义(http://www.imgt.org)基于对所有物种的所有免疫球蛋白和T细胞受体V-REGIONs的IMGT编号(**IMGT®**,国际ImMunoGeneTics**信息系统®**;Lefranc MP等，(1999)Nucleic Acids Res,27(1):209-12;Ruiz M等，(2000)Nucleic Acids Res,28(1):219-21;Lefranc MP(2001)Nucleic Acids Res,29(1):207-9;Lefranc MP(2003)Nucleic Acids Res,31(1):307-10;Lefranc MP等，(2005)Dev Comp Immunol,29(3):185-203;Kaas Q等，(2007)Briefings in Functional Genomics&Proteomics,6(4):253-64)。在本发明中提及的所有互补决定区(CDR)优选按如下进行定义(根据Kabat EA等人的编号，同上引文)：

[0195] LCDR1:24-34,LCDR2:50-56,LCDR3:89-98,HCDR1:26-35,HCDR2:50-65,HCDR3:95-102。

[0196] 可变结构域的"非CDR区"称作框架区(FR)。在本文中VL区的"非CDR区"包含氨基酸序列：1-23(FR1)，35-49(FR2)，57-88(FR3)和99-107(FR4)。在本文中VH区的"非CDR区"包含氨基酸序列：1-25(FR1)，36-49(FR2)，66-94(FR3)和103-113(FR4)。

[0197] 本发明CDR可以包含基于上述定义的"延长的CDR"，具有如下可变结构域残基：LCDR1:24-36,LCDR2:46-56,LCDR3:89-97,HCDR1:26-35,HCDR2:47-65,HCDR3:93-102。这些延长的CDR也根据Kabat等人(同上引文)编号。本文中VL区的"非延长CDR区"包含氨基酸序列：1-23(FR1)，37-45(FR2)，57-88(FR3)和98-大约107(FR4)。本文中VH区的"非延长CDR区"包含氨基酸序列：1-25(FR1)，37-46(FR2)，66-92(FR3)和103-大约113(FR4)。

[0198] 本文使用的术语"Fab"或"FAB"或"Fab区"或"FAB区"包括包含VH、CH1、VL和轻链恒定免疫球蛋白区的多肽。Fab可以指分离的这一区域，或位于全长抗体或抗体片段中的这一区域。

[0199] 本文使用的术语"Fc"或"Fc区"包括这样的多肽，所述多肽包含除第一恒定区免疫球蛋白结构域以外的抗体重链恒定区。因此，Fc指IgA、IgD和IgG的最后2个恒定区免疫球蛋白区域，或IgE和IgM的最后3个恒定区免疫球蛋白区域，和这些结构域N端的柔性铰链。对于

IgA和IgM,Fc可以包括J链。对于IgG,Fc包括免疫球蛋白区域Cgamma2和Cgamma3(Cγ2和Cγ3),和Cgamma1(Cγ1)和Cgamma2(Cγ2)之间的铰链。虽然Fc区的边界可以改变,但人IgG重链Fc区通常定义为包括残基C226或P230至其羧基端,其中根据EU索引编号。Fc可以指分离的这一区域,或位于Fc多肽(例如抗体)环境中的这一区域。

[0200] 术语"免疫球蛋白恒定区"在本文中指,来自人或动物物种的免疫球蛋白或抗体重链恒定区,涵盖所有同种型。优选地,免疫球蛋白恒定区是人源的,选自但不限于:IGHG1 CH1,IGHG2 CH1,IGHG3 CH1,IGHG4 CH1,IGHA1 CH1,IGHA2 CH1,IGHE CH1,IGHEP1 CH1,IGHM CH1,IGHD CH1,IGHGP CH1,IGHG1 CH2,IGHG2 CH2,IGHG3 CH2,IGHG4 CH2,IGHA1 CH2,IGHA2 CH2,IGHE CH2,IGHEP1 CH2,IGHM CH2,IGHD CH2,IGHGP CH2,IGHG1 CH3,IGHG2 CH3,IGHG3 CH3,IGHG4 CH3,IGHA1 CH3,IGHA2 CH3,IGHE CH3,IGHEP1 CH3,IGHM CH3,IGHD CH3,IGHGP CH3,IGHE CH4和IGHM CH4。优选的"免疫球蛋白恒定区"选自:人IGHE CH2,IGHM CH2,IGHG1 CH3,IGHG2 CH3,IGHG3 CH3,IGHG4 CH3,IGHA1 CH3,IGHA2 CH3,IGHE CH3,IGHM CH3,IGHD CH3和IGHGP CH3。最优选的"免疫球蛋白恒定区"选自:人IGHG1 CH3,IGHG2 CH3,IGHG3 CH3,IGHG4 CH3,IGHA1 CH3,IGHA2 CH3,IGHE CH3,IGHM CH3,IGHD CH3和IGHGP CH3。

[0201] 术语"表位结合区"包括具有允许免疫球蛋白分子与一个或多个表位特异性结合的最小氨基酸序列的多肽或其片段。天然抗体具有两个表位结合区,也称作抗原结合位点或抗原结合部位或抗原互补位。天然抗体中的表位结合区局限于VH和/或VL结构域的CDR区内,其中存在介导表位结合的氨基酸。除了天然抗体外,可以工程化构建人工VH结构域或VL结构域或其片段及其组合,提供表位结合区(Holt LJ et al.,(2003)Trends Biotechnol,21(11):484-490;Polonelli L et al.,(2008)PLoS ONE,3(6):e2371)。基于非免疫球蛋白的表位结合区的实例可以见于作为"支架"用于工程化表位结合区的人工蛋白结构域(Binz HK et al.,(2005)Nat Biotechnol,23(10):1257-1268)或肽模拟物(Murali R&Greene MI(2012)Pharmaceuticals,5(2):209-235)。优选地,术语"表位结合区"包括一个或多个重链可变结构域和一个或多个互补轻链可变结构域的组合,其一起形成允许免疫球蛋白分子与一个或多个表位特异性结合的结合位点。在本发明中示例的表位结合区实例包括scFv和FAB。

[0202] 本文中,术语"表位"包括多肽或蛋白或非蛋白分子的片段,其在动物中,优选在哺乳动物中且最优选地在人类中,具有抗原性或免疫原性活性。具有免疫原性活性的表位是可以在动物中引起抗体应答的多肽或蛋白的片段。具有抗原性活性的表位是,通过本领域技术人员熟知的任何方法测定,例如通过免疫测定法测定,可以与抗体或多肽特异性结合的多肽或蛋白的片段。抗原性表位无需一定是免疫原性的。优选地,术语"表位"在本文中指至少约3-5,优选约5-10或15至不超过约1,000(或之间的任何整数)个氨基酸的多肽序列,这些氨基酸定义的序列,自身或作为更大序列的一部分,可以与响应于该序列而产生的抗体结合。对于该片段的长度,没有关键性的上限,其可以包含近乎全长的蛋白序列,或甚至包含一个或多个表位的融合蛋白。用于本发明的表位不限于这样的多肽,所述多肽具有其所源自的亲本蛋白的该部分的确切序列。因此,术语"表位"涵盖与天然序列相同的序列、以及对天然序列的修饰,例如缺失、添加和取代(通常在性质上保守)。蛋白抗原的表位,基于其结构和与表位结合位点的相互作用,可以分为两类,构象表位和线性表位(Goldsby R et

al.,(2003)“抗原(第3章)”Immunology(第5版),New York:W.H.Freeman and Company.pp.57-75,ISBN 0-7167-4947-5)。构象表位由抗原氨基酸序列的不连续部分组成。这些表位基于抗原的3-D表面特征和形状或三级结构,与互补位相互作用。大多数表位是构象表位。相对地,线性表位基于其一级结构与互补位相互作用。线性表位由来自抗原的一段连续氨基酸序列形成。

[0203] 本文使用的术语“异源二聚体免疫球蛋白”或“异源二聚体片段”或“异源二聚体”或“重链异源二聚体”包括,至少包含第一和第二多肽(如第一和第二区)的免疫球蛋白分子或其部分,其中第二多肽的氨基酸序列与第一多肽不同。优选的,异源二聚体免疫球蛋白包含2条多肽链,其中第一链与第二链具有至少一个不同的区域,且其中两条链通过其不同区域组装,即,相互作用。更优选地,异源二聚体免疫球蛋白对至少2个不同配体、抗原或结合位点具有结合特异性,即,是双特异性的。本文使用的异源二聚体免疫球蛋白包括但不限于全长双特异性抗体、双特异性Fab、双特异性F(ab')$_2$、与Fc区融合的双特异性scFv、与Fc区融合的双抗体和与Fc区融合的结构域抗体。

[0204] 本文使用的术语“同源二聚体免疫球蛋白”或“同源二聚体片段”或“同源二聚体”或“重链同源二聚体”包括,至少包含第一和第二多肽(如第一和第二区)的免疫球蛋白分子或其部分,其中第二多肽的氨基酸序列与第一多肽相同。优选的,同源二聚体免疫球蛋白包括2条多肽链,其中第一链与第二链具有至少一个相同的区域,且其中两条链通过其相同区域组装,即,相互作用。优选的,同源二聚体免疫球蛋白片段包含至少2个区域,其中第一区与第二区相同,且其中两个区通过其蛋白质-蛋白质界面组装,即相互作用。

[0205] 对于本发明中包括的所有免疫球蛋白恒定区,可以根据IMGT®(IMGT®;同上引文)编号。

[0206] 对于选自IGHG1、IGHG2、IGHG3和IGHG4的所有的人CH1、CH2、CH3免疫球蛋白重链恒定结构域,可以根据“EU编号体系”编号(Edelman GM等人,(1969)Proc Natl Acad Sci U S A,63(1):78-85)。对于IGHG1的人CH1、铰链、CH2和CH3恒定区,完整的对应性可见于IMGT数据库(IMGT®,同上引文)。

[0207] 对于人κ免疫球蛋白轻链恒定结构域(IGKC),可以根据“EU编号体系”编号(Edelman GM等人,同上引文)。人CK结构域的完整对应性可见于IMGT数据库(IMGT®,同上引文)。

[0208] 对于人λ免疫球蛋白轻链恒定结构域(IGLC1、IGLC2、IGLC3、IGLC6和IGLC7),可以根据“Kabat编号体系”编号(Kabat EA等人,同上引文)。人IGLC区的完整对应性可见于IMGT数据库(IMGT®,同上引文)。

[0209] 本文所述的人IGHG1免疫球蛋白重链恒定区的区边界如下:CH1区[EU编号:118-215]、铰链γ1[EU编号:216-230]、CH2区[EU编号:231-340]和CH3区[EU编号:341-447]。本文所述的人CK区跨残基108至214(EU编号)。本文所述的人IGLC1、IGLC2、IGLC3、IGLC6和IGLC7区跨残基108-215(Kabat编号)。

[0210] 本文使用的术语“氨基酸”或“氨基酸残基”包括天然氨基酸和非天然氨基酸。优选包括天然氨基酸。

[0211] 本文中的术语“修饰”或“氨基酸修饰”包括多肽序列中的氨基酸取代、插入和/或

缺失。本文中术语“氨基酸取代”或“取代”或“氨基酸残基取代”意指，一个氨基酸序列中第一氨基酸残基被第二氨基酸残基替代，其中第一氨基酸残基与第二氨基酸残基不同，即，替代氨基酸残基与被替代的氨基酸不同。例如，取代R94K指这样的变体多肽，其中第94位的精氨酸被赖氨酸替代。例如，94K表示第94位被赖氨酸取代。出于本文的目的，通常用斜线或逗号分开多个取代。例如，“R94K/L78V”或“R94K,L78V”指包含取代R94K和L78V的双重变体。本文使用的术语“氨基酸插入”或“插入”意指在亲代多肽序列的特定位置添加氨基酸。例如，插入-94表示在第94位的插入。本文使用的“氨基酸缺失”或“缺失”意指去除亲代多肽序列的特定位置上的氨基酸。例如，R94-表示缺失第94位的精氨酸。

[0212] 在一些实施方案中，在蛋白A结合中术语“减少”、“降低”或“下降”是指，通过标准本领域已知方法例如本文所述方法检测，与亲本，即未修饰的免疫球蛋白或野生型IgG或具有野生型人IgG Fc区的IgG相比，修饰的免疫球蛋白或其片段与蛋白A的结合，总体减少了至少25%，30%，40%，50%，60%，70%，80%，85%，90%，95%，97%，或99%至多达100%(消除)。在一些实施方案中，这些术语备选地可以指总体减少10倍(即，1log)，100倍(2logs)，1,000倍(或3logs)，10,000倍(或4logs)，或100,000倍(或5logs)。

[0213] 术语“消除”或“废除”蛋白A结合是指，通过标准本领域已知方法例如本文所述方法检测，与亲本，即未修饰的免疫球蛋白或野生型IgG或具有野生型人IgG Fc区的IgG相比，修饰的免疫球蛋白或其片段与蛋白A的结合，总体下降100%，即修饰的免疫球蛋白或其片段与蛋白A的结合完全丧失。

[0214] 类似地，在与亲和试剂的结合中术语“减少”、“降低”或“下降”是指，通过标准本领域已知方法例如本文所述方法检测，与亲本，即未修饰的免疫球蛋白或野生型IgG或具有野生型人IgG Fc区的IgG相比，修饰的免疫球蛋白或其片段与亲和试剂的结合，总体减少了至少25%，30%，40%，50%，60%，70%，80%，85%，90%，95%，97%，或99%至多达100%(消除)。在一些实施方案中，这些术语备选地可以指总体减少10倍(即，1log)，100倍(2logs)，1,000倍(或3logs)，10,000倍(或4logs)，或100,000倍(或5logs)。

[0215] 术语“消除”或“废除”与亲和试剂的结合是指，通过标准本领域已知方法例如本文所述方法检测，与亲本，即未修饰的免疫球蛋白或野生型IgG或具有野生型人IgG Fc区的IgG相比，修饰的免疫球蛋白或其片段与亲和试剂的结合，总体下降100%，即修饰的免疫球蛋白或其片段与亲和试剂的结合完全丧失。

[0216] “双特异性抗体”是对至少两个不同抗原具有结合特异性的单克隆抗体。在一些实施方案中，双特异性抗体是，相对于亲本抗体，在VH区中具有一个或多个氨基酸修饰的双特异性抗体。在一些实施方案中，双特异性抗体可以是人或人源化抗体。双特异性抗体也可以用于将细胞毒性剂定位至表达靶抗原的细胞。这些抗体具有靶-抗原结合臂和结合细胞毒性剂的臂，细胞毒性剂是例如皂草素(saporin)、抗干扰素α、长春花生物碱(vinca alkaloid)、蓖麻毒蛋白A链、氯甲蝶呤或放射性同位素半抗原。双特异性抗体可以制备为全长抗体或抗体片段。制备双特异性抗体的方法是本领域已知的。传统上，双特异性抗体的重组生产基于的是共表达两个免疫球蛋白重链-轻链对，其中两个重链具有不同的特异性(Milstein and Cuello,(1983)Nature,305:537-40)。由于免疫球蛋白重链和轻链的随机组配，这些杂交瘤(四重瘤)产生不同抗体分子的潜在混合物，其中仅一种具有正确的双特异性结构。正确分子的纯化，通常通过亲和色谱步骤进行，十分繁琐且低产率。相似的程序

公开在WO1993/08829和Traunecker et al.,(1991)EMBO J,10:3655-9中。根据不同方法，具有期望结合特异性的抗体可变区(抗体-抗原结合部位)与免疫球蛋白恒定区序列融合。例如融合包含至少部分铰链、CH2和CH3区的免疫球蛋白重链恒定区。在一些实施方案中，含有轻链结合所需位点的第一重链恒定区(CH1)，存在于至少一个融合物中。将编码免疫球蛋白重链融合物、以及如果期望的话，免疫球蛋白轻链的DNA，插入分开的表达载体，共转染合适的宿主生物。在不等比率的三个片段用于构建可以提供最佳产率的实施方案中，这使得可以灵活地调整三个多肽片段的相互比例。然而，当至少两个多肽链的等比率表达可以导致高产率或当比率不是特别重要时，可以将两个或全部三个多肽链的编码序列插入一个表达载体中。

[0217] 双特异性抗体包括交联抗体或"异源缀合物"抗体。例如，异源缀合物中抗体之一可以与亲和素偶联，而另一可以与生物素偶联。这样的抗体已经例如被提出用于将免疫系统细胞靶向不想要的细胞(US4,676,980)和用于治疗HIV感染(WO1991/00360,WO1992/00373和EP03089)。异源缀合物抗体可以使用任何方便的交联方法制备。合适的交联剂是本领域熟知的(参见US4,676,980)，多种交联技术也是已知的。也考虑具有二价以上效价的抗体。例如，可以制备三特异性抗体(参见Tutt A et al.(1991)J.Immunol.147:60-9)。

[0218] 在一些实施方案中，本公开提供了双特异性异源二聚体免疫球蛋白或其片段或双特异性全长抗体，其结合CD3和选自以下的疾病相关抗原：肿瘤抗原、细胞因子、血管生长因子和淋巴-血管生成性生长因子。优选地，双特异性异源二聚体免疫球蛋白或其片段或双特异性抗体结合CD3和选自以下的疾病相关抗原：CCR3、CCR6、CRTH2、PDL1、BLUT1、PirB、CD33、TROP2、CD105、GD2、GD3、CEA、VEGFR1、VEGFR2、NCAM、CD133、CD123、ADAM17、MCSP、PSCA、FOLR1、CD19、CD20、CD38、EpCAM、HER2、HER3、EGFR、PSMA、IgE、整合素a4b1、CCR5、LewisY、FAP、MUC-1、Wue-1、MSP、EGFRvIII、P糖蛋白、AFP、ALK、BAGE蛋白、CD30、CD40、CTLA4、ErbB3、ErbB4、间皮素、OX40、CA125、CAIX、CD66e、cMet、EphA2、HGF/SF、MUC1、磷脂酰丝氨酸、TAG-72、TPBG、β-联蛋白、brc-abl、BRCA1、BORIS、CA9、胱天蛋白酶-8、CDK4、细胞周期蛋白-B1、CYP1B1、ETV6-AML、Fra-1、FOLR1、GAGE-1、GAGE-2、GloboH、磷脂酰肌醇聚糖-3、GM3、gp100、HLA/B-raf、HLA/k-ras、HLA/MAGE-A3、hTERT、LMP2、MAGE1、MAGE2、MAGE3、MAGE4、MAGE6、MAGE12、MART-1、ML-IAP、Muc2、Muc3、Muc4、Muc5、Muc16、MUM1、NA17、NY-BR1、NY-BR62、NY-BR-85、NY-ESO1、p15、p53、PAP、PAX3PAX5、PCTA-1、PLAC1、PRLR、PRAME、RAGE蛋白、Ras、RGS5、Rho、SART-1、SART-3、Steap-1、Steap-2、生存素、TAG-72、TGF-β、TMPRSS2、Tn、TRP-1、TRP-2、酪氨酸酶、uroplakin-3、PSMA。优选地，双特异性异源二聚体免疫球蛋白或其片段或双特异性抗体结合CD3和HER2或CD3和CD38或CD3和OX40或CD3和CD19或CD3和CD20或CD3和爱必妥或CD3和维克替比。

[0219] 蛋白A：蛋白A是细胞壁成分，由几个金黄色葡萄球菌(Staphylococcus aureus)菌株产生，为单个多肽链。蛋白A基因产物由5个同源重复组成，其以串联方式附着到该病原体的细胞壁上。这5个结构域大约58个氨基酸长，命名为EDABC，每一个都显示出免疫球蛋白结合活性(Tashiro M&Montelione GT(1995)Curr.Opin.Struct.Biol.,5(4):471-481)。这5个同源免疫球蛋白结合结构域折叠成三螺旋捆。每个结构域都能够结合来自许多哺乳动物物种的蛋白质，最显著的是IgG(Hober S et al.,(2007)J.Chromatogr.B Analyt.Technol.Biomed.Life Sci.,848(1):40-47)。蛋白A与大多数免疫球蛋白的重链在

Fc区中结合，但在人VH3家族的情况中也可以在Fab区中结合(Jansson B et al,(1998) FEMS Immunol.Med.Microbiol.,20(1):69-78)。蛋白A结合来自各种物种，包括人、小鼠、兔和豚鼠的IgG，但不结合人IgG3(Hober S et al.,(2007)同上引文)。人IgG3不能结合蛋白A，可以由人IgG3 Fc区中H435R和Y436F替代来解释(EU编号，Jendeberg等，(1997) J.Immunol.Methods,201(1):25-34)。除了IgG外，蛋白A还与IgM和IgA相互作用。

[0220] 在人VH亚类中，VH3是唯一结合蛋白A的亚类(Graille M et al.,(2000) Proc.Natl.Acad.Sci.USA 97(10):5399-5404)，蛋白A的所有5个结构域已知都结合该可变结构域亚类(Jansson B et al,(1998)FEMS Immunol.Med.Microbiol.,20(1):69-78)。基于VH3的免疫球蛋白或其片段对生物技术行业至关重要。由于其结合蛋白A的能力有利于其功能性预筛选，故基于VH3的分子已经被广泛地开发，并且由此用于抗体开发的许多基于合成的或供体的噬菌体展示文库或转基因动物技术都基于VH3亚类。此外，由于其良好的表达和稳定性，基于VH3的抗体常优于其他已知的重链可变结构域亚类而被选择。

[0221] 蛋白A以高亲合力结合抗体的能力是其在生物制药中工业规模应用的驱动力。用于在生物制药中生产抗体的蛋白A通常在大肠杆菌中重组生产，并具有与天然蛋白A基于上相同的功能(Liu HF et al.,(2010)MAbs,2(5):480-499)。最通常的，重组蛋白A与固定相色谱树脂结合用于纯化抗体。最佳结合发生在pH8.2，但在中性或生理条件(pH 7.0-7.6)也发生良好的结合。洗脱通常通过将pH迁移至酸性pH(甘氨酸-HCl,pH2.5-3.0)实现。这可以有效地解离大多数蛋白-蛋白和抗体-抗原结合相互作用，而不会永久地影响蛋白质的结构。然而，一些抗体和蛋白会因低pH而被破坏，最好在回收后通过加入十分之一体积的碱性缓冲液例如1M Tris-HCl,pH 8.0进行立即中和，以最小化在低pH条件中的持续时间。

[0222] 有多种商业可得的蛋白A色谱树脂。这些介质之间的主要差别在于支持基质的类型、蛋白A配体修饰、孔径和粒径。这些因素的差异造成在可压缩性、化学和物理稳健性、扩散阻力和吸附剂结合能力方面的差别(Hober S et al.,(2007)，同上引文)。蛋白A色谱树脂的实例包括但不限于用于实施例中的MabSelect SuRe™蛋白A树脂和MabSelect™蛋白A树脂(来自GE Healthcare)。

[0223] 术语“色谱”是指蛋白液相色谱，包括快速蛋白液相色谱(FPLC)——常用于分析或纯化蛋白混合物的一种液相色谱形式。如在其它形式的色谱中一样，由于混合物的不同成分对两个物质——流动液体(流动相)及其通过的多孔相(固定相)——具有不同亲合力，分离是可能的。在FPLC中，流动相是水性溶液或“缓冲液”。缓冲液流速可以在重力流下运行，或通过正排量泵(通常保持在恒定速度)控制，而缓冲液的组成可以通过从两个或两个以上外在贮库抽取不同比率的液体来改变。固定相可以是由珠子组成的树脂，通常为交联琼脂糖，装填在圆柱形玻璃或塑料柱中。取决于应用，可以获得宽范围的珠子大小和表面配体的FPLC树脂。

[0224] “亲和色谱”方法涉及使用亲和试剂作为配体，其与固定相交联，对特定的分子或一类分子具有结合亲合力。配体可以是生物分子，如蛋白配体，或可以是合成的分子。两类配体都倾向于具有良好的特异性。生产中最常用的蛋白配体是亲和试剂蛋白A。在亲和色谱中，当溶液(例如含有目的蛋白的粗制细胞上清液)上样到柱上后，通常靶蛋白被吸附，而允许杂质(其它蛋白、脂质、碳水化合物、DNA、色素等)通过柱子。吸附剂本身通常被装填在色谱柱中；但吸附阶段也可以通过以搅拌浆的形式使用吸附剂以分批结合模式进行。吸附后

的下一阶段是洗涤阶段，其中洗涤吸附剂以去除残余杂质。然后以半纯或纯形式洗脱结合的蛋白质。洗脱通常通过如下方式进行：改变缓冲液或盐组成，以便蛋白质不再与固定化的配体相互作用并被释放。在一些情况中，目的蛋白可以不结合亲和树脂，亲和色谱定向于结合不需要的杂质，由此可以收集未结合的级分以分离目的蛋白。亲和色谱可以在固定床或流化床中进行。

[0225] 术语“梯度模式色谱”是指这样的色谱方法，其中“洗脱”缓冲液(缓冲液B)的比例以逐步或逐渐升高的方式从0％增加到100％。

[0226] 术语“捕获-洗脱模式色谱”或“捕获-洗脱纯化模式”或“捕获-洗脱纯化”是指这样的色谱方法，其中“洗脱”缓冲液(缓冲液B)的比例不是以逐步或逐渐升高的方式从0％增加到100％，而是在捕获和任选地以运行缓冲液(缓冲液A)洗涤步骤后直接地以100％施加。

[0227] 靶向CD3的异源二聚体免疫球蛋白的开发

[0228] 本发明提供结合CD3蛋白复合物的表位结合区，其包含如上所述的重链和轻链CDR，并且还包含重链可变框架区，所述重链可变框架区是人基因IGHV3-23*04(SEQ ID NO:22)的产物或衍生自人基因IGHV3-23*04(SEQ ID NO:22)。重链可变框架区可以包含至少一个来自包含氨基酸序列SEQ ID NO:18的相应鼠抗体OKT3的重链可变区的相应框架区的氨基酸修饰。优选地，氨基酸修饰是氨基酸取代。通常，在框架区内进行不超过7个、优选不超过6个、优选不超过5个、优选不超过4个、更优选不超过3个、甚至更优选不超过2个、最优选不超过1个氨基酸修饰。在一些实施方案中，本公开提供结合CD3蛋白复合物的表位结合区，其中重链可变区的框架区的氨基酸修饰包括在选自以下组的氨基酸位置的氨基酸取代：34、48、49、58、69、71和73，并且其中每个组成员的氨基酸位置根据Kabat编号来指示。优选地，重链可变区的框架区的氨基酸取代选自：I34M、V48I、A49G、R58N、R58Y、I69L、A71T和T73K。重链可变区的框架区的优选氨基酸取代位于选自34、49和71的氨基酸位置。重链可变区的框架区的更优选氨基酸取代选自I34M、A49G和A71T。

[0229] 在另一方面，结合CD3蛋白复合物的第一多肽的表位结合区包含轻链可变框架区，所述轻链可变框架区是选自以下的人基因的产物或衍生自选自以下的人基因：IGKV1-39*01(SEQ ID NO:23)和IGKV3-20*01(SEQ ID NO:24)。轻链可变框架区包含至少一个来自包含氨基酸序列SEQ ID NO:19的相应鼠抗体OKT3的轻链可变区的相应框架区的氨基酸修饰。优选地，氨基酸修饰是氨基酸酸取代。通常，在框架区内进行不超过8个、优选不超过7个、优选不超过6个、优选不超过5个、优选不超过4个、更优选不超过3个、甚至更优选不超过2个、最优选不超过1个的氨基酸修饰。在一些实施方案中，本公开提供结合CD3蛋白复合物的表位结合区，其中轻链可变区序列的框架区的氨基酸修饰包括在选自以下氨基酸位置的氨基酸取代：4、33、34、46、47、66、71和96。优选地，轻链可变区的框架区的氨基酸取代选自：M4L、V33M、A34N、L46R、L47W、R66G、F71Y和P96F。轻链可变区的框架区的优选氨基酸取代位于选自4、46和47的氨基酸位置。轻链可变区的框架区的更优选氨基酸取代选自M4L、L46R、L47W和F71Y。在一些实施方案中，结合CD3蛋白复合物的第一多肽的表位结合区可以包含如上所述的重链可变区序列的框架区的氨基酸修饰和如上所述的轻链可变区序列的框架区的氨基酸修饰。

[0230] 本公开还提供了结合CD3蛋白复合物的抗体或其片段，其包含选自SEQ ID NO:27至38、64-68和359的重链序列，优选选自SEQ ID NO:359的重链序列。本公开还提供了结合

CD3蛋白复合物的抗体或其片段，其包含选自SEQ ID NO:39至47、69至90、360、339和400的轻链序列，优选SEQ ID NO:360的轻链序列。

[0231] 鉴于这些重链和轻链可变区序列每一个都结合CD3蛋白复合物，故可以将这些重链和轻链可变区序列进行"混配"以产生本发明的抗CD3结合分子。可以使用例如实施例中描述的结合测定法，测试该"混配"的抗体的CD3结合。

[0232] 工程化改造免疫球蛋白恒定区以促进异源二聚体形成超过同源二聚体形成

[0233] 本领域已知产生异源二聚体免疫球蛋白的方法，最简单的一个方法依赖于在一个细胞中表达两个不同的免疫球蛋白链(WO95/33844,Lindhofer H&Thierfelder S)。不经工程化改造，该直接方法存在同源二聚体的形成超过目的异源二聚体形成的局限性(Kufer P et al.,(2004)Trends Biotechnol.,22(5):238-244)。当使用增强重链异源二聚体化的互补技术(Merchant AM et al.,(1998)Nat.Biotechnol.,16(7):677-681)时，可以实现较多的异源二聚体产生，但仍有显著量的不期望的同源二聚体生成(Jackman J et al.,(2010) J Biol Chem.,285(27):20850-9,Klein C et al.,(2012)MAbs,4(6):653-63)。因此，本发明利用**BEAT®**技术方法(PCT公布号WO2012/131555)，该方法基于生物模拟的独特概念，其在异源二聚体化方面显示出优于现有技术方法。BEAT技术基于在天然的同源或异源二聚体免疫球蛋白结构域对之间的界面交换来产生新的异源二聚体，该新的异源二聚体可以用作基于Fc的双特异性抗体的构件。

[0234] 一方面，本发明提供包含第一和第二多肽的异源二聚体免疫球蛋白或其片段，其中第一和第二多肽包含具有修饰的CH3结构域的工程化免疫球蛋白恒定区，所述修饰的CH3结构域具有蛋白-蛋白界面，其中第一多肽的蛋白-蛋白界面包含在选自以下位置的氨基酸取代:3,5,7,20,22,26,27,79,81,84,84.2,85.1,86,88和90(**IMGT®**编号)，并且其中第二多肽的蛋白-蛋白界面包含在位置84.4和在选自以下位置的氨基酸取代:3,5,7,20,22,26,27,79,81,84,84.2,85.1,86,88和90(**IMGT®**编号)。

[0235] 在再一实施方案中，本发明提供异源二聚体免疫球蛋白或其片段，其中第一和第二多肽包含具有修饰的CH3结构域的工程化免疫球蛋白恒定区，所述修饰的CH3结构域具有蛋白-蛋白界面，其中第一多肽的蛋白-蛋白界面包含在位置88和在选自下组的位置的氨基酸取代:3,5,7,20,22,26,27,79,81,84,84.2,85.1,86和90(**IMGT®**编号)，并且其中第二多肽的蛋白-蛋白界面包含在位置85.1和/或86以及在选自下组的位置的氨基酸取代:3,5,7,20,22,26,27,79,81,84,84.2,84.4,88和90(**IMGT®**编号)，其中第一工程化免疫球蛋白恒定区中位置88的取代氨基酸残基与第二工程化免疫球蛋白恒定区中位置85.1和/或86的取代氨基酸残基相互作用，其中每个组成员的氨基酸位置根据**IMGT®**编号表示。

[0236] 优选地，第一工程化免疫球蛋白恒定区的蛋白-蛋白界面中位置88的取代氨基酸残基是88W及其保守氨基酸取代，其中氨基酸位置根据**IMGT®**编号给出。更优选地，第一工程化免疫球蛋白恒定区的蛋白-蛋白界面中位置88的取代氨基酸残基是88W，且其中第一工程化免疫球蛋白恒定区的蛋白-蛋白界面中另外的取代氨基酸残基选自:3A,20V,20T,20A,20N,20Q,20E,20S,20K,20W,22A,22G,22T,22L,22I,22V,26R,26Q,26T,26K,26V,26S,26N,26E,79Y,85.1T,85.1M,85.1A,85.1S,85.1R,85.1H,85.1K,85.1F,85.1C,85.1N,85.1W,86S,86I,86T,86H,86Q,86V,86W,86Y,86F和90N,其中氨基酸位置根据**IMGT®**编

号表示。

[0237] 优选地，在第二工程化免疫球蛋白恒定区的蛋白-蛋白界面中在位置85和86进行取代的氨基酸残基选自：85.1A，85.1S，85.1C和86S及其保守氨基酸取代(IMGT®编号)。更优选地，在第二工程化免疫球蛋白恒定区的蛋白-蛋白界面中进行取代的氨基酸残基选自：85.1A，85.1S，85.1C和86S，且其中在第二工程化免疫球蛋白恒定区的蛋白-蛋白界面中进行取代的其他氨基酸残基选自：3E，5A，7F，20T，22V，26T，81D，84L，84.2E，88R和90R及其保守氨基酸取代(IMGT®编号)。

[0238] 在优选的实施方案中，在第一工程化免疫球蛋白恒定区的蛋白-蛋白界面中在位置88进行取代的氨基酸残基为88W，并且其中在第一工程化免疫球蛋白恒定区的蛋白-蛋白界面中进行取代的其他氨基酸残基是：3A、20K、22V、26T、79Y、85.1S、86V和90N，且其中在第二工程化免疫球蛋白恒定区的蛋白-蛋白界面中在位置85.1和86进行取代的氨基酸残基为85.1A、85.1S或85.1A和86S，并且其中在第二工程化免疫球蛋白恒定区的蛋白-蛋白界面中进行取代的其他氨基酸残基是：3E、5A、7F、20T、22V、26T、81D、84L、84.2E、84.4Q、88R和90R(IMGT®编号)。

[0239] 在替代的实施方案中，本发明提供了异源二聚体免疫球蛋白或其片段，其中第一和第二多肽包含具有修饰的CH3结构域的工程化的免疫球蛋白恒定区，所述修饰的CH3结构域具有蛋白-蛋白界面，其中第一多肽的蛋白-蛋白界面包含在位置20和在选自下组的位置的氨基酸取代：3、5、7、22、26、27、79、81、84、84.2、85.1、86、88和90，以及其中第二多肽的蛋白-蛋白界面包含在位置26和在选自下组的位置的氨基酸取代：3、22、27、79、81、84、85.1、86和88，其中在第一工程化免疫球蛋白恒定区中位置20进行取代的氨基酸残基与在第二工程化免疫球蛋白恒定区中位置26进行取代的氨基酸残基相互作用，

[0240] 其中每个组成员的氨基酸位置根据IMGT®编号表示。

[0241] 优选地，在第一工程化免疫球蛋白链的蛋白-蛋白界面中进行取代的氨基酸残基包含位置20和22上的氨基酸残基和任选地在选自以下的位置上的其它氨基酸残基：3、5、7、26、27、79、81、84、84.2、84.4、85.1、86、88和90，其中在第二工程化免疫球蛋白链的蛋白-蛋白界面中进行取代的氨基酸残基包含在位置26和选自3、5、7、20、22、27、79、81、84、84.2、84.4、85.1、86、88和90的其他位置上的氨基酸残基，其中每个组成员的氨基酸位置根据IMGT®编号表示。优选地，在第一工程化的免疫球蛋白链的蛋白-蛋白界面中进行取代的氨基酸残基包含位置20和22上的氨基酸残基、以及任选地在选自以下位置上的其它氨基酸残基：3、5、7、26、27、79、81、84、84.2、84.4、85.1、86、88和90，其中在第二工程化的免疫球蛋白链的蛋白-蛋白界面中进行取代的氨基酸残基包含位置26和86上的氨基酸残基和任选地在选自3、5、7、20、22、27、79、81、84、84.2、84.4、85.1、88和90的其他位置上的氨基酸残基，其中每个组成员的氨基酸位置根据IMGT®编号表示。

[0242] 更优选地，在第一工程化的免疫球蛋白恒定区的蛋白-蛋白界面中位置20上进行取代的氨基酸残基选自20V、20T、20A、20N、20Q、20K、20S、20W和20E，其中在第一工程化的免疫球蛋白恒定区的蛋白-蛋白界面中取代的其他氨基酸残基选自3A、22A、22G、22L、22I、22V、22T、26K、26R、26Q、26T、26V、26S、26N、26E、79Y、85.1W、85.1F、85.1T、85.1M、85.1A、85.1S、85.1R、85.1H、85.1K、85.1C、85.1N、86W、86Y、86S、86I、86H、86Q、86V、86T、86F、88Q、88L、88V、88R、88E、88T、88I、88Y、88K、88W和90N，其中在第二工程化的免疫球蛋白恒定区的

蛋白-蛋白界面中位置26上取代的氨基酸残基选自26T和26E及其保守氨基酸取代，其中氨基酸位置根据IMGT®编号表示。

[0243] 在最优选的实施方案中，在第一工程化的免疫球蛋白恒定区的蛋白-蛋白界面中位置20上取代的氨基酸残基是20K，并且其中在第一工程化的免疫球蛋白恒定区的蛋白-蛋白界面中取代的其他氨基酸残基是3A、22V、26T、79Y、85.1S、86V、88W和90N，并且其中在第二工程化的免疫球蛋白恒定区的蛋白-蛋白界面中位置26上取代的氨基酸残基是26T，其中在第二工程化的免疫球蛋白恒定区的蛋白-蛋白界面中取代的其他氨基酸残基是3E、5A、7F、20T、22V、81D、84L、84.2E、84.4Q、85.1C/S/A、86S、88R和90R(IMGT®编号)。

[0244] 靶向CD3和疾病相关抗原的异源二聚体免疫球蛋白的开发

[0245] 第一步(实施例1)，在基于FAB或scFv片段的同源二聚体免疫球蛋白中测定降低或消除蛋白A结合的取代。已经发现，在具有降低蛋白A结合或消除蛋白A结合的取代的重链中存在VH3亚类的可变重链结构域，会阻碍基于蛋白A的任何差异亲和方法。发现对于基于蛋白质A的差异亲和力方法，这些主要障碍的解决方案在于形成降低或消除蛋白A与VH3亚类结合的框架取代。

[0246] 第二步(实施例2.1)，通过将鼠抗CD3抗体的CDR嫁接到IGVH3-23和IGVK1或IGVK3人种系框架上产生靶向人CD3(ε亚基)的人源化抗体。最好的人源化变体使用G65S或N82aS取代(Kabat编号)消除了在其VH结构域中存在的蛋白A结合位点。这些变体被制备成FAB或scFv片段形式。

[0247] 第三步，产生靶向疾病相关抗原的抗体的抗原结合位点。可将鼠抗体的CDR嫁接到人种系框架IGVH3-23和IGVK1上(实施例2.3、2.4和2.6-2.10)。或者，从噬菌体展示文库分离的抗体的CDR可以基于VH3可变结构域亚类或嫁接到人种系框架IGVH3-23和IGVK1上(实施例2.5和2.6)。使用G65S或N82aS取代(Kabat编号)消除了表位结合区的VH结构域中的蛋白A结合位点。

[0248] 第四步，基于BEAT®技术(如WO2012/131555中所述)制备了异源二聚体抗体，其中来自实施例2.1 2 2的抗CD3抗体和如实施例2.2-2.10中描述的疾病相关抗原的表位结合区以scFv-FAB形式使用，或反之亦然(实施例3.1)。由于同源和异源二聚体物质之间蛋白A结合位点数量的差异可用于通过蛋白A色谱法分离异源二聚体物质，故对本发明的双特异性抗体进行了工程化，导致两个同源二聚体之一不具有蛋白A结合位点，因此不与蛋白A树脂结合。此外，为了改善BEAT抗体的安全谱，通过将两个取代L234A和L235A(EU编号)工程化到Fc区的下铰链区，以降低或消除Fc受体结合。

实施例

[0249] 材料和方法

[0250] 构建用于瞬时哺乳动物细胞表达的表达载体

[0251] 首先编码不同多肽链的cDNA部分或全部由GENEART AG(Regensburg,Germany)合成，并使用标准分子生物学技术修饰。用合适的DNA限制酶消化PCR产物，纯化，连接入之前已经用相同的DNA限制酶消化的修饰的pcDNA3.1质粒(Invitrogen AG,Zug,瑞士)中，所述质粒带有CMV启动子和牛激素多聚腺苷酸化序列(poly(A))。所有的多肽链独立地连接入该表达载体中，其中由鼠源VJ2C前导肽驱动多肽链的分泌。

[0252] 重组蛋白的表达

[0253] 抗体、ScFv-Fc融合蛋白、BEAT抗体和抗原，除非另行说明，否则如下述进行表达。对于瞬时表达，使用聚乙烯亚胺(PEI;Sigma,Buchs,瑞士)，将各工程化链载体以等量共转染至悬浮适应化的HEK293-EBNA细胞(ATCC-LGL标准,Teddington,UK;Cat.No:CRL-10852)中。典型地，100ml悬浮细胞(密度0.8-1.2百万个细胞/ml)用DNA-PEI混合物转染。在将编码各工程化链基因的重组表达载体引入宿主细胞中后，进一步培养细胞4至5天以允许免疫球蛋白构建体分泌到培养基(EX-CELL 293,HEK293-无血清培养基(Sigma)，补充了0.1% pluronic acid,4mM谷氨酰胺和0.25μg/ml遗传霉素)中，产生免疫球蛋白构建体。通过离心制备含有分泌的免疫球蛋白的无细胞培养物上清液，之后无菌过滤并用于进一步的分析。

[0254] 差异蛋白A亲和色谱(实施例1)

[0255] Fc133片段和同源二聚体scFv-Fc免疫球蛋白的纯化

[0256] 捕获-洗脱模式色谱

[0257] 将上清液在纯化前用0.1体积(V)1M Tris-HCl pH8.0进行调节。将蛋白G Sepharose™ 4 Fast Flow(GE Healthcare Europe GmbH,Glattbrugg,瑞士;目录号17-0618-01)加入到调节的上清液中。混合物在4℃下孵育过夜。孵育后，用10CV PBS pH7.4洗涤结合的蛋白质，用4个柱体积(CV)的0.1M甘氨酸，pH3.0洗脱，并用0.1V的1M Tris-HCl pH8.0中和。通过SDS-PAGE(NuPAGE Bis-Tris 4-12%丙烯酰胺,Invitrogen AG,Basel,瑞士)在非还原条件下分析上清液、流通液(flow through)和洗脱级分。

[0258] 梯度模式色谱

[0259] 生成后，使用蛋白G Sepharose™ 4 Fast Flow(上文)、在捕获-洗脱模式色谱中首先纯化含有Fc133片段的细胞培养物上清液。随后将来自捕获-洗脱模式色谱的洗脱物质加载到1ml HiTrap™ MabSelect SuRe™蛋白A柱(蛋白A结合位点突变体)上。柱在0.2M磷酸盐柠檬酸盐缓冲液pH8.0中预平衡，并在**ÄKTApurifier™**色谱系统(柱和仪器都来自GE Healthcare Europe GmbH;柱目录号:11-0034-93)上以1ml/分钟的流速操作。组合不同量的两种缓冲液(运行缓冲液(A):0.2M磷酸盐柠檬酸盐缓冲液pH8.0和洗脱缓冲液(B):0.04M磷酸盐柠檬酸盐缓冲液pH3.0)以pH线性梯度进行洗脱。线性梯度在五个柱体积(CV)中从0%B至100%B。洗脱的级分用0.1V的1M Tris-HCl pH 8.0中和。上清液、流通液和洗脱级分在非还原条件下通过SDS-PAGE(NuPAGE Bis-Tris 4-12%丙烯酰胺,Invitrogen AG,Basel,瑞士)分析。

[0260] 同源二聚体FAB-Fc免疫球蛋白和FAB片段的纯化。

[0261] 生成后，细胞培养物上清液用0.1V的1M Tris-HCl pH 8.0调节。将蛋白L树脂(Genescript,Piscataway,USA)加入到调节的上清液中，并在4℃孵育过夜。孵育后，用10CV的PBS,pH7.4洗涤结合的蛋白质，用4CV的0.1M甘氨酸，pH3.0洗脱，最后用0.1V的1M Tris-HCl pH8.0中和。为了评估蛋白A结合，将蛋白L纯化的FAB在pH8.0(柠檬酸/Na_2HPO_4缓冲液)注射到1ml HiTrap MabSelect™柱(GE Healthcare Europe GmbH,Glattbrugg,瑞士)上。组合不同量的两种缓冲液(运行缓冲液(A):0.2M磷酸盐柠檬酸盐缓冲液pH8.0和洗脱缓冲液(B):0.04M磷酸盐柠檬酸盐缓冲液pH3.0)用pH线性梯度进行洗脱。线性梯度在5CV中从0%B到100%B。洗脱的级分用0.1V的1M Tris-HCl pH8.0中和。通过SDS-PAGE(NuPAGE Bis-Tris 4-12%丙烯酰胺,Invitrogen AG,Basel,瑞士)在非还原条件下分析上清液、流通液和洗脱

级分。

[0262] 基于VH3的同源二聚体FAB-Fc和scFv-Fc免疫球蛋白(在其Fc和VH3结构域中消除了与蛋白A的结合)的纯化和测试。

[0263] 纯化方案包括根据上述程序进行捕获-洗脱模式色谱、然后进行梯度模式色谱。

[0264] 差异蛋白A亲和色谱(实施例1和3)

[0265] 产生后,将无细胞上清液加载到在0.2M磷酸盐柠檬酸盐缓冲液pH6.0中预平衡的1ml HiTrap™ MabSelect SuRe™蛋白A柱上,并在ÄKTApurifier™色谱系统上(两者均来自GE Healthcare Europe GmbH;柱目录号:11-0034-93)以1ml/min流速运行。运行缓冲液是0.2M磷酸盐柠檬酸盐缓冲液pH6。使用20mM柠檬酸钠缓冲液pH4洗脱目的异源二聚体,而使用0.1M甘氨酸pH3.0洗脱同源二聚体物质。

[0266] 通过280nm的OD读数,跟踪洗脱;合并含有目的异源二聚体的级分,用0.1体积的1M Tris pH 8.0(Sigma)中和。

[0267] 通过SDS-PAGE(NuPAGE Bis-Tris 4-12%丙烯酰胺,Invitrogen AG,Basel,瑞士)在非还原条件下分析上清液、流通液和洗脱级分。

[0268] 差示扫描量热法(DSC)

[0269] 使用量热测量,比较抗体的热稳定性。在VP-DSC差式扫描微量热仪(MicroCal-GE Healthcare Europe GmbH,Glattbrugg,瑞士)上,进行量热测量。细胞体积是0.128ml,加热速度是1℃/min,保持过压在64p.s.i..所有蛋白片段均以浓度1-0.5mg/ml在PBS(pH 7.4)中使用。通过与含有相同缓冲液但已经省去了蛋白的重复样品进行比较,估计每个蛋白的摩尔热容。使用标准程序分析了偏摩尔热容和熔解曲线。温谱图进行了基线校正和浓度标化后,使用非二态模型(Non-Two State model)在软件Origin v7.0中进一步分析。

[0270] 人IgG亚类的预期熔解曲线是已知的(Garber E&Demarest SJ(2007)Biochem Biophys Res Commun,355(3):751-7),并且所有曲线均显示出含有三个解折叠过渡,相应于CH2、CH3和FAB结构域的独立解折叠。在4个人IgG亚类中,IGHG1具有最稳定的CH3结构域(~85℃);而其它亚类CH3结构域较不稳定,但已知无一在70℃以下熔解。类似地,已知所有亚类的CH2结构域具有~70℃的熔解温度。

[0271] 通过毛细管凝胶电泳进行的纯度评估(实施例3.2)

[0272] 非还原样品制备

[0273] 将40μg脱盐的蛋白样品缓冲在含有5mM碘乙酰胺(Sigma)的SDS样品缓冲液(Beckman Coulter International S.A.,Nyon,瑞士;IgG Purity Kit,Cat.No:A10663)中。在样品中加入10-kDa内标。样品混合物在70℃加热10分钟。

[0274] 毛细管凝胶电泳

[0275] 样品制备后,在配备有设定在220nm的光电二极管阵列检测器(DAD)的ProteomeLab PA 800(Beckman Coulter International S.A.,Nyon,瑞士)上,运行样品。使用裸露的熔融石英毛细管50μm ID×30.2cm(至检测器的20.2cm有效长度)作为分离介质。样品注射和分离分别在5和15kV恒压进行,在SDS分子量凝胶缓冲液中反转极性。以2Hz的速度记录数据,在分离过程中电流稳定。毛细管和样品被恒温调节在25℃。

[0276] 通过SPR的亲和力测量(实施例1)

[0277] 消除了对蛋白A的结合的FAB片段的SPR检测

[0278] 将编码与IGHG1 Fc片段融合的人HER2细胞外区的cDNA克隆到与上述重链和轻链表达载体相似的表达载体中，使用PEI方法瞬时转染到HEK293E细胞中(参见PCT公开号：WO2012131555)。用0.1V的1M Tris-HCl pH8.0调节上清液，通过蛋白A捕获-洗脱色谱法纯化抗原，如实施例1所述。对于SPR实验，使用单克隆小鼠抗人IgG(Fc)抗体传感器芯片，这允许在正确的方向捕获Fc融合的重组HER2抗原(人抗体捕获试剂盒，目录号BR-1008-39，GE Healthcare Europe GmbH)。测量值记录在BIAcore™2000仪器(GE Healthcare Europe GmbH，Glattbrugg，瑞士)上。将抗HER2 FAB的不同稀释液(50、25、12.5、6.25、3.13、1.57、0.78、0.39nM)以30μl/min注射到传感器芯片上，持续4分钟。对于每次测量，在解离7分钟后，以30μl/min注射3M $MgCl_2$溶液1分钟以进行再生。数据(传感图：fc2-fc1)拟合1:1Langmuir。为了解决在每次测量开始时捕获的HER2-Fc的实验变异，在所有拟合中将Rmax值设置为局部的。测量进行一式两份，包括用于参考的零浓度样品。使用Chi2和残差值来评估实验数据和各结合模型之间拟合的质量。

[0279] 通过SPR的亲和力测量(实施例2和3)

[0280] 使用SPR分析测量不同抗体(鼠源和人源化抗体)结合动力学的结合和解离速度常数。在BIAcore 2000仪器(BIAcore-GE Healthcare Europe GmbH，Glattbrugg，瑞士)上室温测量抗体的结合动力学，用BiaEvaluation软件(4.1版，BIAcore-GE Healthcare Europe GmbH)分析。

[0281] 在CM5传感器芯片(GE Healthcare Europe GmbH，Cat.No:BR-1000-14)上进行测量，所述芯片使用商业胺偶联试剂盒(GE Healthcare Europe GmbH，Cat.No:BR-1000-50)分别偶联了目的配体。蛋白G配体来自Pierce(Thermo Fisher Scientific-Perbio Science S.A.，Lausanne，瑞士，Cat.No:21193)。

[0282] 数据(传感图：fc2-fc1)拟合1:1Langmuir模型(如所示的，有或无传质)。在捕获实验中，为了解决在每次测量开始时的实验变异性，在所有拟合中将Rmax值设置为局部的(local)。解离时间为至少350秒。一式三份进行测量，测量包括零浓度样品作为参照。使用Chi2和残差值来评估实验数据和各结合模型之间拟合的质量。

[0283] 通过FACS在HPB-ALL细胞上的亲合力测量

[0284] HPB-ALL细胞(DSMZ，Braunschweig，德国，Cat.No:ACC483)作为CD3阳性细胞系用于FACS染色。HPB-ALL维持在补充了10％FCS和100U/ml青霉素及100ug/ml链霉素的RPMI 1640中。将嵌合OKT3抗体和人源化变体的100μl系列稀释物与$4x10^5$HPB-all细胞在补充了1％BSA和0.1％叠氮化钠(称作FACS缓冲液)的PBS中于冰上孵育45分钟。无关人IgG1用作同种型对照，并且嵌合OKT3抗体用作阳性对照。洗涤后，细胞与1/200稀释的抗人Fc-PE(EBioscience，Vienna，Austria)在冰上孵育45分钟。然后再次洗涤细胞并重悬在200ul FACS缓冲液中。在FACSCalibur(BD Biosciences，Allschwil，瑞士)上测量了每个样品的相对平均荧光。结果总结在图9中，表示为与嵌合OKT3抗体相比，HBP-ALL的相对染色。

[0285] 用于体外测定法的细胞系

[0286] 人HER2阳性细胞系

[0287] 表达HER2抗原的人细胞已经在PCT公开号WO2010108127中描述。本文使用的HER2阳性人细胞系如下：

[0288] BT474(ATCC-LGL标准品；目录号：HTB-20)

[0289] 培养条件:在150cm^{2}组织培养瓶(TPP,Trasadingen,瑞士;目录号:90150)中补充了10%热灭活FBS、1%青霉素-链霉素(Invitrogen AG,目录号:10378-016)、1%丙酮酸钠溶液(PAA Laboratories,Pasching,Austria;目录号:S11-003)、1%MEM非必需氨基酸(PAA Laboratories,目录号:M11-00dsmz3)和1%GlutaMAX-1(Invitrogen AG,目录号:35050-038)的RPMI培养基。细胞每周传代两次。

[0290] JIMT-1(DSMZ,Braunschweig,德国,目录号:ACC589)

[0291] 培养条件:补充了10%热灭活的FBS、1%青霉素-链霉素(Invitrogen AG,目录号:10378-016)、1%丙酮酸钠溶液(PAA实验室,目录号:S11-003)、1%MEM非必需氨基酸(PAA实验室,目录号:M11-003)和1%GlutaMAX-1(Invitrogen AG,目录号:35050-038)的Dulbeco改良的必需培养基(DMEM(1X))+GlutaMAX-1(Invitrogen AG,目录号:31966-012)。细胞每周传代2-3次。

[0292] MDA-MB-231(ATCC-LGL标准品;目录号:HTB-26)。

[0293] 培养条件:与JIMT-1相同的培养条件。

[0294] HT-1080(ATCC-LGL标准品;目录号:CCL-121)。

[0295] 培养条件:将HT1080细胞在补充了10%热灭活FBS、1%青霉素-链霉素(Invitrogen AG,目录号:10378-016)和1%谷氨酰胺(Invitrogen AG,目录号:25030-024)的EMEM培养基中培养。细胞每周三次(1/6稀释)传代培养。

[0296] NCI-N87(ATCC-LGL标准品;目录号:CRL-5822)。

[0297] 培养条件:将NCI-N87细胞在具有10%热灭活FBS、1%青霉素-链霉素(Invitrogen AG,目录号:10378-016)、1%丙酮酸钠溶液(PAA实验室,Pasching,奥地利;目录号:S11-003)、1%MEM非必需氨基酸(PAA实验室,目录号:M11-00dsmz3)和1%谷氨酰胺(Invitrogen AG,目录号:25030-024)的RPMI 1640培养基中培养。细胞每周两次(1/3稀释)传代。

[0298] 人CD38阳性细胞系

[0299] 表达CD38抗原的人细胞描述在PCT公布号WO2005103083,WO2008047242,WO2011154453和WO2012092612中。在此使用的CD38阳性人细胞系如下:

[0300] 稳定的重组CHO[CD38]细胞

[0301] 在Source Biosciences(Berlin,Germany,Cat.-No.:IRAU37D11,4309086)订购了编码人CD38的基因。人CD38使用引物扩增,在5'端添加kozak序列、起始密码子、及随后的信号肽(鼠V前导序列),并在3'端添加NheI限制性位点。使用NheI和HindIII切割扩增子,克隆至pT1(内部开发的pcDNA3.1(Invitrogen AG)衍生载体)的表达盒中。pT1的表达盒使用两个IRES(内部核糖体进入位点)将目的基因的表达和GFP和PAC(嘌呤霉素抗性基因)的表达连接在一个多顺反子mRNA上。制备质粒的小量制备物,通过DNA测序验证了克隆的CD38开发阅读框。在50ml生物反应器(TubeSpin 50生物反应器,TPP,Trasadingen,瑞士)中,使用聚乙烯亚胺(**JetPEI®**,Polyplus-transfection,Illkirch,法国)转染悬浮CHO-S细胞(Invitrogen AG)。为此目的,将指数生长的细胞接种在OptiMEM培养基(Invitrogen AG,Cat.No.:31985-047)中。向细胞中加入**JetPEI®**:DNA复合物。细胞和**JetPEI®**:DNA复合物在37℃振摇(200RPM)孵育5h进行内吞后,向细胞悬浮液中加入一体积补充了4mM Gln的培养基PowerCHO2(Lonza,distributor RUWAG Lifescience,Bettlach,瑞士,Cat.No:BE12-771Q)。然后,将细胞在摇床上在37℃、5%CO2和80%湿度下孵育。转染后一天,将细胞

以不同浓度接种在96孔板中于含有嘌呤霉素的选择培养基(Sigma,Cat.No:P8833-25mg)中。在静止条件下选择大约14天后,使用TubeSpin 50生物反应器,以悬浮培养物形式扩增46个高GFP表达细胞合并物。一旦成功地适应悬浮后,通过FACS分析细胞的CD38。选择具有同质CD38染色谱的稳定CHO[CD38]克隆,并用于本文中。

[0302] 其它CD38阳性细胞系包括:

[0303] NCI-H929(ATCC-LGL标准品;Cat.No:CRL-9068).

[0304] Namalwa(ATCC-LGL标准品;Cat.No:CRL-1432)

[0305] U266(ATCC-LGL标准品;Cat.No:TIB-196)

[0306] RPMI 8226(ATCC-LGL标准品;Cat.No:CCL-155)

[0307] 培养条件:RPMI 1640培养基,补充10%热灭活的FBS,1%青霉素-链霉素(Invitrogen AG)和1%GlutaMAX-1(Invitrogen AG)

[0308] Raji(ATCC-LGL标准品;Cat.No:CCL-86)

[0309] Daudi(ATCC-LGL标准品;Cat.No:CCL-213)

[0310] 人OX40阳性细胞系

[0311] 表达OX40抗原的人细胞已经在PCT公开号:WO2013008171中描述了。

[0312] 外周血单核细胞(PBMC)和HBP-ALL是人OX40阳性细胞系的实例。

[0313] 本文使用稳定的重组CHO[OX40]细胞。使用与上述用于稳定的重组CHO[CD38]细胞系类似的方案,工程化携带编码人OX40的合成cDNA的重组CHO细胞系。

[0314] 人CD20阳性细胞系

[0315] 表达CD20抗原的人细胞已经在PCT公开号:WO2010095031中描述了。CD20+癌细胞的实例是Daudi癌细胞系(ATCC-LGL标准品;目录号:CCL-213),将这些B淋巴母细胞癌细胞培养在补充有20%FBS和1%P/S;1%L-Glut;1%Na-Pyr和1%NEAA的RPMI 1640培养基(Sigma)中。细胞在37℃和补充5%CO_2下培养。

[0316] 人EGFR阳性细胞系

[0317] 表达EGFR抗原的人细胞已经在PCT公开号:WO2010108127中描述。EGFR+癌细胞的例子是HT-29癌细胞系(ATCC-LGL标准品;目录号:HTB-38),将这些结肠直肠癌细胞培养在补充有10%FBS和1%P/S;1%L-Glut;1%Na-Pyr和1%NEAA的McCoy's 5A培养基(Sigma)中。细胞在37℃和补充5%CO_2下培养。

[0318] 人CD19阳性细胞系

[0319] 表达CD19抗原的人细胞在PCT公开号:WO2010/095031中描述。Namalwa(ATCC-LGL标准品;目录号:CRL-1432)和Raji(ATCC-LGL标准品;目录号:CCL-86)是人CD20阳性细胞系的实例。

[0320] 人膜IgE阳性细胞系

[0321] PCT公开号:WO2010/033736的第71页描述了通过加入白细胞介素-4(IL-4)和抗CD40抗体将人PBMC转化成产生IgE的B细胞的方法。

[0322] 重组靶抗原

[0323] 人CD3γ-ε-Fc融合蛋白

[0324] 首先由GENEART AG(德国雷根斯堡)合成cDNA,其编码通过26个残基肽接头(序列:GSADDAKKDAAKKDDAKKDDAKKDGS;SEQ ID NO:186)与人CD3ε胞外区(UniProt登录号:P07766,

残基22-118(SEQ ID NO:185))融合的人CD3γ胞外区(UniProt登录号:P09693残基23-103(SEQ ID NO:184);UniProt Consortium(2013)Nucleic Acids Res.,41(Database issue):D43-7;http://www.uniprot.org/)。使用标准重叠PCR技术以及从Geneart AG得到的人IgG1 Fc cDNA模板,将该合成基因与人IgG1 Fc部分融合。将得到的cDNA克隆到上述修饰的pcDNA3.1质粒中。

[0325] 对于CD3γ-ε-Fc蛋白(SEQ ID NO:187)的瞬时表达,如上所述,使用聚乙烯亚胺(PEI)将重组载体转染到悬浮适应的HEK-EBNA细胞(ATCC-CRL-10852)中。然后使用重组Streamline rProtein A培养基(GE Healthcare Europe GmbH,Glattbrugg,瑞士),将CD3γ-ε-Fc构建体从无细胞的上清液中纯化,并用于进一步分析。

[0326] 人和猕猴CD3ε1-26_Fc融合蛋白

[0327] 分别从获自GENEART AG的人和猕猴CD3ε胞外区的合成cDNA,PCR扩增编码人CD3ε肽1-26(UniProt登录号:P07766,氨基酸23-48,SEQ ID NO:188)的cDNA和编码猕猴CD3ε肽1-26(UniProt登录号:Q95LI5,氨基酸22-47,SEQ ID NO:189)的cDNA。随后使用标准重叠PCR技术,将扩增产物与人IgG1 Fc部分融合。人IgG1 Fc cDNA模板获自Geneart AG。将得到的cDNA克隆到上述修饰的pcDNA3.1质粒中。

[0328] 对于人和猕猴CD3ε构建体(分别为SEQ ID NO:190和191)的瞬时表达,如上所述使用聚乙烯亚胺(PEI)将重组载体转染入悬浮适应的HEK-EBNA细胞(ATCC-CRL-10852)。然后使用重组Streamline rProtein A培养基(GE Healthcare Europe GmbH,Glattbrugg,瑞士)从无细胞上清液中纯化CD3ε融合构建体,并用于进一步分析。这两种融合蛋白在本文中被称为人和猕猴CD3ε1-26_Fc融合蛋白。

[0329] 人HER2胞外区

[0330] 已经在PCT公开号WO2012131555中描述了HER2可溶性胞外区的制备。制备了融合至多组氨酸标签(本文称为HER2-his)或融合至人IgG1 Fc区(本文称为HER2-Fc)的人HER2可溶性胞外区。

[0331] 人和猕猴CD38胞外区

[0332] 人CD38的cDNA获自Source Biosciences(Erwin-Negelein-Haus,Germany,目录号:IRAU37D11,4309086),PCR扩增其胞外区(UniProt登录号:P28907残基43-300)并克隆到源自pcDNA3.1(Invitrogen AG)的内部表达载体。该表达载体包含在其多克隆位点5'端的kozak序列和起始密码子及随后的鼠VJ2C前导肽和在3'端的6-His标签。融合至6-His-标签的人CD38可溶性胞外区(SEQ ID NO:192)如下表达和纯化:在摇瓶中在37℃、5%CO_2和80%湿度下孵育1体积的含有HEK细胞、0.1%pluronic acid(Invitrogen AG)、表达载体和聚乙烯亚胺(**JetPEI®**,Polyplus-transfection,Illkirch,France)的RPMI 1640培养基(PAA实验室,目录号:E15-039)。4小时后,将1体积的补充有6mM谷氨酰胺的ExCell293培养基加入到混合物中,并进一步孵育共5天。生产后,通过离心并使用0.2μm过滤器过滤,制备无细胞上清液,使用Tris 1M pH8.7将pH调节至7.4(4℃)。将Ni-Sepharose Excell珠(GE Healthcare,目录号:17-3712-03)加入到溶液中并在4℃下搅拌过夜。将溶液装载至Econo-Column(Bio-Rad Laboratories AG,Reinach,瑞士,目录号737-4252)上用于重力流纯化。将珠子在PBS(2x)、20mM咪唑中洗涤,蛋白质在PBS、500mM咪唑中洗脱。合并洗脱的级分并在4℃下用两个透析步骤将缓冲液交换为PBS。使用0.22μm注射过滤器过滤纯化的人CD38胞外

区。

[0333] 使用如上所述的方法，克隆、表达和纯化融合至6-His标签(SEQ ID NO:193)的猕猴CD38抗原的可溶性胞外区。

[0334] 人OX40胞外区

[0335] PCT公开号:WO2013008171中描述了制备人OX40的可溶性胞外区的方法。

[0336] 人EGFR胞外区

[0337] EGFR可溶性胞外区抗原制备的实例已经在PCT公开号:WO2012131555中描述。

[0338] 体外T细胞重定向杀伤试验

[0339] 制备外周血单核细胞

[0340] 为了制备外周血单核细胞(PBMCs)，从瑞士La Chaux-de-Fonds的血液采集中心(Centre de Transfusion Sanguine et Laboratoire de Sérologie,rue Sophie-Mairet 29,CH-2300)，收集了含有人白细胞的血液过滤物。通过用含有10U/ml肝素(liquemin)(Drossapharm AG,Lucern,瑞士)的60ml PBS反洗，从过滤物分离细胞。然后用50mL Blood-Sep-Filter管(Brunschwig,Basel,瑞士)，按照生产商的说明书，纯化PBMCs。800g室温离心(无制动)管子20分钟，从界面收集细胞。用Roswell Park Memorial Institute(RPMI,PAA Laboratories,Pasching,Austria)培养基(无FBS或磷酸缓冲盐水PBS)，洗涤细胞3次。以10e6细胞/mL将PBMCs重悬在RDL培养基(补充了10％热灭活胎牛血清(FBS)和青霉素/链霉素的RPMI)中，37℃在5％CO2温箱中培养过夜，之后用于试验。

[0341] T细胞制备

[0342] 直接在PBMC分离后，使用pan-T细胞分离试剂盒II(Myltenyi Biotec GmbH,Bergisch Gladbach,Germany,Cat.No:130-091-156)，按照生产商的说明书，纯化T细胞。纯化后，以10e6细胞/mL将T细胞重悬在RDL培养基中，37℃在5％CO2温箱中培养过夜。

[0343] 试验读数

[0344] 使用给出高度相当结果的两个不同读出来定量重定向的杀伤。

[0345] 流式细胞术，在此处称作RDL-FACS法，基于Schlereth B等((2005)Cancer Res,65:2882-2889)，Moore PA等((2011)Blood,117(17):4542-51)和Friedrich M等((2012)Mol Cancer Ther,11:2664-2673)中描述的荧光-细胞术。收获靶细胞，计数，洗涤一次，以5x10e6细胞/mL重悬在PBS+1μM羧基荧光素琥珀酰亚胺酯(CFSE,Sigma)中。细胞在37℃孵育15分钟，每5分钟轻柔振荡一次。用RDL培养基洗涤CFSE加载的细胞，并以2x10e5细胞/mL重悬在RDL培养基中。收获PBMCs，计数并以2x10e6细胞/mL重悬在RDL培养基中。在RDL培养基中制备抗体系列稀释物(3x溶液)。将靶细胞(50μl/孔)，T细胞(50μl/孔)和3x抗体溶液(50μl/孔)分配到平底96孔板(TPP,Trasadingen,瑞士)中。效应物:靶比率是10:1。平板在5％CO2温箱中37℃孵育48h。孵育后，300g离心平板3分钟，通过轻敲平板，弃去上清液。用200μl PBS洗涤平板一次，再次离心，并弃去PBS。加入预温的accutase(Invitrogen AG)溶液，37℃孵育平板10分钟。加入100μL RDL培养基后，通过吹打将脱壁的贴壁细胞重悬。将溶液转移到U形底96孔板(TPP)中。300g离心U形底板3分钟。弃去上清液，细胞以1/40稀释度重悬于补充有7-AAD(Becton Dickinson AG,Allschwil,瑞士)的200μl冷FACS缓冲液(PBS+2％FBS+10％Versene)中。立即在Guava easyCyte™流式细胞计数器(Millipore AG,Zug,瑞士)上读取平板。对于每孔，通过门控CFSE阳性7ADD阴性群，使用**Flowjo®**软件(Miltenyi Biotec

GmbH,Bergisch Gladbach,Germany)，确定活靶细胞的绝对数量。使用仅孵育靶细胞的条件作为基线，确定每个样品的比细胞毒性的百分数。使用非线性变斜率回归方法和Prism软件(GraphPad software,La Jolla,CA,USA)，确定EC50值。通过从加有测试抗体的条件下的比细胞毒性百分数中扣除无抗体条件下的比细胞毒性百分数，计算比重定向裂解(RDL)的百分数。

[0346] 细胞存活力方法，在此为RDL-MTS法，基于比色法来评估细胞存活力，参见Bühler P等((2008)Cancer Immunol Immunother,57:43-52,Labrijn AF等((2013)Proc Natl Acad Sci USA,110(13):5145-50)和PCT公布号WO2012143524中的描述。收获靶细胞，计数，洗涤一次，并以2x10e5细胞/ml重悬在RDL培养基中。收获PBMC，计数，并以2x10e6细胞/mL重悬在RDL培养基中。在RDL培养基中制备抗体系列稀释物(3x溶液)。将靶细胞(50μl/孔)，T细胞(50μl/孔)和3x抗体溶液(50μl/孔)分配到平底96孔板(TPP)中。效应物:靶比率是10:1。平板在5%CO2温箱中37℃孵育48h。孵育后，弃去上清液，用200μl PBS洗涤平板3次以移出PBMC，然后每孔加入100μl RDL培养基。使用CellTiter96®试剂盒(Promega AG,Dübendorf,瑞士)，根据厂商说明书，进行读数。简言之，向每孔加入10-20μl MTS试剂，平板在5%CO2温箱中37℃孵育2-6h。然后在BioTek synergy平板读取器(BioTek AG,Luzern,瑞士)上，读取490nm吸光度。使用下面公式计算比杀伤百分数:比杀伤＝100x[(SD-Sp)/(SD-MD)]。SD是在孵育单独靶细胞的自发死亡条件下测量的吸光度。Sp是在每个测试条件(靶细胞+PBMCs+抗体)下测量的吸光度。MD是在以3个冻融循环裂解靶细胞的最大死亡条件下测量的吸光度。通过从加入测试抗体的条件下的比细胞毒性百分数中扣除无抗体条件下的比细胞毒性百分数，计算了比重定向裂解(RDL)百分数。使用非线性变斜率回归法和Prism软件(GraphPad software)，确定EC50值。

[0347] 异种移植物模型

[0348] JIMT-1异种移植物

[0349] 细胞系和试剂

[0350] 从DSMZ(目录号:ACC589)获得乳腺癌JIMT-1细胞系。在补充有10%热灭活的胎牛血清(FBS)(AMIMED,London,UK,目录号:Z10834P)、1%青霉素-链霉素(Invitrogen AG,目录号:10378-016)、1%丙酮酸钠溶液(PAA实验室，目录号:S11-003)、1%MEM非必需氨基酸(PAA实验室，目录号:M11-003)和1%GlutaMAX™-1(Invitrogen AG,目录号:35050-038)的、具有GlutaMAX™-1(Invitrogen AG,目录号:31966-021)的DMEM(1X)中维持细胞。使用StemPro Accutase(Invitrogen AG,目录号:A11105-01)每周传代细胞两次。

[0351] 从来自瑞士La Chaux-de-Fonds的血液采集中心(Centre de Transfusion Sanguine et Laboratoire de Sérologie,rue Sophie-Mairet 29,CH-2300)的含有人白细胞的血液过滤器，收集外周血单核细胞(PMBC)。通过用60ml含有10U/mL肝素(Drossapharm AG,Lucern,瑞士)的PBS反冲洗，将细胞从过滤器中移出。然后用50ml Blood-Sep-Filter Tubes(Brunschwig,Basel,瑞士)，根据制造商的说明，分离PBMC:将管在室温(无制动)下以800g离心20分钟，并从界面收集细胞。将细胞用无FBS的Roswell Park Memorial Institute培养基(RPMI,Invitrogen AG,目录号:21875-091)洗涤3次。将PBMC以10e6细胞/ml重悬于补充有10%FBS(AMIMED)、1%青霉素-链霉素(Invitrogen AG)的RPMI培养基中，并在37℃、5%CO_2下培养过夜。收获靶细胞，计数，洗涤一次，并以5×10e6细胞/

ml重悬于PBS中。

[0352] 小鼠和实验条件

[0353] 在以T细胞、B细胞和自然杀伤细胞缺陷为特征的5周龄免疫缺陷NOD.CB17/AlhnRj-Prkdcscid/Rj(NOD/SCID)雌性小鼠(Janvier Labs,St Berthevin,France)中进行体内实验。小鼠保持在标准的啮齿动物微隔离室笼(20+/-1℃室温、50±10%相对湿度、12小时光暗度节律)中在无菌和标准化的环境条件下。小鼠接受辐射的食物、垫草(bedding)和0.22μm过滤的饮用水。所有实验都是根据瑞士动物保护法,由负责的州政府(Neuchatel Canton,瑞士)批准。根据动物保护法,当肿瘤体积超过2000mm^{3}时,小鼠必须进行安乐死。采用ANOVA单因素方法和Bonferroni参数检验,进行相应治疗组相对于媒介物对照组的平均肿瘤体积的统计学分析。

[0354] 所有小鼠在植入前用VEET霜剂(Reckitt Benckiser AG,Wallisellen,瑞士)在右侧肋去毛。在植入当天和之后每周一次对小鼠拍照和体重测量。对于每只动物,将5×10e6人PBMC与5×10e6JIMT-1乳腺癌细胞在最终体积0.2ml PBS中混合。包括四个不同的PBMC供体。将PBMC/JIMT-1混合物皮下注射到每只NOD/SCID小鼠的右侧肋。对照组使用终体积0.2ml PBS中5×10e6JIMT-1乳腺癌细胞,而不包含任何人PBMC。对于每组10只JIMT-1/PBMC植入动物(每个PBMC供体一组),5只动物在植入后3小时用100μl体积、0.05mg/kg的HER2/CD3-1双特异性抗体静脉内处理。处理每周重复3次,每两天一次、持续两周。每周两次用卡尺在两个垂直维度上测量肿瘤,根据下列公式计算肿瘤体积:肿瘤体积=[(宽2×长)/2]。

[0355] 实施例1:确定在VH3亚类中减少或消除蛋白A结合的突变

[0356] 消除免疫球蛋白恒定区中蛋白A结合的方法是已知的(Lindhofer H.等人,(1995)J Immunol,155(1):219-225;US6,551,592;Jendeberg L.等人,(1997)J Immunol Methods,201(1):25-34;PCT公开号:WO2010151792)。为了评估在全长同源二聚体免疫球蛋白中蛋白A消除方法的应用,制备了基于混合IGHG1-IGHG3 Fc形式的抗HER2同源二聚体免疫球蛋白和相应的Fc 133对照片段。该抗HER2同源二聚体免疫球蛋白的形式与天然存在的抗体类似,由具有抗HER2特异性的FAB片段融合至Fc 133片段组成(来源于天然存在的人IGHG3同种型的Fc序列,其中铰链序列被替代为天然存在的人IGHG1同种型的整个铰链序列,缩写为Fc133,具有SEQ ID NO:1——该名称中的数字按铰链/CH2/CH3的顺序对应于每个结构域的免疫球蛋白γ同种型亚型;相应的全长抗HER2免疫球蛋白在本文中称为抗HER2 FAB-Fc133;具有SEQ ID NO:2的重链和具有SEQ ID NO:3的轻链)。转染后,根据材料和方法部分中描述的方案,通过梯度色谱法分析抗HER2FAB-Fc133同源二聚体和Fc133片段的蛋白A结合。如图3和图4A所示,Fc133片段不结合商业MabSelect SuRe™蛋白A树脂(GE Healthcare Europe GmbH),而抗HER2 FAB-Fc 133同源二聚体能够结合。

[0357] 为了评估FAB恒定结构域的贡献,将上述抗HER2同源二聚体重新制成抗HER2 scFv-Fc分子的形式,其中scFv单元由通过15氨基酸的接头融合的亲本免疫球蛋白可变结构域组成(本文缩写为抗HER2 scFv-Fc133;具有SEQ ID NO:4的重链)。因此,除了缺少CH1和CK恒定结构域外,所得抗HER2 scFv-Fc133同源二聚体与亲本抗HER2 FAB-Fc133同源二聚体免疫球蛋白相同。如图4B所示,与用亲本抗HER2同源二聚体免疫球蛋白观察到的相同,scFv-Fc133同源二聚体显示出蛋白A结合。从此发现,得出:对于在同源二聚体免疫球蛋白Fc部分中消除蛋白A结合的这些方法,抗HER2 FAB片段的可变结构域构成了阻碍这样方法

发挥功效的原因。更重要的是，得出结论：同源二聚体免疫球蛋白的可变结构域内的蛋白A结合将阻止基于蛋白A差异纯化技术制备异源二聚体免疫球蛋白。

[0358] 已知蛋白A的所有五个结构域都结合来自VH3可变结构域亚类的可变重链结构域(Jansson B等人，(1998)FEMS Immunol.Med.Microbiol,20(1):69-78)，该特征已知可以妨碍在全抗体分子的木瓜蛋白酶消化后制备基于VH3的FAB片段，原因是蛋白A不仅结合基于VH3的FAB也结合Fc片段。在所述的同源二聚体抗HER2免疫球蛋白或其scFv-Fc版本中的重链可变结构域属于VH3-23亚类，这解释了为什么这些同源二聚体分子，尽管在其工程化的Fc区没有蛋白A结合位点，仍可以结合蛋白A。

[0359] 基于VH3的免疫球蛋白或其片段对生物技术行业至关重要。基于VH3的分子因为其结合蛋白A的能力有利于其功能性预筛选，而已经获得广泛开发，例如，用于抗体开发的许多合成的或基于供体的噬菌体展示文库或转基因动物技术均基于VH3结构域亚型。此外，由于具有良好的表达和稳定性，基于VH3的分子也常优于其他已知的重链可变结构域亚类而被选择。已经开发了不结合基于VH3的FAB片段的蛋白A重组版本，并由GE Healthcare以商品名MabSelect SuRe™商业化。

[0360] 如上讨论MabSelect SuRe™树脂在本文中用于两种同源二聚体抗HER2免疫球蛋白的蛋白A结合评估，由此得出结论：当使用蛋白A差异纯化技术时，MabSelect SuRe™树脂不适用于制备具有至少一个VH3可变结构域的异源二聚体免疫球蛋白——因为在其Fc区中没有蛋白A结合的同源二聚体物质仍可以通过其VH3结构域结合蛋白A。

[0361] 为了研究可以消除或降低基于VH3的同源二聚体免疫球蛋白或其片段的蛋白A结合的取代，需要分析基于VH3的FAB变体的蛋白A结合。尽管已知MabSelect SuRe™树脂类型缺乏VH3结构域亚类结合，但是称作MabSelect™的另一种商业蛋白A树脂确实结合VH3结构域亚类(也来自GE Healthcare)，并且被选择用于分析基于VH3的FAB变体对蛋白A的结合。

[0362] 通过以下验证了MabSelect™树脂的使用：制备源自前述亲本抗HER2同源二聚体免疫球蛋白(已知具有VH3-23可变结构域亚类)的重组抗HER2 FAB片段(本文缩写为抗-HER2 FAB；具有SEQ ID NO:5的重链和具有SEQ ID NO:3的轻链)，在MabSelect™和MabSelect SuRe™柱上分析所述片段(具有基于VK亚类I的轻链，首先使用蛋白L色谱法在捕获-洗脱模式下纯化FAB片段，之后在MabSelect™或MabSelect SuRe™柱上进行蛋白A梯度色谱，用于两个柱的方案均按照材料和方法部分描述的方案进行)。如图4C所示，基于VH3的抗HER2FAB仅与MabSelect™柱结合，确认MabSelect SuRe™树脂不与基于VH3的FAB片段结合(至少就单体的基于VH3的FAB片段而言，并进一步与先前观察到的具有不结合蛋白A的工程化Fc区的基于VH3的同源二聚体免疫球蛋白的结果形成对比)。相反，抗HER2 FAB显示出与MabSelect™柱的强结合，这使得可以测定与蛋白A不结合或具有降低的结合的基于VH3的FAB变体。

[0363] 为了消除基于VH3的FAB片段中的蛋白A结合，从与蛋白A的D结构域复合的人FAB片段的晶体结构(PDB代码：1DEE；www.pdb.org；Graille M等人，(2000)Proc Natl Acad Sci USA,97(10):5399-5404)，鉴定了VH3结构域中关键的蛋白A结合残基。该分析被用作合理设计的起点，其中基于蛋白A结合性和非蛋白A结合性人源VH亚类之间的序列比较，确定实施的取代的性质。图5显示了每种人重链可变结构域亚类各一个代表性框架的比对。氨基酸位置15、17、19、57、59、64、65、66、68、70、81、82a和82b(Kabat编号)被鉴定为在1DEE结构中蛋

白A的D结构域和基于VH3的FAB片段之间的蛋白-蛋白相互作用界面的一部分。在人VH亚类中，VH3是结合蛋白A的唯一亚类，选择其他亚类中等同氨基酸序列位置的残基作为取代的来源，以期消除或降低蛋白A结合，同时潜在地降低免疫原性——因为这些取代涉及将一个残基替代为在非蛋白A结合性人VH亚类中相同的等同氨基酸位置上的另一个天然存在的残基替换。

[0364] 通过基于标准PCR的诱变技术，在上述抗HER2 FAB片段的序列中引入突变，进行以下取代：G65S(具有SEQ ID NO:6的重链和具有SEQ ID NO:3的轻链)、R66Q(具有SEQ ID NO:7的重链和具有SEQ ID NO:3的轻链)、T68V(具有SEQ ID NO:8的重链和具有SEQ ID NO:3的轻链)、Q81E(具有SEQ ID NO:9的重链和具有SEQ ID NO:3的轻链)、N82aS(具有SEQ ID NO:10的重链和具有SEQ ID NO:3的轻链)和组合R19G/T57A/Y59A(具有SEQ ID NO:11的重链和具有SEQ ID NO:3的轻链)。

[0365] 此外，制备T57A取代(具有SEQ ID NO:12的重链和具有SEQ ID NO:3的轻链)和T57E取代(具有SEQ ID NO:13的重链和具有SEQ ID NO:3的轻链)。先前在WO2010075548中已经证明T57A可以消除蛋白A结合，设计T57E以引入可能破坏VH3-蛋白A相互作用的带电残基。首先使用蛋白L色谱法在捕获-洗脱模式中纯化具有基于VK亚家族I的轻链的FAB突变体，并且如在材料和方法部分中描述的，使用在梯度模式下操作的MabSelect™柱进一步分析蛋白A结合。图6显示仅T57A、T57E、G65S、Q81E、N82aS和组合R19G/T57A/Y59A消除或降低抗HER2 FAB与MAbSelect™树脂的结合。对于消除基于VH3的FAB片段中的蛋白A结合，优选取代G65S、Q81E和N82aS，因为这些突变导致了在非蛋白A结合性VH亚类中的序列等同残基取代，从而潜在地降低免疫原性。

[0366] 抗体亲和力和特异性基本上限于CDR区，然而，框架取代可影响抗体特性，如几种人源化抗体的情况。为了评估上述取代是否可能影响VH3衍生的抗体的特异性和/或亲和力，通过表面等离子共振(SPR)测定了优选FAB突变体中的两种对HER2抗原的结合。如材料和方法部分所述，使用重组HER2抗原进行了SPR测量。当与原始FAB分子(图7)相比时，两个优选的突变体都显示了相同的HER2抗原结合，表明这些取代在特异性或亲和力方面没有影响。因此，预期这些取代可以广泛用于工程化去除VH3衍生的抗体分子中的蛋白A结合，而不显著损失抗原结合。

[0367] 将这些优选取代中的两个引入前述抗HER2同源二聚体免疫球蛋白和抗HER2 scFv-Fc分子中，并测定变体在MabSelect SuRe™树脂上的结合。制备以下变体：抗HER2 scFv(G65S)-Fc 133(具有SEQ ID NO:14的重链)、抗HER2 scFv(N82aS)-Fc 133(具有SEQ ID NO:15的重链)、抗HER2 FAB(G65S)-Fc 133(具有SEQ ID NO:16的重链和具有SEQ ID NO:3的轻链)和抗HER2 FAB(N82aS)-Fc 133(具有SEQ ID NO:17的重链和具有SEQ ID NO:3的轻链)。

[0368] 图8显示了所有四种突变体的MabSelect SuRe™色谱图。所有变体现显示出降低至几乎没有的MabSelect SuRe™柱结合，表明用先前鉴定的取代成功地去除蛋白A结合。更重要的是，得出结论：当与蛋白A差异纯化技术组合时，通过消除或降低基于VH3的FAB对蛋白A的亲和力的取代，可以制备其中存在至少一个VH3可变结构域的异源二聚体免疫球蛋白。

[0369] 实施例2：靶向人CD3抗原、肿瘤相关抗原和炎性细胞表面抗原的抗原结合位点

[0370] 针对人CD3抗原的抗原结合位点

[0371] 选择人CD3ε亚基，以通过双特异性结合来驱动T细胞重定向杀伤。

[0372] 2.1A小鼠OKT3抗体的人源化变体

[0373] 本文使用的抗人CD3ε抗原结合位点来源于小鼠OKT3抗体(Muromonab-CD3，商品名为Orthoclone OKT3，由Janssen-Cilag销售，随后停止；鼠可变重链和轻链结构域分别具有SEQ ID NO:18和19)。将OKT3鼠可变结构域人源化并形成为scFv和FAB片段。

[0374] 人源化遵循Jung S&PlückthunA(1997，Protein Eng，10(8)：959-66)描述的方法，以产生适合于FAB和scFv形式的高度稳定的人源化变体。该方法利用赫赛汀®抗体(rhuMAbHER2、huMAB4D5-8、曲妥珠单抗或商品名赫赛汀®；美国专利公开号5,821,337；分别具有SEQ ID NO:20和21的可变重链和轻链结构域)中的高度稳定的VH/VL结构域对，并遵循在固定的框架区上的人源化过程的工作流程(Almagro JC&Fransson J(2008)，Front Biosci，13:1619-33)。由于赫赛汀®抗体最初来源于高度稳定的人种系框架VH3和VK1家族，所以来自这两个家族的种系框架可以同等地用作固定框架的来源。或者，可以使用人VK3种系轻链框架家族替代VK1，因为它也具有良好的稳定性(Ewert S等人，(2003)J Mol Biol，325:531-553)。除了小鼠抗体之外，可以使用该固定框架方法来设计人抗体以改善稳定性。优选使用分别具有SEQ ID NO:22、23和24的人种系框架IGHV3-23*04、IGKV1-39*01和IGKV3-20*01(根据IMGT®(国际ImMunoGeneTics信息系统提及(Lefranc MP等人(1999)Nucleic Acids Res，27(1)：209-12；Ruiz M等人(2000)Nucleic Acids Res，28(1)：219-21；Lefranc MP(2001)Nucleic Acids Res，29(1)：207-9；Lefranc MP(2003)Nucleic Acids Res，31(1)：307-10；Lefranc MP等人，(2005)Dev Comp Immunol，29(3)：185-203；Kaas Q等人，(2007)Briefings in Functional Genomics&Proteomics，6(4)：253-64；http://www.imgt.org)。

[0375] 为此目的，构建了第一人源化抗体，其中赫赛汀®抗体的可变结构域中的CDR分别被来自小鼠OKT3抗体的CDR替代，并以小鼠OKT3抗体的嵌合体(具有SEQ ID NO:25和26的可变重链和轻链，并且在本文中称为嵌合OKT3抗体)作为参照基准。

[0376] 原型抗体(具有SEQ ID NO:27和39的可变重链和轻链，缩写为VH/VL)在瞬时表达测试中具有增加的生产水平并具有增加的FAB稳定性(如通过差示扫描量热法所测量)，但是不结合HPB-ALL细胞(人CD3ε阳性T细胞肿瘤细胞系)(在FACS实验中通过中值荧光强度评估，参见材料和方法部分)(图9A)。

[0377] 基于该第一对原型可变结构域的3D模型，选择并测试了一组回复突变(从CDR嫁接的赫赛汀®原型至小鼠OKT3序列)：可变重链结构域中I34M，V48I，A49G，R58N，R58Y，I69L，A71T和T73K；和可变轻链中M4L，V33M，A34N，L46R，L47W，R66G，F71Y和P96F(Kabat编号)。注意，R58N取代相应于CDR嫁接的赫赛汀®原型至小鼠OKT3的突变，而R58Y取代相应于CDR嫁接的赫赛汀®原型至人IGHV3-23*04种系的替代。有关组合取代的工程化策略是基于不同取代在如下方面的互补性：不同取代对CDR区和/或可变结构域组装和/或免疫原性的推定影响。

[0378] 在第一个方法中，所有候选物制备为人IgG1抗体形式。根据表达水平、FAB片段的热稳定性、和结合HPB-ALL细胞的能力(通过FACS)，选择最佳变体。使用G65S或N82aS取代，

消除最佳人源化变体在其VH结构域中存在的蛋白A结合位点。需要该工程化步骤以进一步产生无抗CD3同源二聚体的安全的T细胞重定向BEAT抗体。

[0379] 在VH中的回复突变I34M、A49G和A71T以及在VL中的回复突变M4L、L46R、L47W和F71Y使亲和力恢复。可变结构域的最佳组合是VH8/VL4、VH8/VL8、VH11/VL4和VH11/VL8，因为其保留了大部分的亲本细胞结合(图9A-C)。此外，组合VH8/VL8(分别具有SEQ ID NO:48和51的可变结构域)和VH11/VL8(分别具有SEQ ID NO:49和51的可变结构域)具有增强的FAB稳定性(分别为+2.8℃和+1.6℃，图9D)。

[0380] 最后，也将最佳人源化变体重新形成为scFv-Fc融合物形式，并瞬时表达。根据在该形式中的相对亲和力、稳定性、瞬时转染的表达水平，排序变体(图9E)。scFv-Fc融合形式中可变结构域的最佳组合与在抗体形式中鉴定的组合类似：VH8-VL4(具有SEQ ID NO:58的scFv片段)和VH8-VL8(具有SEQ ID NO:59的scFv片段)。两种scFv片段与scFv-Fc融合形式具有良好的热稳定性(图9F)。

[0381] 2.1B小鼠SP34抗体的人源化变体

[0382] 小鼠抗体SP34首先在1985年被描述(Pessano S等人，(1985)EMBO J,4(2):337-44)。它是由杂交瘤产生，所述杂交瘤从使用HPB-ALL细胞的变性蛋白提取物免疫的小鼠获得，该抗体具有人特异性和对猕猴的交叉反应性。人和猕猴CD3ε亚基上的SP34表位是已知的(分别为SEQ ID NO:195和196)。

[0383] 按照以上实施例所述的方法和程序，通过将CDR嫁接到VH3-23和VK3种系框架，工程化构建了鼠SP34抗体的人源化VH和VL结构域(具有SEQ ID NO:60的VH结构域和具有SEQ ID NO:61的VL结构域)。根据其在BEAT抗体形式中的用途，可以使用G65S或N82aS取代(Kabat编号)进一步消除所得的基于VH3的可变结构域对蛋白A的结合。

[0384] 为此目的，构建了第一人源化抗体，其中具有种系VH3重链结构域和种系VK3轻链结构域的人抗体的可变结构域中的CDR分别被来自小鼠SP34抗体的CDR替代。使用所得的人源化抗体作为起点进一步改善亲和力，并以SP34抗体的嵌合体(分别具有SEQ ID NO:62和63的重链和轻链，在本文中称为嵌合SP34抗体)作为参照基准。

[0385] 原型抗体(具有SEQ ID NO:64和69的可变重链和轻链，缩写为VH1/VL1)对人CD3ε 1-26_Fc融合蛋白具有低的结合(通过SPR评估，参见材料和方法部分和图10A)。

[0386] 基于该第一对原型可变结构域的3D模型，选择了一组取代，对应于CDR嫁接的人种系VH3/VK3原型和小鼠SP34序列之间的回复突变(人至小鼠或小鼠至人取代)或对应于合理设计的氨基酸变化。进行了以下改变并测试了各种组合：可变重链结构域中的W100eF和W100eY；以及可变轻链中的A2I、S25A、T27A、G27aA、V27cA、T28A、T29A、S30A、N31A、Y32A、E38Q、F44P、G46L、T51A、N52A、K53A、R54A、P56A、L66G、D69T、F87Y、Q89A、W91F、Y92A、S93A、N94A和Q100G(Kabat编号；参见图10A)。关于组合取代的工程化策略是基于不同取代在如下方面的互补性：对CDR区和/或可变结构域组装和/或免疫原性的推定影响、和/或对哺乳动物细胞中瞬时表达的影响。

[0387] 在第一种方法中，所有候选物形成为人IgG1抗体，并且随后以scFv-Fc融合蛋白形式(图10B)进一步测试，其中一些变体使用G65S消除了在其VH结构域中存在的蛋白A结合位点。根据表达水平和通过SPR测定的与人和猕猴CD3ε 1-26_Fc融合蛋白结合的能力，选择了最佳人源化候选物。

[0388] 令人惊讶的是，当重新形成为scFv时，与IgG1形式相比，SP34嵌合体和H1L21导致了表达的显著丧失(图33)。然而，通过VH中取代W100eY和VL中W91F的组合，增强了scFv-Fc表达(图10B)。

[0389] 为了进一步改善基于CD3的双特异性的可制造性，进一步设计了人源化SP34 scFv。为了鉴定影响SP34 scFv-Fc表达的关键位置，在VL21 CDR中进行丙氨酸扫描。进行以下改变：T27A、G27aA、V27cA、T28A、T29A、S30A、N31A、Y32A、N52A、K53A、R54A、P56A、L90A、Y92A、S93A、N94A和L95A。有趣的是，只有位置29、30(VH1/VL26 SEQ ID NO:351)和95(VH1/VL34 SEQ ID NO:363)的取代导致了scFv-Fc表达的显著增加(图34)，而且这些突变体是保持与人和猕猴CD3ε完全结合的仅有那些突变体。

[0390] 基于这些结果，在轻链中在位置29、30和95测试了更多的取代。

[0391] 由于位置29和30在所有不同的可变轻链家族中显示出高变异性，故通过定点PCR随机突变这两个位置。在产生的所有突变体中，只有取代T29A、T29E、T29S、S30A和S30D在维持与人CD3ε结合的同时，显著改善了瞬时表达水平，图35a和35b。

[0392] 在一个不同的方法中，L95(本领域中已知的规范结构残基)被取代为在可变λ家族中在该相同位置处最常见的残基。进行以下变化：L95A、L95G、L95T、L95S、L95D和L95N。意外的是，只有L95G和L95T在维持与靶标结合的同时显著改善表达(图35c)。

[0393] 基于这项工作，设计了几种基于人源化SP34的BEAT，并测试其表达和与CD3ε的结合。在所有这些构建体中，含有H5L65(SEQ ID NO:394)和H5L67(SEQ ID NO:396)scFv的双特异性显示出，比基于H5L32 scFv的BEAT对照，增加2倍的表达(图36)。

[0394] 通过DSC进一步表征了IgG1或scFv-Fc形式的H5L65和H5L67，并与H5L32、H1L21和SP34嵌合体进行比较。H5L65以IgG1形式显示出优异的热稳定性，但更有意义的是，在scFv-Fc形式中与其他人源化变体相比，增加了5至2℃(表1)。因此，改良抗体的体内稳定性增加了，体外稳定性也增加了。

[0395] 表1

[0396]

SP34 形式	Fab Tm (°C)	IgG (Fab) Tm (°C)	scFvFc Tm (°C)
SP34 嵌合体	66.9	66.7	N/A
hSP34 H1/L21	75.1	73.1	56.7
hSP34 H5/L32	77.5	75.9	59.7
hSP34 H5/L65	**78.1**	**77.3**	**61.6**
hSP34 H5/L67	77.4	76.5	59.9

[0397] 就抗原结合和重组表达而言，重链和轻链可变结构域的优选组合如下：VH1(SEQ

ID NO:101)或VH2(SEQ ID NO:102)或VH3(SEQ ID NO:103)或VH5(SEQ ID NO:104)与轻链结构域VL21(SEQ ID NO:105),VL32(SEQ ID NO:106)、VL65(SEQ ID NO:401)和VL67(SEQ ID NO:402)配对。

[0398] 2.2 HER2

[0399] 重定向T细胞以杀伤HER2阳性癌细胞的双特异性抗体可用于治疗不同形式的人乳腺癌。已经描述了抗HER2抗体(Blumenthal GM等人,(2013)Clin Cancer Res,19(18):4911-6),其中一些目前用于临床或目前在人体临床研究中(Tsang RY&Finn RS(2012)Br J Cancer,106(1):6-13)。

[0400] 本文所用的抗HER2抗原结合位点来自重组人源化抗HER2抗体**赫赛汀®**(见第1.1节),其形式为FAB片段(具有SEQ ID NO:5的FAB重链片段和轻链SEQ ID NO:3)或scFv片段(SEQ ID NO:107)。在一些形式中,使用G65S取代(重链具有SEQ ID NO:108和轻链具有SEQ ID NO:3的FAB片段或具有SEQ ID NO:109的scFv片段)或N82aS取代(重链具有SEQ ID NO:110和轻链具有SEQ ID NO:3的FAB片段或具有SEQ ID NO:111的scFv片段),消除了人源化抗HER2抗体4D5的VH结构域(VH3结构域亚类)中存在的蛋白A结合。

[0401] 2.3 CD38

[0402] CD38是II类跨膜糖蛋白,其正常存在于造血细胞上和实体组织中。CD38也在各种恶性血液学疾病中表达。重定向T细胞以杀伤CD3阳性癌细胞的双特异性抗体可以用于治疗各种恶性血液学疾病,包括多发性骨髓瘤、B细胞慢性淋巴细胞性白血病、B细胞急性淋巴细胞性白血病、**Waldenström**氏巨球蛋白血症、原发性系统性淀粉样变性、套细胞淋巴瘤、前淋巴细胞/髓细胞性白血病、急性髓性白血病、慢性髓性白血病、滤泡性淋巴瘤、NK细胞白血病和浆细胞白血病。几种抗CD38抗体已经被描述为研究试剂或治疗剂候选物(PCT公布号:WO2006099875)。OKT-10和HB-7小鼠杂交瘤是表征最好的抗人CD38抗体(Hoshino S et al.,(1997)J Immunol,158(2):741-7)。

[0403] 在第一种方法中,抗人CD38抗原结合位点可来自小鼠杂交瘤OKT10(分别具有SEQ ID NO:112和113的可变重链和轻链)或HB-7(分别具有SEQ ID NO:114和115的可变重链和轻链)和其人源化版本,其可进一步制备为FAB或scFv片段形式。按照实施例2.1中描述的方法和程序,通过将CDR嫁接到VH3-23和VK1种系框架上,可以工程化构建HB-7杂交瘤的人源化VH和VL结构域。

[0404] 在第二种方法中,根据Almagro JC&Fransson J(Front Biosci,(2008)13:1619-33)描述的所谓最适配人源化方法(best-fit humanization method),通过将CDR嫁接到人IGHV4-59*03和IGKV1-NL1*01种系框架(根据上述**IMGT®**提及)上,工程化构建了HB-7杂交瘤的最适配人源化VH和VL结构域。在计算机上(in silico)研究了具有不同程度的回复突变的人源化VH和VL变体,将一个优选选择的人源化VH和VL瞬时表达为人IgG1形式,并且在本文中称为人源化HB-7最适配VH(SEQ ID NO:116)和VL(SEQ ID NO:117)结构域。引入以下小鼠回复突变:(VH)S35H、I37V、I48L、V67L、V71K、T73N、F78V、Y91F和(VL):M4L、L48I、Y49S、T69K(Kabat编号)。

[0405] 通过FACS,人源化HB-7最适配抗体(具有SEQ ID NO:118的重链和具有SEQ ID NO:119的轻链)染色CHO[CD38]重组细胞(数据未显示)。当通过SPR分析时,人源化HB-7最适配抗体具有与嵌合HB-7抗体(具有SEQ ID NO:120的重链和具有SEQ ID NO:121的轻链)类似

的CD38胞外区结合亲和力(KD分别为3.6和2.5nM;图11A(嵌合)和图11B(人源化))。令人吃惊的是,如从量热谱确定的,人源化HB-7最适配抗体与嵌合HB-7抗体相比,显示显著增强的FAB片段稳定性(+14.6℃)(76.4℃(嵌合)与91.0℃(人源化),图11F)。

[0406] 在第三种方法中,以人CD38胞外结构域和人CD38+细胞免疫小鼠,用于产生针对人CD38的新型杂交瘤候选物。产生杂交瘤的方法是已知的,并且本文使用的方法类似于PCT公开号:WO2013008171中公开的方法。9G7小鼠抗体候选物对人和猕猴CD38两者(分别具有SEQ ID NO:122和123的可变重链和轻链)具有高亲和力。该小鼠抗体首先按照以上实施例所述的方法人源化。使用最适配方法,选择种系VH框架IGHV2-5*09和VK框架IGKV1-33*01(根据上述IMGT®提及)作为人源化过程的起点。CDR嫁接后,第一抗体原型(人IgG1同种型形式,重链SEQ ID NO:124和轻链SEQ ID NO:125)显示出与人CD38的强结合,比小鼠亲本抗体仅低三倍,如通过SPR确定的(具有重链SEQ ID NO:126和轻链SEQ ID NO:127的嵌合9G7抗体;嵌合9G7抗体(数据未显示)和第一人源化原型(数据未显示)的KD分别为0.3nM和1nM)。在抗体可变轻链中引入F36Y回复突变时(Kabat编号),亲和力提高了两倍(所得抗体在本文中称为人源化9G7最适配抗体,其具有重链SEQ ID NO:124和轻链SEQ ID NO:128;人CD38的KD为0.5nM,图11C)。人源化9G7最适配抗体还显示对猕猴CD38抗原的高亲和力(KD为3.2nM,数据未显示),以及比嵌合9G7抗体增强的FAB热稳定性(来自DSC扫描的FAB Tm)(对于人源化9G7最适配抗体和嵌合9G7抗体分别为94℃与82.2℃;参见图11G)。人源化9G7最适配抗体具有SEQ ID NO:129的重链可变结构域和SEQ ID NO:130的轻链可变结构域。

[0407] 此外,通过将CDR嫁接至VH3-23和VK1种系框架上,按照最佳框架方法(best-framework approach),对9G7小鼠抗体进行了人源化。在计算机上研究了具有不同程度回复突变的人源化VH和VL变体,并且将一个优选选择的人源化VH和VL组合瞬时表达为人IgG1抗体(所得抗体在本文中称为人源化9G7最佳框架抗体,其具有重链SEQ ID NO:131和轻链SEQ ID NO:132)。引入以下小鼠回复突变:(VH)A24F、V37I、V48L、S49A、F67L、R71K、N73T、L78V和K94R;和(VL)F36Y(Kabat编号)。该抗体表现出与人CD38和猕猴CD38的强结合,亲和力常数与人源化9G7最适配抗体相似(对人和猕猴CD38的KD分别为0.4和1nM;图11D)。FAB热稳定性(来自DSC扫描的FAB Tm)也非常类似于9G7最适配F36Y人源化变体(89.2℃,参见图11H)。图11J总结了上述不同的人源化9G7抗体。人源化9G7最佳框架抗体具有SEQ ID NO:133的重链可变结构域和SEQ ID NO:134的轻链可变结构域。

[0408] 在第四种方法中,筛选抗体噬菌体文库以产生针对人CD38的另外scFv片段。该文库具有基于天然人V基因的多样性。该供体衍生的抗体噬菌体展示文库使用从48位人供体的血液淋巴细胞扩增的cDNA,48位人供体中70%具有自身免疫性疾病(血管炎、系统性红斑狼疮、脊椎关节病、类风湿性关节炎和硬皮病)。文库构建遵循Schofield等人(2007,Genome Biol.,8(11):R254)描述的方案,其中总多样性为2.53×10e10克隆。如下从该供体衍生的噬菌体展示文库中分离识别人和/或猕猴CD38的ScFv片段。在一系列重复选择循环中在重组来源的人和/或猕猴CD38抗原上分离ScFv片段(参见材料和方法部分)。筛选抗体噬菌体展示文库的方法是已知的(Viti F等人,(2000)Methods Enzymol,326:480-505)。简言之,在与文库孵育后,回收与固定化的抗原结合的噬菌体,而洗去未结合的噬菌体,其中所述固定化抗原之前已经包被在塑料免疫管上(以20μg/ml浓度在PBS中过夜)或捕获在链霉亲和素珠上(当使用抗原的生物素标记形式时,在整个选择过程中以50nM的浓度捕获抗原)。如

Marks等人(Marks JD等人,(1991)J Mol Biol,222(3):581-97)所述,拯救结合的噬菌体,选择过程重复三次。表达来自第二和第三轮淘选的一千多个克隆,并通过针对人和猕猴CD38抗原的ELISA进行分析。对阳性克隆进行DNA测序,并进一步分析一些独特克隆结合人CD38表达细胞系的能力。使用固定在链霉亲和素珠上的人CD38抗原的生物素标记版本进行第一轮淘选和使用固定在链霉亲和素珠上的猕猴CD38抗原的生物素标记版本进行第二轮淘选之后,基于结合人和猕猴CD38的能力,选择了一个优选的scFv片段(克隆号767),其具有SEQ ID NO:135的可变重链序列和SEQ ID NO:136的可变轻链。当形成为人IgG1抗体时,克隆767对人CD38的KD为约300nM(图11E),对猕猴CD38的KD为约1.2μM(数据未显示)(克隆767IgG1抗体在本文中称为人767抗体,其具有重链SEQ ID NO:137和轻链SEQ ID NO:138)。FAB热稳定性(来自DSC扫描的FAB Tm)为70.2℃(图11I)。克隆767VH结构域属于VH3结构域亚类。

[0409] 2.4 OX40

[0410] 靶向CD3和OX40的双特异性抗体将允许靶向和消耗活化的T淋巴细胞。在这种组合中,表达CD3和OX40分子的活化的T淋巴细胞将参与相互杀伤过程,导致快速的细胞消失。双特异性抗体与人CD3和OX40的共结合可以在短时间内实现对病原性T细胞的有效消除。OX40是受体TNFR-超家族的成员,并且在1987年首次被鉴定为在来自大鼠的活化CD4+T细胞上表达的50kDa糖蛋白(Paterson DJ等人,(1987)Mol.Immunol.24:1281-90)。与CD28不同,OX40不在幼稚T细胞上组成型表达,而是在T细胞受体(TCR)结合后被诱导。OX40是第二共刺激分子,活化后24至72小时后表达;其配体OX40L也不在静息的抗原呈递细胞上表达,而在其活化后表达。

[0411] PCT公开号:WO2013008171中公开的小鼠抗人OX40抗体(分别具有SEQ ID NO:139和140的重链和轻链结构域)可以用作抗人OX40抗原结合位点的来源。PCT公开号:WO2013008171中也公开了基于最适配人源化方法的该抗体的人源化版本(分别具有SEQ ID NO:141和142的重链和轻链结构域),并且两种抗体均适于重新制备成BEAT形式。

[0412] 遵循实施例2.1中描述的方法和程序,通过将CDR嫁接至VH3-23和VK1种系框架,工程化构建抗人OX40杂交瘤的人源化VH和VL结构域。根据其在BEAT抗体形式中的用途,使用G65S或N82aS取代(Kabat编号)进一步消除所得的基于VH3的可变结构域对蛋白A的结合。只研究了两个人源化VH和VL结构域,其具有不同程度的回复突变。从亲本抗体可变结构域和类似于第一原型抗体的CDR嫁接的VH3和VK1之间的序列比对和在实施例2.1中描述的方法,鉴定回复突变。这些CDR嫁接的可变结构域没有回复突变,在本文中称为最小嫁接(mingraft)。然后进一步修饰这些序列以包括从先前比对鉴定的所有回复突变,并得到本文称为最大嫁接(maxgraft)的修饰的可变结构域序列。得到的序列总结如下:

[0413] 没有回复突变的人源化和稳定的抗OX40VH;缩写为人源化抗OX40/最小嫁接VH(SEQ ID NO:278)。

[0414] 具有所有可能的回复突变的人源化和稳定的抗OX40VH;缩写为人源化抗OX40/最大嫁接VH(SEQ ID NO:279)。

[0415] 没有回复突变的人源化和稳定的抗OX40VL;缩写为人源化抗OX40/最小嫁接VL(SEQ ID NO:280)。

[0416] 具有所有可能的回复突变的人源化和稳定的抗OX40VL;缩写为人源化抗OX40/最

大嫁接VL(SEQ ID NO:281)。

[0417] 2.5 CD20

[0418] 重定向T细胞以表杀伤达CD20的B细胞的双特异性抗体可用于治疗不同形式的人淋巴瘤癌症。已经将几种抗人CD20抗体描述为研究试剂或治疗候选物。其中最佳表征的抗人CD20抗体有嵌合的利妥昔单抗抗体及其人源化变体(嵌合利妥昔单抗,商品名**Rituxan®**,PCT公开号:WO1994011026;SEQ ID NO:143的小鼠VH结构域和SEQ ID NO:144的VL结构域)。

[0419] 遵循实施例2.1中描述的方法和工作流程,通过CDR嫁接至VH3-23和VK1种系框架上,工程化构建利妥昔单抗嵌合抗体的人源化VH和VL结构域。根据其在BEAT抗体形式中的用途,使用G65S或N82aS取代(Kabat编号)进一步消除所得的基于VH3的可变结构域对蛋白A的结合。研究了两个人源化VH和VL结构域,其具有不同程度的回复突变。从亲本抗体可变结构域和类似于第一原型抗体的CDR嫁接的VH3和VK1之间的序列比对和在实施例2.1中描述的方法,鉴定回复突变。这些CDR嫁接的可变结构域没有回复突变,在本文中称为最小嫁接(mingraft)。然后进一步修饰这些序列以包括从先前比对鉴定的所有回复突变,并得到本文称为最大嫁接(maxgraft)的修饰的可变结构域序列。得到的序列总结如下:

[0420] 没有回复突变的人源化和稳定的利妥昔单抗VH;缩写为人源化利妥昔单抗/最小嫁接VH(SEQ ID NO:282)。

[0421] 具有所有可能的回复突变的人源化和稳定的利妥昔单抗VH;缩写为人源化的利妥昔单抗/最大嫁接VH(SEQ ID NO:283)。

[0422] 没有回复突变的人源化和稳定的利妥昔单抗VL;缩写为人源化的利妥昔单抗/最小嫁接VL(SEQ ID NO:284)。

[0423] 具有所有可能的回复突变的人源化和稳定的利妥昔单抗VL;缩写为人源化的利妥昔单抗/最大嫁接VL(SEQ ID NO:285)。

[0424] 2.6 EGFR

[0425] 重定向T细胞以杀伤EGFR阳性癌细胞的双特异性抗体可用于治疗不同形式的人癌症,优选人胰腺癌和人结肠癌。已经将几种抗人EGFR抗体描述为研究试剂或治疗候选物。其中最佳表征的抗人EGFR抗体是嵌合西妥昔单抗(cetuximab)抗体及其人源化变体。(嵌合西妥昔单抗抗体,商品名为**Erbitux®**,C225,IMC-C225;PCT公开号:WO199640210;具有SEQ ID NO:145的小鼠VH结构域和具有SEQ ID NO:146的小鼠VL结构域)。

[0426] 遵循实施例2.1中描述的方法和工作流程,通过CDR嫁接至VH3-23和VK1种系框架,工程化构建**Erbitux®**嵌合抗体的人源化VH和VL结构域。根据其在BEAT抗体形式中的用途,使用G65S或N82aS取代(Kabat编号)进一步消除所得的基于VH3的可变结构域对蛋白A的结合。研究了两个人源化VH和VL结构域,其具有不同程度的回复突变。从亲本抗体可变结构域和类似于第一原型抗体的CDR嫁接的VH3和VK1之间的序列比对和在实施例2.1中描述的方法,鉴定回复突变。这些CDR嫁接的可变结构域没有回复突变,在本文中称为最小嫁接(mingraft)。然后进一步修饰这些序列以包括从先前比对鉴定的所有回复突变,并得到本文称为最大嫁接(maxgraft)的修饰的可变结构域序列。得到的序列总结如下:

[0427] 没有回复突变的人源化和稳定的爱必妥(Erbitux)VH;缩写为人源化的爱必妥/最小嫁接VH(SEQ ID NO:286)。

[0428] 具有所有可能的回复突变的人源化和稳定的爱必妥VH;缩写为人源化的爱必妥/最大嫁接VH(SEQ ID NO:287)。

[0429] 没有回复突变的人源化和稳定的爱必妥VL;缩写为人源化的爱必妥/最小嫁接VL(SEQ ID NO:288)。

[0430] 具有所有可能的回复突变的人源化和稳定的爱必妥VL;缩写为人源化的爱必妥/最大嫁接VL(SEQ ID NO:289)。

[0431] 另一个良好表征的抗人EGFR抗体是人帕尼单抗(panitumumab)抗体及其人源化变体(人帕尼单抗抗体,商品名**Vectibix®**,PCT公开号:WO2012138997;具有SEQ ID NO:290的小鼠VH结构域和具有SEQ ID NO:291的小鼠VL结构域)。

[0432] 遵循实施例2.1中描述的方法和工作流程,通过CDR嫁接至VH3-23和VK1种系框架,工程化构建**Vectibix®**嵌合抗体的人源化VH和VL结构域。根据其在BEAT抗体形式中的用途,使用G65S或N82aS取代(Kabat编号)进一步消除所得的基于VH3的可变结构域对蛋白A的结合。研究两个人源化VH和VL结构域,其具有不同程度的回复突变。从亲本抗体可变结构域和类似于第一原型抗体的CDR嫁接的VH3和VK1之间的序列比对和在实施例2.1中描述的方法,鉴定回复突变。这些CDR嫁接的可变结构域没有回复突变,在本文中称为最小嫁接(mingraft)。然后进一步修饰这些序列以包括从先前比对鉴定的所有回复突变,并得到本文称为最大嫁接(maxgraft)的修饰的可变结构域序列。得到的序列总结如下:

[0433] 没有回复突变的人源化和稳定的维克替比(Vectibix)VH;缩写为人源化的维克替比/最小嫁接VH(SEQ ID NO:292)。

[0434] 具有所有可能的回复突变的人源化和稳定的维克替比VH;缩写为人源化的维克替比/最大嫁接VH(SEQ ID NO:293)。

[0435] 没有回复突变的人源化和稳定的维克替比VL;缩写为人源化的维克替比/最小嫁接VL(SEQ ID NO:294)。

[0436] 具有所有可能的回复突变的人源化和稳定的维克替比VL;缩写为人源化的维克替比/最大嫁接VL(SEQ ID NO:295)。

[0437] 2.7 CD19

[0438] 重定向T细胞以杀伤表达CD19的B细胞的双特异性抗体可用于治疗不同形式的人血液和骨髓癌。人CD19分子是在人B细胞表面上表达的结构独特的细胞表面受体,所述人B细胞包括但不限于前B细胞、早期发育中的B细胞(即未成熟的B细胞)、终末分化为浆细胞的成熟B细胞和恶性B细胞。CD19由大多数前B急性淋巴母细胞性白血病(ALL)、非霍奇金淋巴瘤、B细胞慢性淋巴细胞性白血病(CLL)、幼淋巴细胞白血病、毛细胞性白血病、普通型急性淋巴细胞性白血病和一些无标志性急性淋巴母细胞性白血病表达(Nadler LM等人(1983)J Immunol,131:244-250;Anderson KC等人,(1984)Blood,63:1424-1433;Loken MR等人(1987)Blood,70:1316-1324;Uckun FM等人(1988)Blood,71:13-29;Scheuermann RH& Racila E(1995)Leuk Lymphoma,18:385-397)。CD19在浆细胞上的表达进一步表明其可以在分化的B细胞肿瘤如多发性骨髓瘤、浆细胞瘤、Waldenstrom's氏肿瘤上表达(Grossbard ML等人(1998)Br J Haematol,102:509-15;Treon SP等人(2003)Semin Oncol,30:248-52)。

[0439] PCT公开号:WO2010/095031中描述的人源化抗人CD19抗体利用VH3-23和VK1可变

结构域框架，并可用于产生如实施例2.1中所述的双特异性抗体。可以使用人源化抗人CD19抗体(其具有SEQ ID NO:296的VH结构域和SEQ ID NO:297的VL结构域)，并且根据其在BEAT抗体形式中的用途，使用G65S或N82aS取代(Kabat编号)进一步消除对蛋白A的结合。

[0440] 2.8 IgE

[0441] 重定向T细胞以杀伤膜结合性IgE阳性B细胞的双特异性抗体可用于治疗不同的炎性疾病如哮喘或纤维化。已经将几种抗人IgE抗体描述为研究试剂或治疗候选物。其中最佳表征的抗人IgE抗体是奥马珠单抗(Omalizumab)抗体(商品名**Xolair®**,USPTO公开号：US6,761,889、US6,329,509和US20080003218A1;Presta LG等人，(1993)J Immunol,151:2623-2632;具有SEQ ID NO:298的人源化VH结构域和具有SEQ ID NO:299的VL结构域)及其变体。

[0442] 遵循实施例2.1中描述的方法和工作流程，通过CDR嫁接至VH3-23和VK1种系框架上，工程化构建奥马珠单抗抗体的人源化VH和VL结构域。根据其在BEAT抗体形式中的用途，使用G65S或N82aS取代(Kabat编号)进一步消除所得的基于VH3的可变结构域对蛋白A的结合。研究两个稳定的VH和VL结构域，其具有不同程度的回复突变。从亲本抗体可变结构域和类似于第一原型抗体的CDR嫁接的VH3和VK1之间的序列比对和在实施例2.1中描述的方法，鉴定回复突变。这些CDR嫁接的可变结构域没有回复突变，在本文中称为最小嫁接(mingraft)。然后进一步修饰这些序列以包括从先前比对鉴定的所有回复突变，并得到本文称为最大嫁接(maxgraft)的修饰的可变结构域序列。得到的序列总结如下：

[0443] 没有回复突变的稳定的奥马珠单抗VH;缩写为稳定的奥马珠单抗/最小嫁接VH(SEQ ID NO:300)。

[0444] 具有所有可能的回复突变的稳定的奥马珠单抗VH;缩写为稳定的奥马珠单抗/最大嫁接VH(SEQ ID NO:301)。

[0445] 没有回复突变的稳定的奥马珠单抗VL;缩写为稳定的奥马珠单抗/最小嫁接VL(SEQ ID NO:302)。

[0446] 具有所有可能的回复突变的稳定的奥马珠单抗VL;缩写为稳定的奥马珠单抗/最大嫁接VL(SEQ ID NO:303)。

[0447] 抗人IgE抗体的另一个实例是小鼠抗体Bsw17(Vogel M等人，(2004)J Mol Biol,341(2):477-89;具有SEQ ID NO:304的小鼠VH结构域和SEQ ID NO:305的小鼠VL结构域)。

[0448] 遵循实施例2.1中描述的方法和工作流程，通过CDR嫁接到VH3-23和VK1种系框架上，工程化构建人源化的Bsw17抗体的人源化VH和VL结构域。根据其在BEAT抗体形式中的用途，使用G65S或N82aS取代(Kabat编号)进一步消除所得的基于VH3的可变结构域对蛋白A的结合。研究两个稳定的VH和VL结构域，两者区别在于不同程度的回复突变。从亲本抗体可变结构域和类似于第一原型抗体的CDR嫁接的VH3和VK1之间的序列比对和在实施例2.1中描述的方法，鉴定回复突变。这些CDR嫁接的可变结构域没有回复突变，在本文中称为最小嫁接(mingraft)。然后进一步修饰这些序列以包括从先前比对鉴定的所有回复突变，并得到本文称为最大嫁接(maxgraft)的修饰的可变结构域序列。得到的序列总结如下：

[0449] 没有回复突变的稳定的Bsw17VH;缩写为稳定的Bsw17/最小嫁接VH(SEQ ID NO:306)。

[0450] 具有所有可能的回复突变的稳定的Bsw17VH;缩写为稳定的Bsw17/最大嫁接VH

(SEQ ID NO:307)。

[0451] 没有回复突变的稳定的Bsw17VL;缩写为稳定的Bsw17/最小嫁接VL(SEQ ID NO:308)。

[0452] 具有所有可能的回复突变的稳定的Bsw17VL;缩写为稳定的Bsw17/最大嫁接VL(SEQ ID NO:309)。

[0453] 实施例3:产生T细胞重靶向性异源二聚体免疫球蛋白

[0454] 3.1**BEAT®**技术和内置纯化系统

[0455] BEAT抗体是基于独特的生物模拟概念的重链异源二聚体,其比"杵臼(knob-into-hole)"技术显示出更优异的异源二聚化(PCT公开号:WO2012131555)。BEAT平台基于天然同源或异源二聚体免疫球蛋白结构域对之间位于3D等同位置的界面氨基酸的交换,以产生新的异源二聚体,其可用作基于Fc的双特异性抗体的构件。该技术允许使用任何类型的抗原结合支架,设计基于Fc的双特异性抗体。本文中使用scFv-FAB形式来设计基于Fc的双特异性抗体,不需要开发共同轻链用于两个抗原结合位点。

[0456] 由于BEAT抗体是重链异源二聚体,因此需要区分两种不同的重链。其在本文中称为BTA和BTB链。本文所用的BTA和BTB链包含抗原结合位点、人IgG1铰链区、源自人IgG1或IgG3同种型的CH2结构域和源自人IgG1或IgG3同种型的修饰的CH3结构域。一些BTA和BTB CH3结构域与PCT公开号:WO2012131555中描述的结构域相同或是其修饰的变体。BTA和BTB CH3结构域选自:(BTA)SEQ ID NO:147、148、149、153、154和155,和(BTB)SEQ ID NO:150、151、152、156、157和158。优选的BTA-BTB CH3结构域对选自:SEQ ID NO:147与SEQ ID NO:150、SEQ ID NO:148与SEQ ID NO:150、SEQ ID NO:149与SEQ ID NO:151、SEQ ID NO:147与SEQ ID NO:152和SEQ ID NO:148与SEQ ID NO:152。最优选的BTA-BTB CH3结构域对选自:SEQ ID NO:147与SEQ ID NO:156、SEQ ID NO:148与SEQ ID NO:156、SEQ ID NO:154与SEQ ID NO:150、和SEQ ID NO:154与SEQ ID NO:152。

[0457] 如上所述,具有与蛋白A不对称结合的BEAT重链异源二聚体,可以使用来自不与蛋白A结合的免疫球蛋白同种型的亲本结构域产生(PCT公开号:WO2012131555)。同源和异源二聚体物之间蛋白A结合位点数量的差异对于通过蛋白A色谱法分离这些分子物类特别有用。为了避免可干扰蛋白A色谱物类分离的残留结合,有必要消除人重链可变结构域的VH3亚类中天然存在的任何次级蛋白A结合位点。当抗原结合位点来自VH3家族时,可以通过G65S或N82aS取代(Kabat编号)实现其蛋白A结合位点的消除。

[0458] 当使用VH3来源的一个抗原结合位点和来自非VH3来源的一个抗原结合位点来制备本发明的双特异性抗体时,VH3来源的抗原结合位点需要位于在其Fc区结合蛋白A的重链上。或者,可以使用N82aS取代或G65S取代或其等价取代,对VH3来源的抗原结合位点进行取代以消除蛋白A结合。当使用一对VH3来源的抗原结合位点制备本发明的双特异性抗体时,唯一的可能性是通过上述氨基酸取代消除至少一个基于VH3的抗原结合位点中的蛋白A结合。优选地,来自本发明的双特异性抗体被工程化以产生没有蛋白A结合位点的两个同源二聚体之一。更优选地,来自本发明的双特异性抗体被工程化,以产生一个没有蛋白A结合位点的同源二聚体、和另一个在蛋白A结合位点数上与感兴趣的异源二聚体实质上不同的(至少一个蛋白A结合位点,优选两个蛋白A结合位点)的同源二聚体。

[0459] 对单特异性抗人CD3ε抗体触发的毒性机制已经有广泛的研究;直接机制与抗体的

亲和力、表位和效价相关，间接毒性机制也已被描述。间接毒性机制由抗人CD3ε抗体的Fc区介导，其与表达Fc受体的免疫细胞相互作用并导致瞬时T细胞活化和细胞因子释放。为了提高安全性的目的，消除了靶向人CD3ε的BEAT抗体在其下铰链区中对Fc受体的结合。使用L234A和L235A取代消除或减少Fc受体结合(EU编号；Strohl WR等人，(2009)Curr Opin Biotechnol,20(6):685-91)；这些取代通常被称为LALA取代。

[0460] 包含至少一个消除了蛋白A结合的VH3结构域的BEAT抗体的实例

[0461] HER2/CD3靶向BEAT抗体的实例

[0462] 抗HER2和抗CD3ε臂可形成为由融合至BEAT链的scFv片段组成的scFv-Fc型重链，或由融合至BEAT链的FAB片段组成的重链(与天然抗体相似)。基于FAB的重链需要与其关联轻链缔合以组装成功能性抗原结合位点。

[0463] 在CH2区引入L234A和L235A取代，并且酌情使用G65S或N82aS取代(Kabat编号)消除残留的蛋白A结合。靶向人HER2抗原和人CD3ε的BEAT抗体的实例形成如下：

[0464] 通过如实施例和2.2中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人HER2臂)，工程化构建了第一BEAT HER2/CD3抗体。该异源二聚体免疫球蛋白的抗人CD3ε臂由与其关联轻链(SEQ ID NO:47)组装的BEAT重链(SEQ ID NO:159)组成，所述BEAT重链(SEQ ID NO:159)包含具有N82aS取代(Kabat编号)的可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区、和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3结构域亚类的重链可变结构域，所以VH结构域被突变以包括N82aS取代，从而去除重链内的任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人HER2臂由BEAT重链(SEQ ID NO:160)组成，所述BEAT重链(SEQ ID NO:160)包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区、基于γ1的BEAT CH3结构域。该双特异性抗体在本文中称为BEAT HER2/CD3-1抗体(图12A的形式A)。

[0465] 使用如实施例2.1和2.2中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人HER2臂)，工程化构建了第二BEAT HER2/CD3抗体。该异源二聚体免疫球蛋白的抗人HER2臂由与其关联轻链(SEQ ID NO:3)组装的BEAT重链(SEQ ID NO:161)组成，所述BEAT重链(SEQ ID NO:161)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1 CH2区、和基于γ1的BEAT CH3结构域。该异源二聚体免疫球蛋白的抗人CD3ε臂由BEAT重链(SEQ ID NO:162)组成，所述BEAT重链(SEQ ID NO:162)包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区、基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3结构域亚类的重链可变结构域，所以突变该VH结构域以包括N82aS取代，从而去除重链内的任何其他蛋白A结合位点。该双特异性抗体在本文中称为BEAT HER2/CD3-2抗体(图12A的形式B)。

[0466] 使用实施例2.1和2.2中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人HER2臂)，工程化构建第三BEAT HER2/CD3抗体。该异源二聚体免疫球蛋白的抗人CD3ε臂由与其关联轻链(SEQ ID NO:47)组装的BEAT重链(SEQ ID NO:163)组成，所述BEAT重链(SEQ ID NO:163)包含具有G65S取代(Kabat编号)的可变重链结构域、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区、和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3结构域亚

类的重链可变结构域，所以突变该VH结构域以包括G65S取代，从而去除重链内的任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人HER2臂由BEAT重链(SEQ ID NO:164)组成，所述BEAT重链(SEQ ID NO:164)包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区、基于γ1的BEAT CH3结构域。如PCT公开号WO 1994029350中所述，使用在Kabat位置重链44(G44C)和轻链100(Q100C)上在重链和轻链结构域之间的工程化二硫键，进一步稳定双特异性抗体的scFV部分。该双特异性抗体在本文中称为BEAT HER2/CD3-3抗体(图12B的形式C)。

[0467] 使用实施例2.1和2.2中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人HER2臂)，工程化构建了第四BEAT HER2/CD3抗体。该异源二聚体免疫球蛋白的抗人CD3ε臂由与其关联轻链(SEQ ID NO:166)组装的BEAT重链(SEQ ID NO:165)组成，所述BEAT重链(SEQ ID NO:165)包含可变重链结构域、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1 CH2区、和基于γ1的BEAT CH3结构域。该组装的重链和轻链包含如PCT公开号WO2008119565中描述的抗人CD3ε抗体(SP34)的人源化版本。该异源二聚体免疫球蛋白的抗人HER2臂由BEAT重链(SEQ ID NO:167)组成，所述BEAT重链(SEQ ID NO:167)包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区、基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3结构域亚类的重链可变结构域，所以突变该VH结构域以包括N82aS取代，从而去除重链内的任何其他蛋白A结合位点。该双特异性抗体在本文中称为BEAT HER2/CD3(SP34)抗体(图12B的形式D)。

[0468] 使用实施例2.1和2.2中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人HER2臂)，工程化构建了第五BEAT HER2/CD3抗体。该异源二聚体免疫球蛋白的抗人CD3ε臂由与其关联轻链(SEQ ID NO:89)组装的BEAT重链(SEQ ID NO:168)组成，所述BEAT重链(SEQ ID NO:168)包含可变重链结构域、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1 CH2区、和基于γ1的BEAT CH3结构域。该双特异性抗体的该臂包含实施例2.1中描述的人源化SP34 VH1/VL21抗体的可变结构域。该异源二聚体免疫球蛋白的抗人HER2臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区、基于γ3的BEAT CH3结构域的BEAT重链(SEQ ID NO:167)组成。该臂等同于上述BEAT HER2/CD3(SP34)抗HER2臂(参见图12B的形式D)。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3结构域亚类的重链可变结构域，所以突变该VH结构域以包括N82aS取代，从而去除重链内的任何其他蛋白A结合位点。该双特异性抗体在本文中称为BEAT HER2/CD3(SP34-κ1)抗体(图12C的形式E)。

[0469] 瞬时表达BEAT HER2/CD3-1、BEAT HER2/CD3-2、BEAT HER2/CD3-3、BEAT HER2/CD3(SP34)和BEAT HER2/CD3(SP34-κ1)抗体，纯化并体外测试其对HER2和CD3ε抗原的亲和力、其稳定性以及其重定向T细胞杀伤的能力。所有BEAT抗体的瞬时表达产量在5-15mg/l培养物上清液的范围内。重要的是，在单个蛋白A色谱步骤后，所有双特异性抗体在其制备物中显示了非常低水平的同源二聚体污染。

[0470] 由于所有这些BEAT抗体都设计成具有包含VH3结构域的两个臂，所以仅消除至少一个VH3结构域中的蛋白A结合已使得可以容易地使用优选的差异纯化方法之一纯化感兴趣的异源二聚体(参见图2E)。图13显示了BEAT HER2/CD3-1抗体的差异蛋白A纯化曲线实

例。图14显示纯化的异源二聚体的毛细管电泳图谱。从该图谱中仅能够鉴定到微小量的同源二聚体污染。没有发现携带FAB部分的重链同源二聚体形式，因为其不结合蛋白A。携带scFv片段的重链同源二聚体形式以微小比例(2.5%)存在，导致在单个蛋白A色谱步骤后异源二聚体含量为97%。在单个蛋白A色谱步骤后BEAT HER2/CD3-2、BEAT HER2/CD3-3、BEAT HER2/CD3(SP34)和BEAT HER2/CD3(SP34-κ1)抗体被纯化至相似水平的均质性和纯度。BEAT HER2/CD3-3抗体在蛋白A色谱后显示出一定比例的二硫键合的异源二聚体聚集体(27%)，其通过阳离子交换色谱法去除。

[0471] 为了进一步证明在基于VH3的重链异源二聚体中消除蛋白A结合对蛋白A色谱后纯度的影响很大，工程化构建不具有前述的N82aS取代的BEAT HER2/CD3-1抗体。图15A和15B分别显示BEAT HER2/CD3-1及其非N82aS取代版本的洗脱蛋白A色谱级分的SDS-PAGE分析。在pH4时，非N82Sa取代版本的洗脱级分显示出对应于携带FAB臂的重链的同源二聚体的额外条带(图15B)，而N82aS取代的BEAT HER2/CD3版本则没有(图15A)，因为携带FAB臂的重链形式在其Fc区(基于人IgG3同种型的Fc区)不结合蛋白A，只能推断：蛋白A的结合由该同源二聚体物中的基于VH3的可变结构域负责。该结果清楚地表明，在基于VH3的重链异源二聚体中消除蛋白A结合的效用。

[0472] BEAT HER2/CD3-1和BEAT HER2/CD3-2抗体对人HER2和人CD3ε抗原具有相似的KD值。对于人HER2抗原，KD值在0.50-2nM的范围内；对于人CD3ε抗原，KD值在1-2μM的范围(使用人CD3γ-ε-Fc构建体通过SPR测量(参见材料和方法部分；图16A和16B)。两种双特异性抗体的DSC谱是相似的，在两种情况下，结合人HER2或人CD3ε的scFv部分保持了其良好的热稳定谱，Tm在68℃范围内。两种抗体中的FAB部分具有82-83℃范围内的Tm(图16C)。

[0473] 使用人源化的赫赛汀VH和VL序列的靶向人HER2抗原和人CD3ε的BEAT抗体的另一个例子按如下形成：使用实施例2.1和2.2中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人HER2抗原结合位点)，工程化BEAT HER2/CD3。

[0474] 该异源二聚体免疫球蛋白的抗人HER2臂由与其关联轻链(SEQ ID NO:3)组装的BEAT重链(SEQ ID NO:310)组成，所述BEAT重链(SEQ ID NO:310)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区、和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包含G65S取代，从而除去重链内任何其他蛋白A结合位点。

[0475] 该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区、基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT HER2/CD3(SP34-κ2)抗体。

[0476] 体外T细胞杀伤测定法

[0477] BEAT HER2/CD3抗体的作用机制是基于：通过桥接细胞毒性T细胞表面上的CD3抗原和靶细胞上表达的HER2抗原，使细胞毒性T细胞杀伤靶向靶细胞。

[0478] 使用基于流式细胞术的方法(本文称为RDL-FACS方法)或基于比色的方法(本文称为RDL-MTS方法)，测量BEAT HER2/CD3-1和BEAT HER2/CD3-2抗体重定向T细胞杀伤的效力。

[0479] 高表达HER2的细胞系JIMT-1(一种赫赛汀®(曲妥珠单抗)抗性乳腺癌细胞系)、高表达HER2的细胞系BT-474(一种赫赛汀®(曲妥珠单抗)敏感性乳腺癌细胞系)和低表达

HER2的乳腺癌细胞系MDA-MB-231分别在人PBMC和系列稀释的BEAT HER2/CD3-1或-2抗体或对照抗体的存在下培养48小时。

[0480] 在这些测定中，来自血液供体的人PBMC被用作细胞毒性T淋巴细胞的来源。在所有测定中使用10:1的效应物对靶细胞比例。以没有抗体处理的样品形式(仅靶细胞和人PBMC)提供阴性对照。在孵育期后使用RDL-FACS或RDL-MTS方法测定细胞毒性(参见材料和方法部分)。结果表明，对照抗体不触发特异性T细胞介导的细胞毒性。相反，BEAT HER2/CD3-1和-2抗体诱导了非常有效的剂量依赖性的肿瘤靶细胞死亡。最大杀伤接近100％。两种读取方法给出了接近的结果。在两种方法之间，供体与供体的差异性解释了EC_{50}的约十倍差异。测量的EC_{50}与靶细胞系的HER2抗原表达水平相关。

[0481] BT-474细胞表达最多的HER2抗原，BEAT HER2/CD3-1和-2抗体的EC_{50}在亚皮摩尔至皮摩尔范围内(分别为0.6和2pM，图17A)。JIMT-1细胞在其细胞表面上具有掩藏的HER2抗原(Nagy P等人(2005)，Cancer Res，65(2):473-482)，因此尽管具有高的HER2表达，但是表现出低的赫赛汀®结合。令人吃惊的是，如通过RDL-MTS方法测量的，BEAT HER2/CD3-1和-2抗体针对JIMT-1细胞均具有皮摩尔范围的EC_{50}(分别为21和16pM，图17B)。当用RDL-FACS方法测量时，BEAT HER2/CD3-1抗体的EC_{50}为1.4pM。低表达HER2的乳腺癌细胞系MDA-MB-231比前两种细胞系敏感性低，两种抗体均显示亚纳摩尔的EC_{50}(两者的值均接近0.2nM；图17C)。当用RDL-FACS方法测量时，BEAT HER2/CD3-1抗体的EC_{50}为0.08nM。总之，这些结果表明，BEAT HER2/CD3-1和-2抗体在重定向T细胞杀伤各种表达HER2的乳腺癌细胞系方面是高度有效的。

[0482] BEAT HER2/CD3(SP34)抗体包含PCT公开号:WO2008119565中描述的抗人CD3ε抗体(SP34)的人源化版本。在体外研究了该BEAT抗体形式将T细胞杀伤重定向到HER2+细胞的能力。两种不同的HER2+细胞系用于杀伤测定，高表达HER2的细胞系(NCI-N87)和低表达HER2的细胞系(HT-1080)(参见材料和方法部分)。图17D-E分别显示BEAT HER2/CD3(SP34)抗体引起的对NCI-N87和HT-1080细胞的T细胞重定向杀伤。测定法使用10:1的效应细胞与靶细胞比例，以及48小时孵育期后RDL-MTS读取方法(参见材料和方法部分)。结果表明，当分别靶向NCI-N87和HT-1080细胞时，BEAT HER2/CD3(SP34)抗体在重定向T细胞杀伤HER2+细胞系方面高度有效，其中EC_{50}为0.35和29pM。

[0483] BEAT HER2/CD3(SP34-κ1)抗体包含实施例2.1中描述的抗人CD3ε抗体(SP34-κ1)VH1/VL21的人源化版本。在体外研究了该BEAT抗体形式将T细胞杀伤重定向到HER2+细胞的能力。两种不同的HER2+细胞系用于杀伤测定，高表达HER2的细胞系(NCI-N87)和低表达HER2的细胞系(HT-1080)(参见材料和方法部分)。图17F-G分别显示BEAT HER2/CD3(SP34-κ1)抗体引起的对NCI-N87和HT-1080细胞的T细胞重定向杀伤。测定法使用10比1的效应细胞与靶细胞比例，以及48小时孵育期后的RDL-MTS读取方法(参见材料和方法部分)。结果表明，BEAT HER2/CD3(SP34-κ1)抗体在重定向T细胞杀伤HER2+细胞系方面高度有效，其中靶向NCI-N87和HT-1080细胞时EC_{50}分别为0.46和338pM。

[0484] 体内功效研究

[0485] JIMT-1异种移植

[0486] 使用JIMT-1/PBMC异种移植模型研究BEAT HER2/CD3-1抗体的体内功效。使用来自血液供体的人PBMC作为细胞毒性T淋巴细胞的来源。赫赛汀®抗性乳腺癌JIMT-1细胞以1:

1的比例与未刺激的人PBMC(四个不同供体)混合,随后皮下注射至免疫缺陷(NOD/SCID)小鼠中。植入后,在两周期间每周三次用BEAT HER2/CD3-1抗体静脉内处理动物。抗体处理于植入后3小时开始,并在此后第2、4、7、9和11天继续进行。

[0487] 为了评估没有PBMC的肿瘤生长,五组中的一组皮下接种不含有人PBMC的5×10e6个JIMT-1细胞,而剩余组皮下注射5×10e6个JIMT-1细胞与来自健康供体的5×10e6个未刺激的人PBMC的混合物。

[0488] 在不存在抗体的情况下,人PBMC对肿瘤生长不显示负面影响(图18A)。在人效应细胞存在下,用BEAT HER2/CD3-1抗体进行处理,在大多数动物中诱导了完全的肿瘤生长抑制(18/20肿瘤,图18B-C)。处理最后一天后18天,只有11%的肿瘤(2/18)再次开始生长。这些数据非常清楚地显示了BEAT HER2/CD3-1抗体的强抗肿瘤功效。

[0489] 靶向CD38/CD3的BEAT抗体的实例

[0490] 抗CD38和抗CD3ε臂可以形成为由融合至BEAT链的scFv片段组成的scFv-Fc型重链,或由融合至BEAT链的FAB片段组成的重链(与天然抗体相似)。基于FAB的重链需要与其关联轻链缔合以组装成功能性抗原结合位点。

[0491] 在CH2区引入L234A和L235A取代,并且酌情地使用G65S或N82aS取代(Kabat编号)消除残留的蛋白A结合。靶向人CD38抗原和人CD3ε的BEAT抗体的实例如下形成:

[0492] 使用人源化HB7最适配VH和VL序列,按如下形成靶向人CD38抗原和人CD3ε的第一BEAT抗体实例:使用实施例2.1和2.3中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人CD38臂),工程化构建了BEAT CD38/CD3抗体。该异源二聚体免疫球蛋白的抗人CD38臂由与其关联的轻链(SEQ ID NO:119)组装的BEAT重链(SEQ ID NO:169)组成,所述BEAT重链(SEQ ID NO:169)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1 CH2区、和基于γ1的BEAT CH3结构域。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区、和基于γ3的BEAT CH3结构域的BEAT重链(SEQ ID NO:162)组成。该重链包含人IgG3 Fc区的一部分,因此不与蛋白A结合,但是由于此处所用的重链具有源自VH3框架的重链可变结构域,所以突变该VH结构域以包括N82aS取代,从而除去重链内的任何其他蛋白A结合位点。该臂等同于上述BEAT HER2/CD3-2的抗CD3ε臂(参见图12A的形式B)。该双特异性抗体在本文中称为BEAT CD38-HB7最适配/CD3抗体(图19的形式A)。

[0493] 瞬时表达BEAT CD38-HB7最适配/CD3抗体,纯化并在体外测试其对CD38和CD3ε抗原的亲和力、其稳定性及其重定向T细胞杀伤的能力。对人CD38抗原,KD值为3.2nM(通过SPR测量;图20A)。该双特异性抗体的DSC谱显示了良好的热稳定谱,scFv部分的Tm约68℃。FAB部分具有约91℃的Tm(图20B)。

[0494] 在类似实施例3.2.1所述的测定法中使用表达CD38的细胞系(参见材料和方法部分)来评估重定向的T细胞杀伤。图21显示了使用BEAT CD38-HB7最适配/CD3抗体对RPMI 8226骨髓瘤细胞的T细胞重定向杀伤。注意,该测定法使用纯化的T细胞作为效应细胞,其中效应细胞与靶细胞的比例为10比1。当用RDL-FACS方法测量时,BEAT CD38-HB7最适配/CD3抗体具有2.2pM的EC_{50}(2个供体的平均值,孵育48小时)。

[0495] 使用人克隆767VH和VL序列的、靶向人CD38抗原和人CD3ε的第二BEAT抗体实例如下形成:使用实施例2.1和2.3中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人

CD38臂)，工程化构建BEAT CD38/CD3抗体。该异源二聚体免疫球蛋白的抗人CD38臂由与其关联的轻链(SEQ ID NO:138)组装的BEAT重链(SEQ ID NO:170)组成，所述BEAT重链(SEQ ID NO:170)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1 CH2区、和基于γ1的BEAT CH3结构域。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区、和基于γ3的BEAT CH3结构域的BEAT重链(SEQ ID NO:171)组成。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变VH结构域以包括G65S取代，从而除去重链内的任何其他蛋白A结合位点。该双特异性抗体在本文中称为BEAT CD38-767/CD3抗体(图19的形式B)。

[0496] 瞬时表达BEAT CD38-767/CD3抗体，纯化并在体外测试其对CD38和CD3ε抗原的亲和力、其稳定性及其重定向T细胞杀伤的能力。在类似实施例3.2.1所述的测定法中使用表达CD38的细胞系(参见材料和方法部分)来评估重定向的T细胞杀伤。图22显示了使用BEAT CD38-767/CD3抗体对Daudi细胞的T细胞重定向杀伤。注意测定法使用人PBMC作为效应细胞，其中效应细胞与靶细胞的比例为10比1。当用RDL-FACS方法测量时，BEAT CD38-767/CD3抗体具有244pM的EC_{50}(3个供体的平均值，孵育24小时)。

[0497] 使用人源化的9G7最佳框架VH和VL序列的靶向人CD38抗原和人CD3ε的BEAT抗体的另一个实例如下形成：使用实施例2.1和2.3中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人CD38抗原结合位点)，工程化构建BEAT CD38/CD3抗体。

[0498] 该异源二聚体免疫球蛋白的抗人CD38臂由与其关联的轻链(SEQ ID NO:132)组装的BEAT重链(SEQ ID NO:312或404)组成，所述BEAT重链(SEQ ID NO:312或404)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区、和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包括G65S取代，从而除去重链内的任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1 CH2区、和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT CD38-9G7最佳框架/CD3(SP34-κ2)抗体。

[0499] 使用人克隆767VH和VL序列的靶向人CD38抗原和人CD3ε的BEAT抗体的另一个实例如下形成：使用实施例2.1和2.3中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人CD38抗原结合位点)，工程化构建BEAT CD38/CD3。

[0500] 该异源二聚体免疫球蛋白的抗人CD38臂由与其关联的轻链(SEQ ID NO:138)组装的BEAT重链(SEQ ID NO:313)组成，所述BEAT重链(SEQ ID NO:313)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区、和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包括G65S取代，从而除去重链内的任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1 CH2区、和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT CD38-767/CD3(SP34-κ2)抗体。

[0501] 靶向OX40/CD3的BEAT抗体的实例

[0502] 抗OX40和抗CD3ε臂可以形成为由融合至BEAT链的scFv片段组成的scFv-Fc型重链，或由融合至BEAT链的FAB片段组成的重链(与天然抗体相似)。基于FAB的重链需要与其关联轻链缔合以组装成功能性抗原结合位点。

[0503] 在CH2区引入L234A和L235A取代，并且酌情地使用G65S或N82aS取代(Kabat编号)消除其中残留的蛋白A结合。靶向人OX40抗原和人CD3ε的BEAT抗体的实例如下形成：

[0504] 使用实施例2.1和2.4中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人OX40臂)，工程化构建BEAT OX40/CD3抗体实例。该异源二聚体免疫球蛋白的抗人OX40臂使用PCT公开号WO2013008171公开的人源化抗人OX40抗体的可变结构域(可变重链和轻链结构域分别具有SEQ ID NO:141和142)，并且由与其关联轻链(SEQ ID NO:173)组装的BEAT重链(SEQ ID NO:172)组成，所述BEAT重链(SEQ ID NO:172)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1 CH2区、和基于γ1的BEAT CH3结构域。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区、和基于γ3的BEAT CH3结构域的BEAT重链(SEQ ID NO:162)组成。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包括N82aS取代，从而除去重链内任何其他蛋白A结合位点。该臂等同于上述BEAT HER2/CD3-2的抗CD3ε臂(参见图12A的形式B)。该双特异性抗体在本文中称为BEAT OX40/CD3抗体(图23)。

[0505] 在体外研究了BEAT OX40/CD3抗体将T细胞杀伤重定向到OX40+细胞的能力。将稳定的重组CHO[OX40]细胞系用于杀伤测定。图24显示BEAT OX40/CD3抗体引起的对稳定的重组CHO[OX40]细胞的T细胞重定向杀伤。测定法使用人PBMC作为效应细胞(其中效应细胞与靶细胞的比例为20比1)，以及48小时孵育期后的RDL-MTS读取方法(参见材料和方法部分)。结果表明，BEAT OX40/CD3抗体在重定向T细胞杀伤稳定的重组CHO[OX40]细胞方面是高度有效的，其中EC_{50}为0.5nM(3个供体的平均值)。

[0506] 使用人源化抗OX40/最大嫁接VH和VL序列的靶向人OX40抗原和人CD3ε的BEAT抗体的另一个实例如下形成：使用实施例2.1和2.4中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人OX40抗原结合位点)，工程化构建BEAT OX40/CD3。

[0507] 该异源二聚体免疫球蛋白的抗人OX40臂由与其关联轻链(SEQ ID NO:315)组装的BEAT重链(SEQ ID NO:314)组成，所述BEAT重链(SEQ ID NO:314)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区、和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包括G65S取代，从而除去重链内的任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1 CH2区、和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT OX40最大嫁接/CD3(SP34-κ2)抗体。

[0508] 使用人源化抗OX40/最小嫁接VH和VL序列的靶向人OX40抗原和人CD3ε的BEAT抗体的另一个实例如下形成：使用实施例2.1和2.4中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人OX40抗原结合位点)，工程化构建BEAT OX40/CD3。

[0509] 该异源二聚体免疫球蛋白的抗人OX40臂由与其关联轻链(SEQ ID NO:317)组装的BEAT重链(SEQ ID NO:316)组成，所述BEAT重链(SEQ ID NO:316)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区、和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包括G65S取代，从而除去重链内的任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1 CH2区、和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT OX40最小嫁接/CD3(SP34-κ2)抗体。

[0510] 靶向CD20/CD3的BEAT抗体实例

[0511] 使用小鼠利妥昔单抗抗体VH和VL序列的靶向人CD20抗原和人CD3ε的BEAT抗体的实例如下形成：

[0512] 使用实施例2.1和2.5中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人CD20臂)，工程化构建BEAT CD20/CD3。

[0513] 使用人源化的利妥昔单抗/最大嫁接VH和VL序列的靶向人CD20抗原和人CD3ε的BEAT抗体的实例如下形成：使用实施例2.1和2.5中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人CD20抗原结合位点)，工程化构建BEAT CD20/CD3。

[0514] 该异源二聚体免疫球蛋白的抗人CD20臂由与其关联轻链(SEQ ID NO:319)组装的BEAT重链(SEQ ID NO:318)组成，所述BEAT重链(SEQ ID NO:318)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包括G65S取代，从而除去重链内任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区、和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。此双特异性抗体在本文中称为BEAT CD20最大嫁接/CD3(SP34-κ2)抗体。

[0515] 使用人源化利妥昔单抗/最小嫁接VH和VL序列的靶向人CD20抗原和人CD3ε的BEAT抗体的另一个实例如下形成：使用实施例2.1和2.5中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人CD20抗原结合位点)，工程化构建BEAT CD20/CD3。

[0516] 该异源二聚体免疫球蛋白的抗人CD20臂由与其关联轻链(SEQ ID NO:321)组装的BEAT重链(SEQ ID NO:320)组成，所述BEAT重链(SEQ ID NO:320)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区、和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包括G65S取代，从而除去重链内任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。这种双特异性抗体在本文中称为BEAT CD20最小嫁接/CD3(SP34-κ2)抗体。

[0517] 靶向EGFR/CD3的BEAT抗体的实例

[0518] 抗EGFR和抗CD3ε臂可以形成由融合至BEAT链的scFv片段组成的scFv-Fc型重链、或由融合至BEAT链的FAB片段组成的重链(与天然抗体相似)。基于FAB的重链需要与其关联的轻链缔合以组装成功能性抗原结合位点。

[0519] 在CH2区引入L234A和L235A取代,并且酌情地使用G65S或N82aS取代(Kabat编号)消除残留的蛋白A结合。靶向人EGFR抗原和人CD3ε的BEAT抗体的实例如下形成:

[0520] 靶向人EGFR和人CD3ε抗原的BEAT抗体的实例如下形成:使用实施例2.1和2.6中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人EGFR臂),工程化构建BEAT EGFR/CD3。该异源二聚体免疫球蛋白的抗人EGFR臂由与其关联轻链(SEQ ID NO:175)组装的BEAT重链(SEQ ID NO:174)组成,所述BEAT重链(SEQ ID NO:174)基于小鼠爱必妥抗体可变结构域(小鼠可变重链和轻链结构域分别具有SEQ ID NO:145和146),包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区和基于γ1的BEAT CH3结构域。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区和基于γ3的BEAT CH3结构域的BEAT重链(SEQ ID NO:171)组成。该重链包含人IgG3 Fc区的一部分,因此不与蛋白A结合,但由于此处所用的重链具有源自VH3框架的重链可变结构域,所以突变该VH结构域以包括G65S取代,从而除去重链内任何其他蛋白A结合位点。该臂等同于上述BEAT CD38-767/CD3的抗CD3ε臂(参见图19的形式B)。该双特异性抗体在本文中称为BEAT EGFR/CD3抗体(图25)。

[0521] 瞬时表达BEAT EGFR/CD3抗体、纯化并在体外测试其重定向T细胞杀伤人EGFR+细胞系的能力。HT-29细胞系用于杀伤测定中。图26显示BEAT EGFR/CD3抗体引起的对HT-29细胞的T细胞重定向杀伤。测定法使用人PBMC作为效应细胞,其中效应细胞与靶细胞的比例为10比1,以及使用48小时孵育期后的RDL-MTS读取方法(参见材料和方法部分)。结果表明,BEAT EGFR/CD3抗体在重定向T细胞杀伤HT-29细胞方面具有很高的效力,其中EC_{50}为70.6pM(4个供体的平均值)。

[0522] 使用人源化的爱必妥/最大嫁接VH和VL序列的靶向人EGFR抗原和人CD3ε的BEAT抗体的另一个实例如下形成:

[0523] 使用实施例2.1和2.6中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人EGFR抗原结合位点),工程化构建BEAT EGFR/CD3。

[0524] 该异源二聚体免疫球蛋白的抗人EGFR臂由与其关联轻链(SEQ ID NO:323)组装的BEAT重链(SEQ ID NO:322)组成,所述BEAT重链(SEQ ID NO:322)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区、和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分,因此不与蛋白A结合,但由于此处所用的重链具有源自VH3框架的重链可变结构域,所以突变该VH结构域以包括G65S取代,从而除去重链内任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。这种双特异性抗体在本文中称为BEAT EGFRcetux-最大嫁接/CD3(SP34-κ2)抗体。

[0525] 使用人源化的爱必妥/最小嫁接VH和VL序列的靶向人EGFR抗原和人CD3ε的BEAT抗体的另一个实例如下形成:

[0526] 使用实施例2.1和2.6中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人

EGFR抗原结合位点)，工程化构建BEAT EGFR/CD3。

[0527] 该异源二聚体免疫球蛋白的抗人EGFR臂由与其关联轻链(SEQ ID NO:325)组装的BEAT重链(SEQ ID NO:324)组成，所述BEAT重链(SEQ ID NO:324)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区、和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包括G65S取代，从而除去重链内任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε部分由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。这种双特异性抗体在本文中称为BEAT EGFRcetux-最小嫁接/CD3(SP34-κ2)抗体。

[0528] 使用人源化的维克替比/最大嫁接VH和VL序列的靶向人EGFR抗原和人CD3ε的BEAT抗体的另一个实例如下形成：使用实施例2.1和2.6中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人EGFR抗原结合位点)，工程化构建BEAT EGFR/CD3。

[0529] 该异源二聚体免疫球蛋白的抗人EGFR臂由与其关联轻链(SEQ ID NO:327)组装的BEAT重链(SEQ ID NO:326)组成，所述BEAT重链(SEQ ID NO:326)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区、和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包括G65S取代，从而除去重链内任何其他蛋白A结合位点。

[0530] 该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT EGFRpani-最大嫁接/CD3(SP34-κ2)抗体。

[0531] 使用人源化的维克替比/最小嫁接VH和VL序列的靶向人EGFR抗原和人CD3ε的BEAT抗体的另一个实例如下形成：使用实施例2.1和2.6中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人EGFR抗原结合位点)，工程化构建BEAT EGFR/CD3。

[0532] 该异源二聚体免疫球蛋白的抗人EGFR臂由其关联轻链(SEQ ID NO:329)组装的BEAT重链(SEQ ID NO:328)组成，所述BEAT重链(SEQ ID NO:328)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区、和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包括G65S取代，从而除去重链内任何其他蛋白A结合位点。

[0533] 该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT EGFRpani-最小嫁接/CD3(SP34-κ2)抗体。

[0534] CD19/CD3 BEAT抗体的实例

[0535] 抗CD19和抗CD3重链可形成为由融合至第一BEAT链的scFv片段组成的scFv-Fc型重链，或由融合至第一BEAT链的FAB片段组成的重链(与天然抗体相似)。基于FAB的重链需

要与其关联轻链结合以组装成功能性抗原结合位点。在CH2区引入L234A和L235A取代，并且酌情使用G65S或N82aS取代(Kabat编号)消除残留的蛋白A结合。使用WO2010095031中描述的抗CD19VH和VL序列的靶向人CD19抗原和人CD3ε的BEAT抗体的实例形成如下：

[0536] 使用实施例2.1和2.7中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人CD19抗原结合位点)，工程化构建BEAT CD19/CD3的实例。

[0537] 该异源二聚体免疫球蛋白的抗人CD19臂由与其关联轻链(SEQ ID NO:331)组装的BEAT重链(SEQ ID NO:330)组成，所述BEAT重链(SEQ ID NO:330)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包括G65S取代，从而去除重链内的任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区、基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT CD19/CD3(SP34-κ2)抗体。

[0538] 在PCT公开号:WO2010/095031中描述的表达CD19的细胞系在类似于实施例3.2.1中所述的测定法中用于评估重定向的T细胞杀伤。

[0539] IgE/CD3 BEAT抗体的实例

[0540] 抗IgE和抗CD3重链可形成为由融合至第一BEAT链的scFv片段组成的scFv-Fc型重链，或由融合至第一BEAT链的FAB片段组成的重链(与天然抗体相似)。基于FAB的重链需要与其关联轻链结合以组装成功能性抗原结合位点。在CH2区引入L234A和L235A取代，并且酌情地使用G65S或N82aS取代(Kabat编号)消除残留的蛋白A结合。

[0541] 使用实施例2.1和2.8中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人IgE抗原结合位点)，工程化构建BEAT IgE/CD3抗体。

[0542] 在PCT公开号:WO2010/033736中描述了在其细胞表面上表达IgE的细胞系，并且可将其用于在类似于实施例3.2.1中描述的测定法中评估重定向的T细胞杀伤。

[0543] 使用稳定化的奥马珠单抗/最大嫁接VH和VL序列的靶向人IgE抗原和人CD3ε的BEAT抗体的实例如下形成：

[0544] 该异源二聚体免疫球蛋白的抗人IgE臂由与其关联轻链(SEQ ID NO:333)组装的BEAT重链(SEQ ID NO:332)组成，所述BEAT重链(SEQ ID NO:332)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分，因此不与蛋白A结合，但是由于此处所用的重链具有源自VH3框架的重链可变结构域，所以突变该VH结构域以包括G65S取代，从而去除重链内的任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区、基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT IgEomali-最大嫁接/CD3(SP34-κ2)抗体。

[0545] 使用稳定化的奥马珠单抗/最小嫁接VH和VL序列的靶向人IgE抗原和人CD3ε的BEAT抗体的另一个实例如下形成：

[0546] 该异源二聚体免疫球蛋白的抗人IgE臂由与其关联轻链(SEQ ID NO:335)组装的

BEAT重链(SEQ ID NO:334)组成,所述BEAT重链(SEQ ID NO:334)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分,因此不与蛋白A结合,但是由于此处所用的重链具有源自VH3框架的重链可变结构域,所以突变该VH结构域以包括G65S取代,从而去除重链内的任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区、基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT IgEomali-最小嫁接/CD3(SP34-κ2)抗体。

[0547] 使用稳定化的Bsw17/最大嫁接VH和VL序列的靶向人IgE抗原和人CD3ε的BEAT抗体的另一个实例如下形成:

[0548] 该异源二聚体免疫球蛋白的抗人IgE臂由与其关联轻链(SEQ ID NO:337)组装的BEAT重链(SEQ ID NO:336)组成,所述BEAT重链(SEQ ID NO:336)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分,因此不与蛋白A结合,但是由于此处所用的重链具有源自VH3框架的重链可变结构域,所以突变该VH结构域以包括G65S取代,从而去除重链内的任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区、基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT IgEbsw17-最大嫁接/CD3(SP34-κ2)抗体。

[0549] 使用稳定化的Bsw17/最小嫁接VH和VL序列的靶向人IgE抗原和人CD3ε的BEAT抗体的另一个实例如下形成:

[0550] 该异源二聚体免疫球蛋白的抗人IgE臂由与其关联轻链(SEQ ID NO:339)组装的BEAT重链(SEQ ID NO:338)组成,所述BEAT重链(SEQ ID NO:338)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区和基于γ3的BEAT CH3结构域。该重链包含人IgG3 Fc区的一部分,因此不与蛋白A结合,但是由于此处所用的重链具有源自VH3框架的重链可变结构域,所以突变该VH结构域以包括G65S取代,从而去除重链内的任何其他蛋白A结合位点。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区、基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT IgE bsw17-最小嫁接/CD3(SP34-κ2)抗体。

[0551] 仅包含一个VH3结构域的BEAT抗体的实例

[0552] 靶向CD38/CD3的BEAT抗体的实例

[0553] 使用人源化的HB7/最适配VH和VL序列的靶向人CD38抗原和人CD3ε的BEAT抗体的实例如下形成:使用实施例2.1和2.3中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人CD38臂),工程化构建BEAT CD38/CD3。该异源二聚体免疫球蛋白的抗人CD38臂由与其关联轻链(SEQ ID NO:119)组装的BEAT重链(SEQ ID NO:176)组成,所述BEAT重链(SEQ ID NO:176)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区和基于γ3的BEAT CH3结构域。因为该重链包含人IgG3 Fc区的一部分并且具有源自非VH3结构域亚类的重链可变结构域,所以该重链不与蛋白A结合。该异源二聚体免疫球蛋

白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区、基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:177)组成。该重链和轻链组装物包含如PCT公开号WO2008119565中描述的抗人CD3ε抗体(SP34)的人源化版本。该BEAT抗体形式在本文中称为BEAT CD38-HB7最适配/CD3(SP34)抗体(图27的形式A)。

[0554] 在体外研究了BEAT CD38-HB7最适配/CD3(SP34)抗体重定向T细胞杀伤CD38+细胞的能力。CD38+B淋巴母细胞系Daudi用于杀伤测定中。图28显示了BEAT CD38-HB7最适配/CD3(SP34)抗体引起的对Daudi细胞的T细胞重定向杀伤。测定法使用人PBMC作为效应细胞,其中效应细胞与靶细胞的比例为10比1,以及使用24小时孵育期后的RDL-FACS读取方法(参见材料和方法部分)。结果表明,BEAT CD38-HB7最适配/CD3(SP34)抗体在重定向T细胞杀伤Daudi CD38+细胞系方面具有高度的功效,其中EC_{50}为1.8pM(3个供体的平均值)。

[0555] 使用人源化的9G7最适配VH和VL序列(分别为SEQ ID NO:129和130)的靶向人CD38抗原和人CD3ε的BEAT抗体的第二个实例如下形成:使用实施例2.1和2.3中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人CD38臂),工程化构建BEAT CD38/CD3。该异源二聚体免疫球蛋白的抗人CD38臂由与其关联轻链(SEQ ID NO:128)组装的BEAT重链(SEQ ID NO:178)组成,所述BEAT重链(SEQ ID NO:178)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区和基于γ3的BEAT CH3结构域。因为该重链包含人IgG3 Fc区的一部分并且具有源自非VH3结构域亚类的重链可变结构域,所以其不与蛋白A结合。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区、基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:179)组成。该双特异性抗体的臂包含实施例2.1中描述的人源化的SP34VH5/VL32抗体的可变结构域。该BEAT抗体形式在本文中称为BEAT CD38-9G7最适配/CD3(SP34-κ2)抗体(图27的形式B)。CD38-9G7最适配/CD3(SP34-κ2)抗体具有针对人CD3 1-26_Fc融合蛋白的18nM KD值(图29)。

[0556] 在体外研究了BEAT CD38-9G7最适配/CD3(SP34-κ2)抗体重定向T细胞杀伤CD38+细胞的能力。CD38+B淋巴母细胞系Daudi用于杀伤测定中。图30显示了BEAT CD38-9G7最适配/CD3(SP34-κ2)抗体引起的对Daudi细胞的T细胞重定向杀伤。测定法使用人PBMC作为效应细胞,其中效应细胞与靶细胞的比例为10比1,以及使用24小时孵育期后的RDL-FACS读取方法(参见材料和方法部分)。结果表明,BEAT CD38-9G7最适配/CD3(SP34-κ2)抗体在重定向T细胞杀伤Daudi CD38+细胞系方面具有高度的功效,其中EC_{50}为2pM(3个供体的平均值)。

[0557] 靶向OX40/CD3的BEAT抗体的实例

[0558] 使用人源化抗-OX40抗体VH和VL序列(PCT公开号:WO2013008171)的靶向人OX40抗原和人CD3ε的BEAT抗体的实例如下形成:

[0559] 使用实施例2.1和2.4中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人OX40抗原结合位点),工程化构建BEAT OX40/CD3。

[0560] 该异源二聚体免疫球蛋白的抗人OX40臂由与其关联轻链(SEQ ID NO:173)组装的BEAT重链(SEQ ID NO:340)组成,所述BEAT重链(SEQ ID NO:340)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3 CH2区和基于γ3的BEAT CH3结构域。因为该重链包含人IgG3 Fc区的一部分并且具有源自非VH3结构域亚类的重链可变结构域,所以其不与蛋白A结合。该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ

1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区、基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文中称为BEAT OX40/CD3(SP34-κ2)抗体)。

[0561] 上述表达人OX40的细胞系用于在类似于实施例3.2.4中描述的测定法中评估重定向的T细胞杀伤。

[0562] 靶向CD20/CD3的BEAT抗体的实例

[0563] 使用小鼠利妥昔单抗抗体VH和VL序列的靶向人CD20抗原和人CD3ε的BEAT抗体的实例如下形成:

[0564] 使用实施例2.1和2.5中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人CD20臂),工程化构建BEAT CD20/CD3。

[0565] 该异源二聚体免疫球蛋白的抗人CD20臂由与其关联轻链(SEQ ID NO:181)组装的BEAT重链(SEQ ID NO:180)组成,所述BEAT重链(SEQ ID NO:180)基于小鼠利妥昔单抗抗体可变结构域(小鼠可变重链和轻链结构域分别具有SEQ ID NO:143和144),包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区和基于γ3的BEAT CH3结构域。因为该重链包含人IgG3 Fc区的一部分并且具有源自非VH3结构域亚类的重链可变结构域,该重链不与蛋白A结合。

[0566] 该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:177)组成。该臂等同于上述BEAT CD38-HB7最适配/CD3的抗CD3ε臂(参见图27的形式A)。该scFv片段包含如PCT公开号:WO2008119565中描述的抗人CD3εSP34抗体的人源化版本(VH和VL结构域分别具有SEQ ID NO:182和183)。该BEAT抗体形式在本文称为BEAT CD20/CD3(SP34)抗体(图31)。

[0567] 瞬时表达BEAT CD20/CD3(SP34)抗体、纯化、并在体外测试其重定向T细胞杀伤人CD20+细胞系的能力。CD38+B淋巴母细胞系Daudi用于杀伤测定中。图32显示了BEAT CD20/CD3(SP34)抗体引起的对Daudi细胞的T细胞重定向杀伤。测定法使用人PBMC作为效应细胞,其中效应细胞与靶细胞的比例为10比1,以及使用24小时孵育期后的RDL-FACS读取方法(参见材料和方法部分)。结果表明,BEAT CD20/CD3(SP34)抗体在重定向T细胞杀伤Daudi细胞方面具有高效力,其中EC_{50}为25pM(3个供体的平均值)。

[0568] 使用嵌合利妥昔单抗抗体VH和VL序列的靶向人CD20抗原和人CD3ε的BEAT抗体的另一个实例如下形成:

[0569] 使用实施例2.1和2.5中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人CD20抗原结合位点),工程化构建BEAT EGFR/CD3。

[0570] 该异源二聚体免疫球蛋白的抗人CD20臂由与其关联轻链(SEQ ID NO:181)组装的BEAT重链(SEQ ID NO:341)组成,所述BEAT重链(SEQ ID NO:341)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区和基于γ3的BEAT CH3结构域。因为该重链包含人IgG3 Fc区的一部分并且具有源自非VH3结构域亚类的重链可变结构域,该重链不与蛋白A结合。

[0571] 该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区和基于γ1的BEAT CH3结构域的BEAT重链(SEQ

ID NO:311)组成。该双特异性抗体在本文称为BEAT CD20/CD3(SP34-κ2)抗体。

[0572] 靶向EGFR/CD3的BEAT抗体的实例

[0573] 使用小鼠爱必妥抗体VH和VL序列的靶向人EGFR抗原和人CD3ε的BEAT抗体的实例如下形成:使用实施例2.1和2.6中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人EGFR抗原结合位点),工程化构建BEAT EGFR/CD3。

[0574] 该异源二聚体免疫球蛋白的抗人EGFR臂由与其关联轻链(SEQ ID NO:175)组装的BEAT重链(SEQ ID NO:342)组成,所述BEAT重链(SEQ ID NO:342)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区和基于γ3的BEAT CH3结构域。因为该重链包含人IgG3 Fc区的一部分并且具有源自非VH3结构域亚类的重链可变结构域,该重链不与蛋白A结合。

[0575] 该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文称为EGFRcetux/CD3(SP34-κ2)抗体。

[0576] 使用人维克替比抗体VH和VL序列的靶向人EGFR抗原和人CD3ε的BEAT抗体的另一个实例如下形成:使用实施例2.1和2.6中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人EGFR抗原结合位点),工程化构建BEAT EGFR/CD3。

[0577] 该异源二聚体免疫球蛋白的抗人EGFR臂由与其关联轻链(SEQ ID NO:344)组装的BEAT重链(SEQ ID NO:343)组成,所述BEAT重链(SEQ ID NO:343)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区和基于γ3的BEAT CH3结构域。因为该重链包含人IgG3 Fc区的一部分并且具有源自非VH3结构域亚类的重链可变结构域,该重链不与蛋白A结合。

[0578] 该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文称为BEAT EGFRpani/CD3(SP34-κ2)抗体。

[0579] 靶向IgE/CD3的BEAT抗体的实例

[0580] 使用人源化奥马珠单抗抗体VH和VL序列的靶向人IgE抗原和人CD3ε的BEAT抗体的实例如下形成:使用实施例2.1和2.8中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人IgE抗原结合位点),工程化构建BEAT IgE/CD3。

[0581] 该异源二聚体免疫球蛋白的抗人IgE臂由与其关联轻链(SEQ ID NO:346)组装的BEAT重链(SEQ ID NO:345)组成,所述BEAT重链(SEQ ID NO:345)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区和基于γ3的BEAT CH3结构域。因为该重链包含人IgG3 Fc区的一部分并且具有源自非VH3结构域亚类的重链可变结构域,该重链不与蛋白A结合。

[0582] 该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文称为BEAT IgEomali/CD3(SP34-κ2)抗体。

[0583] 使用小鼠Bsw17抗体VH和VL序列的靶向人IgE抗原和人CD3ε的BEAT抗体的另一个实例如下形成:使用实施例2.1和2.8中描述的抗原结合位点的组合(分别用于抗人CD3ε和抗人IgE抗原结合位点),工程化构建BEAT IgE/CD3。

[0584] 该异源二聚体免疫球蛋白的抗人IgE臂由与其关联轻链(SEQ ID NO:348)组装的BEAT重链(SEQ ID NO:347)组成，所述BEAT重链(SEQ ID NO:347)包含可变重链区、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ3CH2区和基于γ3的BEAT CH3结构域。因为该重链包含人IgG3 Fc区的一部分并且具有源自非VH3结构域亚类的重链可变结构域，该重链不与蛋白A结合。

[0585] 该异源二聚体免疫球蛋白的抗人CD3ε臂由包含scFv片段、CH1γ1区、γ1铰链区、具有L234A和L235A取代(EU编号)的γ1CH2区和基于γ1的BEAT CH3结构域的BEAT重链(SEQ ID NO:311)组成。该双特异性抗体在本文称为BEAT IgEbsw17/CD3(SP34-κ2)抗体。

[0586] 所述表达膜IgE的细胞系用于类似于以上描述的测定法中评估重定向的T细胞杀伤。

[0587] scFv形式中改进的SP34的功能性等同物

[0588] -双特异性CD38xCD3抗体

[0589] 为了确定为改善表达而对各种SP34 scFv进行的修饰是否也影响其功能性质，即与CD3的结合，在CD38xCD3双特异性背景中测试了这些修饰。作为FAB存在的CD38结合臂包含由SEQ ID NO:133编码的重链可变区和由SEQ ID NO:134编码的轻链可变区。CD3结合臂包含重新格式化scFv的原始小鼠SP34(SEQ ID NO:403)、或修饰的人源化SP34 scFv(包含重链/轻链组合H1/L21(SEQ ID NO:361)、H5/L32(SEQ ID NO:311)、H5/L65(SEQ ID NO:394)和H5/L67(SEQ ID NO:396))。

[0590] 瞬时表达和纯化使用不同SP34版本的每种CD3/CD38 BEAT。在体外测试这些CD3/CD38 BEAT，以比较其重定向T细胞杀伤的能力。Raji CD38表达细胞系(参见材料和方法部分)用于评估重定向的T细胞杀伤。该测定法使用人PBMC作为效应细胞，其中效应细胞与靶细胞的比例为10:1。

[0591] 当用RDL-FACS方法测量时，所有BEAT显示6至10pM的相当EC50(2个供体的平均值，孵育24小时)图37。

[0592] -双特异性CD20xCD3抗体

[0593] 还测试了，包含H5/L65重链/轻链组合(SEQ ID NO:394)的SP34 scFv，当与CD20 FAB结合臂(利妥昔单抗，VH和VL结构域分别具有SEQ ID NO:282和283)联合时，的性质。

[0594] 瞬时表达和纯化CD3/CD20 BEAT，然后在体外测试以比较其重定向T细胞杀伤的能力。Raji CD20表达细胞系(参见材料和方法部分)用于评估重定向的T细胞杀伤。该测定法使用人PBMC作为效应细胞，其中效应细胞与靶细胞的比例为5:1，孵育24小时。

[0595] 当使用RDL-FACS方法测量时，所有BEAT显示与包含H1/L21(SEQ ID NO:361)的CD3xCD20 BEAT(见上文)相当的EC50，37.56pM(4个供体的平均值，24小时孵育)，图38。

[0596] -双特异性EGFRxCD3抗体

[0597] o爱必妥/CD3

[0598] 携带KRAS突变的患者表现出不能得益于单克隆抗体西妥昔单抗(商品名:爱必妥)治疗(Karapetis C等人，N Engl J Med 359:1757(2008)。

[0599] 为了进一步评估改进的SP34 scFv的性质，并且为了确立杀伤EGFR高表达细胞系和克服KRAS抗性的可能性，构建了双特异性爱必妥(重链SEQ ID NO:174和轻链SEQ ID NO:175)-hSP34(SEQ ID NO:394)BEAT，并在KRAS突变的癌细胞系A549(肺)和HCT116(结肠直肠

癌)上进行体外重定向的T细胞裂解测定。此外,为了证明爱必妥-hSP34仅杀伤显示高和中等EGFR表达水平的细胞而非低表达EGFR的细胞,也将低表达EGFR的细胞系MCF-7包括在测定中。使用EGFR PharmDx免疫组化试剂盒(表2),测定了这些细胞系的EGFR状态。

[0600] 表2

[0601]

细胞系	sABC 的均值
A549	182'454
HCT116	44'294
MCF-7	5'644

[0602] 将IvIg以2.5mg/mL加入每孔以阻断爱必妥效应子功能,以更好地模拟爱必妥的作用模式(抑制EGFR信号传导)。如图39所示,爱必妥对任何细胞系均无作用(不可能评估任何EC_{50})。

[0603] 相反,如图40所示,爱必妥/hSP34BEAT可以有效地杀伤KRAS突变的A549和HCT116肿瘤细胞,其中EC50分别为66.97pM和43.3pM,而较低表达EGFR的MCF-7细胞未被有效杀伤。

[0604] o维克替比(Vectibix)

[0605] 为了进一步评估改进的SP34 scFv的性质,以及为了进一步确定可以用双特异性维克替比(VL SEQ ID NO:291和VH SEQ ID NO:290)以FAB-hSP34 BEAT(SEQ ID NO:394)形式杀伤EGFR高表达细胞系并克服KRAS抗性机制。以ScFv形式,在高EGFR表达的肺癌细胞系HCC827以及在KRAS突变的肺癌细胞系A549和结肠直肠癌KRAS阳性细胞系HCT116和SW480上,进行了体外重定向的T细胞裂解测定。

[0606] 为了证明维克替比/hSP34BEAT仅杀伤显示高和中等EGFR表达水平的细胞而不杀伤低EGFR表达的细胞,也将低表达EGFR的细胞系MCF-7包括在杀伤测定中。使用EGFR PharmDx免疫组织化学试剂盒(Dako,Cambridge,UK)测定了细胞系的EGFR状态(图41)。

[0607] 如图42所示,维克替比对所评估的任何细胞系均没有作用(2个供体的平均值,效应物:靶细胞的比例为20:1)。读取:48小时后用MTS)。

[0608] 对于维克替比/hSP34BEAT,使用10:1的效应物:靶细胞比例,数据为4个供体的平均值呈现。

[0609] 如图43所示,维克替比/hSP34BEAT可以有效地杀伤高表达EGFR的细胞系HCC827和KRAS突变的A549、HCT116和SW480肿瘤细胞,其中EC50分别为0.1557、0.6127、0.3406和6.986pM,而较低EGFR表达的MCF-7细胞没有被有效地杀伤(EC50=627.4pM)。

[0610] 表3

[0611]

EC_{50}s	HCC827	HCT116	SW480	MCF7
Vectibix-CD3	0.1457	0.3406	6.986	627.4
EC_{50} (X) / EC_{50} (HCC827) Vectibix-CD3	/	2.3	47.9	4303

[0612] 在表3中,测定了4个供体的平均EC 50,并计算了治疗窗口(EC_{50}(X)/EC_{50}(HCC827)维克替比-CD3)。

序列表

<110> 格兰马克药品股份有限公司(Glenmark Ph 臂aceuticals S.A.)
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35 40 45
Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Va. Asp Gly Val Glu Val
50 55 60
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe
65 70 75 80
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
85 90 95
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100 105 110
Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
115 120 125
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
130 135 140
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
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Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro
165 170 175
Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
180 185 190
Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met
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35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

[0001]

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215 220
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270
Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His
275 280 285
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg
290 295 300
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335
Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
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Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380
Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met
385 390 395 400
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415
Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His
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35 40 45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
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Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
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Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
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Phe Asn Arg Gly Glu Cys

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35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
130 135 140
Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala
145 150 155 160
Ser Gln Asp Val Asn Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
165 170 175
Lys Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Thr
180 185 190
Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu
195 200 205
Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln
210 215 220

[0003]

Gln His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
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Ile Lys Arg Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys Pro Pro
245 250 255
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
260 265 270
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
275 280 285
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys
290 295 300
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305 310 315 320
Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val
325 330 335
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
340 345 350
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys
355 360 365
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
370 375 380
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385 390 395 400
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu
405 410 415
Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe
420 425 430
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
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Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
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Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
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Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
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Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
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35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Ser Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
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85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125
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130 135 140
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145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
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35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Gln Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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100 105 110
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115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
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[0005]

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20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Val Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190
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20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

<210> 10
<211> 223
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 10 - 抗-HER2 FAB N82aS 重链
<400> 10

[0006]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

<210> 11
<211> 223
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 11 - 抗 HER2 FAB K19G/T57A/Y59A 重链
<400> 11

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Gly Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr

20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Ala Arg Ala Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

<210> 12
<211> 223
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 12 - 抗-HER2 FAB T57A 重链
<400> 12
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30

[0007]

Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Ala Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220
<210> 13
<211> 223
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 13 - 抗-HER2 FAB T57E 重链
<400> 13
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

```
        35                  40                  45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Glu Arg Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
    210                 215                 220
<210> 14
<211> 475
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 14 ~ 抗-HER2 scFv(G65S)-Fc 133 重链
<400> 14
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
            20                  25                  30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
    50                  55                  60
Lys Ser Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110
Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
    130                 135                 140
Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala
145                 150                 155                 160
Ser Gln Asp Val Asn Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
                165                 170                 175
Lys Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Thr
            180                 185                 190
Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu
        195                 200                 205
Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln
    210                 215                 220
Gln His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
225                 230                 235                 240
Ile Lys Arg Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys Pro Pro
                245                 250                 255
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
            260                 265                 270
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
        275                 280                 285
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys
    290                 295                 300
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305                 310                 315                 320
Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val
                325                 330                 335
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[0008]

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
340 345 350
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys
355 360 365
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
370 375 380
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385 390 395 400
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu
405 410 415
Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe
420 425 430
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
435 440 445
Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe
450 455 460
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470 475

<210> 15
<211> 475
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 15 - 抗-HER2 scFv(NS2aS)-Fc 133 重链
<400> 15

[0009]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
130 135 140
Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala
145 150 155 160
Ser Gln Asp Val Asn Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
165 170 175
Lys Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Thr
180 185 190
Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu
195 200 205
Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln
210 215 220
Gln His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
225 230 235 240
Ile Lys Arg Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys Pro Pro
245 250 255
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
260 265 270
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
275 280 285
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys
290 295 300
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305 310 315 320
Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val
325 330 335
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
340 345 350
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys
355 360 365
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu

370 375 380
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385 390 395 400
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu
405 410 415
Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe
420 425 430
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
435 440 445
Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe
450 455 460
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470 475

<210> 16
<211> 450
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 16 - 抗-HER2 FAB(G6S)-Fc 133 重链
<400> 16
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Ser Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215 220
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270
Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His
275 280 285
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg
290 295 300
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335
Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
355 360 365
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380
Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met
385 390 395 400
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

[0010]

Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His
420 425 430
Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445
Gly Lys
450
<210> 17
<211> 450
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 17 - 抗-HER2 FAB(N82aS)-Fc 139 重链
<400> 17
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
[0011] Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215 220
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270
Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His
275 280 285
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg
290 295 300
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335
Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
355 360 365
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380
Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met
385 390 395 400
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415
Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His
420 425 430
Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445
Gly Lys
450
<210> 18

<211> 119
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 18 - OKT3 重链 轻链可变结构域
<400> 18
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60
Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Thr Leu Thr Val Ser Ser
115
<210> 19
<211> 106
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 19 - OKT3 轻链 轻链可变结构域
<400> 19
Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15
Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30
Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser
50 55 60
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu
65 70 75 80
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95
Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn
100 105

[0012]

<210> 20
<211> 120
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 20 - 赫塞汀 重链 轻链可变结构域
<400> 20
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser
115 120
<210> 21
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 21 - 赫塞汀 轻链 轻链可变结构域

<400> 21
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 22
<211> 98
<212> PRT
<213> 智人(Homo sapiens)
<400> 22
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Lys

[0013]

<210> 23
<211> 95
<212> PRT
<213> 智人(Homo sapiens)
<400> 23
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro
85 90 95

<210> 24
<211> 96
<212> PRT
<213> 智人(Homo sapiens)
<400> 24
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
85 90 95

<210> 25
<211> 449
<212> PRT
<213> 人工序列

[0014]

<220>
<223> SEQ ID NO: 25 - 嵌合 OKT3 重链 IgG1
<400> 25
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60
Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 26
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 26 - 嵌合 OKT3 人 kappa 轻链
<400> 26
Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15
Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser
50 55 60
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu
65 70 75 80
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95
Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

<210> 27
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 27 - OKT3 人源化重链 具有 VH 结构域
<400> 27

[0015]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Arg Tyr Ala Asn Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys

325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 28
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 28 - OKT3 人源化重链 具有 VH1 结构域
<400> 28
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380

[0016]

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 29
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 29 - OKT3 人源化重链 具有 VH2 结构域
<400> 29
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly

[0017]

435 440 445
Lys

<210> 30
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 30 - OKT3 人源化重链 具有 VH3 结构域
<400> 30
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Thr Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
[0018] Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 31
<211> 449
<212> PRT
<213> 人工序列

<220>
<223> SEQ ID NO: 31 - OKT3 人源化重链 具有VH4结构域
<400> 31
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Thr Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
[0019] Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 32
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 32 - OKT3 人源化重链具有VH5结构域
<400> 32
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

[0020]

```
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35                  40                  45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Arg Tyr Ala Asp Ser Val
        50                  55                  60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445
Lys

<210> 33
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 33 - OKT3 人源化重链 具有 VH6 结构域
<400> 33
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1                   5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Arg Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
```

[0021]

```
                85                  90                  95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300
Val Ser Val Leu Thr Val Leu His Glu Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445
Lys
```

<210> 34
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 34 OKT3 人源化重链 具有VH7 结构域
<400> 34

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1                   5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140
```

[0022]

```
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445
Lys
```

<210> 35
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 35 – OKT3 人源化重链 具有VH3 结构域
<400> 35

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
```

195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

[0023]

<210> 36
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 36 - OKT3 人源化重链 具有 VH9 结构域
<400> 36
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Ser Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 37
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 37 - OKT3 人源化重链 具有 VH10 结构域
<400> 37
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Ser Arg Ala Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu

[0024]

305 310 315 320
Cys Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 38
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 38 - OKT3 人源化重链 具有 VH11 结构域
<400> 38
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Thr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
[0025]
Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 39
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 39 - OKT3 人源化轻链 具有 VL 结构域
<400> 39
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val
20 25 30
Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

[0026]

<210> 40
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 40 - OKT3 人源化轻链 具有 VL1 结构域
<400> 40
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val
20 25 30
Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Arg Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

<210> 41
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 41 - OKT3 人源化轻链 具有VL2 结构域
<400> 41
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val
20 25 30
Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Cys Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Cys Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

[0027]

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

<210> 42
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 42 - OKT3 人源化轻链 具有VL3 结构域
<400> 42
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val
20 25 30
Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Cys Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Arg Ser Gly Thr Asp Cys Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Cys Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210
<210> 43
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 43 - OKT3 人源化轻链 具有 VL4 结构域
<400> 43
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val
20 25 30
Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Cys Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Arg Ser Gly Thr Asp Cys Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Cys Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

[0028]

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210
<210> 44
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 44 - OKT3 人源化轻链 具有 VL5 结构域
<400> 44
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30
Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Cys Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Arg Ser Gly Thr Asp Cys Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Cys Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

<210> 45
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 45 ~ OKT3 人源化轻链 具有VL6 结构域
<400> 45
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val
20 25 30
Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Arg Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

[0029]

<210> 46
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 46 ~ OKT3 人源化轻链 具有VL7 结构域
<400> 46
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30
Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Arg Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210
<210> 47
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 47 - OKT3 人源化轻链 具有 VL8 结构域
<400> 47
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val
20 25 30
Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
[0030] Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210
<210> 48
<211> 119
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 48 - OKT3 人源化 VH8 结构域
<400> 48
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115
<210> 49
<211> 119
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 49 - OKT3 人源化 VH11 结构域
<400> 49
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr

20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 50
<211> 106
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 50 - OKT3 人源化 VL4 结构域
<400> 50
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val
20 25 30
Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Arg Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

[0031]

<210> 51
<211> 106
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 51 - OKT3 人源化 VL8 结构域
<400> 51
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val
20 25 30
Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 52
<211> 472
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 52 - scFv 片段：小鼠 OKT3 - 人 IgG1 Fc 融合蛋白
<400> 52
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60
Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

[0032]

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125
Ser Gly Gly Gly Gly Ser Gln Ile Val Leu Thr Gln Ser Pro Ala Ile
130 135 140
Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser
145 150 155 160
Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser
165 170 175
Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
180 185 190
Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
195 200 205
Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
210 215 220
Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn
225 230 235 240
Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
245 250 255
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
260 265 270
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
275 280 285
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
290 295 300
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
305 310 315 320
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
325 330 335
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
340 345 350
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
355 360 365
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
370 375 380
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
385 390 395 400
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
405 410 415
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
420 425 430
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
435 440 445
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
450 455 460
Ser Leu Ser Leu Ser Pro Gly Lys
465 470

<210> 53
<211> 477
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 53 - scFv片段 人源化OKT3 VH5-VL3 - 人IgG1 Fc 融合蛋白
<400> 53
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

[0033]

```
Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
        115                 120                 125
Ser Gly Gly Gly Gly Ser Gly Gly Gly Ala Ser Asp Ile Gln Leu Thr
    130                 135                 140
Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
145                 150                 155                 160
Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val Ala Trp Tyr Gln Gln
                165                 170                 175
Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp Thr Ser Lys Leu
            180                 185                 190
Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp
        195                 200                 205
Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
    210                 215                 220
Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr Phe Gly Gln Gly Thr
225                 230                 235                 240
Lys Val Glu Ile Lys Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys
                245                 250                 255
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
            260                 265                 270
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
        275                 280                 285
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
    290                 295                 300
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
305                 310                 315                 320
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
                325                 330                 335
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
            340                 345                 350
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
        355                 360                 365
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
    370                 375                 380
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
385                 390                 395                 400
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
                405                 410                 415
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
            420                 425                 430
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
        435                 440                 445
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
    450                 455                 460
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465                 470                 475
<210> 54
<211> 477
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 54 - scFv 片段 人源化 OKT3 VH6-VL4 - 人
      IgG1 Fc 融合蛋白
<400> 54
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1                   5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Arg Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110
Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
        115                 120                 125
Ser Gly Gly Gly Gly Ser Gly Gly Gly Ala Ser Asp Ile Gln Leu Thr
    130                 135                 140
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[0034]

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Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
145                 150                 155                 160
Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val Ala Trp Tyr Gln Gln
                165                 170                 175
Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu
            180                 185                 190
Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp
        195                 200                 205
Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
    210                 215                 220
Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr Phe Gly Gln Gly Thr
225                 230                 235                 240
Lys Val Glu Ile Lys Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys
                245                 250                 255
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
            260                 265                 270
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
        275                 280                 285
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
    290                 295                 300
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
305                 310                 315                 320
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
                325                 330                 335
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
            340                 345                 350
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
        355                 360                 365
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
    370                 375                 380
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
385                 390                 395                 400
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
                405                 410                 415
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
            420                 425                 430
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
        435                 440                 445
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
    450                 455                 460
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465                 470                 475
<210> 55
<211> 477
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 55 - scFv片段: 人源化OKT3 VH6-VL5 - 人
      IgG1 Fc 融合蛋白
<400> 55
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Arg Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110
Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
        115                 120                 125
Ser Gly Gly Gly Gly Ser Gly Gly Gly Ala Ser Asp Ile Gln Leu Thr
    130                 135                 140
Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
145                 150                 155                 160
Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln
                165                 170                 175
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Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp Thr Ser Lys Leu
180 185 190
Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp
195 200 205
Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
210 215 220
Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr Phe Gly Gln Gly Thr
225 230 235 240
Lys Val Glu Ile Lys Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys
245 250 255
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
260 265 270
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
275 280 285
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
290 295 300
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
305 310 315 320
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
325 330 335
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
340 345 350
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
355 360 365
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
370 375 380
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
385 390 395 400
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
405 410 415
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
420 425 430
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
435 440 445
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
450 455 460

[0035]

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470 475

<210> 56
<211> 477
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 56 - scFv片段 人源化OKT3 VH8-VL4 - 人IgG1 Fc 融合蛋白
<400> 56
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Thr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125
Ser Gly Gly Gly Gly Ser Gly Gly Gly Ala Ser Asp Ile Gln Leu Thr
130 135 140
Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
145 150 155 160
Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val Ala Trp Tyr Gln Gln
165 170 175
Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu
180 185 190
Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp
195 200 205

Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
210 215 220
Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr Phe Gly Gln Gly Thr
225 230 235 240
Lys Val Glu Ile Lys Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys
245 250 255
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
260 265 270
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
275 280 285
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
290 295 300
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
305 310 315 320
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
325 330 335
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
340 345 350
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
355 360 365
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
370 375 380
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
385 390 395 400
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
405 410 415
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
420 425 430
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
435 440 445
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
450 455 460
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470 475

[0036]

<210> 57
<211> 477
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 57 - scFv 片段 人源化 OKT3 VH8-VL8 - 人 IgG1 Fc 融合蛋白
<400> 57
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125
Ser Gly Gly Gly Gly Ser Gly Gly Gly Ala Ser Asp Ile Gln Leu Thr
130 135 140
Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
145 150 155 160
Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val Ala Trp Tyr Gln Gln
165 170 175
Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu
180 185 190
Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
195 200 205
Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
210 215 220
Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr Phe Gly Gln Gly Thr
225 230 235 240

Lys Val Glu Ile Lys Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys
245 250 255
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
260 265 270
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
275 280 285
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
290 295 300
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
305 310 315 320
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
325 330 335
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
340 345 350
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
355 360 365
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
370 375 380
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
385 390 395 400
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
405 410 415
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
420 425 430
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
435 440 445
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
450 455 460
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470 475

<210> 58
<211> 245
<212> PRT
<213> 人工序列
<220>

[0037] <223> SEQ ID NO: 58 – scFv片段 人源化OKT3 VH6-VL4

<400> 58
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125
Ser Gly Gly Gly Gly Ser Gly Gly Gly Ala Ser Asp Ile Gln Leu Thr
130 135 140
Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
145 150 155 160
Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val Ala Trp Tyr Gln Gln
165 170 175
Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu
180 185 190
Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp
195 200 205
Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
210 215 220
Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr Phe Gly Gln Gly Thr
225 230 235 240
Lys Val Glu Ile Lys
245

<210> 59
<211> 245
<212> PRT

<213> 人工序列
<220>
<223> SEQ ID NO: 59 - scFv 片段 人源化 OKT3 VH8-VL8
<400> 59
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125
Ser Gly Gly Gly Gly Ser Gly Gly Gly Ala Ser Asp Ile Gln Leu Thr
130 135 140
Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
145 150 155 160
Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val Ala Trp Tyr Gln Gln
165 170 175
Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu
180 185 190
Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
195 200 205
Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
210 215 220
Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr Phe Gly Gln Gly Thr
225 230 235 240
Lys Val Glu Ile Lys
245

[0038]

<210> 60
<211> 125
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 60 - 小鼠 抗-人 CD3 ε SP34 VH 结构域
<400> 60
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Lys Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Ile
65 70 75 80
Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
115 120 125
<210> 61
<211> 109
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 61 - 小鼠 抗-人 CD3 ε SP34 VL 结构域
<400> 61
Gln Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu
1 5 10 15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly
35 40 45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala
65 70 75 80
Gln Thr Glu Asp Glu Ala Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn
85 90 95
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> 62
<211> 455
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 62 - 该合SP34 重链 IgG1
<400> 62

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Lys Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Ile
65 70 75 80
Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr
115 120 125
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
130 135 140
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145 150 155 160
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
165 170 175
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
195 200 205
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
210 215 220
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445
Leu Ser Leu Ser Pro Gly Lys

[0039]

450 455

<210> 63
<211> 216
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 63 - 嵌合 SP34 轻链 (小鼠 V lambda - 人 lambda 恒定结构域)
<400> 63

Gln Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu
1 5 10 15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly
35 40 45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala
65 70 75 80
Gln Thr Glu Asp Glu Ala Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn
85 90 95
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Arg Thr Val
100 105 110
Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys
115 120 125
Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg
130 135 140
Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn
145 150 155 160
Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser
165 170 175
Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
180 185 190
Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
195 200 205
Lys Ser Phe Asn Arg Gly Glu Cys
210 215

[0040]

<210> 64
<211> 456
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 64 - SP34 人源化重链 具有 VH1 结构域
<400> 64

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr
115 120 125
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
130 135 140
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145 150 155 160
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
165 170 175
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
195 200 205
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
210 215 220

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445
Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 65
<211> 455
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 65 - SP34 人源化重链 具有 VH2 结构域

[0041]

<400> 65
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr
115 120 125
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
130 135 140
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145 150 155 160
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
165 170 175
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
195 200 205
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
210 215 220
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp

275 280 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445
Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 66
<211> 455
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 66 - SF34 人源化重链 具有 VH3 结构域
<400> 66

[0042]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr
115 120 125
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
130 135 140
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145 150 155 160
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
165 170 175
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
195 200 205
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
210 215 220
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445
Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 67
<211> 455
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 67 - SP34 人源化重链 具有 VH4 结构域
<400> 67

[0043]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Phe Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr
115 120 125
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
130 135 140
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145 150 155 160
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
165 170 175
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
195 200 205
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
210 215 220
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys

385 390 395 400
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445
Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 68
<211> 455
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 68 - SP34 人源化重链具有 VH5 结构域
<400> 68

[0044]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Cyr
85 90 95
Cyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr
115 120 125
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
130 135 140
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145 150 155 160
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
165 170 175
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
195 200 205
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
210 215 220
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Cyr
290 295 300
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445

Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 69
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 69 - SP34 人源化轻链 具有 VL1 结构域
<400> 69
Glu Ala Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Gln Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

[0045]

<210> 70
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 70 - SP34 人源化轻链 具有 VL2 结构域
<400> 70
Glu Ala Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 71
<211> 216
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 71 - SP34 人源化轻链 具有 VL3 结构域
<400> 71
Glu Ala Val Val Thr Gln Ser Ala Thr Leu Ser Val Ser Pro Gly Glu
1 5 10 15
Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly
35 40 45
Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu
65 70 75 80
Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Gln Leu Trp Tyr Ser Asn
85 90 95
Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val
100 105 110
Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys
115 120 125
Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg
130 135 140
Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn
145 150 155 160
Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser
165 170 175
Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
180 185 190
Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
195 200 205
Lys Ser Phe Asn Arg Gly Glu Cys
210 215

[0046]

<210> 72
<211> 216
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 72 - SP34 人源化轻链 具有 VL1 结构域
<400> 72
Glu Ala Val Val Thr Gln Ser Ala Thr Leu Ser Val Ser Pro Gly Glu
1 5 10 15
Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly
35 40 45
Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu
65 70 75 80
Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser Asn
85 90 95
Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val
100 105 110
Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys
115 120 125
Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg
130 135 140
Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn
145 150 155 160
Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser
165 170 175
Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
180 185 190
Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
195 200 205
Lys Ser Phe Asn Arg Gly Glu Cys
210 215
<210> 73
<211> 217

<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 73 - SP34 人源化轻链 具有 VL5 结构域
<400> 73
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215
<210> 74
<211> 217
<212> PRT
[0047] <213> 人工序列
<220>
<223> SEQ ID NO: 74 - SP34 人源化轻链 具有 VL6 结构域
<400> 74
Glu Ala Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Pro Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215
<210> 75
<211> 217
<212> PRT
<213> 人工序列

<220>
<223> SEQ ID NO: 75 - SP34 人源化轻链 具有 VL7 结构域
<400> 75
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Pro Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215
<210> 76
<211> 217
<212> PRT
<213> 人工序列
<220>

[0048]

<223> SEQ ID NO: 76 - SP34 人源化轻链 具有 VL8 结构域
<400> 76
Glu Ala Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Gly Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215
<210> 77
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 77 - SP34 人源化轻链 具有 VL9 结构域

<400> 77
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Gly Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215
<210> 78
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 78 - SP34 人源化轻链 具有 VL10 结构域
[0049] <400> 78
Glu Ala Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Glu Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215
<210> 79
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 79 - SP34 人源化轻链 具有 VL11 结构域
<400> 79
Glu Ala Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly

1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Gly Ser Gly Thr Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 80
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 80 - SP34 人源化轻链 具有 VL12 结构域
<400> 80

[0050]

Glu Ala Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Thr Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 81
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 81 - SP34 人源化轻链 具有 VL13 结构域
<400> 81

Glu Ala Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Thr Gly Ala Val Thr Thr

20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 82
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 82 - SP34 人源化轻链 具有 VL14 结构域
<400> 82
Glu Ala Va. Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30

[0051]

Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Leu Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 83
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 83 - SP34 人源化轻链 具有 VL15 结构域
<400> 83
Glu Ala Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Phe Arg

35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 84
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 84 - SP34 人源化轻链 具有 VL16 结构域
<400> 84
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg

[0052]

35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Thr Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 85
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 85 - SP34 人源化轻链 具有 VL17 结构域
<400> 85
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg

50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215
<210> 86
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 86 - SP34 人源化轻链 具有 VL18 结构域
<400> 86
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60

[0053]

Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215
<210> 87
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 87 - SP34 人源化轻链 具有 VL19 结构域
<400> 87
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Thr Glu Ala Thr Leu Thr Ile Ser Ser

65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 88
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 88 - SP34 人源化轻链 具有 VL20 结构域
<400> 88
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Thr Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80

[0054]

Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 89
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 89 - SP34 人源化轻链 具有 VL21 结构域
<400> 89
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser

85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 90
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 90 - SP34 人源化轻链 具有VL22 结构域
<400> 90
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 91
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 91 - scFv 片段 人源化 SP34 VH2-VL21 - 人 IgG1 Fc 融合蛋白
<400> 91
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95

[0055]

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
[0056] Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 92
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 92 - scFv 片段 人源化 SP34 VH3-VL23 - 人 IgG1 Fc 融合蛋白
<400> 92
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110

[0057]

Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 93
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 93 - scFv片段 人源化SP34 VH4-VL23 - 人IgG1 Fc 融合蛋白
<400> 93
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Phe Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

[0058]

<210> 94
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 94 - scFv片段 人源化SP34 VH5-VL23 - 人IgG1 Fc 融合蛋白
<400> 94
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Glu Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140

[0059]

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Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
                165                 170                 175
Asn Trp Val Glu Glu Lys Pro Gly Glu Ala Phe Arg Gly Leu Ile Gly
            180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
        195                 200                 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
    210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225                 230                 235                 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
                245                 250                 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
            260                 265                 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    290                 295                 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
    370                 375                 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385                 390                 395                 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
            420                 425                 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
        435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly Lys
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<210> 95
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 95 - scFv 片段 人源化 SP34 VH1-VL27 - 人 IgG1 Fc 融合蛋白
<400> 95

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1                 5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
            20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
    130                 135                 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
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Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Ala Ala Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
[0060]
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 96
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 96 - scFv 片段：人源化 SP34 VH1-VL28 - 人 IgG1 Fc 融合蛋白
<400> 96
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175

Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Ala Ala Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
[0061] Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 97
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 97 - scFv 片段 人源化 SP34 VH1-VL29 - 人 IgG1 Fc 融合蛋白
<400> 97
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190

Gly Ala Asn Lys Ala Ala Ala Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

[0062]

<210> 98
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 98 — scFv片段 人源化SP34 VH1-VL30 — 人IgG1 Fc 融合蛋白
<400> 98
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205

Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Ala Ala Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

[0063]

<210> 99
<211> 482
<212> PRT
<213> 人工序列
<220>
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<400> 99
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1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220

Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

[0064]

<210> 100
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 100 - scFv 片段 人源化 SP34 VH5-VL32 - 人 IgG1 Fc 融合蛋白
<400> 100
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala Ala Asn Tyr Ala
165 170 175
Asn Trp Val Glu Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

[0065]

<210> 101
<211> 125
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 101 - SP34 人源化VH1 结构域
<400> 101
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125
<210> 102
<211> 125
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 102 - SP34 人源化VH2 结构域
<400> 102
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125
<210> 103
<211> 125
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 103 - SP34 人源化 VH3 结构域
<400> 103
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125
<210> 104
<211> 125
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 104 - SP34 人源化 VH5 结构域
<400> 104

[0066]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125
<210> 105
<211> 110
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 105 - SP34 人源化 VL21 结构域
<400> 105
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys

100 105 110

<210> 106
<211> 110
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 106 - SP34 人源化 VL32 结构域
<400> 106
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala
20 25 30
Ala Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 107
<211> 242
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 107 - 人源化抗-HER2 抗体 4D5 - scFv 片段
<400> 107
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
130 135 140
Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala
145 150 155 160
Ser Gln Asp Val Asn Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
165 170 175
Lys Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Gly
180 185 190
Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu
195 200 205
Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln
210 215 220
Gln His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
225 230 235 240
Ile Lys

[0067]

<210> 108
<211> 120
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 108 - 人源化抗-HER2 抗体 4D5 - FAB 片段
重链 (VH-CH1)具有 VH G65S 取代
<400> 108
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr

20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Ser Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 109
<211> 242
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 109 - 人源化抗-HER2 抗体 4D5 - scFv 片段
具有 VH:G65S 取代
<400> 109
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Ser Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
130 135 140
Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala
145 150 155 160
Ser Gln Asp Val Asn Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
165 170 175
Lys Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Gly
180 185 190
Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu
195 200 205
Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln
210 215 220
Gln His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
225 230 235 240
Ile Lys

<210> 110
<211> 120
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 110 - 人源化抗-HER2 抗体 4D5 - FAB 片段
重链 (VH-VH1) 具有 VH:N82aS 取代
<400> 110
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

[0068]

85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser
115 120
<210> 111
<211> 242
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 111 - 人源化抗-HER2 抗体 4D5 - scFv 片段
具有 VH:N82aS 取代
<400> 111
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
130 135 140
Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala
145 150 155 160
Ser Gln Asp Val Asn Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
165 170 175
Lys Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Gly
180 185 190
Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu
195 200 205
Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln
210 215 220
Gln His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
225 230 235 240
Ile Lys

[0069]

<210> 112
<211> 109
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 112 - OKT10 小鼠 VH 结构域
<400> 112
Gln Val Glu Leu Val Glu Ser Gly Gly Ser Leu Lys Leu Ser Cys Ala
1 5 10 15
Ala Ser Gly Phe Asp Phe Ser Arg Ser Trp Met Asn Trp Val Arg Gln
20 25 30
Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Glu Ile Asn Pro Asp Ser
35 40 45
Ser Thr Ile Asn Tyr Thr Thr Ser Leu Lys Asp Lys Phe Ile Ile Ser
50 55 60
Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Thr Lys Val Arg
65 70 75 80
Ser Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Tyr Gly Asn Trp Phe
85 90 95
Pro Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105
<210> 113
<211> 108
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 113 - OKT10 小鼠 VL 结构域

<400> 113
Asp Ile Leu Met Thr Gln Ser Gln Lys Ile Met Pro Thr Ser Val Gly
1 5 10 15
Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Asp Thr Asn
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
35 40 45
Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
65 70 75 80
Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr Asp Ser Tyr Pro Leu
85 90 95
Thr Phe Gly Ala Gly Thr Lys Leu Asp Leu Lys Arg
100 105
<210> 114
<211> 119
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 114 - HB-7 小鼠 VH 结构域
<400> 114
Lys Val Gln Leu Gln Glu Ser Gly Pro Ser Leu Val Gln Pro Ser Gln
1 5 10 15
Arg Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Ile Ser Tyr
20 25 30
Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Arg Gly Gly Ser Thr Asp Tyr Asn Ala Ala Phe Met
50 55 60
Ser Arg Leu Ser Ile Thr Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80
Lys Met Asn Ser Leu Gln Ala Asp Asp Thr Ala Ile Tyr Phe Cys Ala
85 90 95
Lys Thr Leu Ile Thr Thr Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110
[0070] Thr Thr Val Thr Val Ser Ser
115
<210> 115
<211> 106
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 115 - HB-7 小鼠 VL 结构域
<400> 115
Asp Ile Glu Leu Thr Gln Ser Pro Ser Ser Phe Ser Val Ser Leu Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Asp Ile Tyr Asn Arg
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Asn Ala Pro Arg Leu Leu Ile
35 40 45
Ser Gly Ala Thr Ser Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Lys Asp Tyr Thr Leu Ser Ile Thr Ser Leu Gln Thr
65 70 75 80
Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Tyr Trp Ser Thr Pro Thr
85 90 95
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105
<210> 116
<211> 119
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 116 - 人源化 HB-7 最适配 VH 结构域
<400> 116
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ile Ser Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Arg Gly Gly Ser Thr Asp Tyr Asn Ala Ala Phe Met
50 55 60
Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Gln Val Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Phe Cys Ala
85 90 95
Lys Thr Leu Ile Thr Thr Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 117
<211> 106
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 117 – 人源化 HB-7 最适配 VL 结构域
<400> 117
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr Asn Arg
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Ser Gly Ala Thr Ser Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Lys Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Trp Ser Tar Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 118
<211> 449
<212> PRT
<213> 人工序列
<220>
[0071] <223> SEQ ID NO: 118 – 人源化 HB-7 最适配重链
<400> 118
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ile Ser Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Arg Gly Gly Ser Thr Asp Tyr Asn Ala Ala Phe Met
50 55 60
Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Gln Val Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Phe Cys Ala
85 90 95
Lys Thr Leu Ile Thr Thr Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 119
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 119 - 人源化 HB-7 最适配轻链
<400> 119
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr Asn Arg
20 25 30
[0072] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Ser Gly Ala Thr Ser Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Lys Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Trp Ser Thr Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210
<210> 120
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 120 - 嵌合 HB-7 重链 IgG1
<400> 120
Lys Val Gln Leu Gln Glu Ser Gly Pro Ser Leu Val Gln Pro Ser Gln
1 5 10 15
Arg Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Ile Ser Tyr
20 25 30
Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Arg Gly Gly Ser Thr Asp Tyr Asn Ala Ala Phe Met
50 55 60
Ser Arg Leu Ser Ile Thr Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80
Lys Met Asn Ser Leu Gln Ala Asp Asp Thr Ala Ile Tyr Phe Cys Ala
85 90 95
Lys Thr Leu Ile Thr Thr Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Glu Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335

[0073]

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 121
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 121 - 嵌合 HB-7 人 kappa 轻链
<400> 121
Asp Ile Glu Leu Thr Gln Ser Pro Ser Ser Phe Ser Val Ser Leu Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Asp Ile Tyr Asn Arg
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Asn Ala Pro Arg Leu Leu Ile
35 40 45
Ser Gly Ala Thr Ser Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Lys Asp Tyr Thr Leu Ser Ile Thr Ser Leu Gln Thr
65 70 75 80
Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Tyr Trp Ser Thr Pro Thr
85 90 95
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro

100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

<210> 122
<211> 121
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 122 - 9G7 小鼠 VH 结构域
<400> 122
Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Pro Ser Gln
1 5 10 15
Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Leu Ser Leu Ser Thr Ser
20 25 30
Gly Lys Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ala
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Ser Asn Gln Val
65 70 75 80
Phe Leu Lys Ile Ala Ser Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg Ile Glu Leu Gly Arg Ser Tyr Val Met Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

[0074]

<210> 123
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 123 - 9G7 小鼠 VL 结构域
<400> 123
Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15
Asp Arg Val Ser Ile Ser Cys Lys Ala Ser Gln Asp Val Ile Thr Ser
20 25 30
Val Ala Trp Phe Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala
65 70 75 80
Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His Tyr Thr Ile Pro Leu
85 90 95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

<210> 124
<211> 451
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 124 - 人源化 9G7 最适配重链
<400> 124
Gln Val Thr Leu Lys Glu Ser Gly Pro Thr Leu Val Lys Pro Thr Gln
1 5 10 15
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Leu Ser Leu Ser Thr Ser
20 25 30
Gly Lys Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

[0075]

```
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ala
    50                  55                  60
Leu Lys Ser Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
                85                  90                  95
Cys Ala Arg Ile Glu Leu Gly Arg Ser Tyr Val Met Asp Tyr Trp Gly
            100                 105                 110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115                 120                 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
    130                 135                 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
        195                 200                 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
    210                 215                 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225                 230                 235                 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
                245                 250                 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
            260                 265                 270
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
        275                 280                 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
    290                 295                 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305                 310                 315                 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
                325                 330                 335
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
            340                 345                 350
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
        355                 360                 365
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    370                 375                 380
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385                 390                 395                 400
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
                405                 410                 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
            420                 425                 430
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
        435                 440                 445
Pro Gly Lys
    450
<210> 125
<211> 214
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 125 - 人源化 9G7 最适配第一原型轻
      链
<400> 125
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1                   5                   10                  15
Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Val Ile Thr Ser
            20                  25                  30
Val Ala Trp Phe Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45
Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Ile Pro Leu
                85                  90                  95
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Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210

<210> 126
<211> 451
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 126 - 嵌合 9G7 重链 IgG1
<400> 126

[0076]

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Pro Ser Gln
1 5 10 15
Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Leu Ser Leu Ser Thr Ser
20 25 30
Gly Lys Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ala
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Ser Asn Gln Val
65 70 75 80
Phe Leu Lys Ile Ala Ser Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg Ile Glu Leu Gly Arg Ser Tyr Val Met Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225 230 235 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
355 360 365
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro

385 390 395 400
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445
Pro Gly Lys
450

<210> 127
<211> 214
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 127 - 嵌合 9G7 人 kappa 轻链
<400> 127
Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15
Asp Arg Val Ser Ile Ser Cys Lys Ala Ser Gln Asp Val Ile Thr Ser
20 25 30
Val Ala Trp Phe Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala
65 70 75 80
Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His Tyr Thr Ile Pro Leu
85 90 95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210

[0077]

<210> 128
<211> 214
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 128 - 人源化 9G7 最适配轻链 (原型
轻链 具有 T36Y 取代)
<400> 128
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Val Ile Thr Ser
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Ile Pro Leu
85 90 95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210

<210> 129
<211> 121
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 129 - 人源化 9G7 最适配 VH 结构域
<400> 129
Gln Val Thr Leu Lys Glu Ser Gly Pro Thr Leu Val Lys Pro Thr Gln
1 5 10 15
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Leu Ser Leu Ser Thr Ser
20 25 30
Gly Lys Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ala
50 55 60
Leu Lys Ser Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg Ile Glu Leu Gly Arg Ser Tyr Val Met Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 130
<211> 107
<212> PRT
<213> 人工序列
<220>

[0078]

<223> SEQ ID NO: 130 - 人源化 9G7 最适配 VL 结构域
<400> 130
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Val Ile Thr Ser
20 25 30
Val Ala Trp Phe Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Ile Pro Leu
85 90 95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 131
<211> 451
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 131 - 人源化 9G7 最佳框架重链
<400> 131
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Leu Ser Leu Ser Thr Ser
20 25 30
Gly Lys Gly Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ala
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Ile Glu Leu Gly Arg Ser Tyr Val Met Asp Tyr Trp Gly

[0079]

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                100                 105                 110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
            115                 120                 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
        130                 135                 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
        195                 200                 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
    210                 215                 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225                 230                 235                 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
                245                 250                 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
            260                 265                 270
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
        275                 280                 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
    290                 295                 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305                 310                 315                 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
                325                 330                 335
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
            340                 345                 350
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
        355                 360                 365
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    370                 375                 380
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385                 390                 395                 400
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
                405                 410                 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
            420                 425                 430
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
        435                 440                 445
Pro Gly Lys
    450
<210> 132
<211> 214
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 132 - 人源化 9G7 最佳框架轻链
<400> 132
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1                   5                   10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ile Thr Ser
            20                  25                  30
Val Ala Trp Phe Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45
Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Ile Pro Leu
                85                  90                  95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160
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Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210

<210> 133
<211> 121
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 133 - 人源化 9G7 最佳框架 VH 结构域
<400> 133
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Leu Ser Leu Ser Thr Ser
20 25 30
Gly Lys Gly Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ala
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Ile Glu Leu Gly Arg Ser Tyr Val Met Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 134
<211> 107
<212> PRT
<213> 人工序列
<220>

[0080]

<223> SEQ ID NO: 134 - 人源化 9G7 最佳框架 VL 结构域
<400> 134
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ile Thr Ser
20 25 30
Val Ala Trp Phe Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Ile Pro Leu
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 135
<211> 118
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 135 - 人 克隆 767 VH 结构域
<400> 135
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Glu Gly Arg Thr Gly Tyr Phe Asp Tyr Trp Gly Gln Gly Thr

100 105 110
Leu Val Thr Val Ser Ser
115
<210> 136
<211> 111
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 136 - 人 克隆 767 VL 结构域
<400> 136
Gln Ser Val Leu Thr Gly Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Ser Thr Ser Asn Ile Gly Thr Asn
20 25 30
Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Arg Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Arg
85 90 95
Ser Gly Val Tyr Ala Phe Gly Thr Gly Thr Lys Val Thr Val Leu
100 105 110
<210> 137
<211> 448
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 137 - 人 767 重链
<400> 137
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Glu Gly Arg Thr Gly Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130 135 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
225 230 235 240
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260 265 270
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
290 295 300
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr

[0081]

[0082]

325 330 335
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
340 345 350
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
355 360 365
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385 390 395 400
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
405 410 415
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
420 425 430
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 138
<211> 218
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 138 - 人 767 轻链
<400> 138
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Ser Thr Ser Asn Ile Gly Thr Asn
20 25 30
Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Arg Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Arg
85 90 95
Ser Gly Val Tyr Ala Phe Gly Thr Gly Thr Lys Val Thr Val Leu Arg
100 105 110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 139
<211> 118
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 139 - 小鼠 抗-人 OX40 抗体 VH 结构域 来自 WO2013/008171
<400> 139
Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Pro Ser Gln
1 5 10 15
Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Thr Ala
50 55 60
Leu Lys Ser Gly Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80
Phe Leu Lys Ile Ala Ser Val Asp Thr Thr Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg Ile Asp Trp Asp Gly Phe Ala Tyr Trp Gly Glu Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 140
<211> 106
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 140 - 小鼠 抗-人 OX40 抗体VL 结构域 来自 WO2013/008171
<400> 140
Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1 5 10 15
Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45
Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Asn Arg Val Glu Ala Glu
65 70 75 80
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Trp Thr
85 90 95
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 141
<211> 118
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 141 - 人源化抗-人 OX40 抗体VH 结构域 来自 WO2013/008171
<400> 141
Gln Val Thr Leu Lys Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15
Thr Leu Thr Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45
Trp Ile Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Thr Ala
50 55 60
Leu Lys Thr Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg Ile Asp Trp Asp Gly Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser
115

<210> 142
<211> 106
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 142 - 人源化抗-人 OX40 抗体VL 结构域 来自 WO2013/008171
<400> 142
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Pro Trp Ile Tyr
35 40 45
Ala Thr Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
65 70 75 80
Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Trp Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 143
<211> 121

[0083]

<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 143 - rituximab 小鼠 VH 结构域
<400> 143
Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Ser Thr Tyr Cyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly
100 105 110
Ala Gly Thr Thr Val Thr Val Ser Ala
115 120
<210> 144
<211> 108
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 144 - rituximab 小鼠 VL 结构域
<400> 144
Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1 5 10 15
Glu Lys Val Thr Met Glu Thr Thr Cys Arg Ala Ser Ser Ser Val Ser
20 25 30
Cyr Ile His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp
35 40 45
Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu
65 70 75 80
Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro
85 90 95
Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

[0084]

<210> 145
<211> 119
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 145 - cetuximab 小鼠 VH 结构域
<400> 145
Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
1 5 10 15
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Asn Tyr
20 25 30
Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Thr Pro Phe Thr
50 55 60
Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80
Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95
Arg Ala Leu Thr Tyr Cyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ala
115
<210> 146
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 146 - cetuximab 小鼠 VL 结构域
<400> 146

Asp Ile Leu Leu Thr Gln Ser Pro Val Ile Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Ser Ile Gly Thr Asn
20 25 30
Ile His Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
35 40 45
Lys Tyr Ala Ser Glu Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser
65 70 75 80
Glu Asp Ile Ala Asp Tyr Tyr Cys Gln Gln Asn Asn Asn Trp Pro Thr
85 90 95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

<210> 147
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 147 - BTA CH3 NO: 1 原始 BTA 11
<400> 147
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
1 5 10 15
Glu Leu Thr Lys Asn Gln Val Lys Leu Val Cys Leu Val Thr Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Tyr Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
50 55 60
Ser Leu Val Ser Trp Leu Asn Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105

[0085]

<210> 148
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 148 - BTA CH3 NO: 2 BTA FTO 11
<400> 148
Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu Pro Pro Ser Arg Asp
1 5 10 15
Glu Leu Thr Lys Asn Gln Val Lys Leu Val Cys Leu Val Thr Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Tyr Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
50 55 60
Ser Leu Val Ser Trp Leu Asn Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105

<210> 149
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 149 - BTA CH3 NO: 3 BTA FTO 33 411D
<400> 149
Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu Pro Pro Ser Arg Glu
1 5 10 15
Glu Met Thr Lys Asn Gln Val Lys Leu Val Cys Leu Val Thr Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe
50 55 60
Ser Leu Val Ser Trp Leu Asp Val Asp Lys Ser Arg Trp Gln Gln Gly

65 70 75 80
Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105
<210> 150
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 150 - BTB CH3 NO: 1 原始 BTB
<400> 150
Gly Gln Pro Arg Glu Pro Glu Val Ala Thr Phe Pro Pro Ser Arg Asp
1 5 10 15
Glu Leu Thr Lys Asn Gln Val Thr Leu Val Cys Leu Val Tar Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Lys Thr Asp Pro Pro Leu Leu Glu Ser Asp Gly Ser Phe
50 55 60
Ala Leu Ser Ser Arg Leu Arg Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105
<210> 151
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 151 - BTB CH3 NO: 2 BTB 401R 11
<400> 151
Gly Gln Pro Arg Glu Pro Glu Val Ala Thr Phe Pro Pro Ser Arg Asp
1 5 10 15
[0086] Glu Leu Thr Lys Asn Gln Val Thr Leu Val Cys Leu Val Tar Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Lys Thr Asp Pro Pro Leu Leu Glu Ser Arg Gly Ser Phe
50 55 60
Ala Leu Ser Ser Arg Leu Arg Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105
<210> 152
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 152 - BTB CH3 NO: 3 BTB 401G 11
<400> 152
Gly Gln Pro Arg Glu Pro Glu Val Ala Thr Phe Pro Pro Ser Arg Asp
1 5 10 15
Glu Leu Thr Lys Asn Gln Val Thr Leu Val Cys Leu Val Tar Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Lys Thr Asp Pro Pro Leu Leu Glu Ser Gln Gly Ser Phe
50 55 60
Ala Leu Ser Ser Arg Leu Arg Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105
<210> 153
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> SEQ ID NO: 153 - BTA CH3 NO: 4 BTA 11_FTO_N411T
<400> 153
Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu Pro Pro Ser Arg Asp
1 5 10 15
Glu Leu Thr Lys Asn Gln Val Lys Leu Val Cys Leu Val Thr Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Tyr Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
50 55 60
Ser Leu Val Ser Trp Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105
<210> 154
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 154 - BTA CH3 NO: 5 BTA 33_FTO
<400> 154
Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu Pro Pro Ser Arg Glu
1 5 10 15
Glu Met Thr Lys Asn Gln Val Lys Leu Val Cys Leu Val Thr Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe
50 55 60
Ser Leu Val Ser Trp Leu Asn Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80
Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105

[0087]

<210> 156
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 155 - BTA CH3 NO: 6 BTA 33_FTO_N411T
<400> 155
Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu Pro Pro Ser Arg Glu
1 5 10 15
Glu Met Thr Lys Asn Gln Val Lys Leu Val Cys Leu Val Thr Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe
50 55 60
Ser Leu Val Ser Trp Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80
Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105
<210> 156
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 156 - BTB CH3 No: 4 BTB 33_D401Q
<400> 156
Gly Gln Pro Arg Glu Pro Glu Val Ala Thr Phe Pro Pro Ser Arg Glu
1 5 10 15
Glu Met Thr Lys Asn Gln Val Thr Leu Val Cys Leu Val Thr Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu
35 40 45

Asn Asn Tyr Asn Thr Asp Pro Pro Leu Leu Glu Ser Gln Gly Ser Phe
50 55 60
Ala Leu Ser Ser Arg Leu Arg Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80
Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105

<210> 157
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 157 - BTB CH3 No: 5 BTB 11_D401Q_R411T
<400> 157
Gly Gln Pro Arg Glu Pro Glu Val Ala Thr Phe Pro Pro Ser Arg Asp
1 5 10 15
Glu Leu Thr Lys Asn Gln Val Thr Leu Val Cys Leu Val Thr Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Lys Thr Asp Pro Pro Leu Leu Glu Ser Gln Gly Ser Phe
50 55 60
Ala Leu Ser Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105

<210> 158
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 158 - BTB CH3 No: 6 BTB 33_D401Q_R411T
<400> 158

[0088]

Gly Gln Pro Arg Glu Pro Glu Val Ala Thr Phe Pro Pro Ser Arg Glu
1 5 10 15
Glu Met Thr Lys Asn Gln Val Thr Leu Val Cys Leu Val Thr Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Asn Thr Asp Pro Pro Leu Leu Glu Ser Gln Gly Ser Phe
50 55 60
Ala Leu Ser Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80
Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105

<210> 159
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 159 - BEAT HER2/CD3-1 抗体 FAB 重链 (CD3 ε 臂- 人源化 OKT3 具有 N82aS 取代)
<400> 159
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

[0089]

```
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro
225                 230                 235                 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270
Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val
    290                 295                 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335
Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr
            340                 345                 350
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val
        355                 360                 365
Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380
Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu
385                 390                 395                 400
Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys
                405                 410                 415
Ser Arg Trp Gln Glu Gly Asn Ile Phe Ser Cys Ser Val Met His Glu
            420                 425                 430
Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445
Lys.

<210> 160
<211> 475
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 160 - BEAT HER2/CD3-1 抗体 scFv 重链 (HER2
      臂)
<400> 160
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
            20                  25                  30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110
Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
    130                 135                 140
Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala
145                 150                 155                 160
```

[0090]

Ser Gln Asp Val Asn Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
165 170 175
Lys Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Gly
180 185 190
Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu
195 200 205
Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln
210 215 220
Gln His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
225 230 235 240
Ile Lys Arg Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys Pro Pro
245 250 255
Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro
260 265 270
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
275 280 285
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
290 295 300
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305 310 315 320
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
325 330 335
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
340 345 350
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
355 360 365
Gly Gln Pro Arg Glu Pro Glu Val Ala Thr Phe Pro Pro Ser Arg Asp
370 375 380
Glu Leu Thr Lys Asn Gln Val Thr Leu Val Cys Leu Val Thr Gly Phe
385 390 395 400
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
405 410 415
Asn Asn Tyr Lys Thr Asp Pro Pro Leu Leu Glu Ser Asp Gly Ser Phe
420 425 430
Ala Leu Ser Ser Arg Leu Arg Val Asp Lys Ser Arg Trp Gln Gln Gly
435 440 445
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
450 455 460
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470 475

<210> 161
<211> 450
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 161 - BEAT HER2/CD3-2 抗体 FAB 重链 (HER2 臂)
<400> 161
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Asn Gly Pro Ser Val
115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215 220
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
225 230 235 240
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
290 295 300
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr
340 345 350
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Lys Leu
355 360 365
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Val
385 390 395 400
Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp
405 410 415
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445
Gly Lys
450

[0091]

<210> 162
<211> 477
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 162 - BEAT HER2/CD3-2 抗体 scFv 重链 (CD3 ε 链- 人源化 OKT3 具有 N82aS 取代)
<400> 162
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125
Ser Gly Gly Gly Gly Ser Gly Gly Gly Ala Ser Asp Ile Gln Leu Thr
130 135 140
Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
145 150 155 160
Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Val Ala Trp Tyr Gln Gln
165 170 175
Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu
180 185 190
Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
195 200 205
Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
210 215 220
Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr Phe Gly Gln Gly Thr
225 230 235 240

Lys Val Glu Ile Lys Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys
245 250 255
Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu
260 265 270
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
275 280 285
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
290 295 300
Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
305 310 315 320
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val Val Ser Val Leu
325 330 335
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
340 345 350
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
355 360 365
Thr Lys Gly Gln Pro Arg Glu Pro Glu Val Ala Thr Phe Pro Pro Ser
370 375 380
Arg Glu Glu Met Thr Lys Asn Gln Val Thr Leu Val Cys Leu Val Thr
385 390 395 400
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Glu
405 410 415
Pro Glu Asn Asn Tyr Asn Thr Asp Pro Pro Leu Leu Glu Ser Asp Gly
420 425 430
Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp Lys Ser Arg Trp Gln
435 440 445
Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
450 455 460
Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470 475

<210> 163
<211> 449
<212> PRT
<213> 人工序列
<220>

[0092] <223> SEQ ID NO: 163 - BEAT HER2/CD3-3 抗体 FAB 重链(CD3 ε臂- 人源化 OKT3 具有 G65S 取代)

<400> 163
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Ser Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val
355 360 365
Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 161
<211> 474
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 164 - BEAT HER2/CD3-3 抗体 scFv 重链 (HER2 臂具有额外的二硫键)
<400> 164
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
[0093] Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
130 135 140
Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala
145 150 155 160
Ser Gln Asp Val Asn Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
165 170 175
Lys Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Gly
180 185 190
Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu
195 200 205
Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln
210 215 220
Gln His Tyr Thr Thr Pro Pro Thr Phe Gly Cys Gly Thr Lys Val Glu
225 230 235 240
Ile Lys Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys Pro Pro Cys
245 250 255
Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
260 265 270
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
275 280 285
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
290 295 300
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
305 310 315 320

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
325 330 335
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
340 345 350
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
355 360 365
Gln Pro Arg Glu Pro Glu Val Ala Thr Phe Pro Pro Ser Arg Asp Glu
370 375 380
Leu Thr Lys Asn Gln Val Thr Leu Val Cys Leu Val Thr Gly Phe Tyr
385 390 395 400
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
405 410 415
Asn Tyr Lys Thr Asp Pro Pro Leu Leu Glu Ser Gln Gly Ser Phe Ala
420 425 430
Leu Ser Ser Arg Leu Arg Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
435 440 445
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
450 455 460
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470

<210> 165
<211> 455
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 165 — BEAT HER2/CD3(SP34) 抗体FAB 重链(CD3 ε臂- 人源化 SP34 VH 来自 WO2008/119565)
<400> 165

[0094]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Lys Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Ala Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Ile Ser Tyr Trp
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
115 120 125
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
130 135 140
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145 150 155 160
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
165 170 175
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
195 200 205
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
210 215 220
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240
Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350

Glu Pro Ala Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365
Asn Gln Val Lys Leu Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp
370 375 380
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Tyr
385 390 395 400
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser
405 410 415
Trp Leu Asn Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445
Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 166
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 166 - BEAT HER2/CD3(SP34) 抗体 FAB 轻链(CD3 ε 臂- 人源化 SP34 VL 来自 WO2008/119565)
<400> 166
Gln Thr Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Ser Gly
20 25 30
Asn Tyr Pro Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
35 40 45
Leu Ile Gly Gly Thr Lys Phe Leu Ala Pro Gly Thr Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val
65 70 75 80
Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Val Leu Trp Tyr Ser Asn
85 90 95
Arg Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

[0095]

<210> 167
<211> 474
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 167 - BEAT HER2/CD3(SP34) 抗体 scFv 重链 (HER2 臂具有 N82aS 取代)
<400> 167
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
130 135 140
Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala
145 150 155 160
Ser Gln Asp Val Asn Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
165 170 175
Lys Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Gly
180 185 190
Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu
195 200 205
Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln
210 215 220
Gln His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
225 230 235 240
Ile Lys Gly Gly Gly Gly Thr Asp Lys Thr His Thr Cys Pro Pro Cys
245 250 255
Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
260 265 270
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
275 280 285
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys Trp
290 295 300
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
305 310 315 320
Glu Gln Tyr Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu
325 330 335
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
340 345 350
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly
355 360 365
Gln Pro Arg Glu Pro Glu Val Ala Thr Phe Pro Pro Ser Arg Glu Glu
370 375 380
Met Thr Lys Asn Gln Val Thr Leu Val Cys Leu Val Thr Gly Phe Tyr
385 390 395 400
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn
405 410 415
Asn Tyr Asn Thr Asp Pro Pro Leu Leu Glu Ser Gln Gly Ser Phe Ala
420 425 430
Leu Ser Ser Arg Leu Arg Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
435 440 445
Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr
450 455 460
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470

[0096]

<210> 168
<211> 455
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 168 - BEAT HER2/CD3(SP34-Kappa1) 抗体FAB 重链(CD3 ε臂- 人源化SP34 VH1)
<400> 168
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr
115 120 125
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
130 135 140

[0097]

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Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145                 150                 155                 160
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
                165                 170                 175
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
            180                 185                 190
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
        195                 200                 205
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
    210                 215                 220
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225                 230                 235                 240
Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                245                 250                 255
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
            260                 265                 270
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
        275                 280                 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
    290                 295                 300
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305                 310                 315                 320
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
                325                 330                 335
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
            340                 345                 350
Glu Pro Ala Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
        355                 360                 365
Asn Gln Val Lys Leu Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp
    370                 375                 380
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Tyr
385                 390                 395                 400
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser
                405                 410                 415
Trp Leu Asn Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
            420                 425                 430
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
        435                 440                 445
Leu Ser Leu Ser Pro Gly Lys
    450                 455
<210> 169
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 169 - DEAT CD38 HB7 最适配/CD3 抗体 PAD 重
      链 (CD38 臂- 人源化 HB-7 最适配)
<400> 169
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1               5                   10                  15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ile Ser Tyr
            20                  25                  30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45
Gly Val Ile Trp Arg Gly Gly Ser Thr Asp Tyr Asn Ala Ala Phe Met
    50                  55                  60
Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Gln Val Ser Leu
65                  70                  75                  80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Phe Cys Ala
                85                  90                  95
Lys Thr Leu Ile Thr Thr Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly
            100                 105                 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190
```

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Lys Leu Val
355 360 365
Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

[0098]

<210> 170
<211> 448
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 170 - BEAT CD38-767/CD3 抗体 FAB 重链 (CD38 臂- 人 克隆 767)
<400> 170
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Glu Gly Arg Thr Gly Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130 135 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser
225 230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260 265 270
Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val Val
290 295 300
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
325 330 335
Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu
340 345 350
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val Cys
355 360 365
Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380
Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu Asp
385 390 395 400
Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys Ser
405 410 415
Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala
420 425 430
Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 171
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 171 - BEAT CD38-767/CD3 抗体 scFv 重链(CD3 ε 鼠- 人源化 OKT3 具有 G65S 取代)
<400> 171

[0099]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Lys Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Ala Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Ile Ser Tyr Trp
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val
130 135 140
Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu
145 150 155 160
Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Ser Gly Asn Tyr Pro Asn
165 170 175
Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
180 185 190
Thr Lys Phe Leu Ala Pro Gly Thr Pro Ala Arg Phe Ser Gly Ser Leu
195 200 205
Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val Gln Pro Glu Asp
210 215 220
Glu Ala Glu Tyr Tyr Cys Val Leu Trp Tyr Ser Asn Arg Trp Val Phe
225 230 235 240
Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300

```
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Ala
    370                 375                 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385                 390                 395                 400
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
            420                 425                 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
        435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly Lys
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<210> 172
<211> 448
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 172 BEAT OX40/CD3 抗体 FAD 重链 (OX40 臂具有人源化抗-人 OX40 VH 结构域 来自 WO2013/008171)

<400> 172

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Gln Val Thr Leu Lys Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1               5                   10                  15
Thr Leu Thr Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser
            20                  25                  30
Gly Met Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35                  40                  45
Trp Ile Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Thr Ala
    50                  55                  60
Leu Lys Thr Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
                85                  90                  95
Cys Ala Arg Ile Asp Trp Asp Gly Phe Ala Tyr Trp Gly Gln Gly Thr
            100                 105                 110
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125
Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
    210                 215                 220
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser
225                 230                 235                 240
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                245                 250                 255
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            260                 265                 270
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        275                 280                 285
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    290                 295                 300
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305                 310                 315                 320
```

[0100]

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
325 330 335
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu
340 345 350
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Lys Leu Val Cys
355 360 365
Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380
Asn Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Val Leu Asp
385 390 395 400
Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys Ser
405 410 415
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
420 425 430
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 173
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 173 ~ BEAT OX40/CD3 抗体 FAB 轻链 (OX40 臂具有人源化抗-人 OX40 VL 结构域 来自 WO2013/008171)
<400> 173
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Pro Trp Ile Tyr
35 40 45
Ala Thr Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
65 70 75 80
Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Trp Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

[0101]

<210> 174
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 174 ~ BEAT EGFR/CD3 抗体 FAB 重链 (EGFR 臂具有小鼠 Erbitux VH 结构域)
<400> 174
Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
1 5 10 15
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Asn Tyr
20 25 30
Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Thr Pro Phe Thr
50 55 60
Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80
Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95

Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Lys Leu Val
355 360 365
Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
[0102] Ser Asn Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 175
<211> 214
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 175 - BEAT EGFR/CD3 抗体 FAB 轻链 (EGFR 臂具有小鼠 Erbitux VL 结构域)
<400> 175
Asp Ile Leu Leu Thr Gln Ser Pro Val Ile Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Ser Ile Gly Thr Asn
20 25 30
Ile His Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
35 40 45
Lys Tyr Ala Ser Glu Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser
65 70 75 80
Glu Asn Ile Ala Asp Tyr Tyr Cys Gln Gln Asn Asn Asn Trp Pro Thr
85 90 95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210

<210> 176
<211> 449
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 176 - BEAT CD38-HB7最适配/CD3(SP34)抗体FAB 重链 (CD38臂- 人源化HB-7 最适配)
<400> 176

[0103]

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ile Ser Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Arg Gly Gly Ser Thr Asp Tyr Asn Ala Ala Phe Met
50 55 60
Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Gln Val Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Phe Cys Ala
85 90 95
Lys Thr Leu Ile Thr Thr Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val
355 360 365
Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys
405 410 415
Ser Arg Trp Gln Glu Gly Asn Ile Phe Ser Cys Ser Val Met His Glu
420 425 430

Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
Lys

<210> 177
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 177 - BEST CD38-HB7最适配/CD3(SP34) 抗体scFv 重链 (CD3臂- 人源化SP34 VH/YL 结构域 来自 WO2008/119565)
<400> 177
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Lys Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Ala Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Ile Ser Tyr Trp
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val
130 135 140
Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu
145 150 155 160
Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Ser Gly Asn Tyr Pro Asn
165 170 175
Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
180 185 190
Thr Lys Phe Leu Ala Pro Gly Thr Pro Ala Arg Phe Ser Gly Ser Leu
195 200 205
Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val Gln Pro Glu Asp
210 215 220
Glu Ala Glu Tyr Tyr Cys Val Leu Trp Tyr Ser Asn Arg Trp Val Phe
225 230 235 240
Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Ala
370 375 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385 390 395 400
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
420 425 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro

[0104]

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465                 470                 475                 480
Gly Lys

<210> 178
<211> 451
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 178 - BEAT CD38-9G7 最适配/CD3(SP34)抗体 FAB 重
      链 (CD38 臂- 人源化 9G7 最适配 VH)
<400> 178
Gln Val Thr Leu Lys Glu Ser Gly Pro Thr Leu Val Lys Pro Thr Gln
1               5                  10                  15
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Leu Ser Leu Ser Thr Ser
            20                  25                  30
Gly Lys Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35                  40                  45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ala
    50                  55                  60
Leu Lys Ser Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
                85                  90                  95
Cys Ala Arg Ile Glu Leu Gly Arg Ser Tyr Val Met Asp Tyr Trp Gly
            100                 105                 110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115                 120                 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
    130                 135                 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
        195                 200                 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
    210                 215                 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225                 230                 235                 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
                245                 250                 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
            260                 265                 270
Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val
        275                 280                 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe
    290                 295                 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305                 310                 315                 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
                325                 330                 335
Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val
            340                 345                 350
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys
        355                 360                 365
Leu Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    370                 375                 380
Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro
385                 390                 395                 400
Met Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val
                405                 410                 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met
            420                 425                 430
His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser
        435                 440                 445
Pro Gly Lys
    450
<210> 179
<211> 482
<212> PRT
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[0105]

<213> 人工序列
<220>
<223> SEQ ID NO: 179 - BEAT CD38-9G7 最适配/CD3(SP34-Kappa2) 抗体 scFv 重链 (CD3 臂- 人源化 SP34 VH5/VL32)v
<220>
<221> misc_feature
<222> (213)..(213)
<223> Xaa 可以是任何天然氨基酸
<400> 179

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Xaa Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Ala
370 375 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385 390 395 400
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
420 425 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 180

[0106]

<211> 451
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 180 - BEAT CD20/CD3 抗体 FAB 重链
<400> 180
Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly
100 105 110
Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser
115 120 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240
[0107] Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270
Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val
275 280 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe
290 295 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335
Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val
340 345 350
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys
355 360 365
Leu Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380
Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro
385 390 395 400
Met Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val
405 410 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met
420 425 430
His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445
Pro Gly Lys
450
<210> 181
<211> 215
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 181 - BEAT CD20/CD3 抗体 FAB 轻链
<400> 181
Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly

1 5 10 15
Glu Lys Val Thr Met Glu Thr Thr Cys Arg Ala Ser Ser Ser Val Ser
20 25 30
Tyr Ile His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp
35 40 45
Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu
65 70 75 80
Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro
85 90 95
Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala
100 105 110
Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115 120 125
Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130 135 140
Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145 150 155 160
Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165 170 175
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180 185 190
Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195 200 205
Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 182
<211> 109
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 182 - 人源化 SP34 VH 结构域 来自 WO2008/119565
<400> 182

[0108]

Gln Thr Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Ser Gly
20 25 30
Asn Tyr Pro Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
35 40 45
Leu Ile Gly Gly Thr Lys Phe Leu Ala Pro Gly Thr Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val
65 70 75 80
Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Val Leu Trp Tyr Ser Asn
85 90 95
Arg Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> 183
<211> 109
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 183 - 人源化 SP34 VL 结构域 来自 WO2008/119565
<400> 183
Gln Thr Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Ser Gly
20 25 30
Asn Tyr Pro Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
35 40 45
Leu Ile Gly Gly Thr Lys Phe Leu Ala Pro Gly Thr Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val
65 70 75 80
Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Val Leu Trp Tyr Ser Asn
85 90 95
Arg Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> 184
<211> 94
<212> PRT

<213> 人工序列
<220>
<223> SEQ ID NO: 184 - 人 CD3 γ 胞外区
<400> 184
Glu Ser Ile Lys Gly Asn His Leu Val Lys Val Tyr Asp Tyr Gln Glu
1 5 10 15
Asp Gly Ser Val Leu Leu Thr Cys Asp Ala Glu Ala Lys Asn Ile Thr
20 25 30
Trp Phe Lys Asp Gly Lys Met Ile Gly Phe Leu Thr Glu Asp Lys Lys
35 40 45
Lys Trp Asn Leu Gly Ser Asn Ala Lys Asp Pro Arg Gly Met Tyr Gln
50 55 60
Cys Lys Gly Ser Gln Asn Lys Ser Lys Pro Leu Gln Val Tyr Tyr Arg
65 70 75 80
Met Cys Gln Asn Cys Ile Glu Leu Asn Ala Ala Thr Ile Ser
85 90
<210> 185
<211> 104
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 185 - 人 CD3 ε 胞外区
<400> 185
Asp Gly Asn Glu Glu Met Gly Gly Ile Thr Gln Thr Pro Tyr Lys Val
1 5 10 15
Ser Ile Ser Gly Thr Thr Val Ile Leu Thr Cys Pro Gln Tyr Pro Gly
20 25 30
Ser Glu Ile Leu Trp Gln His Asn Asp Lys Asn Ile Gly Gly Asp Glu
35 40 45
Asp Asp Lys Asn Ile Gly Ser Asp Glu Asp His Leu Ser Leu Lys Glu
50 55 60
Phe Ser Glu Leu Glu Gln Ser Gly Tyr Tyr Val Cys Tyr Pro Arg Gly
65 70 75 80
Ser Lys Pro Glu Asp Ala Asn Phe Tyr Leu Tyr Leu Arg Ala Arg Val
85 90 95
Cys Glu Asn Cys Met Glu Met Asp
100

[0109]

<210> 186
<211> 26
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 186 - 26-残基 肽接头
<400> 186
Gly Ser Ala Asp Asp Ala Lys Lys Asp Ala Ala Lys Lys Asp Asp Ala
1 5 10 15
Lys Lys Asp Asp Ala Lys Lys Asp Gly Ser
20 25
<210> 187
<211> 435
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 187 - 人 CD3 γ-ε-Fc 融合蛋白
<400> 187
Gln Ser Ile Lys Gly Asn His Leu Val Lys Val Tyr Asp Tyr Gln Glu
1 5 10 15
Asp Gly Ser Val Leu Leu Thr Cys Asp Ala Glu Ala Lys Asn Ile Thr
20 25 30
Trp Phe Lys Asp Gly Lys Met Ile Gly Phe Leu Thr Glu Asp Lys Lys
35 40 45
Lys Trp Asn Leu Gly Ser Asn Ala Lys Asp Pro Arg Gly Met Tyr Gln
50 55 60
Cys Lys Gly Ser Gln Asn Lys Ser Lys Pro Leu Gln Val Tyr Tyr Arg
65 70 75 80
Met Gly Ser Ala Asp Asp Ala Lys Lys Asp Ala Ala Lys Lys Asp Asp
85 90 95
Ala Lys Lys Asp Asp Ala Lys Lys Asp Gly Ser Gln Asp Gly Asn Glu
100 105 110
Glu Met Gly Gly Ile Thr Gln Thr Pro Tyr Lys Val Ser Ile Ser Gly
115 120 125
Thr Thr Val Ile Leu Thr Cys Pro Gln Tyr Pro Gly Ser Glu Ile Leu

130 135 140
Trp Gln His Asn Asp Lys Asn Ile Gly Gly Asp Glu Asp Asp Lys Asn
145 150 155 160
Ile Gly Ser Asp Glu Asp His Leu Ser Leu Lys Glu Phe Ser Glu Leu
165 170 175
Glu Gln Ser Gly Tyr Tyr Val Cys Tyr Pro Arg Gly Ser Lys Pro Glu
180 185 190
Asp Ala Asn Phe Tyr Leu Tyr Leu Arg Ala Arg Val Gly Gly Gly Gly
195 200 205
Thr Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
210 215 220
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
225 230 235 240
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
245 250 255
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
260 265 270
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
275 280 285
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
290 295 300
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
305 310 315 320
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
325 330 335
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
340 345 350
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
355 360 365
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
370 375 380
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
385 390 395 400
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
405 410 415
[0110] Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
420 425 430
Ser Pro Gly
435

<210> 188
<211> 26
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 188 - 人 CD3 ε 1-26 氨基酸序列
<400> 188
Gln Asp Gly Asn Glu Glu Met Gly Gly Ile Thr Gln Thr Pro Tyr Lys
1 5 10 15
Val Ser Ile Ser Gly Thr Thr Val Ile Leu
20 25

<210> 189
<211> 26
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 189 - 狨猴 CD3 ε 1-26 氨基酸序列
<400> 189
Gln Asp Gly Asn Glu Glu Met Gly Ser Ile Thr Gln Thr Pro Tyr Glu
1 5 10 15
Val Ser Ile Ser Gly Thr Thr Val Ile Leu
20 25

<210> 190
<211> 258
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 190 - 人 CD3 ε 1-26 Fc 融合蛋白
<400> 190
Gln Asp Gly Asn Glu Glu Met Gly Gly Ile Thr Gln Thr Pro Tyr Lys
1 5 10 15
Val Ser Ile Ser Gly Thr Thr Val Ile Leu Gly Gly Gly Gly Thr Asp

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            20                  25                  30
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
        35                  40                  45
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
    50                  55                  60
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
65                  70                  75                  80
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
                85                  90                  95
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
            100                 105                 110
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
        115                 120                 125
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
    130                 135                 140
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
145                 150                 155                 160
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
                165                 170                 175
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
            180                 185                 190
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
        195                 200                 205
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
    210                 215                 220
Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His
225                 230                 235                 240
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
                245                 250                 255
Gly Lys
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[0111]

<210> 191
<211> 258
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 191 – 猕猴 CD3 ε 1-26 Fc 融合蛋白
<400> 191

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Gln Asp Gly Asn Glu Glu Met Gly Ser Ile Thr Gln Thr Pro Tyr Gln
1               5                   10                  15
Val Ser Ile Ser Gly Thr Thr Val Ile Leu Gly Gly Gly Gly Thr Asp
            20                  25                  30
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
        35                  40                  45
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
    50                  55                  60
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
65                  70                  75                  80
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
                85                  90                  95
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
            100                 105                 110
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
        115                 120                 125
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
    130                 135                 140
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
145                 150                 155                 160
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
                165                 170                 175
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
            180                 185                 190
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
        195                 200                 205
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
    210                 215                 220
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
225                 230                 235                 240
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
                245                 250                 255
Gly Lys
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<210> 192
<211> 264
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 192 - 人 CD38 胞外区 融合到多聚组氨酸标签- 氨基酸序列
<400> 192
Val Pro Arg Trp Arg Gln Gln Trp Ser Gly Pro Gly Thr Thr Lys Arg
1 5 10 15
Phe Pro Glu Thr Val Leu Ala Arg Cys Val Lys Tyr Thr Glu Ile His
20 25 30
Pro Glu Met Arg His Val Asp Cys Gln Ser Val Trp Asp Ala Phe Lys
35 40 45
Gly Ala Phe Ile Ser Lys His Pro Cys Asn Ile Thr Glu Glu Asp Tyr
50 55 60
Gln Pro Leu Met Lys Leu Gly Thr Gln Thr Val Pro Cys Asn Lys Ile
65 70 75 80
Leu Leu Trp Ser Arg Ile Lys Asp Leu Ala His Gln Phe Thr Gln Val
85 90 95
Gln Arg Asp Met Phe Thr Leu Glu Asp Thr Leu Leu Gly Tyr Leu Ala
100 105 110
Asp Asp Leu Thr Trp Cys Gly Glu Phe Asn Thr Ser Lys Ile Asn Tyr
115 120 125
Gln Ser Cys Pro Asp Trp Arg Lys Asp Cys Ser Asn Asn Pro Val Ser
130 135 140
Val Phe Trp Lys Thr Val Ser Arg Arg Phe Ala Glu Ala Ala Cys Asp
145 150 155 160
Val Val His Val Met Leu Asn Gly Ser Arg Ser Lys Ile Phe Asp Lys
165 170 175
Asn Ser Thr Phe Gly Ser Val Glu Val His Asn Leu Gln Pro Glu Lys
180 185 190
Val Gln Thr Leu Glu Ala Trp Val Ile His Gly Gly Arg Glu Asp Ser
195 200 205
Arg Asp Leu Cys Gln Asp Pro Thr Ile Lys Glu Leu Glu Ser Ile Ile
210 215 220
Ser Lys Arg Asn Ile Gln Phe Ser Cys Lys Asn Ile Tyr Arg Pro Asp
225 230 235 240
Lys Phe Leu Gln Cys Val Lys Asn Pro Glu Asp Ser Ser Cys Thr Ser
245 250 255
Glu Ile His His His His His His
260

[0112]

<210> 193
<211> 264
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 193 - 狒狒 CD38 胞外区融合到多聚组氨酸标签- 氨基酸序列
<400> 193
Val Pro Arg Trp Arg Gln Gln Trp Ser Gly Ser Gly Thr Thr Ser Arg
1 5 10 15
Phe Pro Glu Thr Val Leu Ala Arg Cys Val Lys Tyr Thr Glu Val His
20 25 30
Pro Glu Met Arg His Val Asp Cys Gln Ser Val Trp Asp Ala Phe Lys
35 40 45
Gly Ala Phe Ile Ser Lys Tyr Pro Cys Asn Ile Thr Glu Glu Asp Tyr
50 55 60
Gln Pro Leu Val Lys Leu Gly Thr Gln Thr Val Pro Cys Asn Lys Thr
65 70 75 80
Leu Leu Trp Ser Arg Ile Lys Asp Leu Ala His Gln Phe Thr Gln Val
85 90 95
Gln Arg Asp Met Phe Thr Leu Glu Asp Met Leu Leu Gly Tyr Leu Ala
100 105 110
Asp Asp Leu Thr Trp Cys Gly Glu Phe Asn Thr Phe Glu Ile Asn Tyr
115 120 125
Gln Ser Cys Pro Asp Trp Arg Lys Asp Cys Ser Asn Asn Pro Val Ser
130 135 140
Val Phe Trp Lys Thr Val Ser Arg Arg Phe Ala Glu Thr Ala Cys Gly
145 150 155 160
Val Val His Val Met Leu Asn Gly Ser Arg Ser Lys Ile Phe Asp Lys
165 170 175

Asn Ser Thr Phe Gly Ser Val Glu Val His Asn Leu Gln Pro Glu Lys
180 185 190
Val Gln Ala Leu Glu Ala Trp Val Ile His Gly Gly Arg Glu Asp Ser
195 200 205
Arg Asp Leu Cys Gln Asp Pro Thr Ile Lys Glu Leu Glu Ser Ile Ile
210 215 220
Ser Lys Arg Asn Ile Arg Phe Phe Cys Lys Asn Ile Tyr Arg Pro Asp
225 230 235 240
Lys Phe Leu Gln Cys Val Lys Asn Pro Glu Asp Ser Ser Cys Leu Ser
245 250 255
Gly Ile His His His His His His
260

<210> 194
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 194 - 小鼠 抗- 人 CD3 ε OKT3 CDR H1
<400> 194
Gly Tyr Thr Phe Thr Arg Tyr Thr
1 5

<210> 195
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 195 - 小鼠 抗- 人 CD3 ε OKT3 CDR H2
<400> 195
Ile Asn Pro Ser Arg Gly Tyr Thr
1 5

<210> 196
<211> 12
<212> PRT
<213> 人工序列
<220>
[0113] <223> SEQ ID NO: 196 - 小鼠 抗- 人 CD3 ε OKT3 CDR H3
<400> 196
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asn Tyr
1 5 10

<210> 197
<211> 5
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 197 - 小鼠 抗- 人 CD3 ε OKT3 CDR L1
<400> 197
Ser Ser Val Ser Tyr
1 5

<210> 198
<211> 3
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 198 - 小鼠 抗- 人 CD3 ε OKT3 CDR L2
<400> 198
Asp Thr Ser
1

<210> 199
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 199 - 小鼠 抗- 人 CD3 ε OKT3 CDR L3
<400> 199
Gln Gln Trp Ser Ser Asn Pro Pro Thr
1 5

<210> 200
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 200 - 小鼠 抗- 人 CD3 ε SP34 CDR H1

<400> 200
Gly Phe Thr Phe Asn Thr Tyr Ala
1 5
<210> 201
<211> 10
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 201 - 小鼠 抗- 人 CD3 ε SP34 CDR H2
<400> 201
Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr
1 5 10
<210> 202
<211> 16
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 202 - 小鼠 抗- 人 CD3 ε SP34 CDR H3
<400> 202
Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr
1 5 10 15
<210> 203
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 203 - 小鼠 抗- 人 CD3 ε SP34 CDR L1
<400> 203
Thr Gly Ala Val Thr Thr Ser Asn Tyr
1 5
<210> 204
<211> 3
<212> PRT
<213> 人工序列
<220>
[0114] <223> SEQ ID NO: 204 - 小鼠 抗- 人 CD3 ε SP34 CDR L2
<400> 204
Gly Thr Asn
1
<210> 205
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 205 - 小鼠 抗- 人 CD3 ε SP34 CDR L3
<400> 205
Ala Leu Trp Tyr Ser Asn Leu Trp Val
1 5
<210> 206
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 206 - 赫赛汀 (trastuzumab)CDR H1
<400> 206
Gly Phe Asn Ile Lys Asp Thr Tyr
1 5
<210> 207
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 207 - 赫赛汀 (trastuzumab) CDR H2
<400> 207
Ile Tyr Pro Thr Asn Gly Tyr Thr
1 5
<210> 208
<211> 13
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 208 - 赫赛汀 (trastuzumab) CDR H3

<400> 208
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr
1 5 10
<210> 209
<211> 6
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 209 - 赫赛汀 (trastuzumab) CDR L1
<400> 209
Gln Asp Val Asn Thr Ala
1 5
<210> 210
<211> 3
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 210 - 赫赛汀 (trastuzumab) CDR L2
<400> 210
Ser Ala Ser
1
<210> 211
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 211 - 赫赛汀 (trastuzumab) CDR L3
<400> 211
Gln Gln His Tyr Thr Thr Pro Pro Thr
1 5
<210> 212
<211> 8
<212> PRT
<213> 人工序列
<220>

[0115]

<223> SEQ ID NO: 212 - 小鼠 抗- 人 CD38 HB-7 CDR H1
<400> 212
Gly Phe Ser Leu Ile Ser Tyr Gly
1 5
<210> 213
<211> 7
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 213 - 小鼠 抗- 人 CD38 HB-7 CDR H2
<400> 213
Ile Trp Arg Gly Gly Ser Thr
1 5
<210> 214
<211> 13
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 214 - 小鼠 抗- 人 CD38 HB-7 CDR H3
<400> 214
Ala Lys Thr Leu Ile Thr Thr Gly Tyr Ala Met Asp Tyr
1 5 10
<210> 215
<211> 6
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 215 - 小鼠 抗- 人 CD38 HB-7 CDR L1
<400> 215
Glu Asp Ile Tyr Asn Arg
1 5
<210> 216
<211> 3
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 216 - 小鼠 抗- 人 CD38 HB-7 CDR L2

<400> 216
Gly Ala Thr
1
<210> 217
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 217 - 小鼠 抗- 人 CD38 HB-7 CDR L3
<400> 217
Gln Gln Tyr Trp Ser Thr Pro Thr
1 5
<210> 218
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 218 - 小鼠 抗- 人 CD38 OKT10 CDR H1
<400> 218
Gly Phe Asp Phe Ser Arg Ser Trp
1 5
<210> 219
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 219 - 小鼠 抗- 人 CD38 OKT10 CDR H2
<400> 219
Ile Asn Pro Asp Ser Ser Thr Ile Val
1 5
<210> 220
<211> 9
<212> PRT
<213> 人工序列
<220>
[0116] <223> SEQ ID NO: 220 - 小鼠 抗- 人 CD38 OKT10 CDR H3
<400> 220
Ala Arg Tyr Gly Asn Trp Phe Pro Tyr
1 5
<210> 221
<211> 6
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 221 - 小鼠 抗- 人 CD38 OKT10 CDR L1
<400> 221
Gln Asn Val Asp Thr Asn
1 5
<210> 222
<211> 3
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 222 - 小鼠 抗- 人 CD38 OKT10 CDR L2
<400> 222
Ser Ala Ser
1
<210> 223
<211> 15
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 223 - 小鼠 抗- 人 CD38 OKT10 CDR L3
<400> 223
Gln Gln Tyr Asp Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys
1 5 10 15
<210> 224
<211> 10
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 224 - 小鼠 抗- 人 CD38 9G7 CDR H1

<400> 224
Gly Leu Ser Leu Ser Thr Ser Gly Lys Gly
1 5 10
<210> 225
<211> 7
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 225 - 小鼠 抗- 人 CD38 9G7 CDR H2
<400> 225
Ile Trp Trp Asp Asp Asp Lys
1 5
<210> 226
<211> 13
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 226 - 小鼠 抗- 人 CD38 9G7 CDR H3
<400> 226
Ala Arg Ile Glu Leu Gly Arg Ser Tyr Val Met Asp Tyr
1 5 10
<210> 227
<211> 6
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 227 - 小鼠 抗- 人 CD38 9G7 CDR L1
<400> 227
Gln Asp Val Ile Thr Ser
1 5
<210> 228
<211> 3
<212> PRT
<213> 人工序列
<220>
[0117] <223> SEQ ID NO: 228 - 小鼠 抗- 人 CD38 9G7 CDR L2
<400> 228
Ser Ala Ser
1
<210> 229
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 229 - 小鼠 抗- 人 CD38 9G7 CDR L3
<400> 229
Gln Gln His Tyr Thr Ile Pro Leu Thr
1 5
<210> 230
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 230 - 人 抗- 人 CD38 767 CDR H1
<400> 230
Gly Phe Thr Phe Ser Ser Tyr Trp
1 5
<210> 231
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 231 - 人 抗- 人 CD38767 CDR H2
<400> 231
Ile Lys Gln Asp Gly Ser Glu Lys
1 5
<210> 232
<211> 11
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 232 - 人 抗- 人 CD38767 CDR H3

<400> 232
Ala Arg Glu Gly Arg Thr Gly Tyr Phe Asp Tyr
1 5 10
<210> 233
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 233 - 人 抗- 人 CD38767 CDR L1
<400> 233
Thr Ser Asn Ile Gly Thr Asn Tyr
1 5
<210> 234
<211> 3
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 234 - 人 抗- 人 CD38767 CDR L2
<400> 234
Arg Asn Asp
1
<210> 235
<211> 12
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 235 - 人 抗- 人 CD38767 CDR L3
<400> 235
Ala Ala Trp Asp Asp Ser Arg Ser Gly Val Tyr Ala
1 5 10
<210> 236
<211> 10
<212> PRT
<213> 人工序列
<220>
[0118] <223> SEQ ID NO: 236 - 小鼠 抗-人 OX40 CDR H1 来自 WO2013/008171
<400> 236
Gly Phe Ser Leu Ser Thr Ser Gly Met Gly
1 5 10
<210> 237
<211> 7
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 237 - 小鼠 抗-人 OX40 CDR H2 来自 WO2013/008171
<400> 237
Ile Trp Trp Asp Asp Asp Lys
1 5
<210> 238
<211> 10
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 238 - 小鼠 抗-人 OX40 CDR H3 来自 WO2013/008171
<400> 238
Ala Arg Ile Asp Trp Asp Gly Phe Ala Tyr
1 5 10
<210> 239
<211> 5
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 239 - 小鼠 抗-人 OX40 CDR L1 来自 WO2013/008171
<400> 239
Ser Ser Val Ser Tyr
1 5
<210> 240
<211> 3
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 240 - 小鼠 抗-人 OX40 CDR L2 来自 WO2013/008171

<400> 240
Ala Thr Ser
1
<210> 241
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 241 - 小鼠 抗-人 OX40 CDR L3 来自 WO2013/008171
<400> 241
Gln Gln Trp Ser Ser Asn Pro Trp Thr
1 5
<210> 242
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 242 - Rituxan (rituximab) CDR H1
<400> 242
Gly Tyr Thr Phe Thr Ser Tyr Asn
1 5
<210> 243
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 243 - Rituxan (rituximab) CDR H2
<400> 243
Ile Tyr Pro Gly Asn Gly Asp Thr
1 5
<210> 244
<211> 14
<212> PRT
<213> 人工序列
<220>
[0119] <223> SEQ ID NO: 244 - Rituxan (rituximab) CDR H3
<400> 244
Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val
1 5 10
<210> 245
<211> 7
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 245 - Rituxan (rituximab) CDR L1
<400> 245
Ala Ser Ser Ser Val Ser Tyr
1 5
<210> 246
<211> 3
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 246 - Rituxan (rituximab) CDR L2
<400> 246
Ala Thr Ser
1
<210> 247
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 247 - Rituxan (rituximab) CDR L3
<400> 247
Gln Gln Trp Thr Ser Asn Pro Pro Thr
1 5
<210> 248
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 248 - Erbitux (cetuximab) CDR H1

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<400> 248
Gly Phe Ser Leu Thr Asn Tyr Gly
1 5
<210> 249
<211> 7
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 249 - Erbitux (cetuximab) CDR H2
<400> 249
Ile Trp Ser Gly Gly Asn Thr
1 5
<210> 250
<211> 13
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 250 - Erbitux (cetuximab) CDR H3
<400> 250
Ala Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr
1 5 10
<210> 251
<211> 6
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 251 - Erbitux (cetuximab) CDR L1
<400> 251
Gln Ser Ile Gly Thr Asn
1 5
<210> 252
<211> 3
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 252 - Erbitux (cetuximab) CDR L2
<400> 252
Tyr Ala Ser
1
<210> 253
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 253 - Erbitux (cetuximab) CDR L3
<400> 253
Gln Gln Asn Asn Asn Trp Pro Thr Thr
1 5
<210> 254
<211> 10
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 254 - Vectibix (panitumumab) CDR H1
<400> 254
Gly Gly Ser Val Ser Ser Gly Asp Tyr Tyr
1 5 10
<210> 255
<211> 7
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 255 - Vectibix (panitumumab) CDR H2
<400> 255
Ile Tyr Tyr Ser Gly Asn Thr
1 5
<210> 256
<211> 11
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 256 - Vectibix (panitumumab) CDR H3

<400> 256
Val Arg Asp Arg Val Thr Gly Ala Phe Asp Ile
1 5 10
<210> 257
<211> 6
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 257 - Vectibix (panitumumab) CDR L1
<400> 257
Gln Asp Ile Ser Asn Tyr
1 5
<210> 258
<211> 3
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 258 - Vectibix (panitumumab) CDR L2
<400> 258
Asp Ala Ser
1
<210> 259
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 259 - Vectibix (panitumumab) CDR L3
<400> 259
Gln His Phe Asp His Leu Pro Leu Ala
1 5
<210> 260
<211> 8
<212> PRT
<213> 人工序列
<220>
[0121] <223> SEQ ID NO: 260 - 小鼠 抗-人 CD19 CDR H1 来自 WO2010/095031
<400> 260
Gly Val Ser Leu Pro Asp Tyr Gly
1 5
<210> 261
<211> 7
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 261 - 小鼠 抗-人 CD19 CDR H2 来自 WO2010/095031
<400> 261
Ile Trp Gly Ser Glu Thr Thr
1 5
<210> 262
<211> 14
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 262 - 小鼠 抗-人 CD19 CDR H3 来自 WO2010/095031
<400> 262
Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr
1 5 10
<210> 263
<211> 6
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 263 - 小鼠 抗-人 CD19 CDR L1 来自 WO2010/095031
<400> 263
Gln Asp Ile Ser Lys Tyr
1 5
<210> 264
<211> 3
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 264 - 小鼠 抗-人 CD19 CDR L2 来自 WO2010/095031

<400> 264
His Thr Ser
1
<210> 265
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 265 - 小鼠 抗-人 CD19 CDR L3 来自 WO2010/095031
<400> 265
Gln Gln Gly Ala Thr Leu Pro Tyr Thr
1 5
<210> 266
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 266 - Bsw17 CDR H1
<400> 266
Gly Phe Thr Phe Ser Ser Tyr Ala
1 5
<210> 267
<211> 7
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 267 - Bsw17 CDR H2
<400> 267
Ile Ser Ser Gly Asn Ile Ile
1 5
<210> 268
<211> 12
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 268 - Bsw17 CDR H3
<400> 268
Thr Arg Gly Arg Ser Thr Tyr Gly Gly Phe Asp His
1 5 10
<210> 269
<211> 5
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 269 - Bsw17 CDR L1
<400> 269
Ser Ser Val Thr Phe
1 5
<210> 270
<211> 3
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 270 - Bsw17 CDR L2
<400> 270
Asp Thr Ser
1
<210> 271
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 271 - Bsw17 CDR L3
<400> 271
Gln His Trp Ser Gly Asn Pro Leu Thr
1 5
<210> 272
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 272 - Onalizumab CDR H1

[0122]

<400> 272
Gly Tyr Ser Ile Thr Ser Gly Tyr Ser
1 5
<210> 273
<211> 7
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 273 - Omalizumab CDR H2
<400> 273
Ile Thr Tyr Asp Gly Ser Thr
1 5
<210> 274
<211> 14
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 274 - Omalizumab CDR H3
<400> 274
Ala Arg Gly Ser His Tyr Phe Gly His Trp His Phe Ala Val
1 5 10
<210> 275
<211> 10
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 275 - Omalizumab CDR L1
<400> 275
Gln Ser Val Asp Tyr Asp Gly Asp Ser Tyr
1 5 10
<210> 276
<211> 3
<212> PRT
<213> 人工序列
<220>

[0123]

<223> SEQ ID NO: 276 - Omalizumab CDR L2
<400> 276
Ala Ala Ser
1
<210> 277
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 277 - Omalizumab CDR L3
<400> 277
Gln Gln Ser His Glu Asp Pro Tyr Thr
1 5
<210> 278
<211> 118
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 278 - 人源化抗-OX40/mingraft VH 结构域
<400> 278
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Val Ser Ala Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Ala Asp Ser
50 55 60
Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Ile Asp Trp Asp Gly Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser
115
<210> 279

<211> 118
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 279 - 人源化抗-OX40/maxgraft VH 结构域
<400> 279
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Gly Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Thr Ala
50 55 60
Leu Lys Ser Gly Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyc
85 90 95
Cys Ala Arg Ile Asp Trp Asp Gly Phe Ala Tyr Trp Gly Glu Gly Thr
100 105 110
Leu Val Thr Val Ser Ser
115
<210> 280
<211> 106
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 280 - 人源化抗 OX40/mingraft VL 结构域
<400> 280
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Leu
20 25 30
Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45
Ala Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Trp Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105
<210> 281
<211> 106
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 281 - 人源化抗-OX40/maxgraft VL 结构域
<400> 281
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Trp Ile Tyr
35 40 45
Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Tyr Thr Leu Thc Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Trp Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105
<210> 282
<211> 121
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 282 - 人源化 Rituximab/mingraft VH 结构域
<400> 282
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

[0124]

1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Asn Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Tyr Pro Gly Asn Gly Asp Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 283
<211> 121
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 283 - 人源化Rituximab/maxgraft VH 结构域
<400> 283
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Asn Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Ala Thr Leu Ser Ala Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

[0125]

<210> 284
<211> 108
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 284 - 人源化Rituximab/mingraft VL 结构域
<400> 284
Asp Ile Glu Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ala Ser Ser Ser Val Ser
20 25 30
Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Ala Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80
Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro
85 90 95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 285
<211> 108
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 285 - 人源化Rituximab/maxgraft VL 结构域
<400> 285
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Leu Ser Ala Ser Ser Ser Val Ser
20 25 30
Tyr Leu Asn Trp Phe Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Trp
35 40 45

Ile Tyr Ala Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80
Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro
85 90 95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 286
<211> 119
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 286 - 人源化 Erbitux/mingraft VH 结构域
<400> 286
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Asn Tyr
20 25 30
Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Trp Ser Gly Gly Asn Thr Tyr Tyr Ala Asp Ser Val Lys
50 55 60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 287
<211> 119
<212> PRT
<213> 人工序列
<220>

[0126]

<223> SEQ ID NO: 287 - 人源化 Erbitux/maxgraft VH 结构域
<400> 287
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Asn Tyr
20 25 30
Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Ala Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Thr Pro Phe Thr
50 55 60
Gly Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 288
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 288 - 人源化 Erbitux/mingraft VL 结构域
<400> 288
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Thr Asn
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Tyr Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Asn Asn Asn Trp Pro Thr

85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105
<210> 289
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 289 - 人源化 Erbitux/maxgraft VL 结构域
<400> 289
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Thr Asn
20 25 30
Ile His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Lys Tyr Ala Ser Glu Ser Ile Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Asn Asn Asn Trp Pro Thr
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105
<210> 290
<211> 119
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 290 - Vectibix VH 结构域
<400> 290
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Val Ser Ser Gly
20 25 30
Asp Tyr Tyr Trp Thr Trp Ile Arg Gln Ser Pro Gly Lys Gly Leu Glu
35 40 45
Trp Ile Gly His Ile Tyr Tyr Ser Gly Asn Thr Asn Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Ile Asp Thr Ser Lys Thr Gln Phe
65 70 75 80
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ile Tyr Tyr
85 90 95
Cys Val Arg Asp Arg Val Thr Gly Ala Phe Asp Ile Trp Gly Gln Gly
100 105 110
Thr Met Val Thr Val Ser Ser
115
<210> 291
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 291 - Vectibix VL 结构域
<400> 291
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Ile Ala Thr Tyr Phe Cys Gln His Phe Asp His Leu Pro Leu
85 90 95
Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105
<210> 292
<211> 119
<212> PRT
<213> 人工序列

[0127]

<220>
<223> SEQ ID NO: 292 - 人源化 Vectibix/mingraft VH 结构域
<400> 292
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gly Ser Val Ser Ser Gly
20 25 30
Asp Tyr Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Val Ser Ala Ile Tyr Tyr Ser Gly Asn Thr Tyr Tyr Ala Asp Ser
50 55 60
Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Val Arg Asp Arg Val Thr Gly Ala Phe Asp Ile Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115
<210> 293
<211> 119
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 293 - 人源化 Vectibix/maxgraft VH 结构域
<400> 293
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Gly Ser Val Ser Ser Gly
20 25 30
Asp Tyr Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Ile Gly Ala Ile Tyr Tyr Ser Gly Asn Thr Tyr Tyr Ala Asp Ser
50 55 60
Val Lys Gly Arg Leu Thr Ile Ser Ile Asp Thr Ser Lys Asn Thr Phe
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Val Arg Asp Arg Val Thr Gly Ala Phe Asp Ile Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115
<210> 294
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 294 - 人源化 Vectibix/mingraft VL 结构域
<400> 294
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Phe Asp His Leu Pro Leu
85 90 95
Ala Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105
<210> 295
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 295 - 人源化 Vectibix/maxgraft VL 结构域
<400> 295
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

[0128]

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Ile Ala Thr Tyr Phe Cys Gln His Phe Asp His Leu Pro Leu
85 90 95
Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 296
<211> 120
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 296 - 抗-人 CD19 VH 结构域 来自 WO2010/095031
<400> 296
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ser Leu Pro Asp Tyr
20 25 30
Gly Val Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys
50 55 60
Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser
115 120

[0129]

<210> 297
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 297 - 抗-人 CD19 VL 结构域 来自 WO2010/095031
<400> 297
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Lys Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Ile Lys Leu Leu Ile
35 40 45
Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Ala Thr Leu Pro Tyr
85 90 95
Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
100 105

<210> 298
<211> 121
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 298 - Omalizomab VH 结构域
<400> 298
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr Ser Gly
20 25 30
Tyr Ser Trp Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
35 40 45
Val Ala Ser Ile Thr Tyr Asp Gly Ser Thr Asn Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Phe Tyr

65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Ser His Tyr Phe Gly His Trp His Phe Ala Val Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser
115 120
<210> 299
<211> 111
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 299 - Omalizumab VL 结构域
<400> 299
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30
Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
35 40 45
Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser Gly Val Pro Ser
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80
Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser His
85 90 95
Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110
<210> 300
<211> 121
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 300 - 稳定化的 Omalizumab/maxgraft VH 结构域
<400> 300
[0130] Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr Ser Gly
20 25 30
Tyr Ser Trp Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
35 40 45
Val Ala Ser Ile Thr Tyr Asp Gly Ser Thr Asn Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Phe Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Ser His Tyr Phe Gly His Trp His Phe Ala Val Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser
115 120
<210> 301
<211> 121
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 301 - 稳定化的 Omalizumab/mingraft VH 结构域
<400> 301
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Ile Thr Ser Gly
20 25 30
Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
35 40 45
Val Ser Ala Ile Thr Tyr Asp Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Ser His Tyr Phe Gly His Trp His Phe Ala Val Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 302
<211> 111
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 302 - 稳定化的 Omalizumab/maxgraft VL 结构域
<400> 302
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp Tyr Asn
20 25 30
Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
35 40 45
Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser Gly Val Pro Ser
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80
Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser His
85 90 95
Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 303
<211> 111
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 303 - 稳定化的 Omalizumab/mingraft VL 结构域
<400> 303
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
35 40 45
Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80
Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser His
85 90 95
Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

[0131]

<210> 304
<211> 118
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 304 - Bsw17 小鼠 VH 结构域
<400> 304
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Phe Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45
Ala Ser Ile Ser Ser Gly Asn Ile Ile Tyr Tyr Pro Asp Asn Val Lys
50 55 60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Val Arg Asn Ile Leu Tyr Leu
65 70 75 80
Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Thr
85 90 95
Arg Gly Arg Ser Thr Tyr Gly Gly Phe Asp His Trp Gly Gln Gly Thr
100 105 110
Thr Leu Thr Val Ser Ser
115

<210> 305
<211> 106
<212> PRT
<213> 人工序列
<220>

<223> SEQ ID NO: 305 - Bsw17 小鼠 VL 结构域
<400> 305
Glu Leu Val Met Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15
Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Thr Phe Ile
20 25 30
His Trp Tyr Arg Gln Lys Ser Gly Thr Ser Pro Lys Gly Trp Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Thr Met Glu Ala Glu
65 70 75 80
Asp Ala Ala Thr Tyr Tyr Cys Gln His Trp Ser Gly Asn Pro Leu Thr
85 90 95
Phe Gly Thr Gly Thr Lys Leu Glu Leu Lys
100 105
<210> 306
<211> 118
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 306 - 人源化 Bsw17/mingraft VH 结构域
<400> 306
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Ser Ser Gly Asn Ile Ile Tyr Tyr Ala Asp Ser Val Lys
50 55 60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr
85 90 95
Arg Gly Arg Ser Thr Tyr Gly Gly Phe Asp His Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser
115

[0132]

<210> 307
<211> 118
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 307 - 人源化 Bsw17/maxgraft VH 结构域
<400> 307
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Ser Ile Ser Ser Gly Asn Ile Ile Tyr Tyr Pro Asp Asn Val Lys
50 55 60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr
85 90 95
Arg Gly Arg Ser Thr Tyr Gly Gly Phe Asp His Trp Gly Gln Gly Thr
100 105 110
Thr Val Thr Val Ser Ser
115
<210> 308
<211> 106
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 308 - 人源化 Bsw17/mingraft VL 结构域
<400> 308
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Thr Phe Leu

20 25 30
Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45
Asp Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln His Trp Ser Gly Asn Pro Leu Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 309
<211> 106
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 309 - 人源化 Bsw17/maxgraft VL 结构域
<400> 309
Asp Leu Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Thr Phe Leu
20 25 30
Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Trp Leu Leu Ile Tyr
35 40 45
Asp Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Met Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln His Trp Ser Gly Asn Pro Leu Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 310
<211> 449
<212> PRT
[0133] <213> 人工序列
<220>
<223> SEQ ID NO: 310 - BEAT HER2/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-HER2 FAB 臂具有 G6SS 取代 Bf23 LALA)
<400> 310
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60
Lys Ser Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215 220
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
225 230 235 240
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270
Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His
275 280 285
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg
290 295 300
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335
Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr
340 345 350
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu
355 360 365
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380
Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met
385 390 395 400
Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp
405 410 415
Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His
420 425 430
Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445
Gly

<210> 311
<211> 481
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 311 ~ BEAT 抗体 scFv 重链
SP34-Kappa2(抗-CD3 ε 臂- 人源化 SP34 VH5/VL32 BC11
LALA)
<400> 311

[0134]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala Ala Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu

290 295 300

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Ala
370 375 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385 390 395 400
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
420 425 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly

<210> 312
<211> 450
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 312 - BEAT CD38-9G7 最佳框架/CD3(SP34-Kappa2)抗体 FAB 重链 (抗-CD38 FAB 臂具有 G65S 取代 BT33 LALA)
<400> 312

[0135]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Leu Ser Leu Ser Thr Ser
20 25 30
Gly Lys Gly Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ala
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Ile Glu Leu Gly Arg Ser Tyr Val Met Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270
Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val
275 280 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335
Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val
340 345 350
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys
355 360 365
Leu Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380
Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro
385 390 395 400
Met Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val
405 410 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met
420 425 430
His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445
Pro Gly
450

<210> 313
<211> 447
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 313 - BEAT CD38-767/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-CD38 FAB 臂具有 G65S 取代 BT33 LALA)
<400> 313

[0136]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60
Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Glu Gly Arg Thr Gly Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130 135 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser
225 230 235 240
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260 265 270
Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val Val
290 295 300
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
325 330 335
Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu
340 345 350

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val Cys
355 360 365
Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380
Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu Asp
385 390 395 400
Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys Ser
405 410 415
Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala
420 425 430
Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

<210> 314
<211> 447
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 314 - BFAT OX40maxgraft/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-OX40 maxgraft FAB 臂具有 G65S 取代 BT33 LALA)

<400> 314
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Gly Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Thr Ala
50 55 60
Leu Lys Ser Gly Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Ile Asp Trp Asp Gly Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130 135 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser
225 230 235 240
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260 265 270
Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val Val
290 295 300
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
325 330 335
Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu
340 345 350
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val Cys
355 360 365
Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380
Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu Asp
385 390 395 400
Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys Ser

[0137]

405 410 415
Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala
420 425 430
Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445
<210> 315
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 315 - BEAT OX40maxgraft/CD3(SP34-Kappa2) 抗体 FAB 轻链 (抗 OX40 maxgraft FAB臂 LC)
<400> 315
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Trp Ile Tyr
35 40 45
Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Trp Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
[0138] Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210
<210> 316
<211> 447
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 316 - BEAT OX40mingraft/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-OX40 mingraft FAB臂具有 G65S 取代 BT33 LALA)
<400> 316
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Val Ser Ala Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Ala Asp Ser
50 55 60
Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Ile Asp Trp Asp Gly Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130 135 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser
225 230 235 240
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260 265 270
Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val Val
290 295 300
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
325 330 335
Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu
340 345 350
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val Cys
355 360 365
Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380
Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu Asp
385 390 395 400
Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys Ser
405 410 415
Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala
420 425 430
Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

[0139]

<210> 317
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 317 - BEAT OX40mingraft/CD3(SP34-kappa2) 抗体FAB 轻链 (抗-OX40 mingraft FAB臂 LC)
<400> 317
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Leu
20 25 30
Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45
Ala Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Trp Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210
<210> 318
<211> 450

<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 318 - BEAT CD20maxgraft/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-CD20 maxgraft FAB 臂具有 C65S 取代 BT33 LALA)
<400> 318
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Asn Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Ser Arg Ala Thr Leu Ser Ala Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270
Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val
275 280 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe
290 295 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335
Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val
340 345 350
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys
355 360 365
Leu Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380
Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro
385 390 395 400
Met Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val
405 410 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met
420 425 430
His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445
Pro Gly
450

[0140]

<210> 319
<211> 215
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 319 - BEAT CD20maxgraft/CD3(SP34-Kappa2) 抗体 FAB 轻链 (抗-CD20 maxgraft FAB 臂 LC)

<400> 319
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Leu Ser Ala Ser Ser Ser Val Ser
20 25 30
Tyr Leu Asn Trp Phe Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Trp
35 40 45
Ile Tyr Ala Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80
Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro
85 90 95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
100 105 110
Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115 120 125
Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130 135 140
Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145 150 155 160
Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165 170 175
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180 185 190
Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195 200 205
Ser Phe Asn Arg Gly Glu Cys
210 215
<210> 320
<211> 450
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 320 - BEAT CD20mingraft/CD3(SP34-Kappa2) 抗体FAB 重链 (抗-CD20 mingraft FAB臂具有G65S 取代 BT33 LALA)

[0141]

<400> 320
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Asn Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Tyr Pro Gly Asn Gly Asp Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val
275 280 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe
290 295 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335
Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val
340 345 350
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys
355 360 365
Leu Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380
Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro
385 390 395 400
Met Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val
405 410 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met
420 425 430
His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445
Pro Gly
450

<210> 321
<211> 215
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 321 - BEAT CD20mingraft/CD3(SP34-Kappa2) 抗体 FAB 轻链 (抗-CD20 mingraft FAB 臂 LC)
<400> 321
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ala Ser Ser Ser Val Ser
20 25 30
Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Ala Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80
Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro
85 90 95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
100 105 110
Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115 120 125
Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130 135 140
Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145 150 155 160
Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165 170 175
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180 185 190
Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195 200 205
Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 322
<211> 448
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 322 - BEAT EGFRcetux-maxgraft/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-EGFR cetuximab maxgraft FAB 臂 具有 Q65S 取代 BT33 LALA)
<400> 322
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Asn Tyr

[0142]

[0143]

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            20                  25                  30
Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45
Gly Ala Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Thr Pro Phe Thr
    50                  55                  60
Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65                  70                  75                  80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95
Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly
            100                 105                 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro
225                 230                 235                 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270
Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val
    290                 295                 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335
Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr
            340                 345                 350
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val
        355                 360                 365
Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380
Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu
385                 390                 395                 400
Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys
                405                 410                 415
Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu
            420                 425                 430
Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445
<210> 323
<211> 214
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 323 - BEAT EGFRcetux-maxgraft/CD3(SP34-Kappa2)
      抗体FAB 轻链 (抗-EGFR cetuximab maxgraft FAB 臂)
<400> 323
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1                   5                   10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Thr Asn
            20                  25                  30
Ile His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45
Lys Tyr Ala Ser Glu Ser Ile Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Asn Asn Asn Trp Pro Thr
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85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210

<210> 324
<211> 448
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 321 - BEAT EGFRcetux-mingraft/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-EGFR cetuximab mingraft FAB 臂 具有 G65S 取代 BT33 LALA)
<400> 324

[0144]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Asn Tyr
20 25 30
Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Trp Ser Gly Gly Asn Thr Tyr Tyr Ala Asp Ser Val Lys
50 55 60
Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val
355 360 365

Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Ser Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Lys Leu Asn Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

<210> 325
<211> 214
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 325 - BEAT EGFRcetux-mingraft/CD3(SP34-Kappa2) 抗体FAB 轻链 (抗 EGFR cetuximab mingraft FAB 臂)
<400> 325
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Thr Asn
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Tyr Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Asn Asn Asn Trp Pro Thr
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210

[0145]

<210> 326
<211> 448
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 326 - BEAT EGFRpani-maxgraft/CD3(SP34-Kappa2) 抗体FAB 重链 (抗-EGFR panitumumab maxgraft FAB臂具有G65S取代 ST33 LALA)
<400> 326
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Gly Ser Val Ser Ser Gly
20 25 30
Asp Tyr Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Ile Gly Ala Ile Tyr Tyr Ser Gly Asn Thr Tyr Tyr Ala Asp Ser
50 55 60
Val Lys Ser Arg Leu Thr Ile Ser Ile Asp Thr Ser Lys Asn Thr Phe
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Val Arg Asp Arg Val Thr Gly Ala Phe Asp Ile Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu

[0146]

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    130                 135                 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro
225                 230                 235                 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270
Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val
    290                 295                 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335
Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr
            340                 345                 350
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val
        355                 360                 365
Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380
Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu
385                 390                 395                 400
Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys
                405                 410                 415
Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu
            420                 425                 430
Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445
<210> 327
<211> 214
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 327 - BEAT EGFRpani-maxgraft/CD3(SP34-Kappa2) 抗体
      FAB 轻链 (抗-EGFR panitumumab maxgraft FAB 臂)
<400> 327
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1                   5                   10                  15
Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45
Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Ile Ala Thr Tyr Phe Cys Gln His Phe Asp His Leu Pro Leu
                85                  90                  95
Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
```

195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 328
<211> 448
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 328 - BEAT EGFRpani-mingraft/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-EGFR panitumumab mingraft FAB 臂具有 G65S 取代 BT33 LALA)
<400> 328

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gly Ser Val Ser Ser Gly
20 25 30
Asp Tyr Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Val Ser Ala Ile Tyr Tyr Ser Gly Asn Thr Tyr Tyr Ala Asp Ser
50 55 60
Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Val Arg Asp Arg Val Thr Gly Ala Phe Asp Ile Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

[0147]

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val
355 360 365
Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Ser Gly Gln Pro Glu Asn Asn Tyr Thr Thr Pro Pro Met Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

<210> 329
<211> 214
<212> PRT
<213> 人工序列

<220>
<223> SEQ ID NO: 329 - BEAT EGFRpani-mingraft/CD3(SP34-Kappa2) 抗体 FAB 轻链 (抗-EGFR panitumumab mingraft FAB 臂)
<400> 329
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Phe Asp His Leu Pro Leu
85 90 95
Ala Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210
<210> 330
<211> 449
<212> PRT
[0148] <213> 人工序列
<220>
<223> SEQ ID NO: 330 - BEAT CD19/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-CD19 FAB 臂具有 G65S 取代 BT33 LALA)
<400> 330
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ser Leu Pro Asp Tyr
20 25 30
Gly Val Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys
50 55 60
Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215 220
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
225 230 235 240
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270
Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His
275 280 285
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg
290 295 300
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335
Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr
340 345 350
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
355 360 365
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380
Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Thr Thr Pro Pro Met
385 390 395 400
Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp
405 410 415
Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His
420 425 430
Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445
Gly

<210> 331
<211> 214
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 331 - BEAT CD19/CD3(SP34-Kappa2) 抗体 FAB 轻链 (抗-CD19 FAB 臂)
<400> 331

[0149]

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Lys Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Ile Lys Leu Leu Ile
35 40 45
Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Ala Thr Leu Pro Tyr
85 90 95
Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210
<210> 332
<211> 450
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 332 - BEAT IgEomali-maxgraft/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-IgE omalizumab maxgraft FAB 臂具有 G65S 取代 BT33 LALA)
<400> 332
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

[0150]

```
1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr Ser Gly
            20                  25                  30
Tyr Ser Trp Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
        35                  40                  45
Val Ala Ser Ile Thr Tyr Asp Gly Ser Thr Asn Tyr Ala Asp Ser Val
    50                  55                  60
Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Phe Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Gly Ser His Tyr Phe Gly His Trp His Phe Ala Val Trp Gly
            100                 105                 110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115                 120                 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
    130                 135                 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
        195                 200                 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
    210                 215                 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225                 230                 235                 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
                245                 250                 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
            260                 265                 270
Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val
        275                 280                 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe
    290                 295                 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305                 310                 315                 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
                325                 330                 335
Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val
            340                 345                 350
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys
        355                 360                 365
Leu Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    370                 375                 380
Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro
385                 390                 395                 400
Met Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val
                405                 410                 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met
            420                 425                 430
His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser
        435                 440                 445
Pro Gly
    450
<210> 333
<211> 218
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 333 - BEAT IgEomali-maxgraft/CD3(SP34-Kappa2) 抗体
      FAB 轻链 (抗-IgE omalizumab maxgraft FAB 臂)
<400> 333
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp Tyr Asp
            20                  25                  30
Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
        35                  40                  45
Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser Gly Val Pro Ser
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50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80
Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser His
85 90 95
Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105 110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 334
<211> 450
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 334 - BEAT IgEomal1-mingraft/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-IgE omalizumab mingraft FAB 臂具有 G65S 取代 BT33 LALA)
<400> 334
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Ile Thr Ser Gly
20 25 30
Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
35 40 45
Val Ser Ala Ile Thr Tyr Asp Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Ser His Tyr Phe Gly His Trp His Phe Ala Val Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270
Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val
275 280 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe
290 295 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335

[0151]

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val
340 345 350
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys
355 360 365
Leu Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380
Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro
385 390 395 400
Met Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val
405 410 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met
420 425 430
His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445
Pro Gly
450

<210> 335
<211> 218
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 335 - BEAT IgEomali-mingraft/CD3(SP34-Kappa2) 抗体 FAB 轻链 (抗-IgE omalizumab mingraft FAB 臂)
<400> 335
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
35 40 45
Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80
Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser His
85 90 95
[0152] Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105 110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 336
<211> 447
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 336 - BEAT IgEbsw17-maxgraft/CD3(SP34-Kappa2) 抗体 FAD 重链 (抗 IgE omalizumab maxgraft FAD 臂具有 G65S 取代 BT33 LALA)
<400> 336
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Ser Ile Ser Ser Gly Asn Ile Ile Tyr Tyr Pro Asp Asn Val Lys
50 55 60
Ser Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Cyr Cys Thr

85 90 95
Arg Gly Arg Ser Thr Tyr Gly Gly Phe Asp His Trp Gly Gln Gly Thr
100 105 110
Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130 135 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser
225 230 235 240
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260 265 270
Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val Val
290 295 300
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
325 330 335
Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu
340 345 350
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val Cys
355 360 365
Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380
Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu Asp
385 390 395 400
Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys Ser
405 410 415
Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala
420 425 430
Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

[0153]

<210> 337
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 337 - BEAT IgEbsw17-maxgraft/CD3(SP34-Kappa2) 抗体 FAB 轻链 (抗-IgE omalizumab maxgraft FAB 臂)
<400> 337
Asp Leu Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Thr Phe Leu
20 25 30
Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Trp Leu Leu Ile Tyr
35 40 45
Asp Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Met Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln His Trp Ser Gly Asn Pro Leu Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu

145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

<210> 338
<211> 447
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 338 ~ BEAT IgEbsw17-mingraft/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-IgE omalizumab mingraft FAB 臂具有 G65S 取代 BT33 LALA)
<400> 338

[0154]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Ser Ser Gly Asn Ile Ile Tyr Tyr Ala Asp Ser Val Lys
50 55 60
Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr
85 90 95
Arg Gly Arg Ser Thr Tyr Gly Gly Phe Asp His Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130 135 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser
225 230 235 240
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260 265 270
Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val Val
290 295 300
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
325 330 335
Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu
340 345 350
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val Cys
355 360 365
Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380
Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu Asp
385 390 395 400
Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys Ser
405 410 415
Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala
420 425 430

Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

<210> 339
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 339 - BEAT IgEbsw17-mingraft/CD3(SP34-Kappa2) 抗体 FAB 轻链 (抗-IgE omalizumab mingraft FAB 臂)
<400> 339
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Thr Phe Leu
20 25 30
Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45
Asp Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80
Asp Phe Ala Thr Tyr Tyr Cys Gln His Trp Ser Gly Asn Pro Leu Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

[0155]

Asn Arg Gly Glu Cys
210

<210> 340
<211> 447
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 340 - BEAT OX40/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-OX40 1D4 FAB 臂 BT33 LALA)
<400> 340
Gln Val Thr Leu Lys Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15
Thr Leu Thr Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45
Trp Ile Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Thr Ala
50 55 60
Leu Lys Thr Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg Ile Asp Trp Asp Gly Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130 135 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser
225 230 235 240
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260 265 270
Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val Val
290 295 300
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
325 330 335
Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu
340 345 350
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val Cys
355 360 365
Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380
Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu Asp
385 390 395 400
Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys Ser
405 410 415
Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala
420 425 430
Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

<210> 341
<211> 450
<212> PRT
<213> 人工序列
<220>

[0156] <223> SEQ ID NO: 341 - BEAT CD20/CD3(SP34-Kappa2) 抗体FAB 重链 (抗-CD20 rituximab FAB 臂BI33 LALA)

<400> 341
Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly
100 105 110
Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser
115 120 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val
275 280 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe
290 295 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335
Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val
340 345 350
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys
355 360 365
Leu Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380
Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro
385 390 395 400
Met Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val
405 410 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met
420 425 430
His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445
Pro Gly
450

<210> 342
<211> 448
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 342 - BEAT EGFRcetux/CD3(SP34 Kappa2) 抗体 FAB 重链 (抗-EGFR cetuximab FAB 臂 BT33 LALA)
<400> 342

Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
1 5 10 15
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Asn Tyr
20 25 30
Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Thr Pro Phe Thr
50 55 60
Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80
Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95
Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

[0157]

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val
355 360 365
Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

<210> 343
<211> 448
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 343 - BEAT EGFRpan1/CD3(SP34-Kappa2) 抗体FAB 重链 (抗-EGFR pan1 tumumab FAB 臂 BT33 LALA)
<400> 343

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Val Ser Ser Gly
20 25 30
Asp Tyr Tyr Trp Thr Trp Ile Arg Gln Ser Pro Gly Lys Gly Leu Glu
35 40 45
Trp Ile Gly His Ile Tyr Tyr Ser Gly Asn Thr Asn Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Ile Asp Thr Ser Lys Thr Gln Phe
65 70 75 80
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ile Tyr Tyr
85 90 95
[0158]
Cys Val Arg Asp Arg Val Thr Gly Ala Phe Asp Ile Trp Gly Gln Gly
100 105 110
Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val
355 360 365
Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380

Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

<210> 344
<211> 214
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 344 - BEAT EGFRpan1/CD3(SP34-Kappa2) 抗体 FAB 轻链 (抗-EGFR panitumumab FAB 臂)
<400> 344
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Ile Ala Thr Tyr Phe Cys Gln His Phe Asp His Leu Pro Leu
85 90 95
Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210

[0159]

<210> 345
<211> 450
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 345 - BEAT IgEomali/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-IgE omalizumab FAB 臂 BT33 LALA)
<400> 345
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr Ser Gly
20 25 30
Tyr Ser Trp Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
35 40 45
Val Ala Ser Ile Thr Tyr Asp Gly Ser Thr Asn Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Phe Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Ser His Tyr Phe Gly His Trp His Phe Ala Val Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270
Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val
275 280 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe
290 295 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335
Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val
340 345 350
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys
355 360 365
Leu Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380
Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro
385 390 395 400
Met Leu Asp Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val
405 410 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met
420 425 430
His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

[0160]

Pro Gly
450

<210> 346
<211> 218
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 346 - BEAT IgEomali/CD3(SP34-Kappa2) 抗体 FAB 轻链 (抗-IgE omalizumab FAB 臂)
<400> 346
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30
Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
35 40 45
Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser Gly Val Pro Ser
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80
Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser His
85 90 95
Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105 110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 347
<211> 447
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 347 - BEAT IgEbsw17/CD3(SP34-Kappa2) 抗体 FAB 重链 (抗-IgE Bsw17 FAB 臂 BT33 LALA)
<400> 347
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Phe Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45
Ala Ser Ile Ser Ser Gly Asn Ile Ile Tyr Tyr Pro Asp Asn Val Lys
50 55 60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Val Arg Asn Ile Leu Tyr Leu
65 70 75 80
Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Thr
85 90 95
Arg Gly Arg Ser Thr Tyr Gly Gly Phe Asp His Trp Gly Gln Gly Thr
100 105 110
Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130 135 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser
225 230 235 240
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260 265 270
Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Phe Arg Val Val
290 295 300
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
325 330 335
Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Ala Val Tyr Thr Leu
340 345 350
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Lys Leu Val Cys
355 360 365
Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380
Ser Gly Gln Pro Glu Asn Asn Tyr Tyr Thr Thr Pro Pro Met Leu Asp
385 390 395 400
Ser Asp Gly Ser Phe Ser Leu Val Ser Trp Leu Asn Val Asp Lys Ser
405 410 415
Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met His Glu Ala
420 425 430
Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

<210> 348
<211> 213
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 348 - BEAT IgEbsw17/CD3(SP34-Kappa2) 抗体 FAB

[0161]

轻链 (抗-IgE Bsw17 FAB 臂)

<400> 348

Glu Leu Val Met Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15
Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Thr Phe Ile
20 25 30
His Trp Tyr Arg Gln Lys Ser Gly Thr Ser Pro Lys Gly Trp Ile Tyr
35 40 45
Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Thr Met Glu Ala Glu
65 70 75 80
Asp Ala Ala Thr Tyr Tyr Cys Gln His Trp Ser Gly Asn Pro Leu Thr
85 90 95
Phe Gly Thr Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

<210> 349
<211> 482
<212> PRT
<213> 人工序列
<220>

[0162] <223> SEQ ID NO: 349 - scFv片段 人源化SP34 VHI-VL24 - 人IgG1 Fc 融合蛋白

<400> 349

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Ala Ala Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270

```
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    290                 295                 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
    370                 375                 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385                 390                 395                 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
            420                 425                 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
        435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly Lys
```

<210> 350
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 350 - scFv片段 人源化SP34 VH1-VL25 - 人IgG1 Fc 融合蛋白

[0163]

<400> 350

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
            20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
    130                 135                 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Ala Ala Thr Ser Asn Tyr Ala
                165                 170                 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
            180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
        195                 200                 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
    210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val
225                 230                 235                 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
                245                 250                 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
            260                 265                 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        275                 280                 285
```

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asn
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 351
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 351 - scFv片段 人源化SP34 VH1-VL26 - 人IgG1 Fc 融合蛋白
<400> 351

[0164]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala Ala Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 352
<211> 8
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 352 - 人源化抗- 人 CD3 - ε SP34 VH5 CDR H1
<400> 352
Gly Phe Thr Phe Asn Thr Tyr Ala
1 5

[0165]

<210> 353
<211> 10
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 353 - 人源化抗- 人 CD3 - ε SP34 VH5 CDR H2
<400> 353
Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr
1 5 10
<210> 354
<211> 16
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 354 - 人源化抗- 人 CD3 - ε SP34 VH5 CDR H3
<400> 354
Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe Ala Tyr
1 5 10 15
<210> 355
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 355 - 人源化抗- 人 CD3 - ε SP34 VL32 CDR L1
<400> 355
Thr Gly Ala Val Thr Ala Ala Asn Tyr
1 5
<210> 356
<211> 3
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 356 - 人源化抗- 人 CD3 - ε SP34 VL32 CDR L2
<400> 356

Gly Ala Asn
1

<210> 357
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 357 - 人源化抗- 人 CD3 e SP34 VL32 CDR L3
<400> 357
Ala Leu Phe Tyr Ser Asn Leu Trp Val
1 5

<210> 358
<211> 119
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 358 - OKT3 人源化 VH9 结构域
<400> 358
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Ala Asp Ser Val
50 55 60
Lys Ser Arg Phe Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

[0166]

<210> 359
<211> 455
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 359 - SP34 人源化 IgG1 重链 具有 VH5
<400> 359
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr
115 120 125
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
130 135 140
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145 150 155 160
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
165 170 175
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
195 200 205
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
210 215 220
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
355 360 365
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445
Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 360
<211> 218
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 360 - SP34 人源化轻链 具有 VL32
<400> 360

[0167]

Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala
20 25 30
Ala Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Lys Arg
100 105 110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 361
<211> 452
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 361 - scFv 片段 人源化 SP34 VH1-VL21 人 IgG1 Fc 融合蛋白
<400> 361
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

[0168]

```
1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
            20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
    130                 135                 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
                165                 170                 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
            180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
        195                 200                 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
    210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val
225                 230                 235                 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
                245                 250                 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
            260                 265                 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    290                 295                 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
    370                 375                 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385                 390                 395                 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
            420                 425                 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
        435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly Lys
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<210> 362
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 362 - scFv 片段 人源化 SP34 VH1-VL33 - 人 IgG1 Fc 融合蛋白
<400> 362

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
```

[0169]

```
            20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
    130                 135                 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala Ala Asn Tyr Ala
                165                 170                 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
            180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
        195                 200                 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
    210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Ala Phe Tyr Ser Asn Leu Trp Val
225                 230                 235                 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
                245                 250                 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
            260                 265                 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    290                 295                 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
    370                 375                 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385                 390                 395                 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
            420                 425                 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
        435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly Lys
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<210> 363
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 363 - scFv片段 人源化SP34 VH1-VL34 - 人IgG1 Fc 融合蛋白

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<400> 363
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
            20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
```

[0170]

```
          35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
      50                  55                  60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                  85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
             100                 105                 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
         115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
     130                 135                 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala Ala Asn Tyr Ala
                 165                 170                 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
             180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
         195                 200                 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
     210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Ala Trp Val
225                 230                 235                 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
                 245                 250                 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
             260                 265                 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
         275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
     290                 295                 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                 325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
             340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
         355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
     370                 375                 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385                 390                 395                 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                 405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
             420                 425                 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
         435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
     450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly Lys

<210> 364
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 364 - scFv 片段 人源化 SP34 VH3-VL35 - 人 IgG1 Fc 融合蛋白 (T29A)
<400> 364
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1                   5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
             20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
         35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
```

50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

[0171]

<210> 365
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 365 - scFv片段 人源化SP34 VH3-VL36 - 人IgG1 Fc 融合蛋白 (T29V)
<400> 365
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr

65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Val Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
[0172] 355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 366
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 366 - scFv 片段 人源化SP34 VH3-VL37 - 人 IgG1 Fc 融合蛋白 (T29C)
<400> 366
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr

```
                85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
            100                 105                 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
    130                 135                 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Cys Ser Asn Tyr Ala
                165                 170                 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
            180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
        195                 200                 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
    210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225                 230                 235                 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
                245                 250                 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
            260                 265                 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    290                 295                 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
    370                 375                 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385                 390                 395                 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
            420                 425                 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
        435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly Lys
```

[0173]

<210> 367
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 367 - scFv 片段 人源化 SP34 VH3-VL38 - 人 IgG1 Fc 融合蛋白(T290)
<400> 367

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
            20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
```

100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Gly Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
[0174] Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 368
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 368 - scFv 片段 人源化 SP34 VH3-VL39 - 人 IgG1 Fc 融合蛋白 (T29S)
<400> 368
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly

115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ser Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

[0175]

<210> 369
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 369 - scFv 片段, 人源化 SP34 VH3-VL40 - 人 IgG1 Fc 融合蛋白 (T29L)
<400> 369
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val

130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Leu Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Glu Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

[0176]

<210> 370
<211> 482
<212> PRT
<213> 人工产列
<220>
<223> SEQ ID NO: 370 - scFv 片段 人源化 SP34 VH3-VL4I - 人 IgG1 Fc 融合蛋白 (T29F)
<400> 370
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr

145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Phe Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
[0177] Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 371
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 371 - scFv 片段 人源化 SP34 VH3-VL42 - 人 IgG1 Fc 融合蛋白 (T29M)
<400> 371
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Met Ser Asn Tyr Ala

[0178]

```
                165                 170                 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
            180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
        195                 200                 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
    210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225                 230                 235                 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
                245                 250                 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
            260                 265                 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    290                 295                 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
    370                 375                 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385                 390                 395                 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
            420                 425                 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
        435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly Lys
```

<210> 372
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 372 - scFv片段 人源化SP34 VH3-VL43 - 人IgG1 Fc 融合蛋白 (T20P)
<400> 372

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
            20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
            100                 105                 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
    130                 135                 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Pro Ser Asn Tyr Ala
                165                 170                 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
```

[0179]

```
            180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
        195                 200                 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
    210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225                 230                 235                 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
                245                 250                 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
            260                 265                 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    290                 295                 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
    370                 375                 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385                 390                 395                 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
            420                 425                 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
        435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly Lys
```

<210> 373
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 373 - scFv片段, 人源化SP34 VH3-VL44 - 人IgG1 Fc 融合蛋白 (T29E)
<400> 373

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1                 5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
            20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
            100                 105                 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
    130                 135                 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Glu Ser Asn Tyr Ala
                165                 170                 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
            180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
```

195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

[0180]

<210> 374
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 374 - scFv 片段 人源化 SP34 VH3-VL45 - 人 IgG1 Fc 融合蛋白 (T29R)
<400> 374
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Arg Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu

```
    210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225                 230                 235                 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
                245                 250                 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
            260                 265                 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    290                 295                 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
    370                 375                 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385                 390                 395                 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
            420                 425                 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
        435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly Lys
```

[0181]

<210> 375
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 375 - scFv 片段 人源化SP34 VH3-VL46 - 人 IgG1 Fc 融合蛋白 (T290)
<400> 375

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
            20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
            100                 105                 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
    130                 135                 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Gln Ser Asn Tyr Ala
                165                 170                 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
            180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
        195                 200                 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
    210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
```

225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 376
<211> 482
<212> PRT
[0182] <213> 人工序列
<220>
<223> SEQ ID NO: 376 - scFv 片段 人源化 SP34 VH3-VL47 - 人 IgG1 Fc 融合蛋白 (S30A)
<400> 376
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ala Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp

245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 377
<211> 482
<212> PRT
<213> 人工序列
<220>
[0183] <223> SEQ ID NO: 377 - scFv 片段 人源化SP34 VH3-VL48 - 人 IgG1 Fc 融合蛋白 (S30I)
<400> 377
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ile Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly

260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 378
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 378 - scFv 片段 人源化SP34 VH3-VL49 - 人IgG1 Fc 融合蛋白 (S90L)

[0184]

<400> 378
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Leu Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile

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        275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    290                 295                 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
    370                 375                 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385                 390                 395                 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
            420                 425                 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
        435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly Lys
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<210> 379
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 379 - scFv 片段 人源化 SP34 VH3-VL50 - 人 IgG1 Fc 融合蛋白(S306)
<400> 379

[0185]

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
            20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
            100                 105                 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
    130                 135                 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Gly Asn Tyr Ala
                165                 170                 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
            180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
        195                 200                 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
    210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225                 230                 235                 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
                245                 250                 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
            260                 265                 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
```

290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 380
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 380 - scFv 片段 人源化 SP34 VH3-VL5I - 人 IgG1 Fc 融合蛋白 (S30D)
<400> 380
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Asp Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His

[0186]

305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 381
<211> 462
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 381 - scFv片段 人源化SP34 VH3-VL52 - 人IgG1 Fc 融合蛋白 (S30Q)
<400> 381
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Gln Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg

[0187]

325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 382
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 382 - scFv 片段 人源化 SP34 VH3-VL53 - 人 IgG1 Fc 融合蛋白 (S300)
<400> 382
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Met Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys

[0188]

340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 383
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 383 - scFv片段 人源化SP34 VH3-VL54 - 人IgG1 Fc 融合蛋白 (S30H)
<400> 383
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr His Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu

[0189]

355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 384
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 384 - scFv片段 人源化SI34 VH3-VL55 - 人IgG1 Fc 融合蛋白(S30F)
<400> 384
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Ser Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Phe Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Leu Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr

[0190]

370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Glu Glu Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 365
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 385 - scFv片段：人源化SP34 VH5-VL56 - 人IgG1 Fc 融合蛋白 (L95C)
<400> 385
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Gly Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu

[0191]

385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 386
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 386 - scFv片段 人源化SP34 VH5-VL57 - 人IgG1 Fc 融合蛋白 (L93S)
<400> 386
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Ser Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp

[0192]

405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 387
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 387 - scFv 片段：人源化SP34 VH5-VL58 - 人IgG1 Fc 融合蛋白(L95T)
<400> 387
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Thr Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val

[0193]

420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 388
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 388 - scFv片段 人源化SP34 VH5-VL59 - 人IgG1 Fc 融合蛋白 (L95D)
<400> 388
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
[0194] Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Asp Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp

435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 389
<211> 482
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 389 - scFv 片段：人源化 SP34 VH5-VL60 - 人 IgG1 Fc 融合蛋白 (L95N)
<400> 389
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Asn Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His

[0195]

450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly Lys

<210> 390
<211> 481
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 390 - DEAT 抗体 scFv 重链 SP34(抗-CD3 ε 臂- 人源化 SP34 VH5/VL61 BT11 LALA)
<400> 390
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala Ala Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Ala Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Ala
370 375 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385 390 395 400
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
420 425 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
435 440 445
Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro

[0196]

465 470 475 480
Gly

<210> 391
<211> 481
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 391 - BEAT 抗体 scFv 重链 SP34(抗-CD3 ε 臂- 人源化 SP34 VH5/VL62 BT11 LALA)
<400> 391
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Glu Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Ala Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Glu Pro Arg Glu Pro Glu Val Ala
370 375 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385 390 395 400
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
420 425 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly

[0197]

<210> 392
<211> 481
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 392 - BEAT 抗体 scFv 重链 SP34(抗-CD3 ε 臂- 人源化 SP34 VH5/VL63 BI11 LALA)
<400> 392

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ser Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Ala Trp Val
225 230 235 240
Phe Gly Glu Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Ala
370 375 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385 390 395 400
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
420 425 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly

[0198]

<210> 393

<211> 481
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 393 - BEA1 抗体 scFv 重链 SP34(抗-CD3 ε 臂- 人源化 SP34 VH5/VL64 BT11 LALA)
<400> 393
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Glu Ser Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Gly Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Ala
370 375 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385 390 395 400
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
420 425 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly

[0199]

<210> 394
<211> 481
<212> PRT

<213> 人工序列
<220>
<223> SEQ ID NO: 394 - BEAT 抗体 scFv 重链 SP34(抗-CD3 ε 暨- 人源化 SP34 VH5/VL65 BT11 LALA)版本 3
<400> 394

[0200]

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
            20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
            100                 105                 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
    130                 135                 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Glu Ser Asn Tyr Ala
                165                 170                 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
            180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
        195                 200                 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
    210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Thr Trp Val
225                 230                 235                 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
                245                 250                 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
            260                 265                 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    290                 295                 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Ala
    370                 375                 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385                 390                 395                 400
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
            420                 425                 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
        435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly
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<210> 395
<211> 481
<212> PRT
<213> 人工序列
<220>

<223> SEQ ID NO: 395 - BEAT 抗体 scFv 重链 SP34(抗-CD3 ε 臂- 人源化 SP34 VH5/VL66 BT11 LALA)

<400> 395

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala Ala Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Gly Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Ala
370 375 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385 390 395 400
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
420 425 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly

<210> 396
<211> 481
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 396 - BEAT 抗体 scFv 重链 SP34(抗-CD3 ε 臂- 人源化 SP34 VH5/VL67 BT11 LALA)版本 4

[0201]

<400> 396
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Va. Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala Ala Asn Tyr Ala
165 170 175
Asn Trp Va. Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Thr Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
260 265 270
Pro Ser Va. Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Ala
370 375 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385 390 395 400
Val Cys Leu Val Tyr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
420 425 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly

<210> 397
<211> 481
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 397 - BEAT 抗体 scFv 重链 SP34(抗-CD3 e 臂 人源化 SP34 VH5/VL68 DT11 LALA)
<400> 397
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

[0202]

[0203]

1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
130 135 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145 150 155 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Glu Ala Asn Tyr Ala
165 170 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
180 185 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
195 200 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
210 215 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Thr Trp Val
225 230 235 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
245 250 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
260 265 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Ala
370 375 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385 390 395 400
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
420 425 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
435 440 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480
Gly

<210> 398
<211> 481
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 398 - BEAT 抗体 scFv 重链 SP34(抗-CD3 ε 臂- 人源化 SP34 VH5/VL09 BT11 LALA)
<400> 398
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr

[0204]

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                20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35                  40                  45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
        50                  55                  60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe
            100                 105                 110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Val
    130                 135                 140
Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly Glu Arg Ala Thr
145                 150                 155                 160
Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Glu Asp Asn Tyr Ala
                165                 170                 175
Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly
            180                 185                 190
Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
        195                 200                 205
Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu
    210                 215                 220
Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser Asn Thr Trp Val
225                 230                 235                 240
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Thr Asp
                245                 250                 255
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly
            260                 265                 270
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        275                 280                 285
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    290                 295                 300
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305                 310                 315                 320
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                325                 330                 335
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            340                 345                 350
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        355                 360                 365
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Ala
    370                 375                 380
Thr Phe Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Thr Leu
385                 390                 395                 400
Val Cys Leu Val Thr Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                405                 410                 415
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Asp Pro Pro Leu
            420                 425                 430
Leu Glu Ser Gln Gly Ser Phe Ala Leu Ser Ser Arg Leu Arg Val Asp
        435                 440                 445
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    450                 455                 460
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465                 470                 475                 480
Gly
<210> 399
<211> 217
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 399 SP34 人源化轻链 具有 VL65 结构域
<400> 399
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1               5                   10                  15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Glu
            20                  25                  30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
        35                  40                  45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
```

50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser
85 90 95
Asn Thr Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100 105 110
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205
Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215
<210> 400
<211> 216
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 400 - SP34 人源化轻链 具有 VL67 结构域
<400> 400
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala
20 25 30
Ala Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg

[0205]

50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser
85 90 95
Asn Thr Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Thr Val
100 105 110
Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys
115 120 125
Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg
130 135 140
Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn
145 150 155 160
Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser
165 170 175
Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
180 185 190
Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
195 200 205
Lys Ser Phe Asn Arg Gly Glu Cys
210 215
<210> 401
<211> 110
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 401 - SP34 人源化 VH65 结构域
<400> 401
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Glu
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser

65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser
85 90 95
Asn Thr Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110
<210> 402
<211> 110
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 402 - SP34 人源化 VL67 结构域
<400> 402
Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Thr Gly Ala Val Thr Ala
20 25 30
Ala Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg
35 40 45
Gly Leu Ile Gly Gly Ala Asn Lys Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ser Gly Asp Glu Ala Thr Leu Thr Ile Ser Ser
65 70 75 80
Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Ala Leu Phe Tyr Ser
85 90 95
Asn Thr Trp Val Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110
<210> 403
<211> 481
<212> PRT
<213> 人工序列
<220>
<223> SEQ ID NO: 403 - scFv 片段 小鼠 SP34 人 IgG1 Fc 融合蛋白
<400> 403
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Lys Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Glu Ser Ile
65 70 75 80
Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Gly Gly Gly
115 120 125
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val
130 135 140
Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr Val Thr Leu
145 150 155 160
Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
165 170 175
Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly Leu Ile Gly Gly
180 185 190
Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu
195 200 205
Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Thr Glu Asp
210 215 220
Glu Ala Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val Phe
225 230 235 240
Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gly Gly Gly Thr Asp Lys
245 250 255
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
260 265 270
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
275 280 285
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
290 295 300
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn

[0206]

[0207]

305 310 315 320
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
325 330 335
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
340 345 350
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
355 360 365
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
370 375 380
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
385 390 395 400
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
405 410 415
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
420 425 430
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
435 440 445
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
450 455 460
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
465 470 475 480
Lys

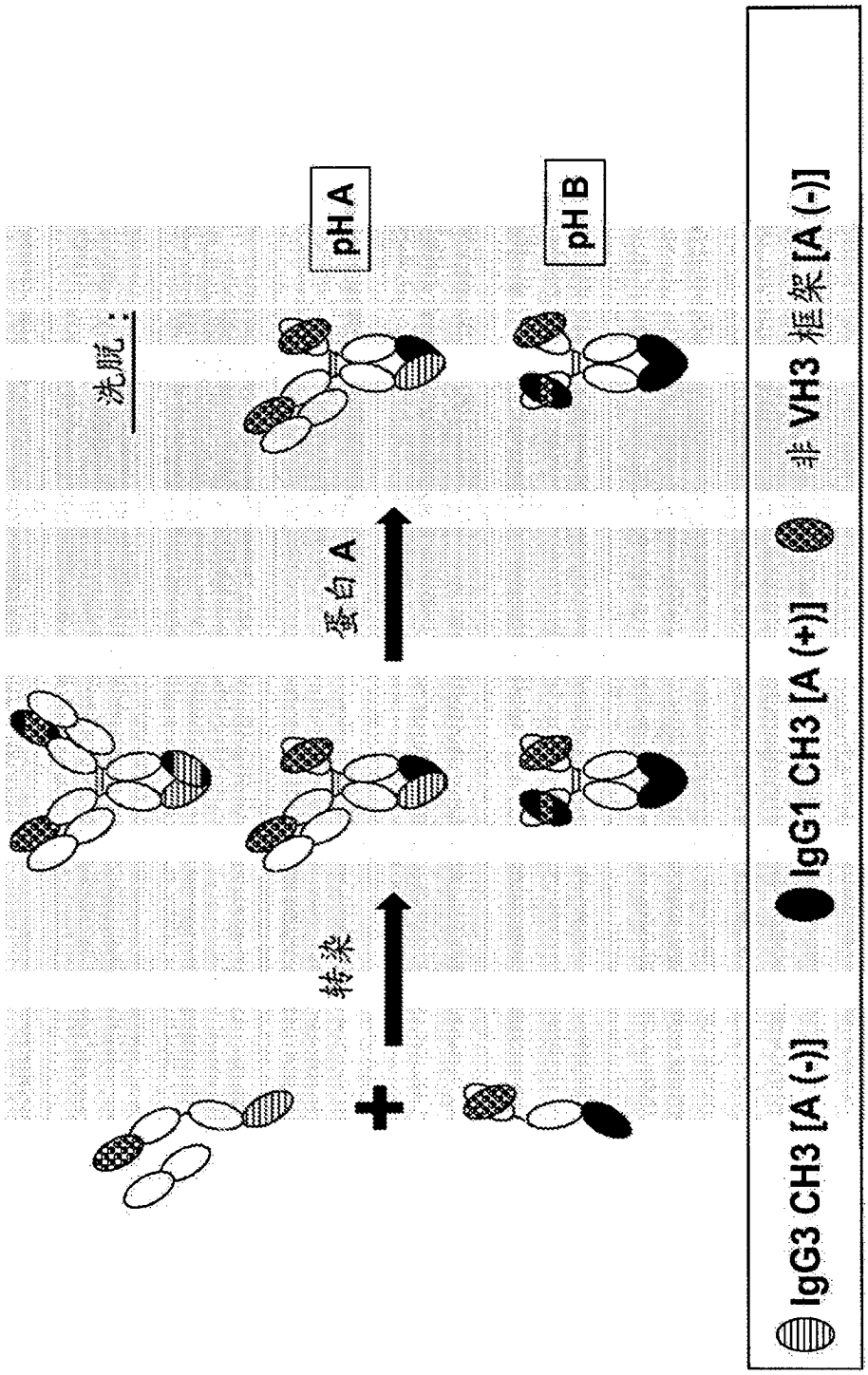


图1

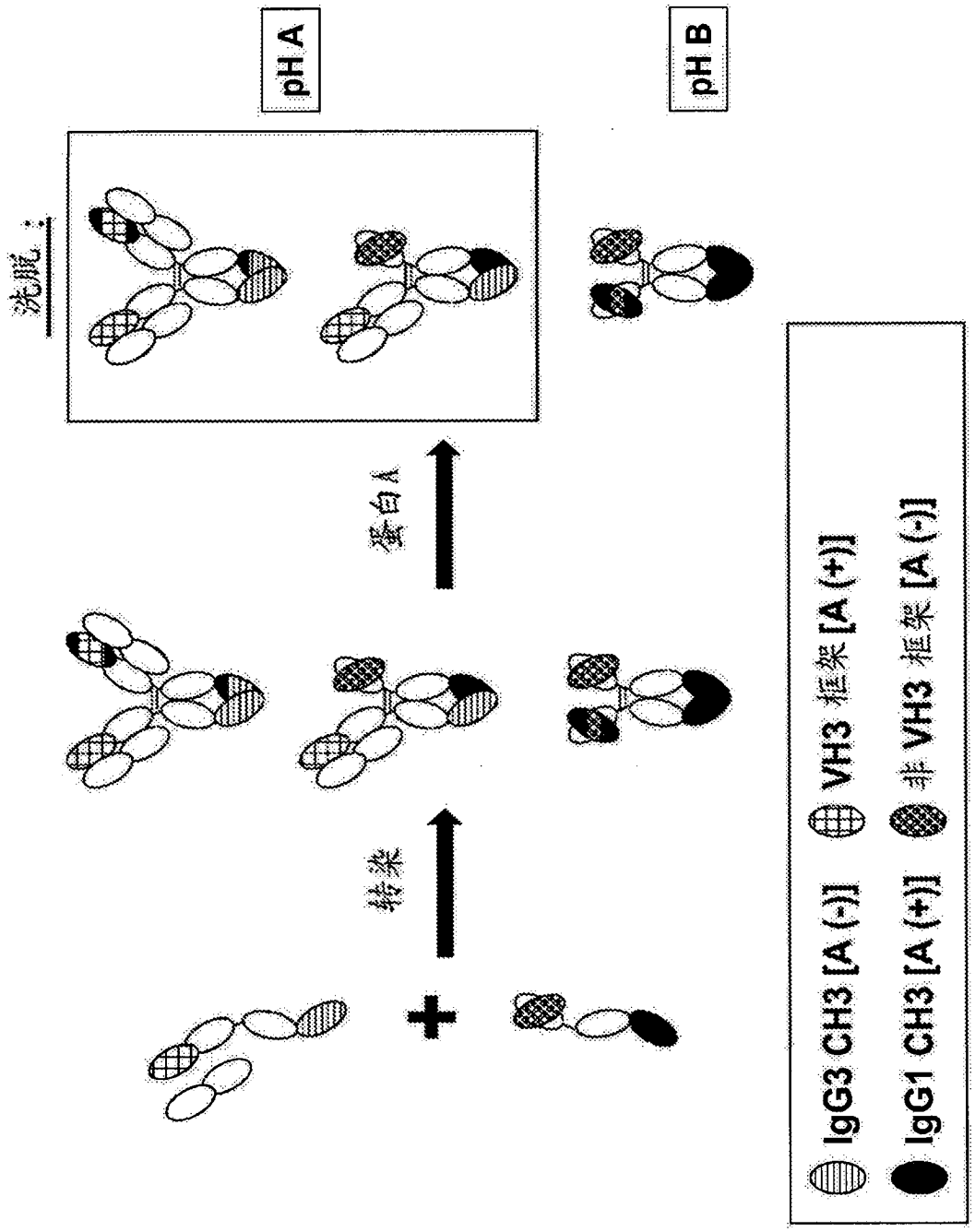


图2A

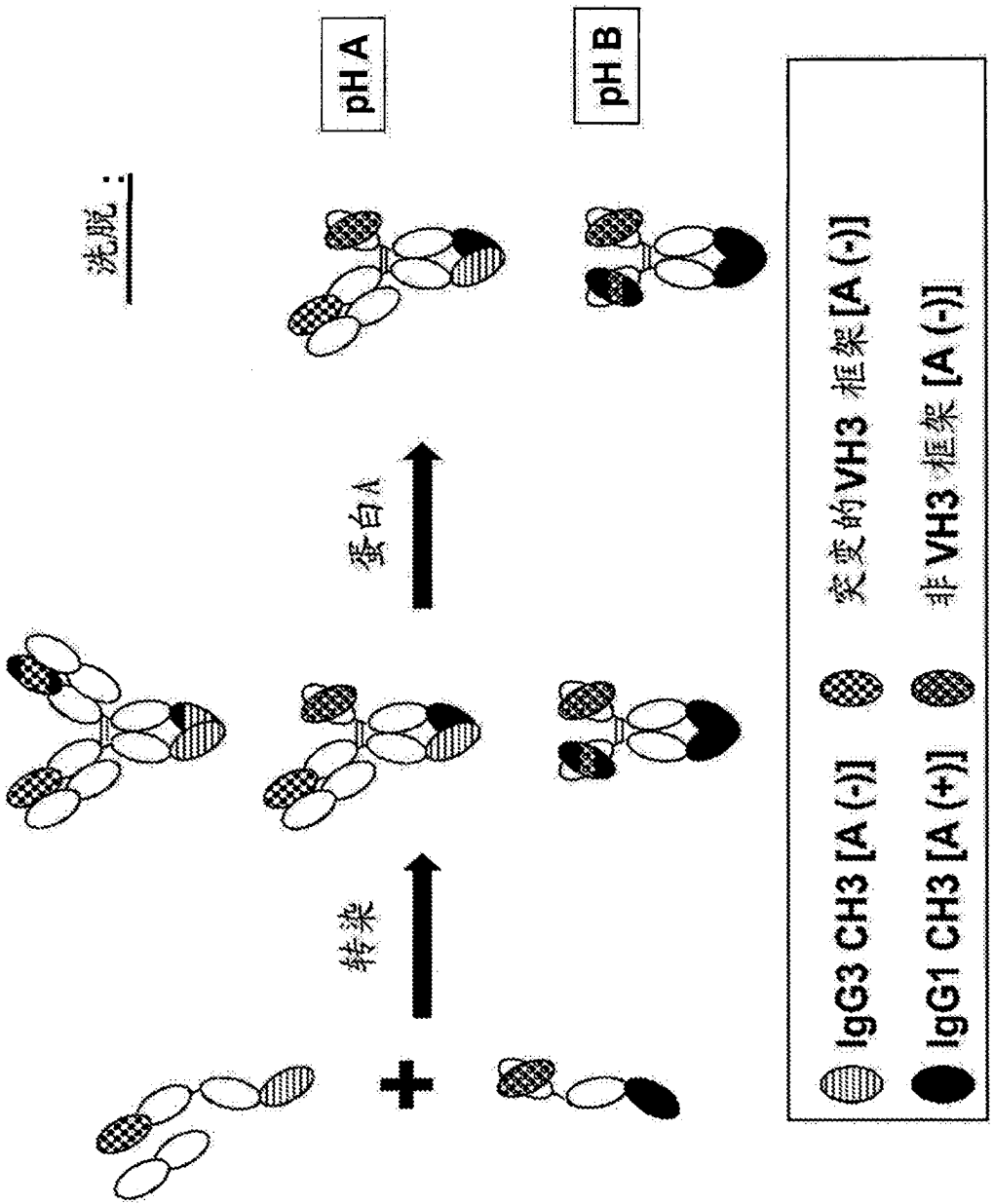


图2B

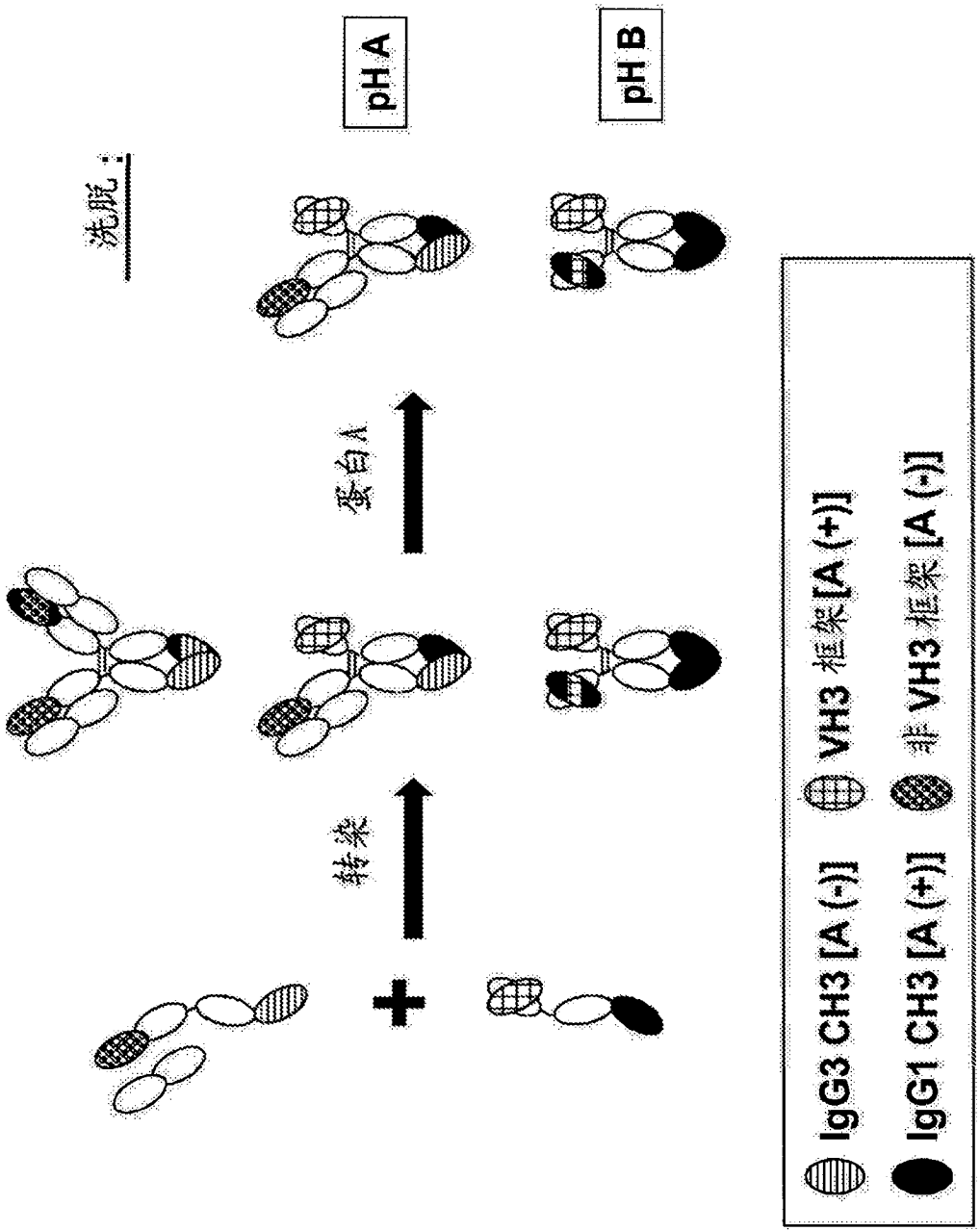


图2C

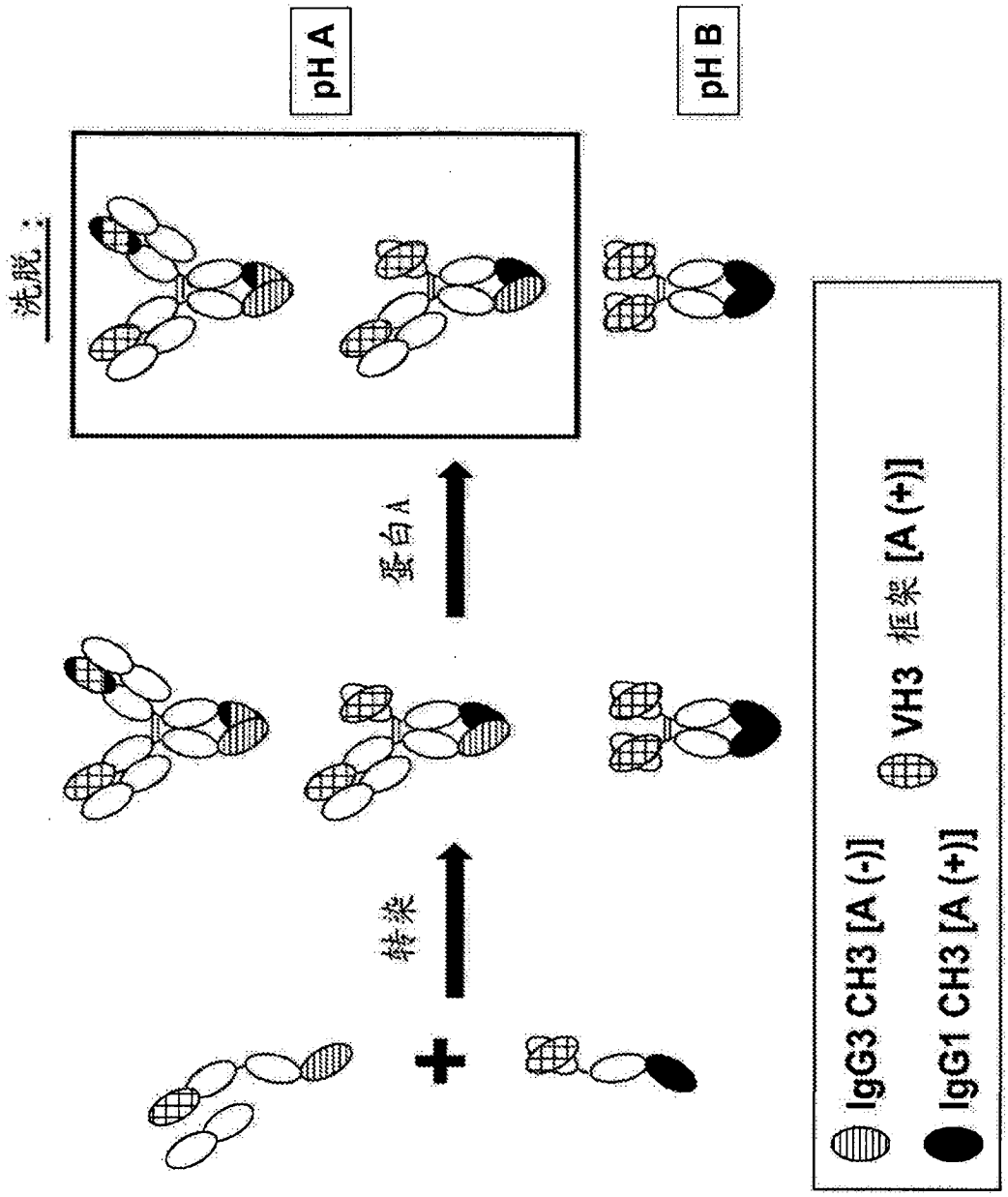


图2D

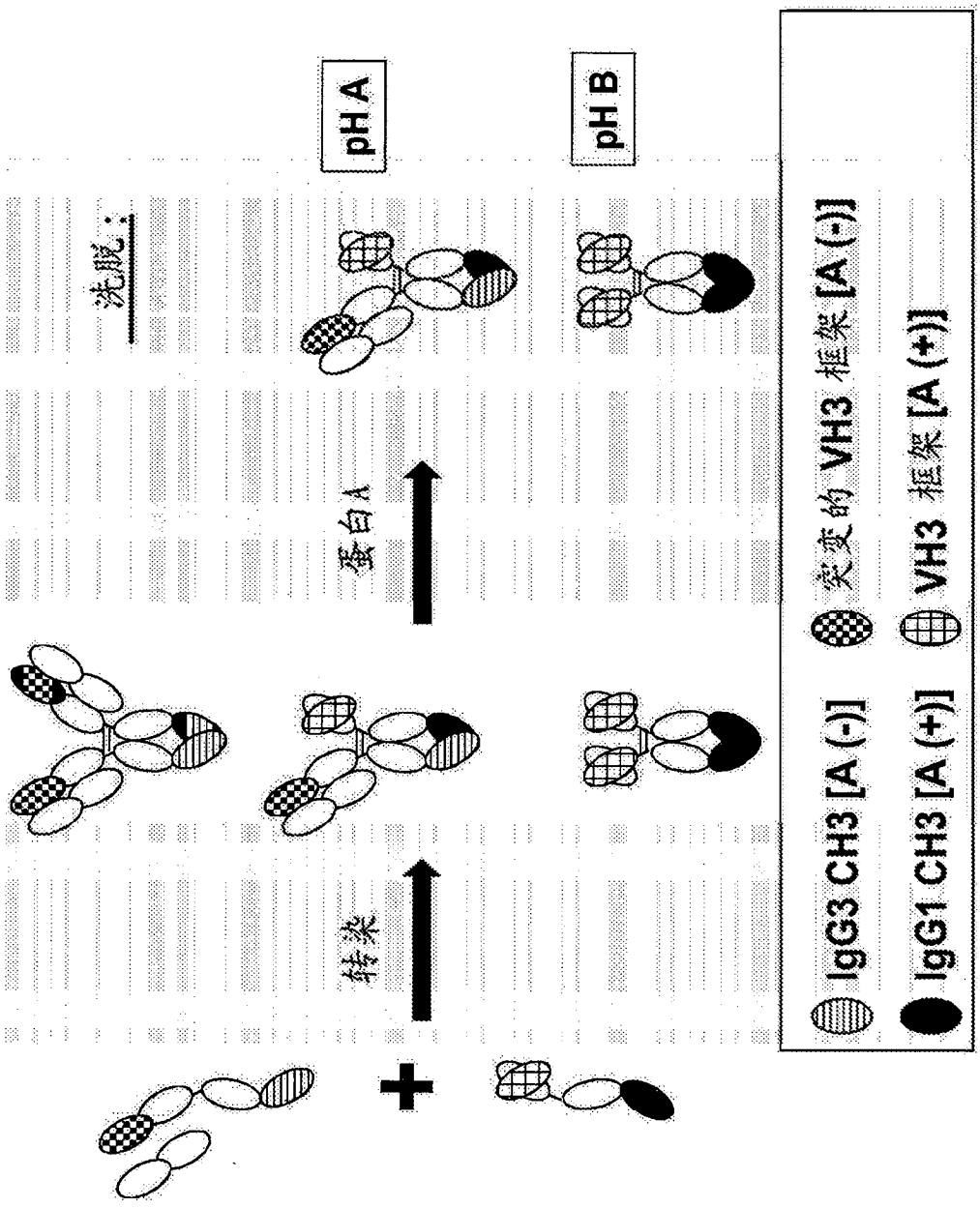


图2E

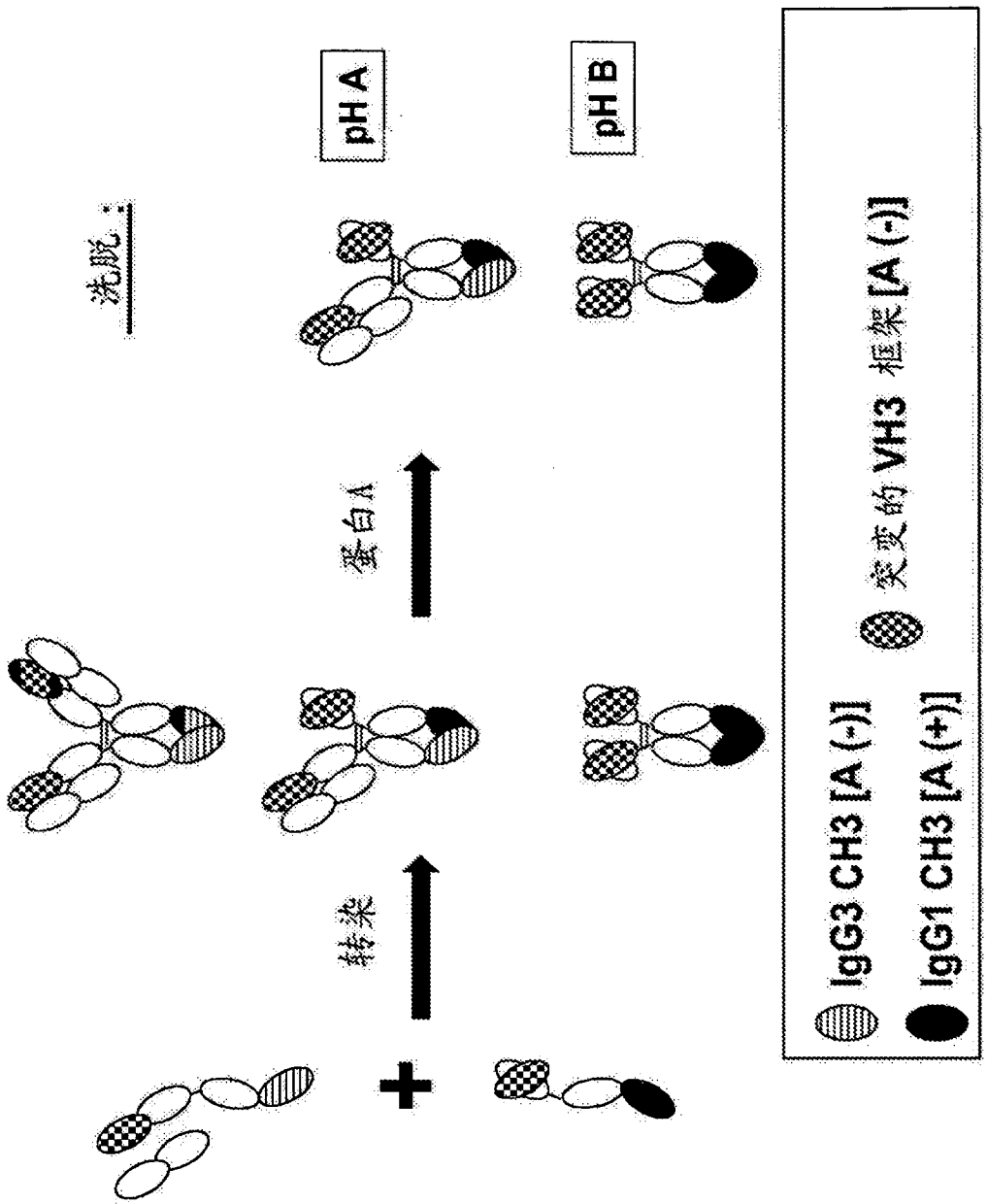


图2F

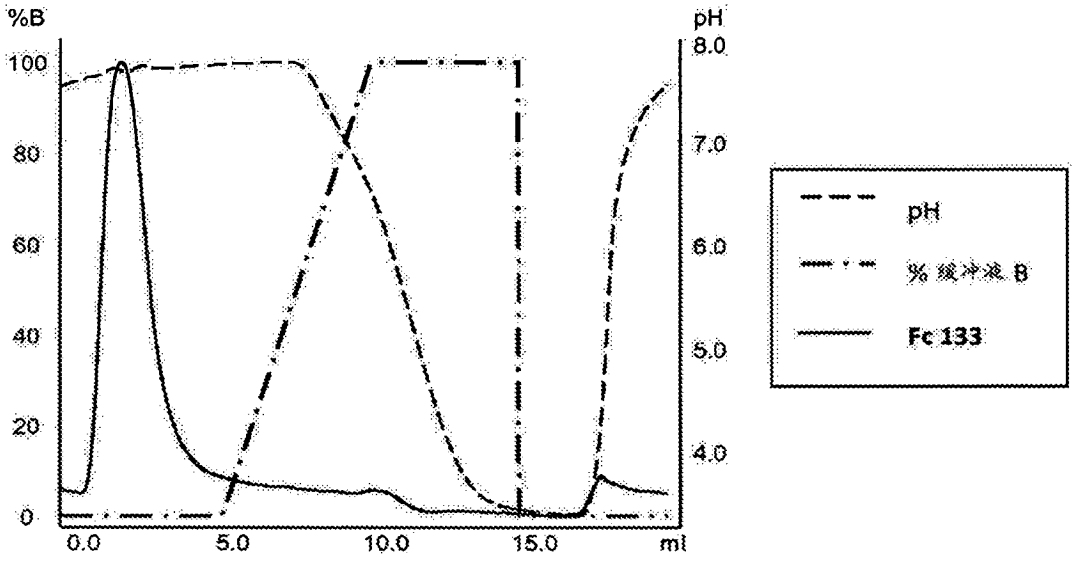

%B
100
80
60
40
20
0
pH
8.0
7.0
6.0
5.0
4.0
0.0
5.0
10.0
15.0
ml
pH
% 缓冲液 B
Fc 133

图3

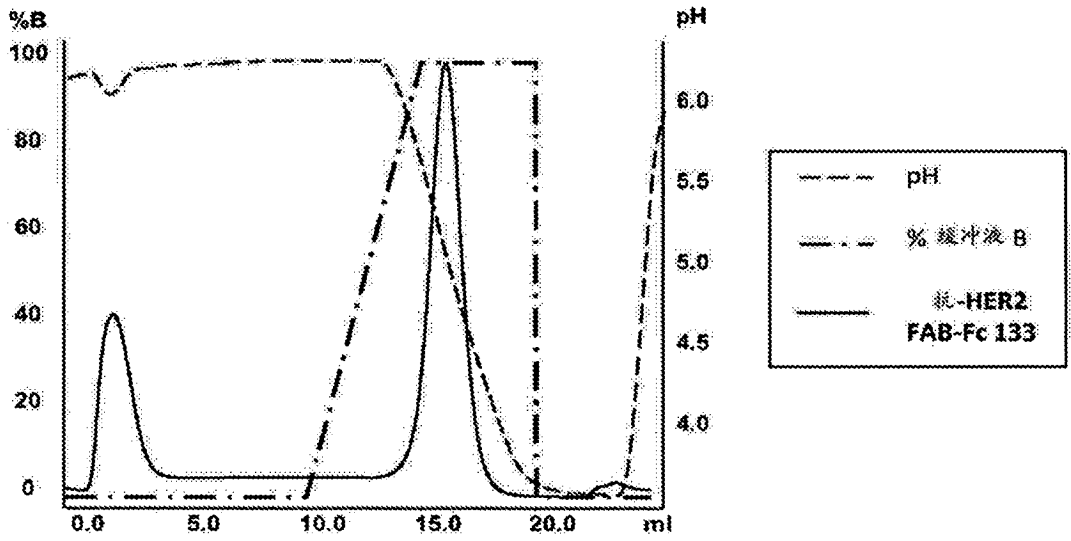

%B
100
80
60
40
20
0
pH
6.0
5.5
5.0
4.5
4.0
0.0
5.0
10.0
15.0
20.0
ml
pH
% 缓冲液 B
抗-HER2
FAB-Fc 133

图4A

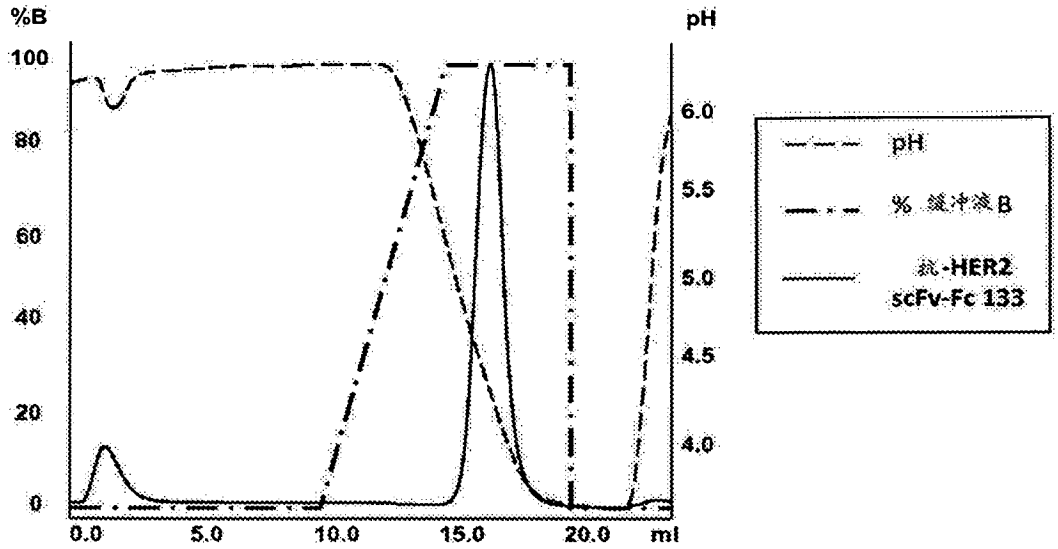

%B
pH
100
80
60
40
20
0
6.0
5.5
5.0
4.5
4.0
0.0
5.0
10.0
15.0
20.0
ml
pH
% 缓冲液B
抗-HER2 scFv-Fc 133

图4B

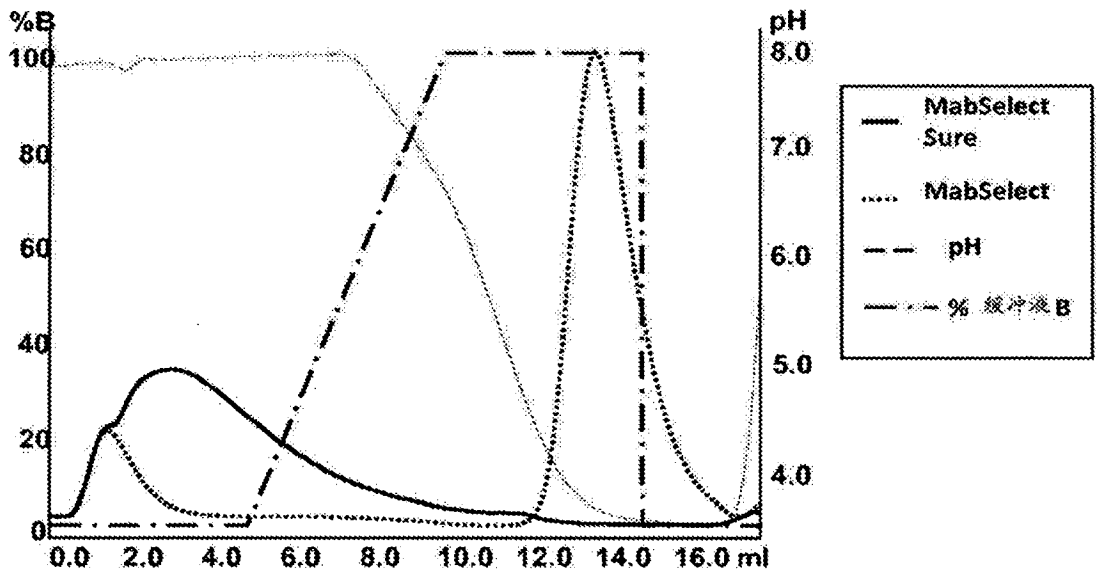

%B
pH
100
80
60
40
20
0
8.0
7.0
6.0
5.0
4.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0 ml
MabSelect Sure
MabSelect
pH
% 缓冲液B

图4C

```
                                10        20        30        40        50
                     ....|....|....|....|....|....|....|....|....|....|....|....|
                     12345678901234567890123456789012345AB67890123456789012345678890
Kabat 编号
人    IGHV1-3        QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAMH..WVRQAPGQRLEWMGW
人    IGHV2-26       QVTLKESGPVLVKPTETLTLTCTVSGFSLSNARMGVSWIRQPPGKALEWLAH
人    IGHV3-23       EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS..WVRQAPGKGLEWVSA
人    IGHV4-28       QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWG.WIRQPPGKGLEWIGY
人    IGHV5-51       EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIG..WVRQMPGKGLEWMGI
人    IGHV6-1        QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGR
人    IGHV7-4-1      QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMN..WVRQAPGQGLEWMGW

                                60        70        80        90
                     ....|....|....|....|....|....|....|....|....|....|....|....|
                     12AB3456789012345678901234567890123456789012ABC345678901234 5
Kabat 编号
人    IGHV1-3        INA.GNGNTKYSQKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCAR.
人    IGHV2-26       IF..SNDEKSYSTSLKSRLTISKDTSKSQVVLTMTNMDPVDTATYYCARI
人    IGHV3-23       ISG.SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK.
人    IGHV4-28       IY..YSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYYCAR.
人    IGHV5-51       IYP.GDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCAR.
人    IGHV6-1        TYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCAR.
人    IGHV7-4-1      INT.NTGNPTYAQGFTGRFVFSLDTSVSTAYLQICSLKAEDTAVYYCAR.
```

图5

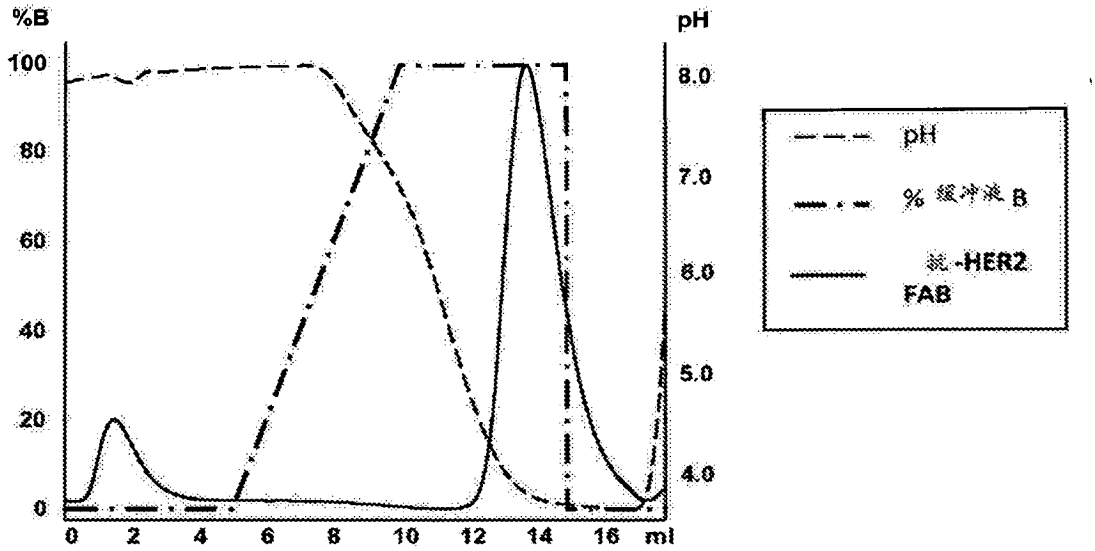

%B
pH
100
80
60
40
20
0
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% 缓冲液 B
抗-HER2 FAB

图6A

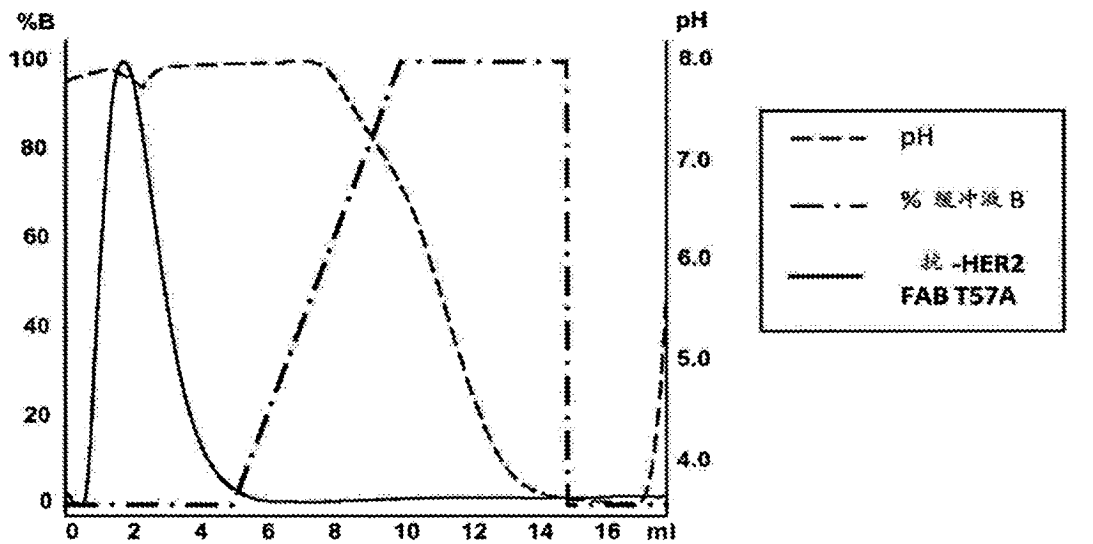

%B
pH
100
80
60
40
20
0
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% 缓冲液 B
抗-HER2 FAB T57A

图6B

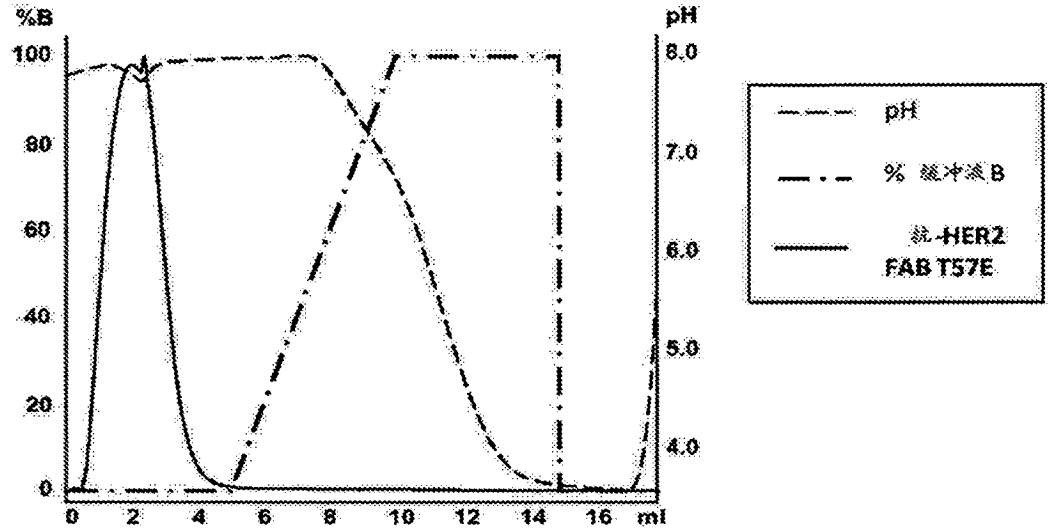

%B
100
80
60
40
20
0
pH
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% 缓冲液B
抗-HER2 FAB T57E

图6C

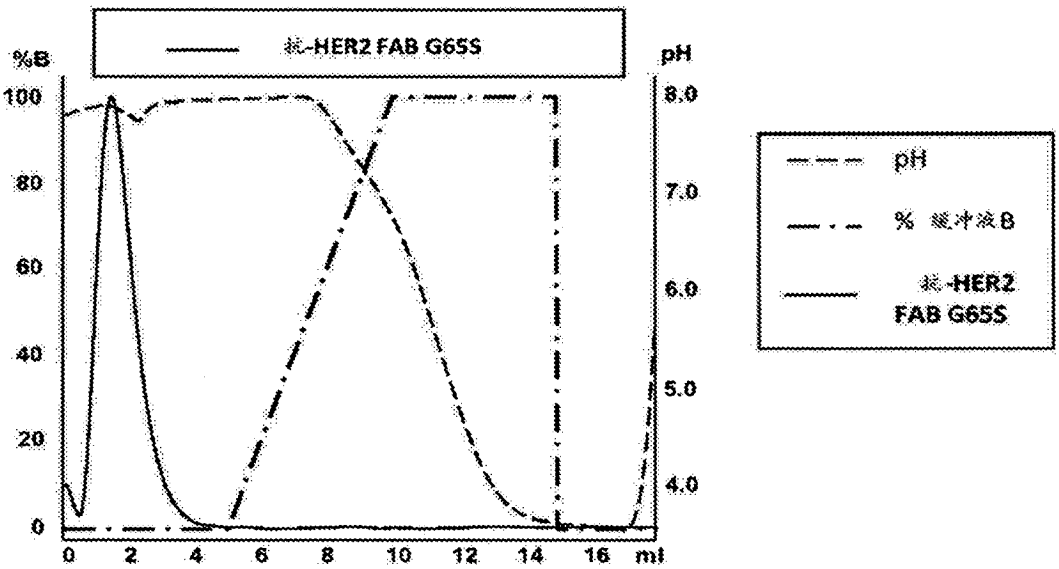

抗-HER2 FAB G65S
%B
100
80
60
40
20
0
pH
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% 缓冲液B
抗-HER2 FAB G65S

图6D

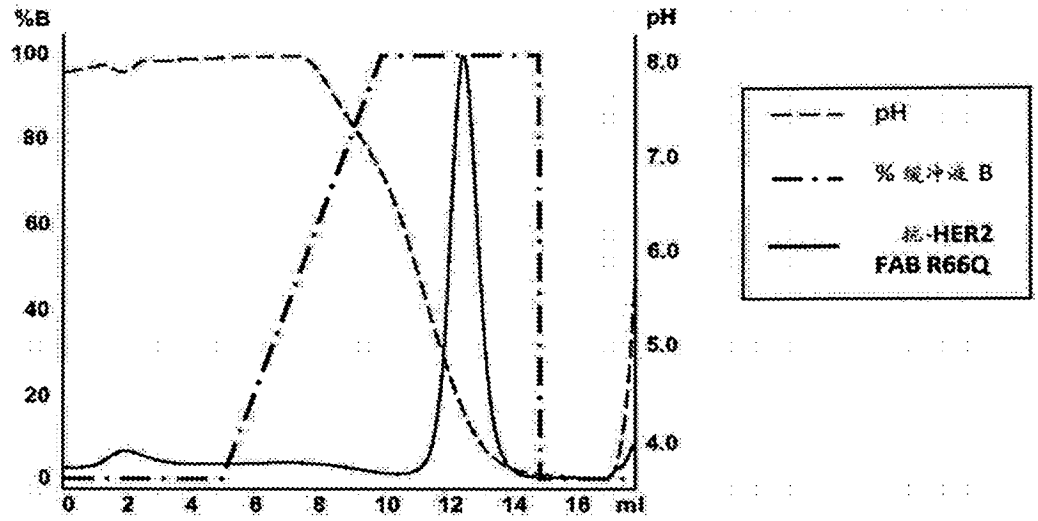

%B
100
80
60
40
20
0
pH
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% 缓冲液 B
抗-HER2
FAB R66Q

图6E

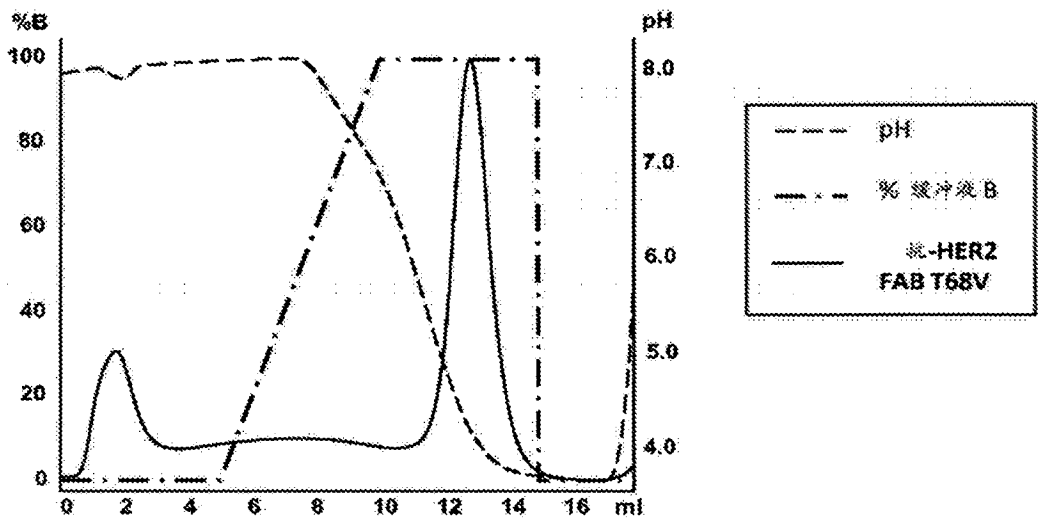

%B
100
80
60
40
20
0
pH
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% 缓冲液 B
抗-HER2
FAB T68V

图6F

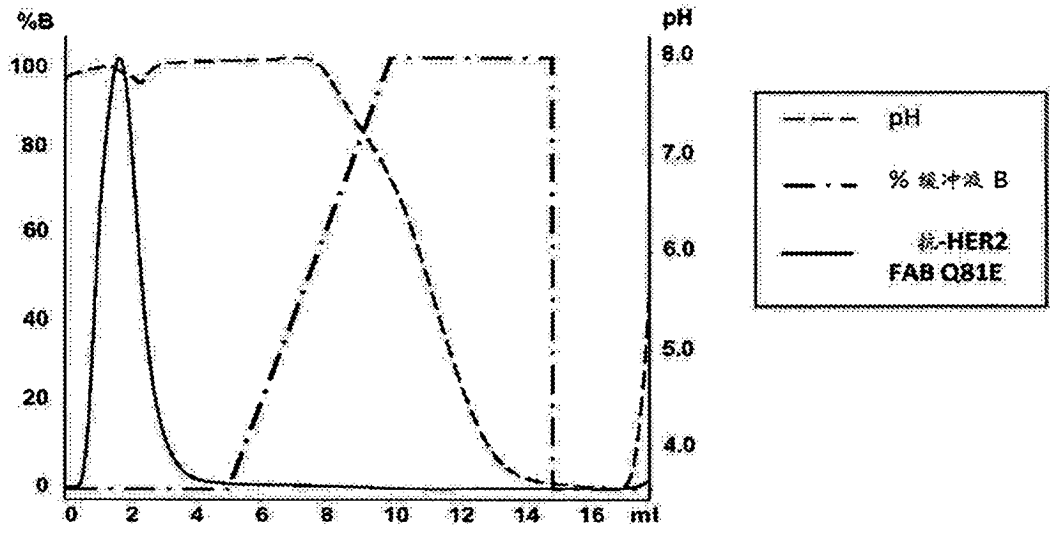

%B
100
80
60
40
20
0
pH
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% 缓冲液 B
抗-HER2
FAB Q81E

图6G

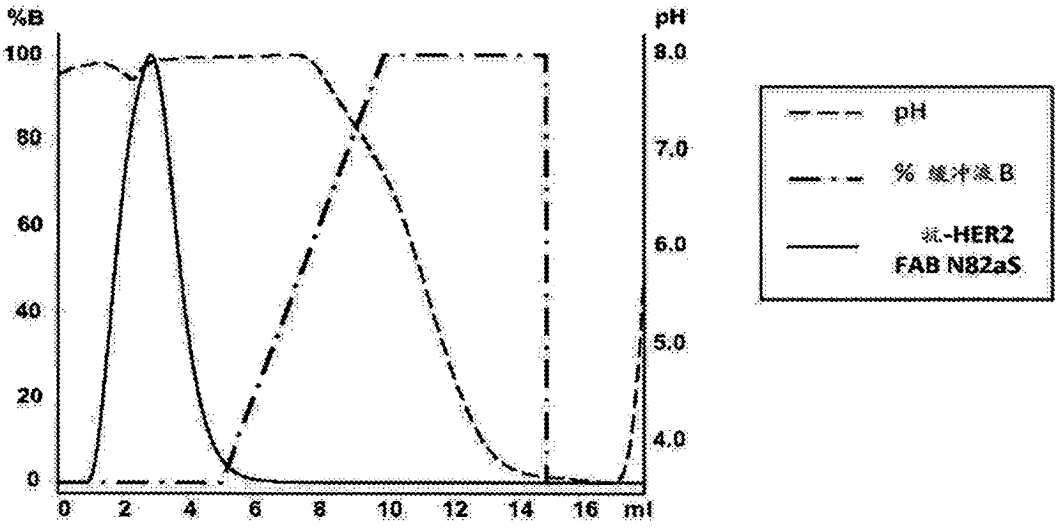

%B
100
80
60
40
20
0
pH
8.0
7.0
6.0
5.0
4.0
0 2 4 6 8 10 12 14 16 ml
pH
% 缓冲液 B
抗-HER2
FAB N82aS

图6H

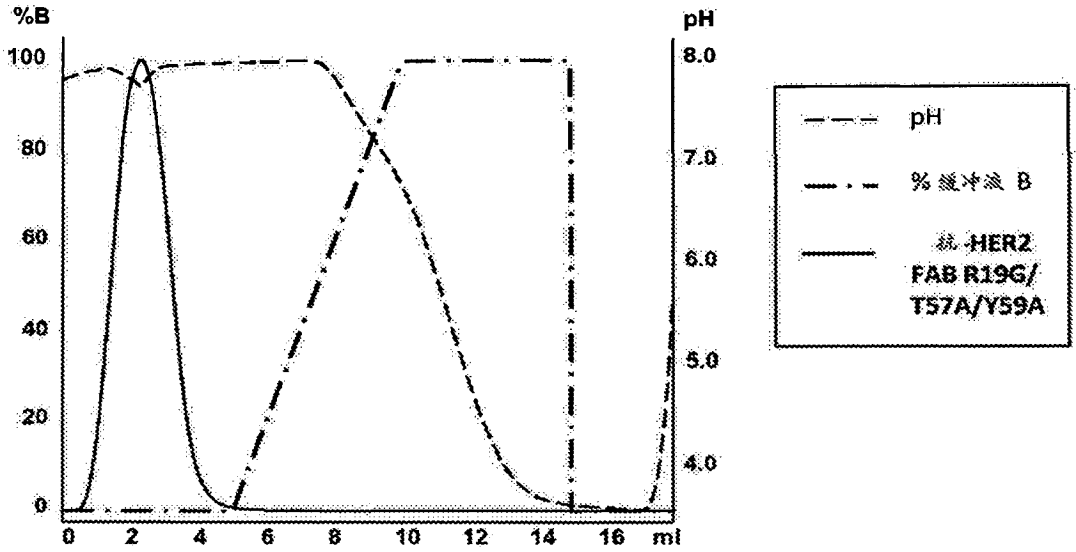


图6I

	KD (pM)
抗-HER2 FAB	153 ± 9
抗-HER2 FAB N82aS	162 ± 19
抗-HER2 FAB G65S	142 ± 30

图7

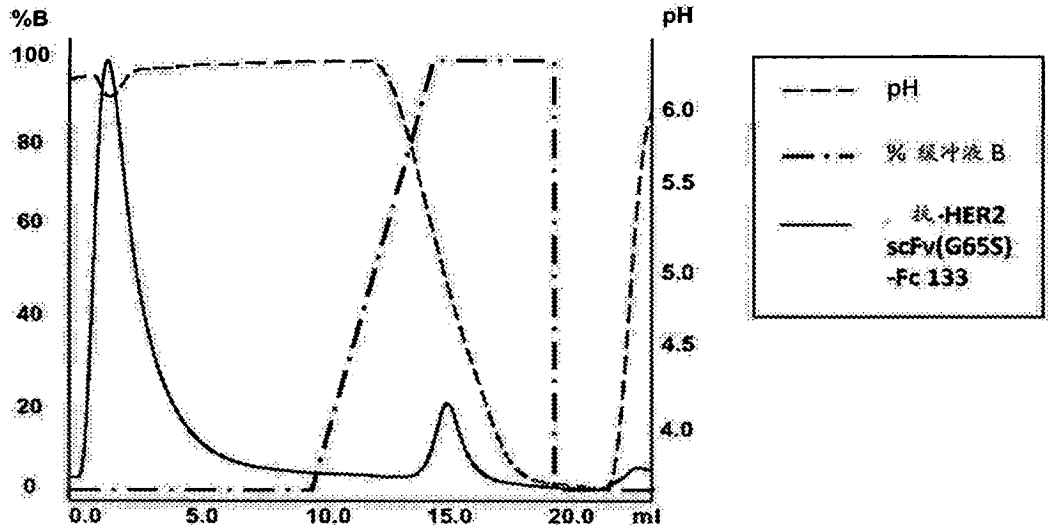

%B
100
80
60
40
20
0
pH
6.0
5.5
5.0
4.5
4.0
0.0
5.0
10.0
15.0
20.0
ml
pH
% 缓冲液 B
抗-HER2 scFv(G65S) -Fc 133

图8A

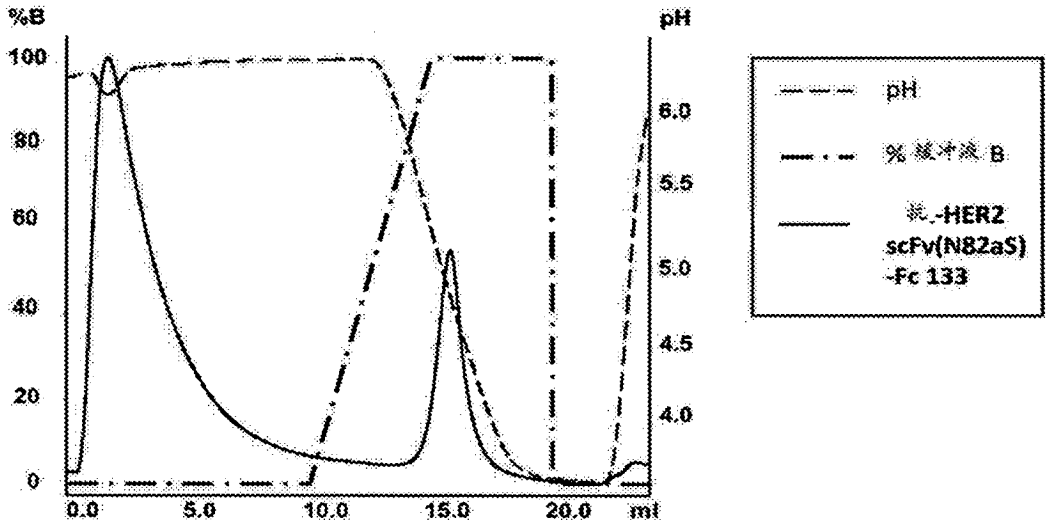

%B
100
80
60
40
20
0
pH
6.0
5.5
5.0
4.5
4.0
0.0
5.0
10.0
15.0
20.0
ml
pH
% 缓冲液 B
抗-HER2 scFv(N82aS) -Fc 133

图8B

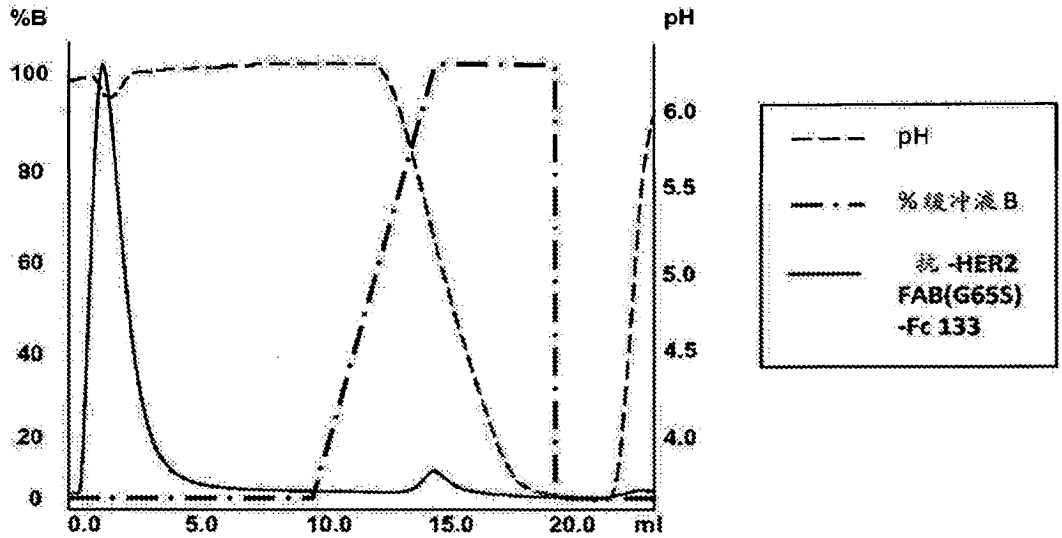

%B
pH
100
80
60
40
20
0
6.0
5.5
5.0
4.5
4.0
0.0
5.0
10.0
15.0
20.0
ml
pH
% 缓冲液 B
抗-HER2 FAB(G65S)-Fc 133

图8C

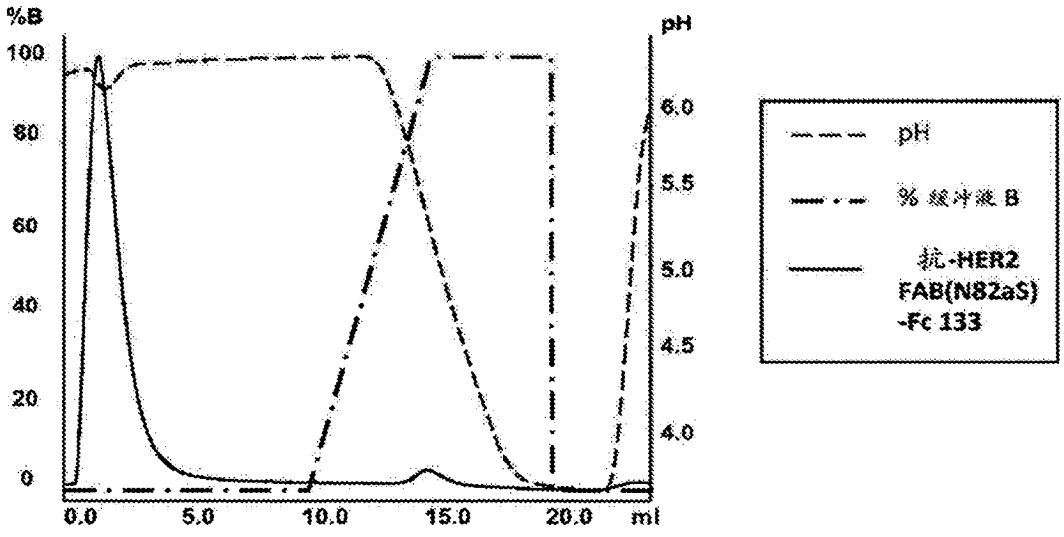

%B
pH
100
80
60
40
20
0
6.0
5.5
5.0
4.5
4.0
0.0
5.0
10.0
15.0
20.0
ml
pH
% 缓冲液 B
抗-HER2 FAB(N82aS)-Fc 133

图8D

抗体	回复突变 VH / VL	SEQ ID NO H / L 链	瞬时表达 (mg/L)	FAB Tm (°C)	HPB-ALL 染色 相对于 OKT3 嵌合体
嵌合 OKT3	N.A.	25/26	20	80.7	+++
VH/VL	-/-	27/39	50	88.1	-
VH/VL2	-/ M4L	27/41	47	90.6	-
VH/VL3	-/ M4L-F71Y	27/42	43	89.8	+
VH1/VL1	A49G / F71Y	28/40	22	90.1	-
VH1/VL2	A49G / M4L	28/41	42	90.5	-
VH1/VL3	A49G / M4L-F71Y	28/42	40	90.7	+
VH2/VL1	I34M-A49G / F71Y	29/40	51	89.5	-
VH2/VL2	I34M-A49G / M4L	29/41	43	90.1	-
VH2/VL3	I34M-A49G / M4L- F71Y	29/42	42.5	89.5	+
VH3/VL1	A49G-A71T / F71Y	30/40	33.5	89.7	-
VH3/VL2	A49G-A71T / M4L	30/41	42	90.4	-
VH3/VL3	A49G-A71T / M4L- F71Y	30/42	56.5	89.8	+

图9A

抗体	回复突变	SEQ ID NO H / L 链	瞬时表达 (mg/l)	FAB Tm (°C)	HPB-ALL 染色相对于 OKT3 嵌合体
VH4/VL2	I34M-A49G-A71T/M4L	31/41	20	88.4	++
VH4/VL3	I34M-A49G-A71T/M4L-F71Y	31/42	34	88.7	++
VH5/VL2	I34M-A49G-I69L-A71T-T73K/M4L	32/41	40	87	++
VH5/VL3	I34M-A49G-I69L-A71T-T73K/M4L-F71Y	32/42	36	87.2	++
VH5/VL4	I34M-A49G-I69L-A71T-T73K/M4L-L46R-L47W-F71Y	32/43	12	80.3	+++
VH5/VL6	I34M-A49G-I69L-A71T-T73K/M4L-L46R-L47W-F71Y-P96F	32/45	15.6	78.6	+++
VH5/VL7	I34M-A49G-I69L-A71T-T73K/M4L-V33M-A34N-F71Y-P96F	32/46	35.4	84.1	++
VH6/VL3	I34M-V48I-A49G-I69L-A71T-T73K/M4L-F71Y	33/42	23	88.3	++
VH6/VL4	I34M-V48I-A49G-I69L-A71T-T73K/M4L-L46R-L47W-F71Y	33/43	14	80.8	+++
VH6/VL5	I34M-V48I-A49G-I69L-A71T-T73K/M4L-V33M-A34N-F71Y	33/44	26	86.1	++
VH6/VL6	I34M-V48I-A49G-I69L-A71T-T73K/M4L-L46R-L47W-F71Y-P96F	33/45	14.8	79.1	+++
VH6/VL7	I34M-V48I-A49G-I69L-A71T-T73K/M4L-V33M-A34N-F71Y-P96F	33/46	32	85.3	++

图9B

抗体	回复突变	SEQ ID NO H / L 链	瞬时表达 (mg/l)	FAB Tm (°C)	HPB-ALL 染色相对于 OKT3 缺合体
VH6/VL8	I34M-V48I-A49G-I69L-A71T-T73K / M4L-L46R-L47W-R66G-F71Y	33/47	7	80.6	+++
VH7/VL3	I34M-A49G-R58N-I69L-A71T-T73K / M4L-F71Y	34/42	21	86.1	++
VH7/VL4	I34M-A49G-R58N-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	34/43	25	80.5	+++
VH7/VL5	I34M-A49G-R58N-I69L-A71T-T73K / M4L-V33M-A34N-F71Y	34/44	26	84.12	++
VH8/VL4	I34M-V48I-A49G-R58Y-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	35/43	7	80.9	+++
VH8/VL8	I34M-V48I-A49G-R58Y-I69L-A71T-T73K / M4L-L46R-L47W-R66G-F71Y	35/47	23	83.5	+++
VH9/VL8	I34M-V48I-A49G-R58Y-G65S-I69L-A71T-T73K / M4L-L46R-L47W-R66G-F71Y	36/47	13	82	+++
VH10/VL4	I34M-V48I-A49G-R58Y-G65S-F67A-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	37/43	7	78.6	+++
VH10/VL8	I34M-V48I-A49G-R58Y-G65S-F67A-I69L-A71T-T73K / M4L-L46R-L47W-R66G-F71Y	37/47	10	80.4	+++
VH11/VL4	I34M-V48I-A49G-R58Y-I69L-A71T-T73K-N82aS / M4L-L46R-L47W-F71Y	38/43	8	80.3	+++
VH11/VL8	I34M-V48I-A49G-R58Y-I69L-A71T-T73K-N82aS / M4L-L46R-L47W-R66G-F71Y	38/47	15	82.3	+++

图9C

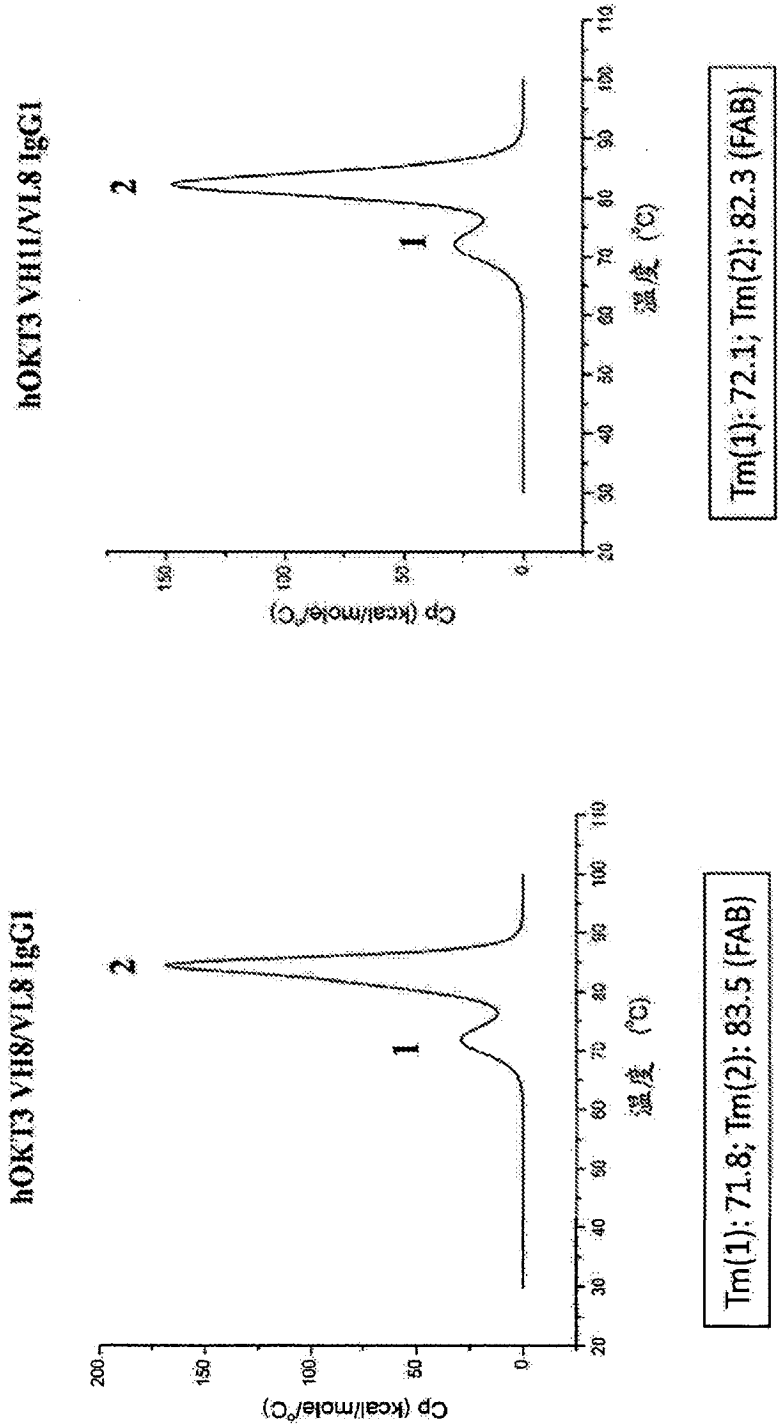

hOKT3 VH11/VL8 IgG1
1
2
Cp (kcal/mole/°C)
温度 (°C)
Tm(1): 72.1; Tm(2): 82.3 (FAB)
hOKT3 VH8/VL8 IgG1
1
2
Cp (kcal/mole/°C)
温度 (°C)
Tm(1): 71.8; Tm(2): 83.5 (FAB)

图9D

scFv-Fc 融合物	SEQ ID NO	瞬时表达 (mg/L)	scFv Tm (°C)	HPB-ALL 染色，相对于 OKT3 嵌合体
小鼠 OKT3	52	0.35		++
VH5-VL3	53	21	71.2	+
VH6-VL4	54	29	65.3	++
VH6-VL5	55	27	71.2	++
VH8-VL4	56	28	66.4	+++
VH8-VL8	57	28	69.2	+++

图9E

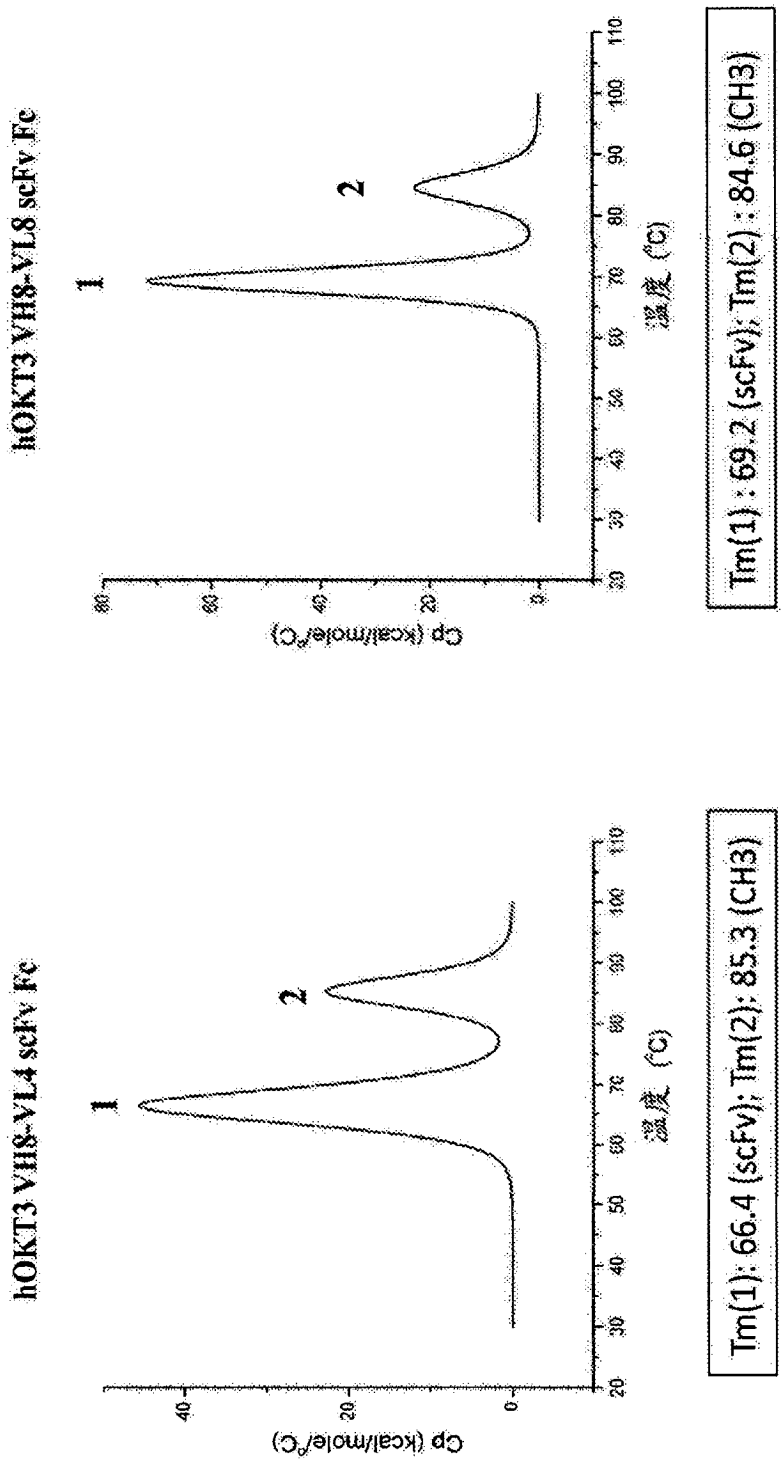
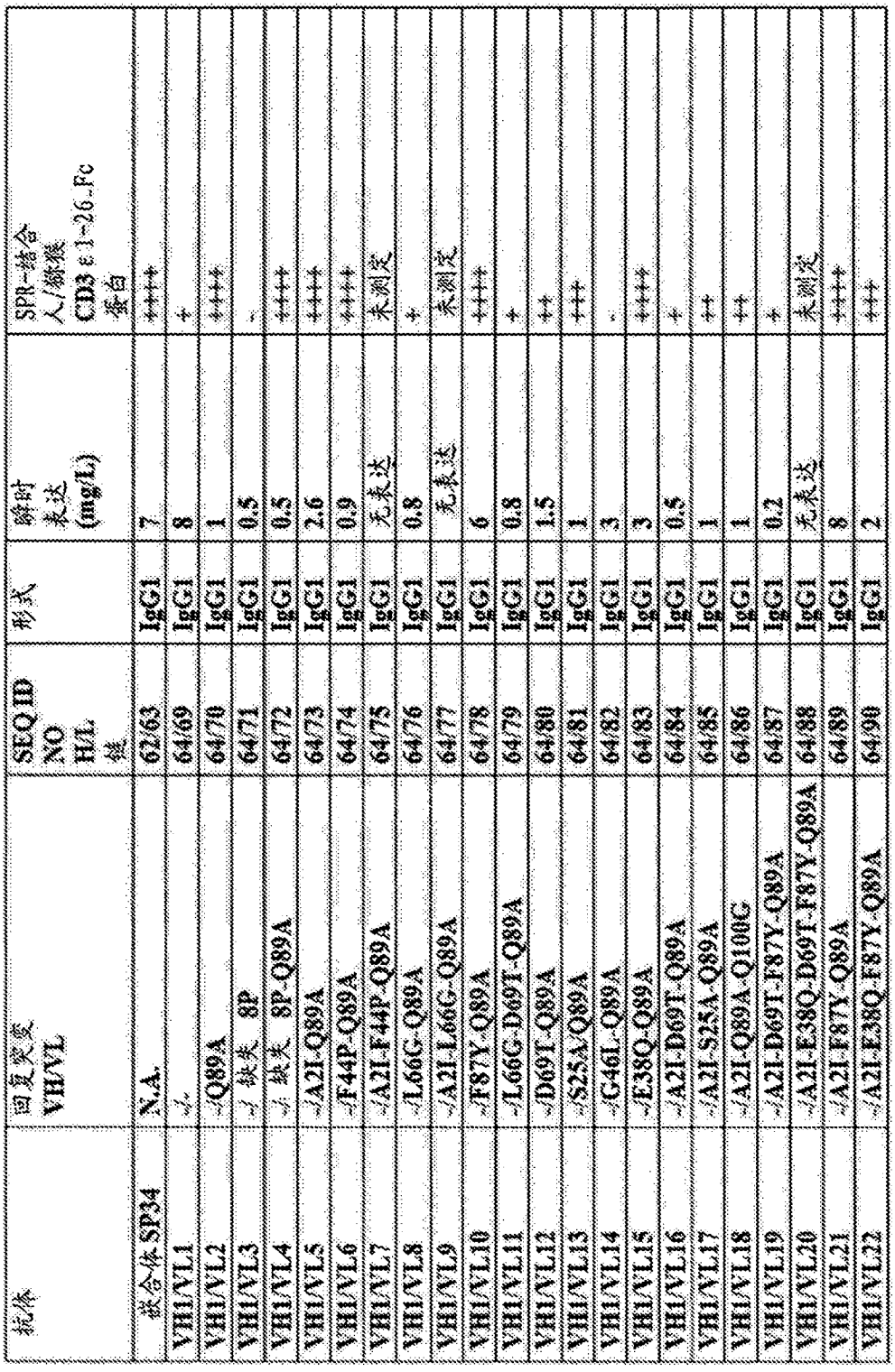
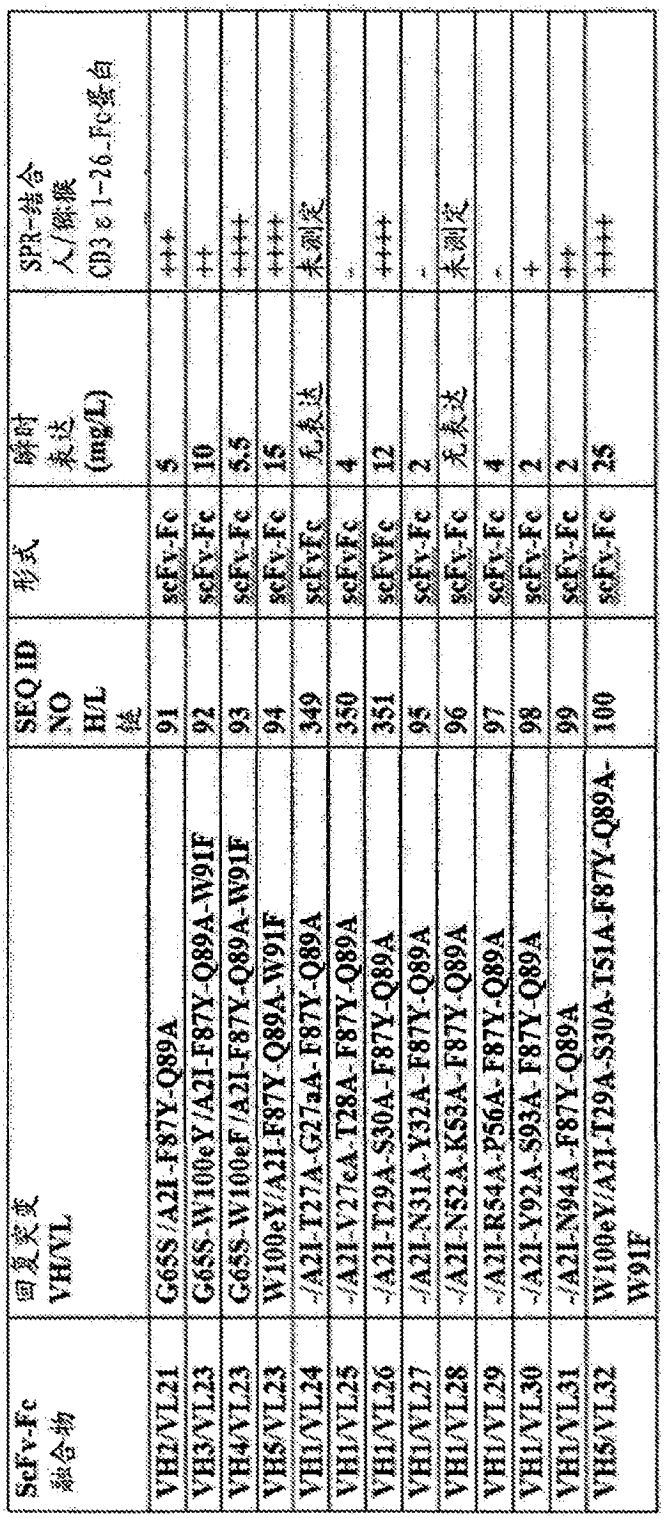

hOKT3 VH8-VL8 scFv Fc
1
2
Cp (kcal/mole/°C)
温度 (°C)
Tm(1) : 69.2 (scFv); Tm(2) : 84.6 (CH3)
hOKT3 VH8-VL4 scFv Fc
1
2
Cp (kcal/mole/°C)
温度 (°C)
Tm(1): 66.4 (scFv); Tm(2): 85.3 (CH3)

图9F



构体	回复突变 VH/VL	SEQ ID NO H/L 链	形式	瞬时表达 (mg/L)	SPR-结合 人/猕猴 CD3 ε 1-26-Fc 蛋白
嵌合体SP34	N.A.	62/63	IgG1	7	++++
VH1/VL1	-/-	64/69	IgG1	8	+
VH1/VL2	-/Q89A	64/70	IgG1	1	++++
VH1/VL3	-/ 缺失 8P	64/71	IgG1	0.5	-
VH1/VL4	-/ 缺失 8P-Q89A	64/72	IgG1	0.5	++++
VH1/VL5	-/A2I-Q89A	64/73	IgG1	2.6	++++
VH1/VL6	-/F44P-Q89A	64/74	IgG1	0.9	++++
VH1/VL7	-/A2I-F44P-Q89A	64/75	IgG1	无表达	未测定
VH1/VL8	-/L66G-Q89A	64/76	IgG1	0.8	+
VH1/VL9	-/A2I-L66G-Q89A	64/77	IgG1	无表达	未测定
VH1/VL10	-/F87Y-Q89A	64/78	IgG1	6	++++
VH1/VL11	-/L66G-D69T-Q89A	64/79	IgG1	0.8	+
VH1/VL12	-/D69T-Q89A	64/80	IgG1	1.5	++
VH1/VL13	-/S25A/Q89A	64/81	IgG1	1	+++
VH1/VL14	-/G46L-Q89A	64/82	IgG1	3	-
VH1/VL15	-/E38Q-Q89A	64/83	IgG1	3	++++
VH1/VL16	-/A2I-D69T-Q89A	64/84	IgG1	0.5	+
VH1/VL17	-/A2I-S25A-Q89A	64/85	IgG1	1	++
VH1/VL18	-/A2I-Q89A-Q100G	64/86	IgG1	1	++
VH1/VL19	-/A2I-D69T-F87Y-Q89A	64/87	IgG1	0.2	+
VH1/VL20	-/A2I-E38Q-D69T-F87Y-Q89A	64/88	IgG1	无表达	未测定
VH1/VL21	-/A2I-F87Y-Q89A	64/89	IgG1	8	++++
VH1/VL22	-/A2I-E38Q-F87Y-Q89A	64/90	IgG1	2	+++

图10A



ScFv-Fc 融合物	回复突变 VH/VL	SEQ ID NO H/L 链	形式	瞬时表达 (mg/L)	SPR-结合 人/猕猴 CD3ε 1-26_Fc蛋白
VH2/VL21	G65S /A2I-F87Y-Q89A	91	scFv-Fc	5	+++
VH3/VL23	G65S-W100eY/A2I-F87Y-Q89A-W91F	92	scFv-Fc	10	++
VH4/VL23	G65S-W100eF /A2I-F87Y-Q89A-W91F	93	scFv-Fc	5.5	++++
VH5/VL23	W100eY/A2I-F87Y-Q89A-W91F	94	scFv-Fc	15	++++
VH1/VL24	-/A2I-T27A-G27aA-F87Y-Q89A	349	scFvFc	无表达	未测定
VH1/VL25	-/A2I-V27cA-T28A-F87Y-Q89A	350	scFvFc	4	-
VH1/VL26	-/A2I-T29A-S30A-F87Y-Q89A	351	scFvFc	12	++++
VH1/VL27	-/A2I-N31A-Y32A-F87Y-Q89A	95	scFv-Fc	2	-
VH1/VL28	-/A2I-N52A-K53A-F87Y-Q89A	96	scFv-Fc	无表达	未测定
VH1/VL29	-/A2I-R54A-P56A-F87Y-Q89A	97	scFv-Fc	4	-
VH1/VL30	-/A2I-Y92A-S93A-F87Y-Q89A	98	scFv-Fc	2	+
VH1/VL31	-/A2I-N94A-F87Y-Q89A	99	scFv-Fc	2	++
VH5/VL32	W100eY/A2I-T29A-S30A-T51A-F87Y-Q89A-W91F	100	scFv-Fc	25	++++

图10B

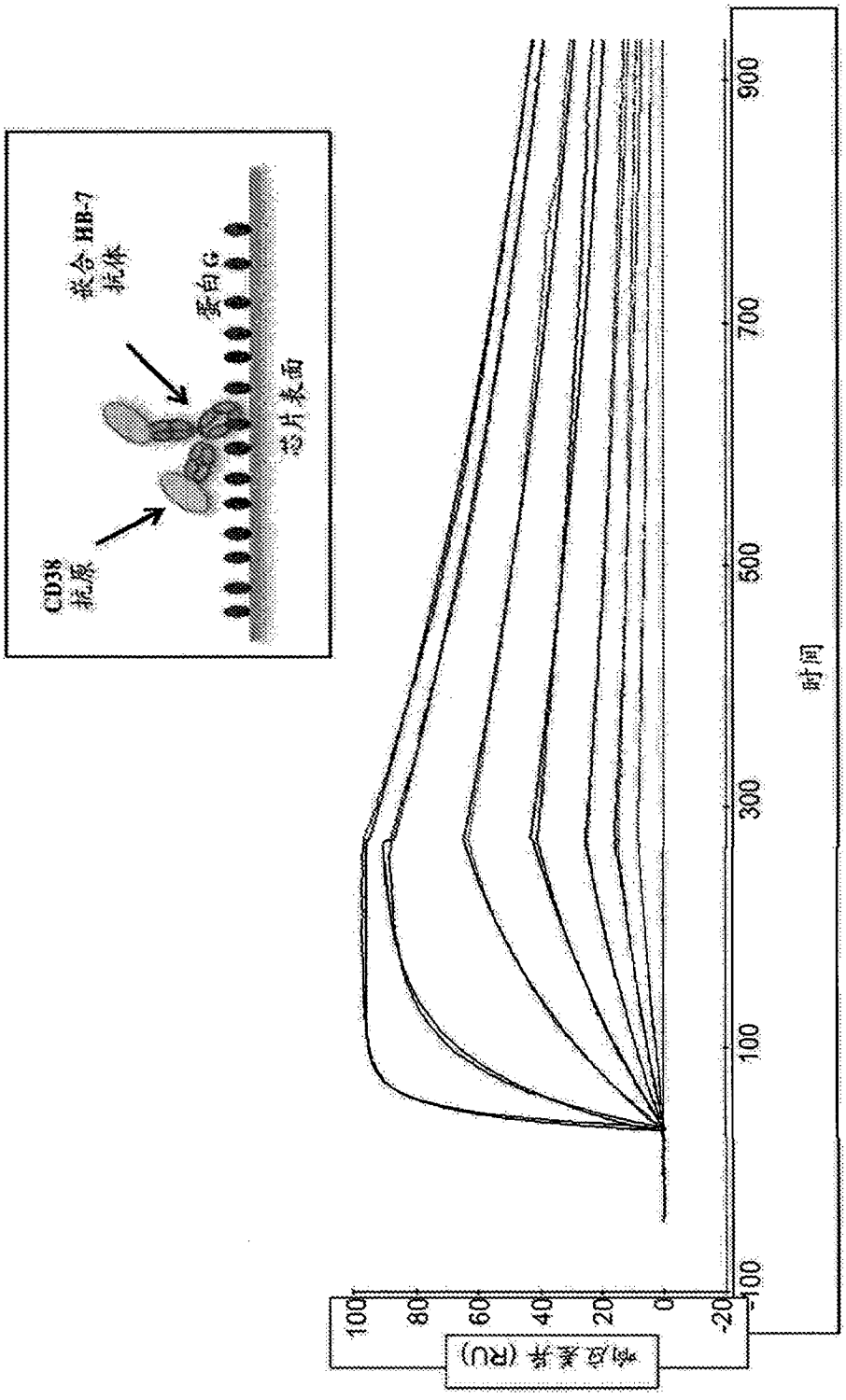

CD38
抗原
来合 HB-7
抗体
蛋白G
芯片表面
100
80
60
40
20
0
-20
-100
100
300
500
700
900
时间

图11A

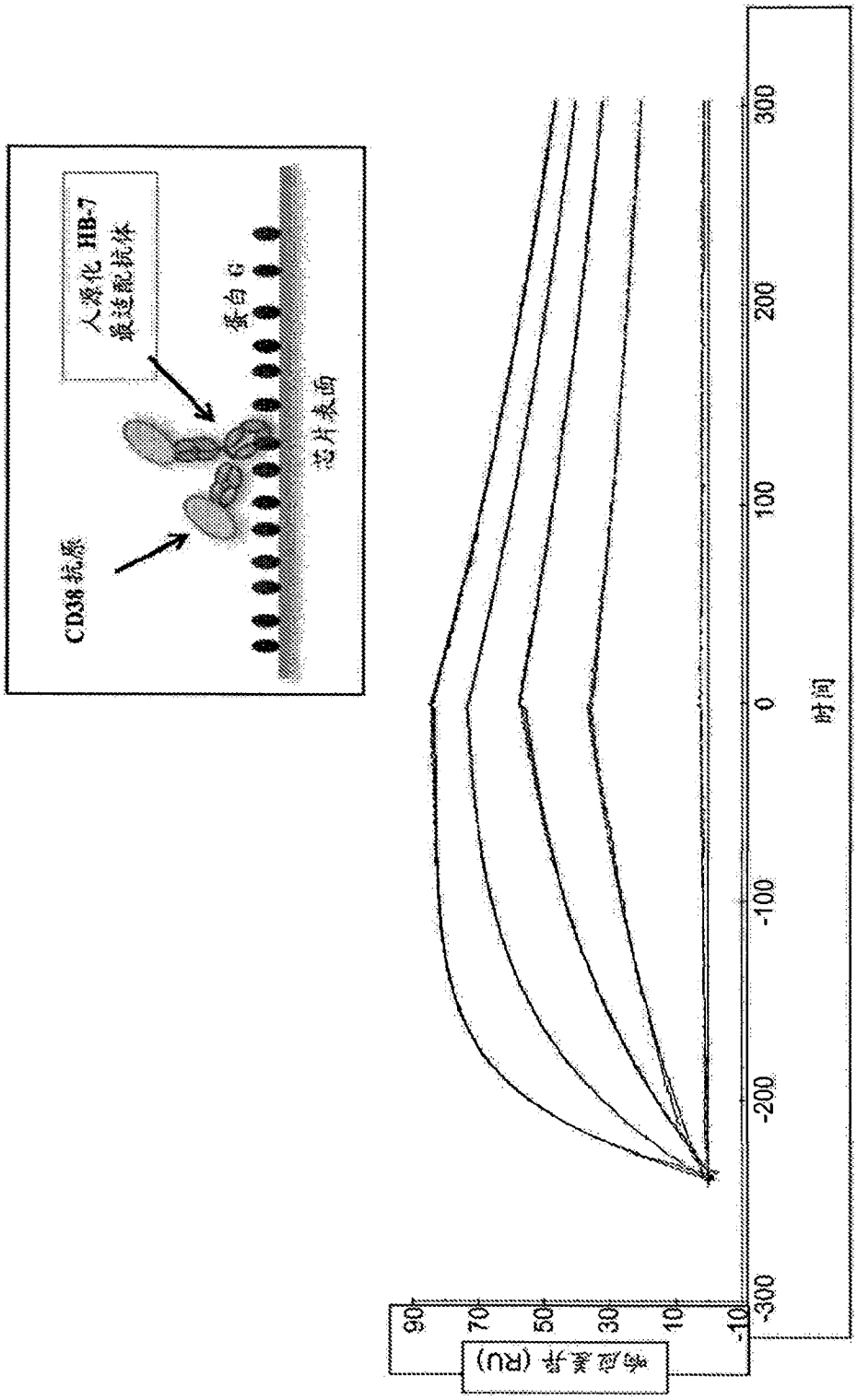

CD38 抗原
人源化 HB-7
最适配抗体
蛋白 G
芯片表面
90
70
50
30
10
-10
-300
-200
-100
0
100
200
300
时间

图11B

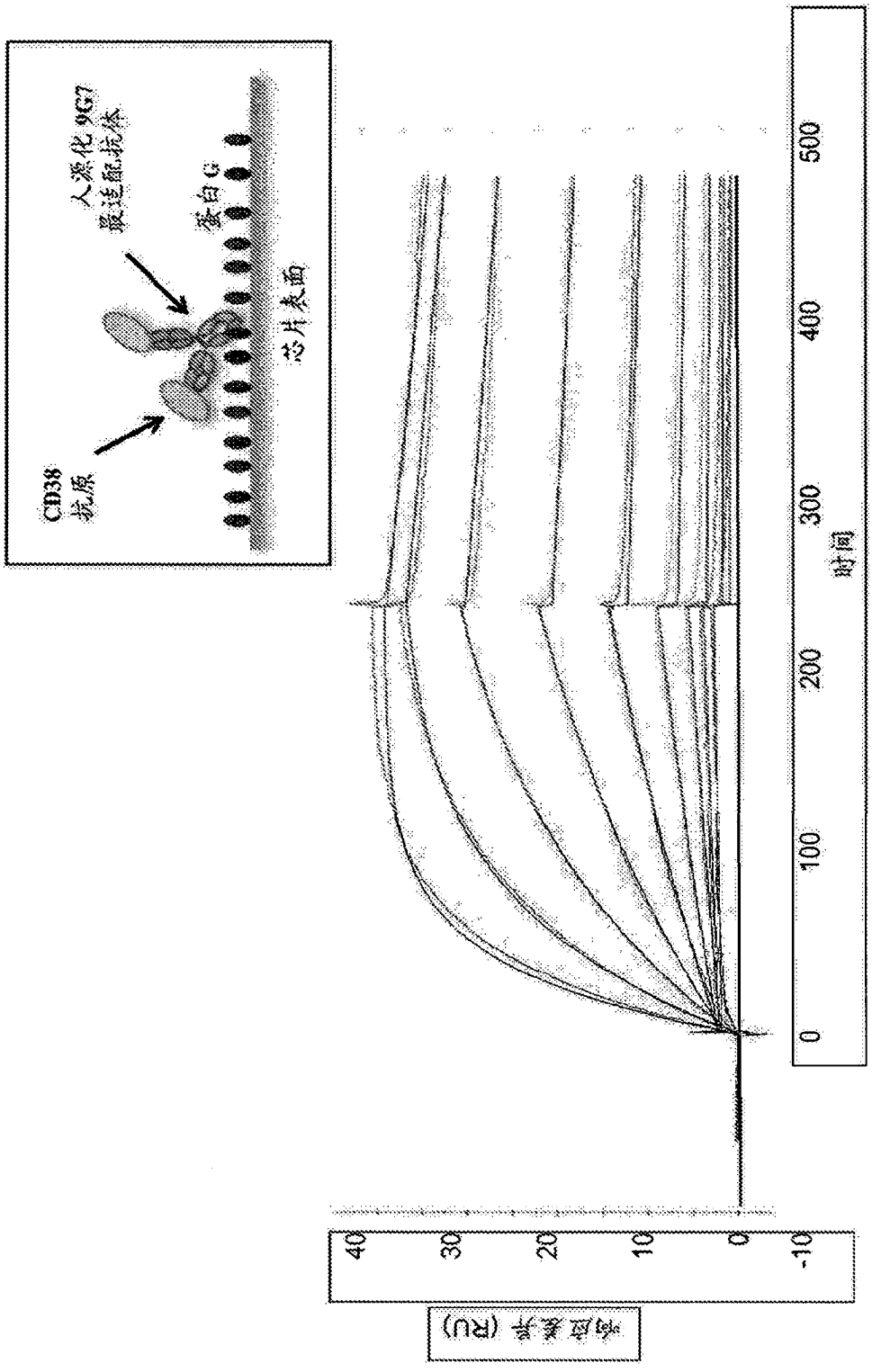

人源化 9G7
最适配抗体
蛋白 G
芯片表面
CD38
抗原
反应差异 (RU)
40
30
20
10
0
-10
0
100
200
300
400
500
时间

图11C

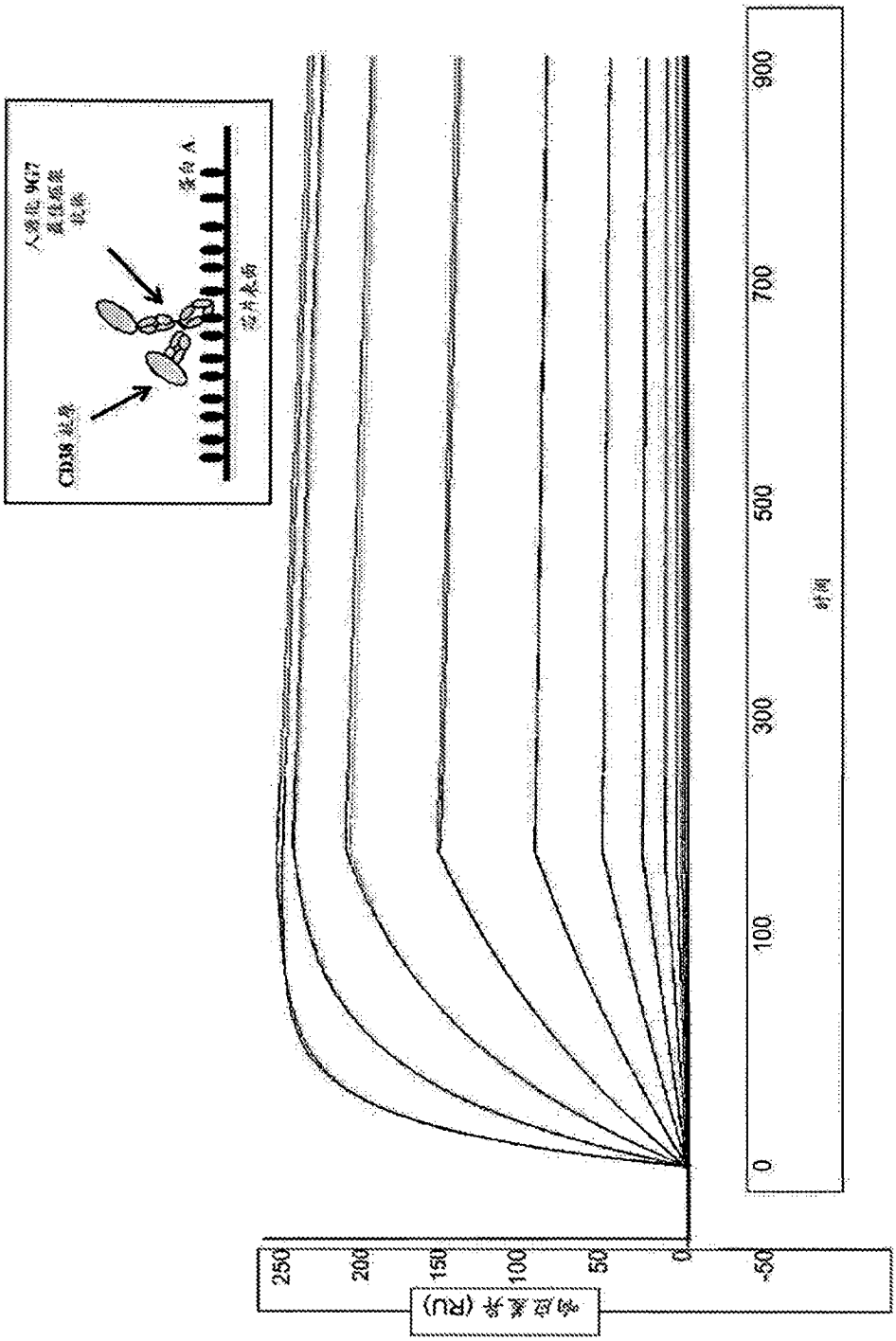
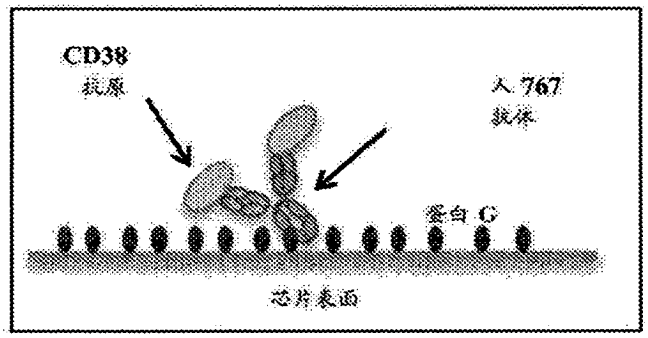

CD38 抗原
蛋白 A
0
100
300
500
700
900
时间
250
200
150
100
50
0
-50
响应差异 (RU)

图11D



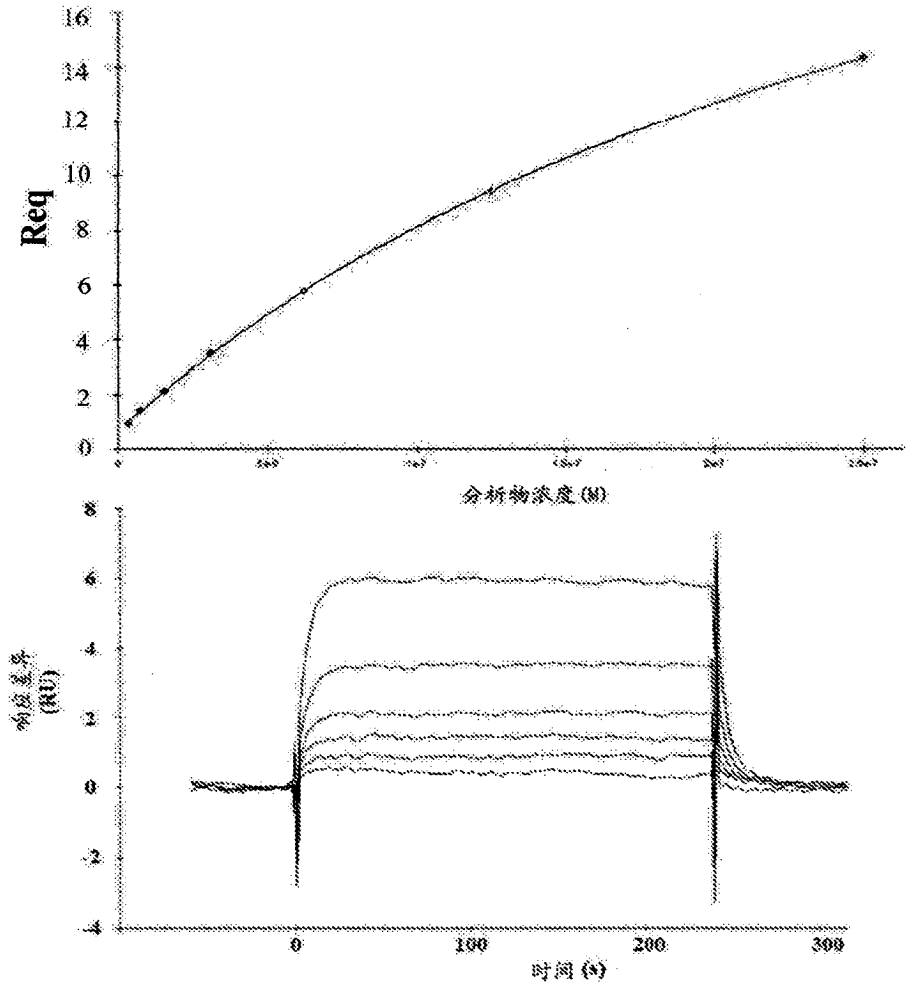


图11E

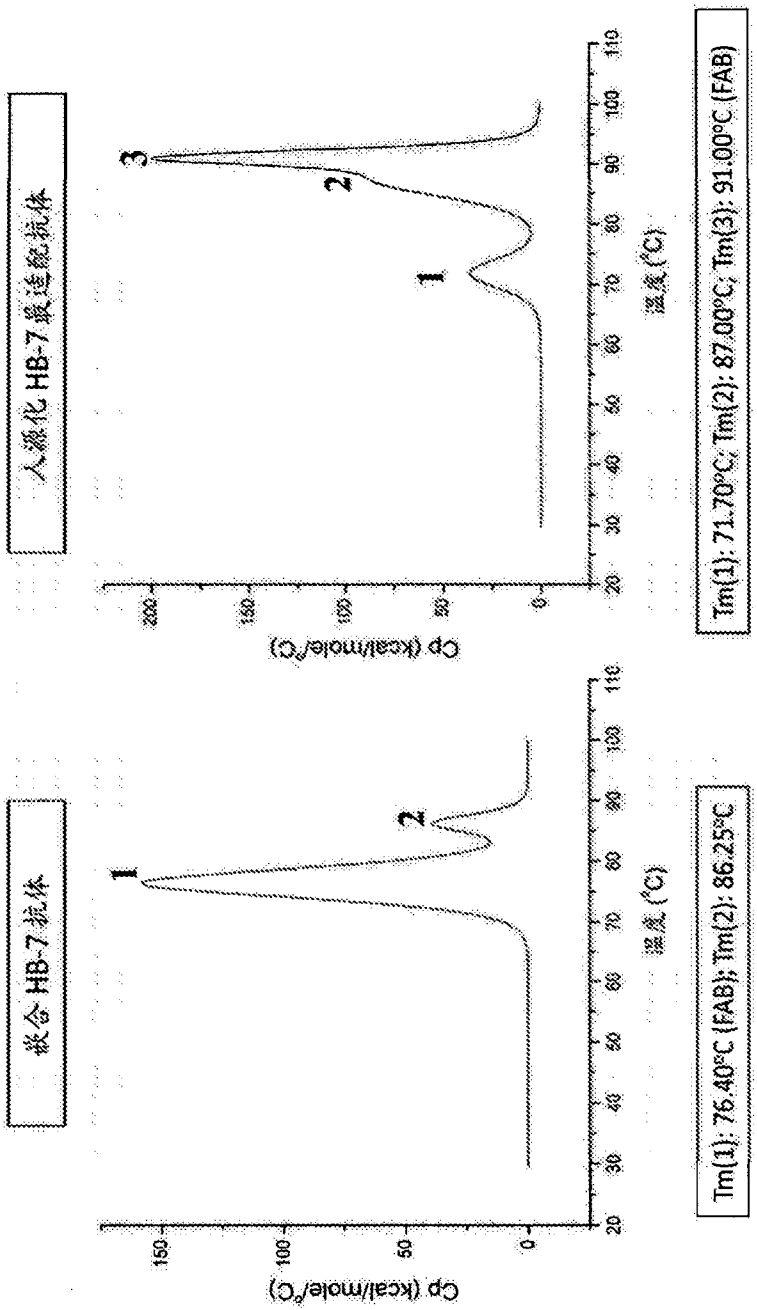

人源化 HB-7 最适配抗体
Tm(1): 71.70°C; Tm(2): 87.00°C; Tm(3): 91.00°C (FAB)
嵌合 HB-7 抗体
Tm(1): 76.40°C (FAB); Tm(2): 86.25°C
Cp (kcal/mole/°C)
温度 (°C)

图11F

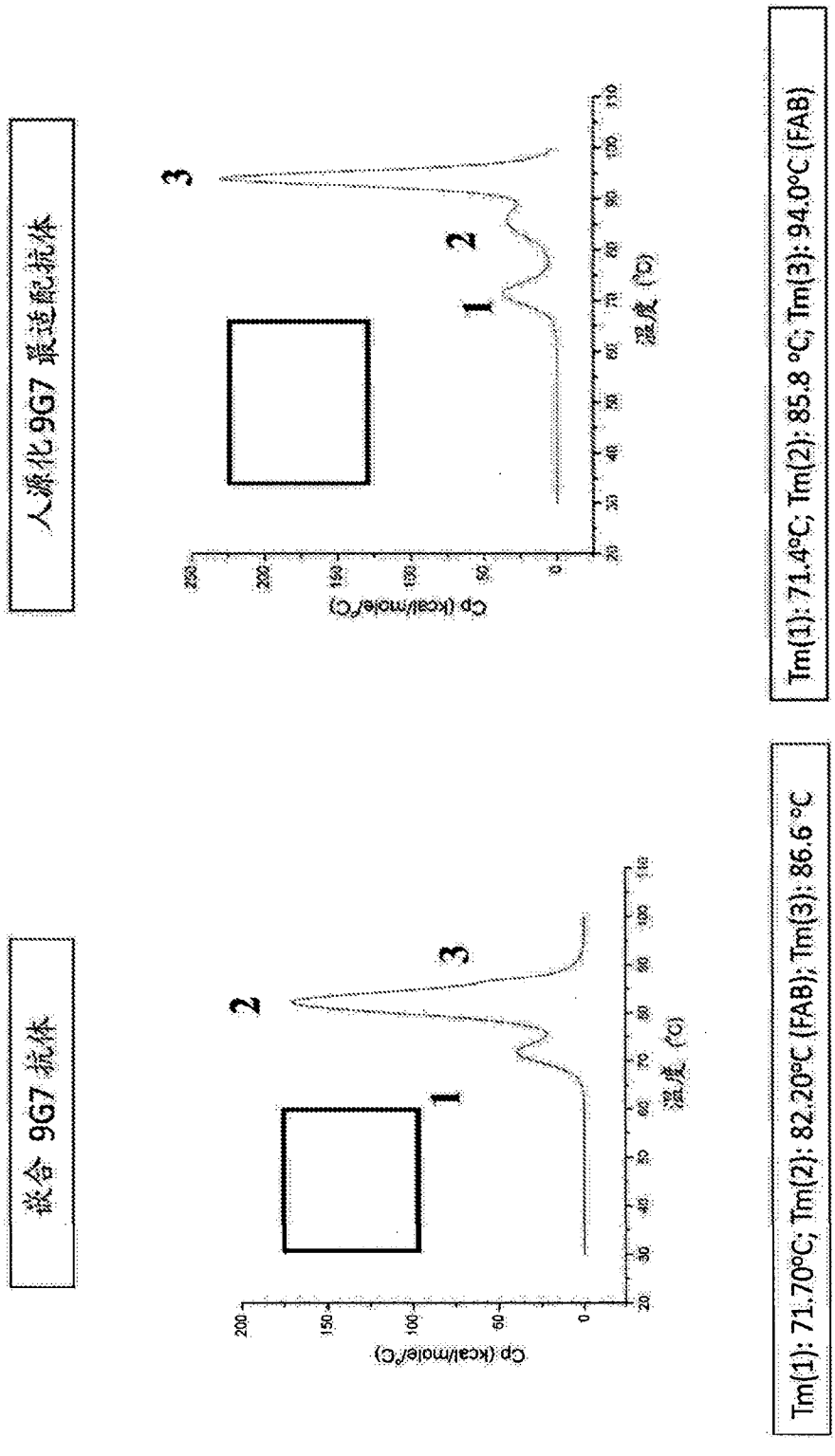

人源化9G7 最适配抗体
Tm(1): 71.4°C; Tm(2): 85.8 °C; Tm(3): 94.0°C (FAB)
嵌合 9G7 抗体
Tm(1): 71.70°C; Tm(2): 82.20°C (FAB); Tm(3): 86.6 °C
Cp (kcal/mole/°C)
温度 (°C)
1
2
3

图11G

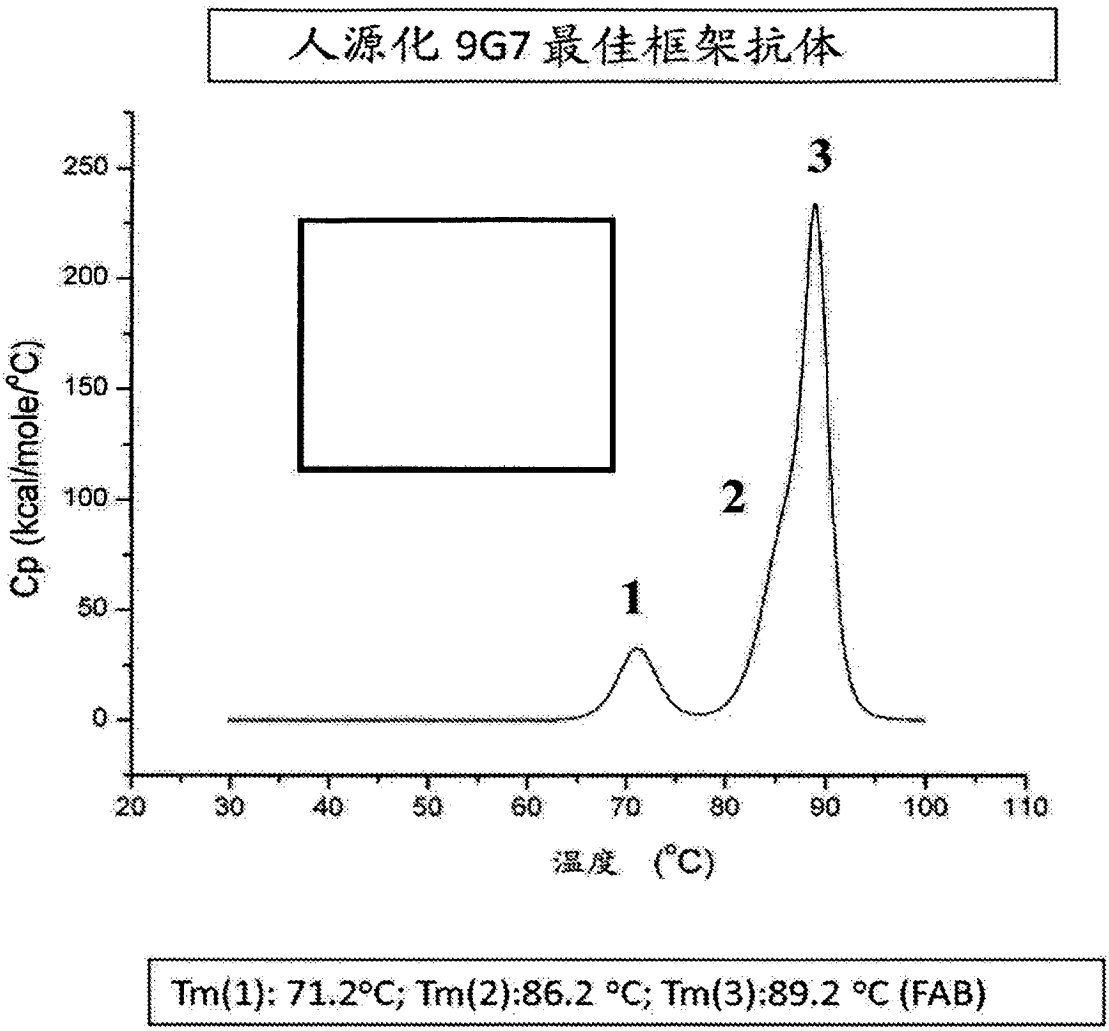

人源化 9G7 最佳框架抗体
Cp (kcal/mole/°C)
250
200
150
100
50
0
20
30
40
50
60
70
80
90
100
110
温度 (°C)
1
2
3
Tm(1): 71.2°C; Tm(2):86.2 °C; Tm(3):89.2 °C (FAB)

图11H

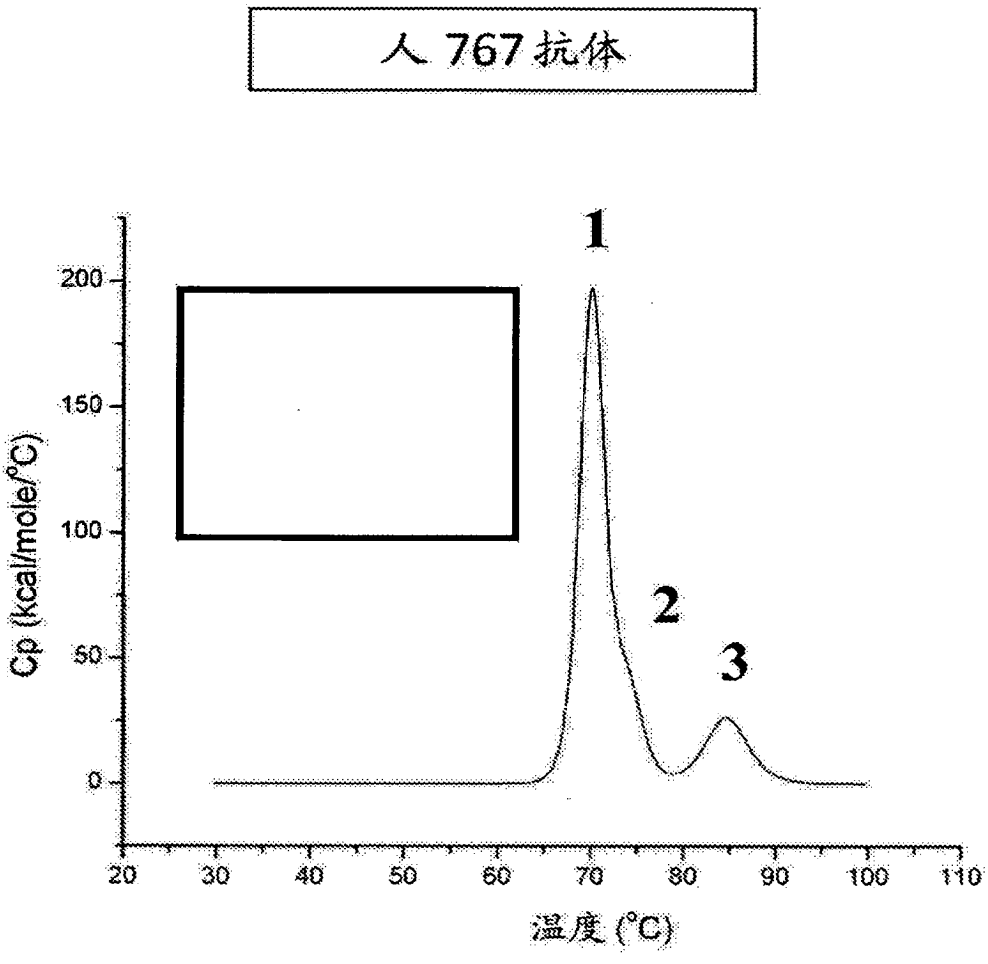
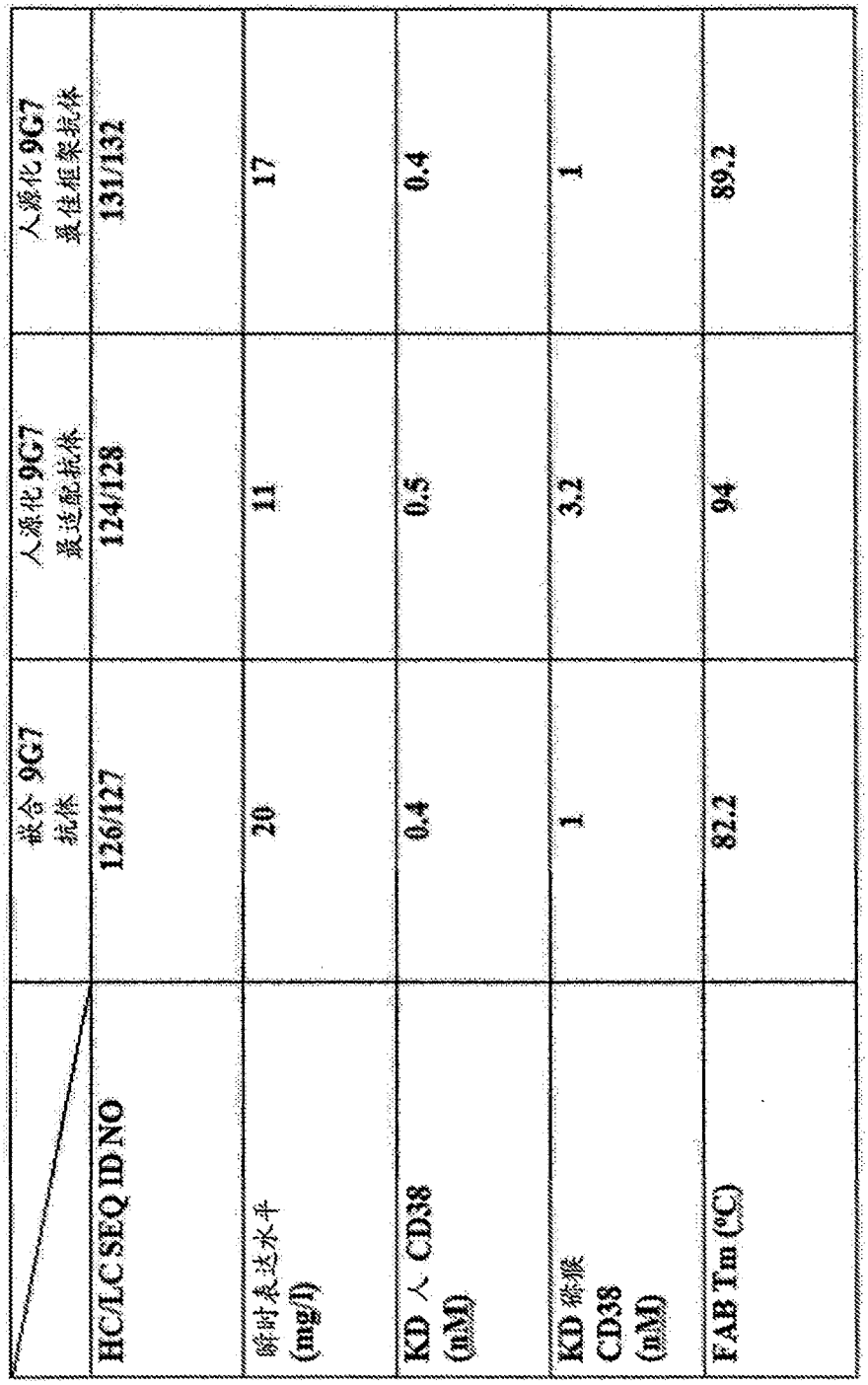

人 767 抗体
1
2
3
200
150
100
50
0
Cp (kcal/mole/°C)
20
30
40
50
60
70
80
90
100
110
温度 (°C)
Tm(1): 70.2°C (FAB); Tm(2):74.1 °C; Tm(3):84.7 °C

图11I



	嵌合 9G7 抗体	人源化 9G7 最适配抗体	人源化 9G7 最佳框架抗体
HC/LC SEQ ID NO	126/127	124/128	131/132
瞬时表达水平 (mg/l)	20	11	17
KD 人 CD38 (nM)	0.4	0.5	0.4
KD 猕猴 CD38 (nM)	1	3.2	1
FAB Tm (°C)	82.2	94	89.2

图11J

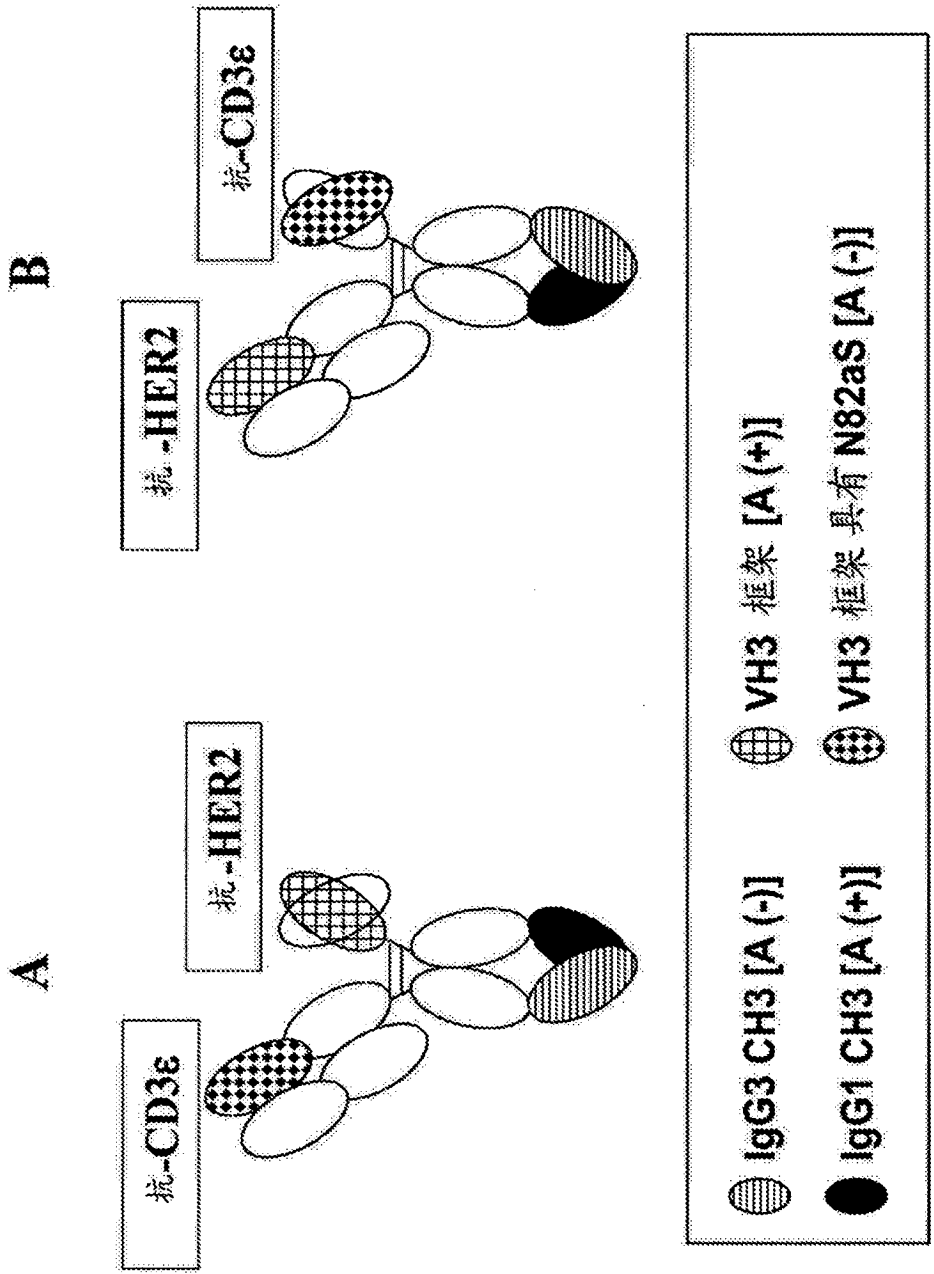

A
抗-CD3ε
抗-HER2
B
抗-CD3ε
抗-HER2
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 框架 [A (+)]
VH3 框架 具有 N82aS [A (-)]

图12A

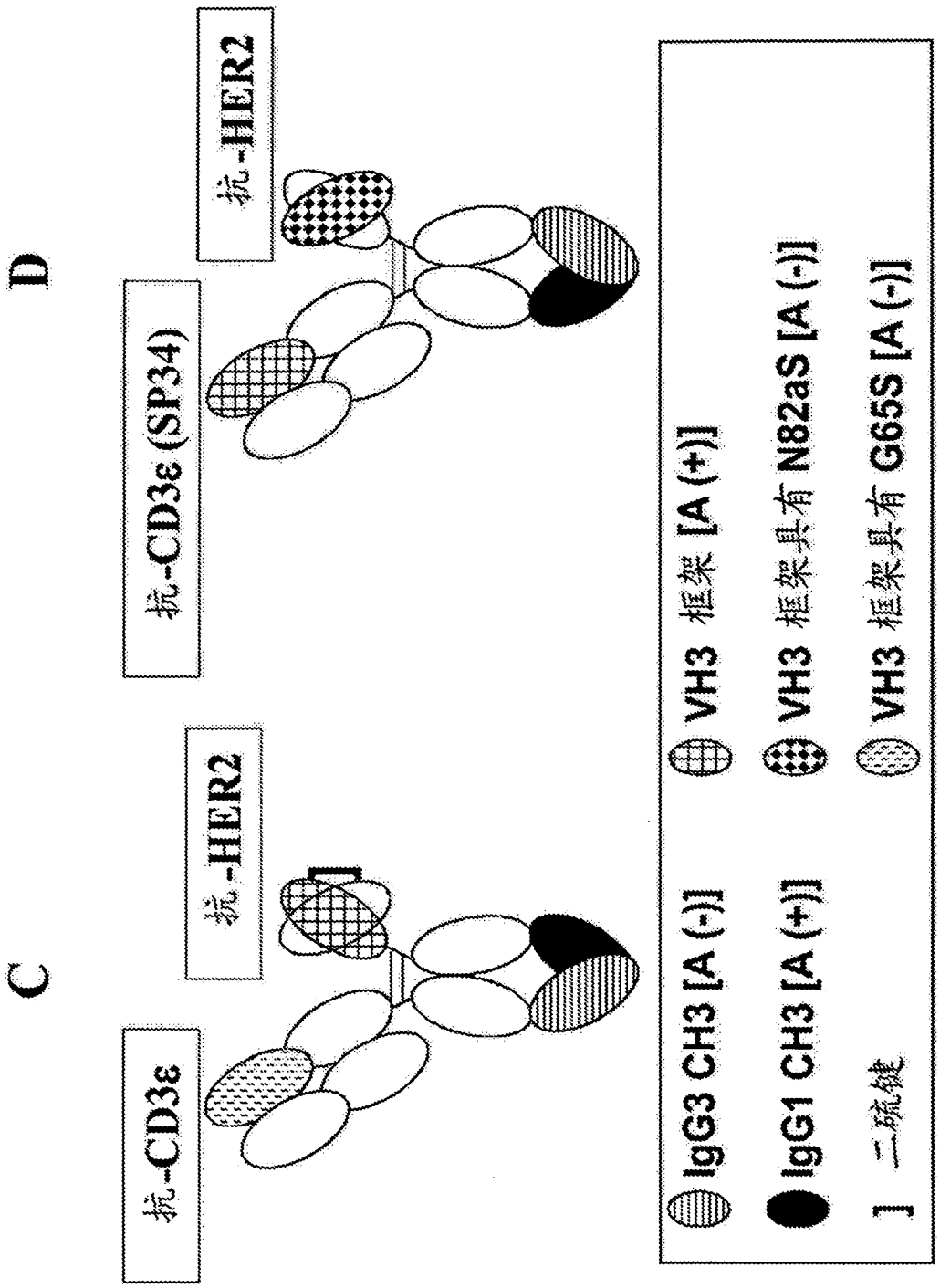

C
抗-CD3ε
抗-HER2
D
抗-CD3ε (SP34)
抗-HER2
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
二硫键
VH3 框架 [A (+)]
VH3 框架具有 N82aS [A (-)]
VH3 框架具有 G65S [A (-)]

图12B

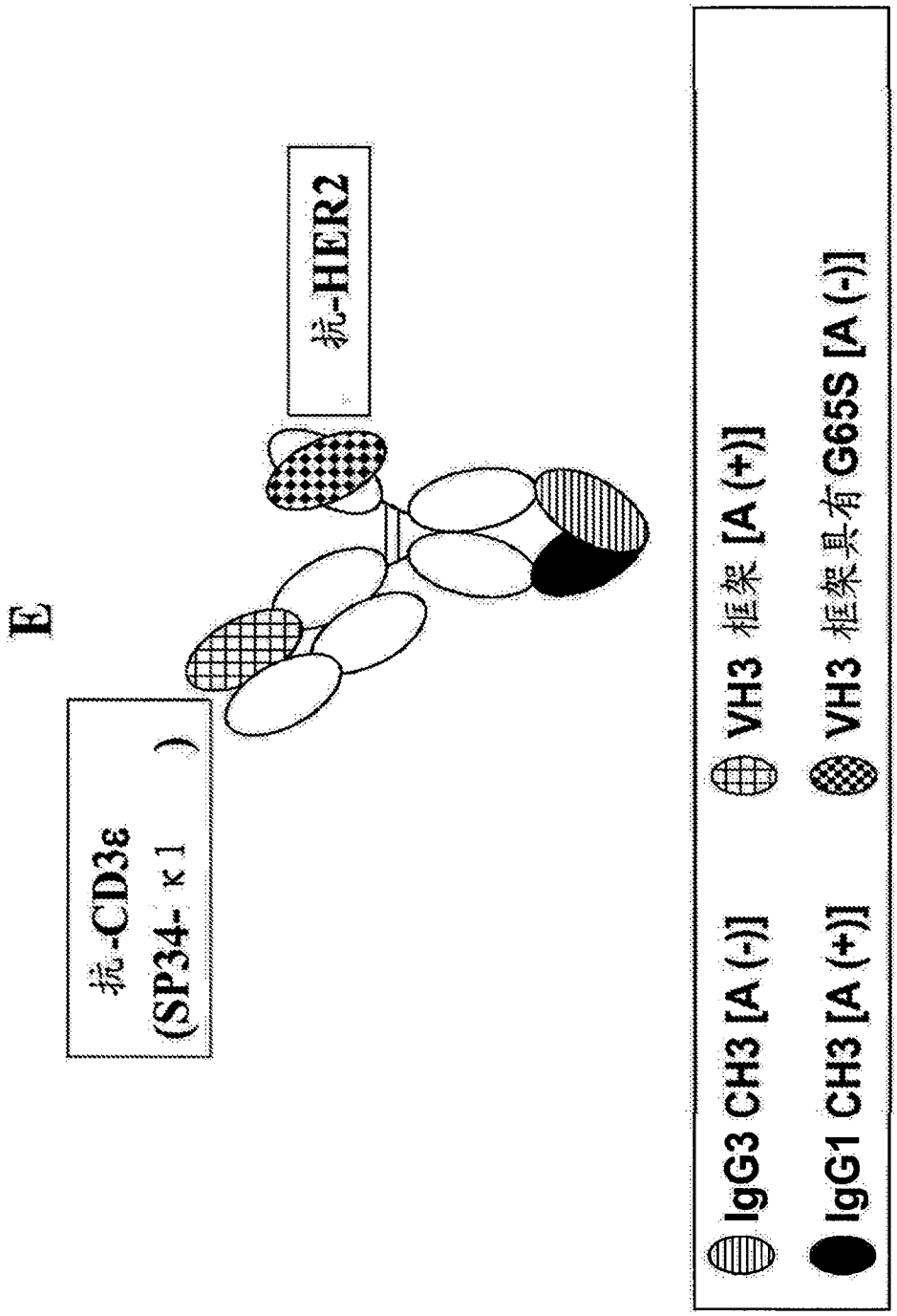

E
抗-CD3ε
(SP34-κ1)
抗-HER2
IgG3 CH3 [A (-)]
VH3 框架 [A (+)]
IgG1 CH3 [A (+)]
VH3 框架具有G65S [A (-)]

图12C

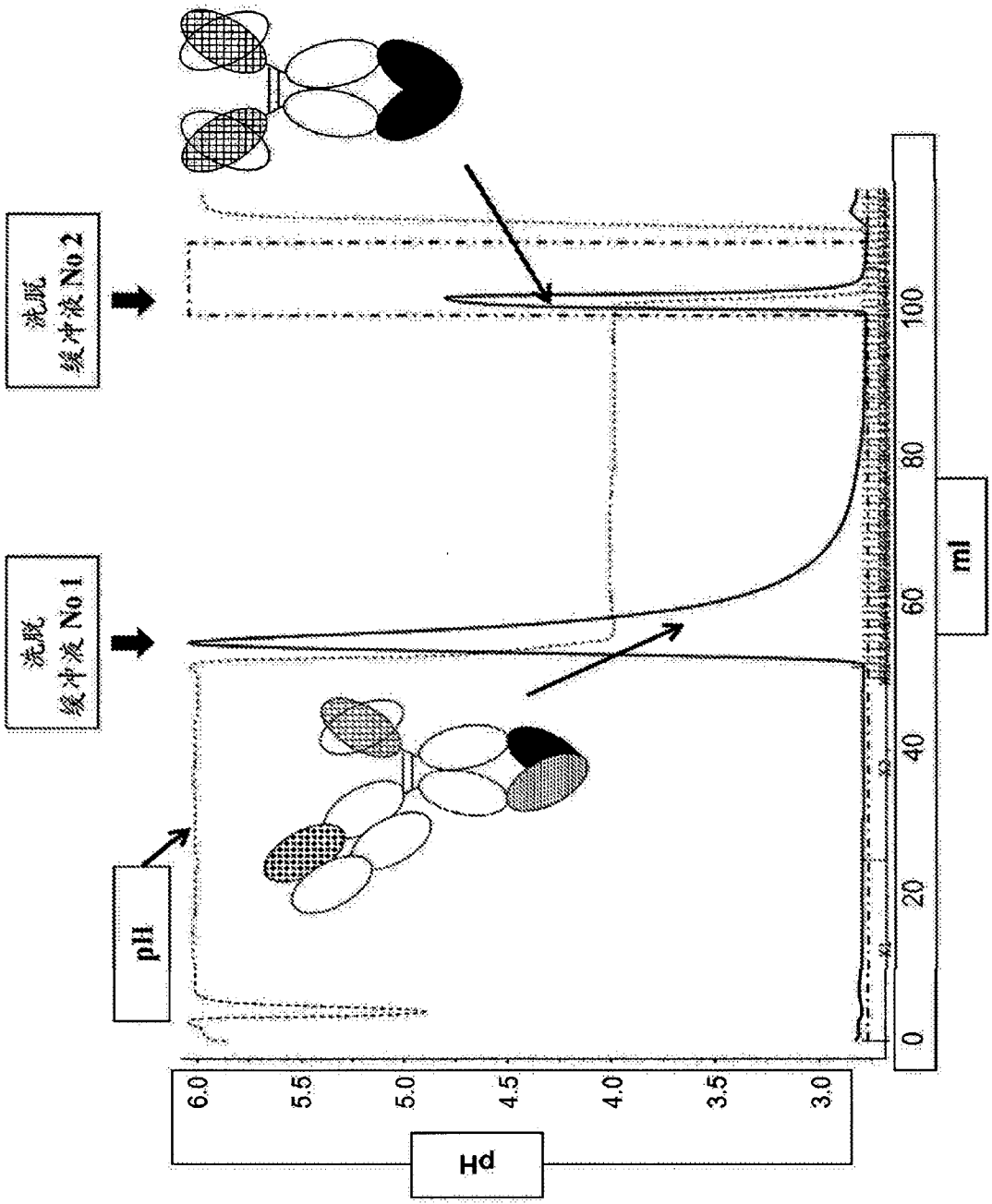
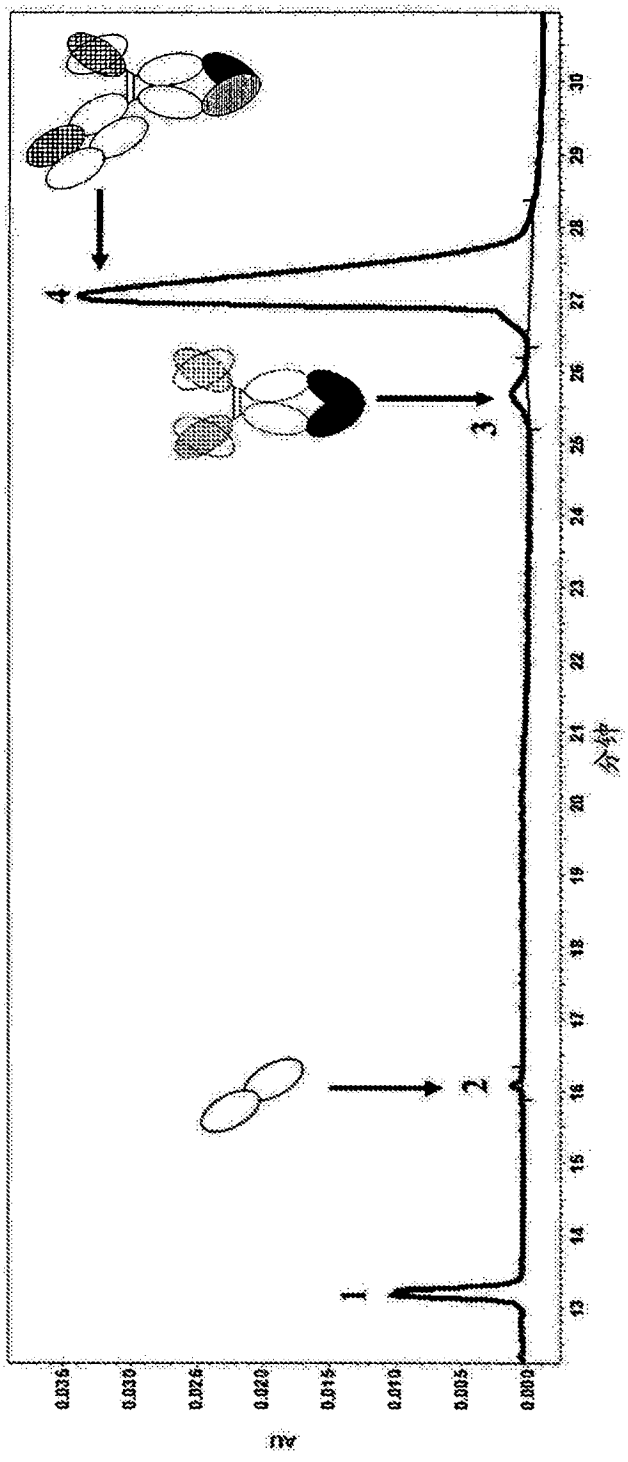
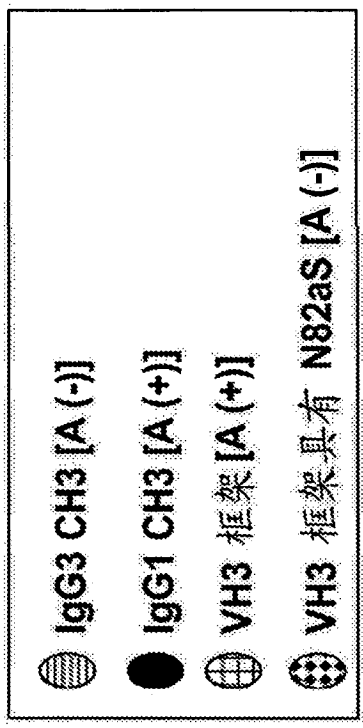


图13





峰	物类	% 总 AUC
1	内标	N.A.
2	轻链 (25 kDa)	0.5
3	同源二聚体(100 kDa)	2.5
4	异源二聚体 (125 kDa)	97

图14

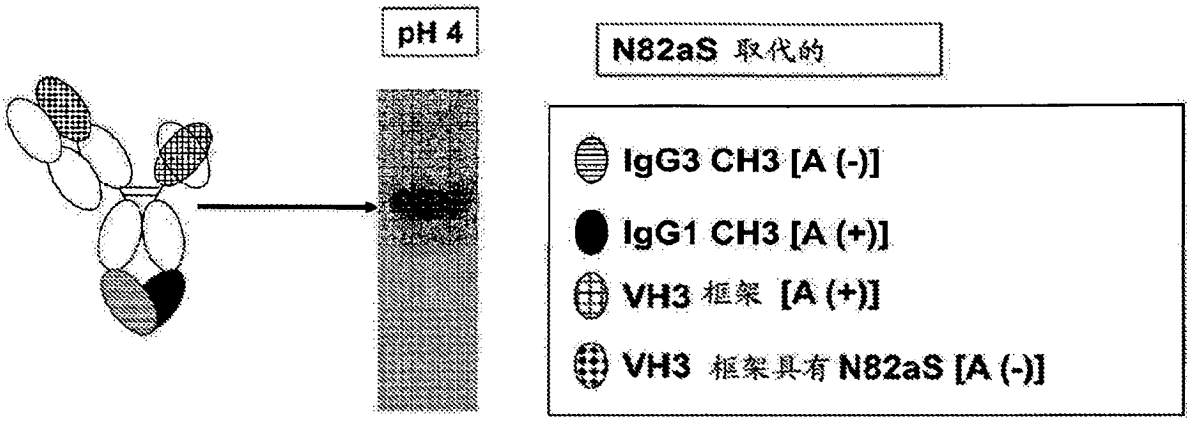

pH 4
N82aS 取代的
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 框架 [A (+)]
VH3 框架具有 N82aS [A (-)]

图15A

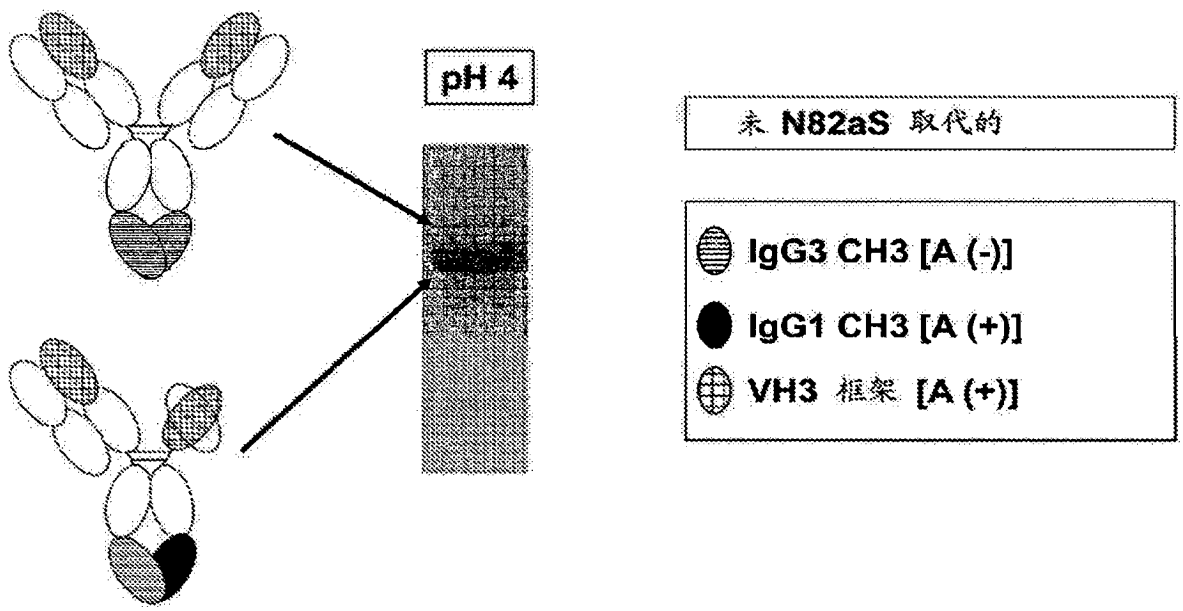

pH 4
未 N82aS 取代的
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 框架 [A (+)]

图15B

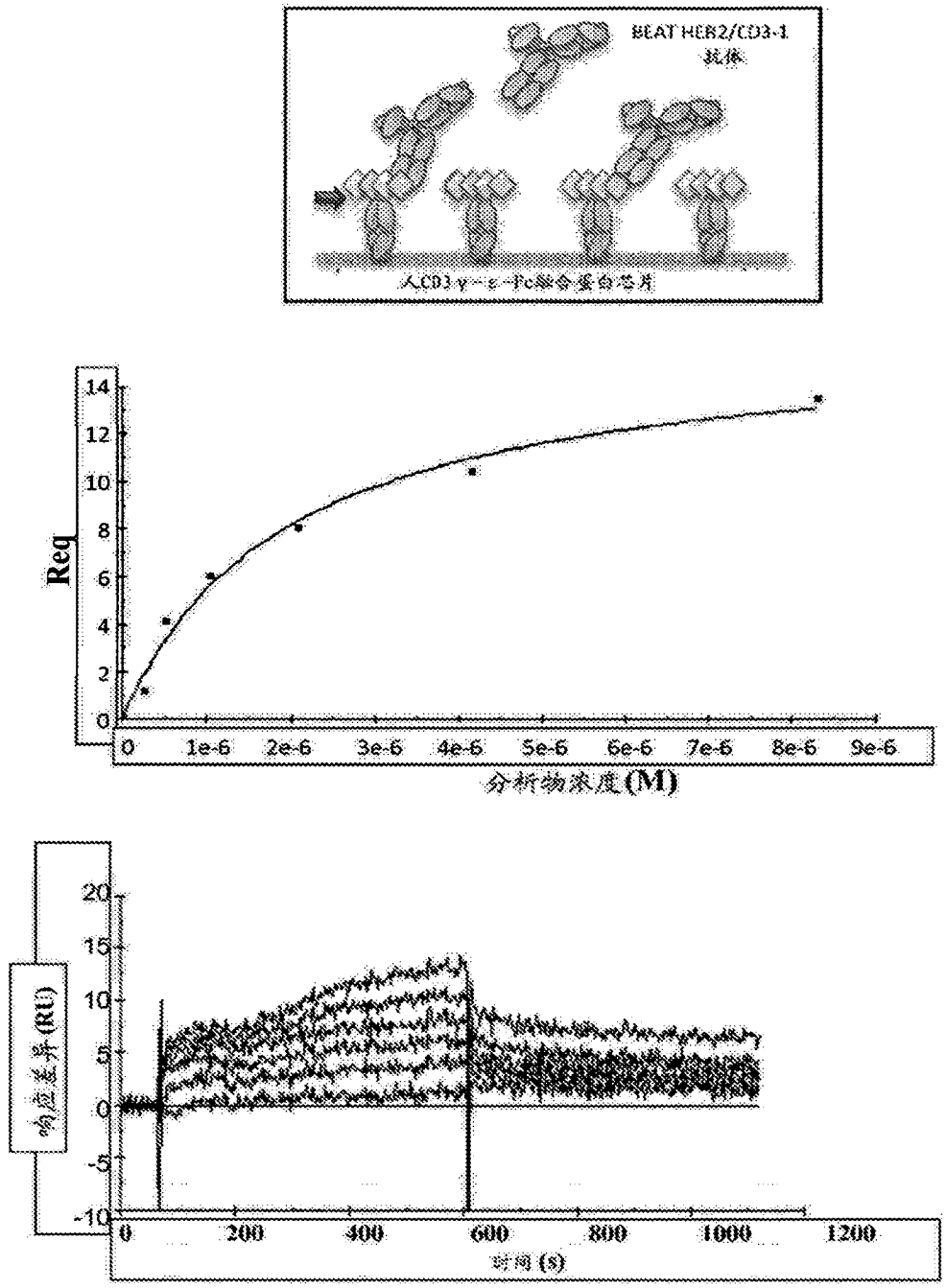

BEAT HER2/CD3-1
抗体
人CD3γ-ε-Fc融合蛋白芯片
Req
0
2
4
6
8
10
12
14
0
1e-6
2e-6
3e-6
4e-6
5e-6
6e-6
7e-6
8e-6
9e-6
分析物浓度(M)
响应差异(RU)
20
15
10
5
0
-5
-10
0
200
400
600
800
1000
1200
时间(s)

图16A

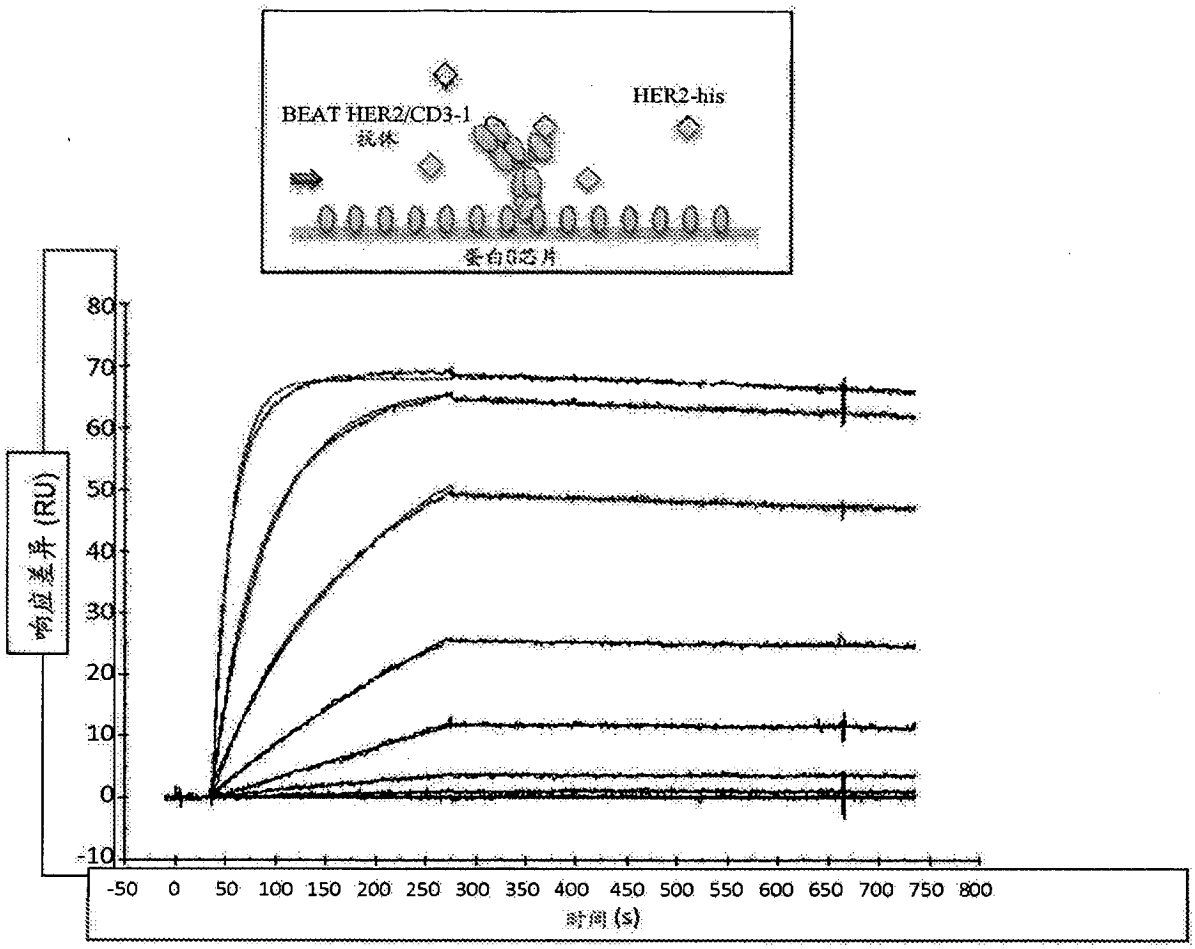

BEAT HER2/CD3-1
抗体
HER2-his
蛋白质芯片
响应差异 (RU)
80
70
60
50
40
30
20
10
0
-10
-50 0 50 100 150 200 250 300 350 400 450 500 550 600 650 700 750 800
时间 (s)

图16B

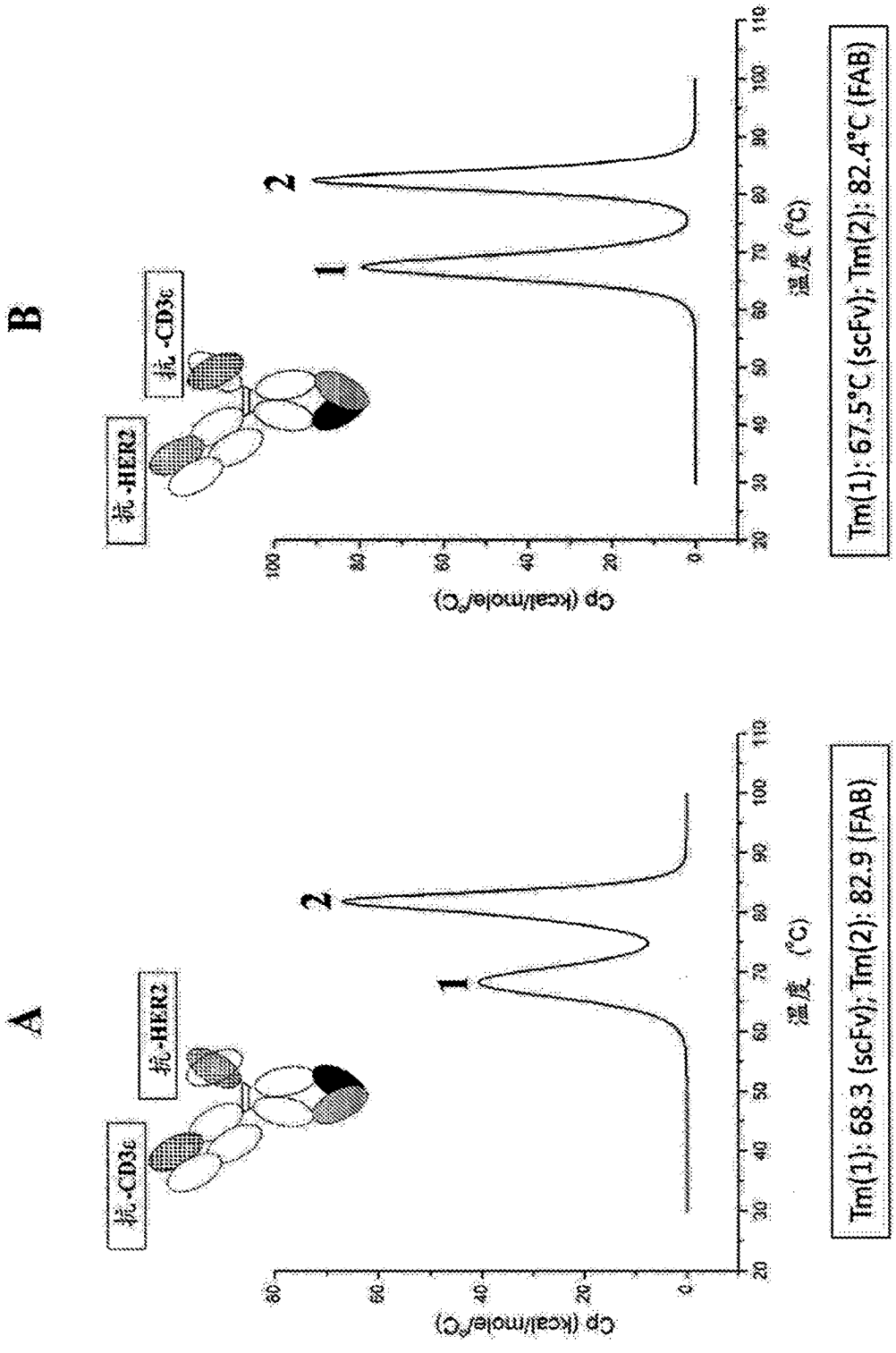

A
抗-CD3ε
抗-HER2
1
2
Cp (kcal/mole/°C)
温度 (°C)
Tm(1): 68.3 (scFv); Tm(2): 82.9 (FAB)
B
抗-CD3ε
抗-HER2
1
2
Cp (kcal/mole/°C)
温度 (°C)
Tm(1): 67.5°C (scFv); Tm(2): 82.4°C (FAB)

图16C

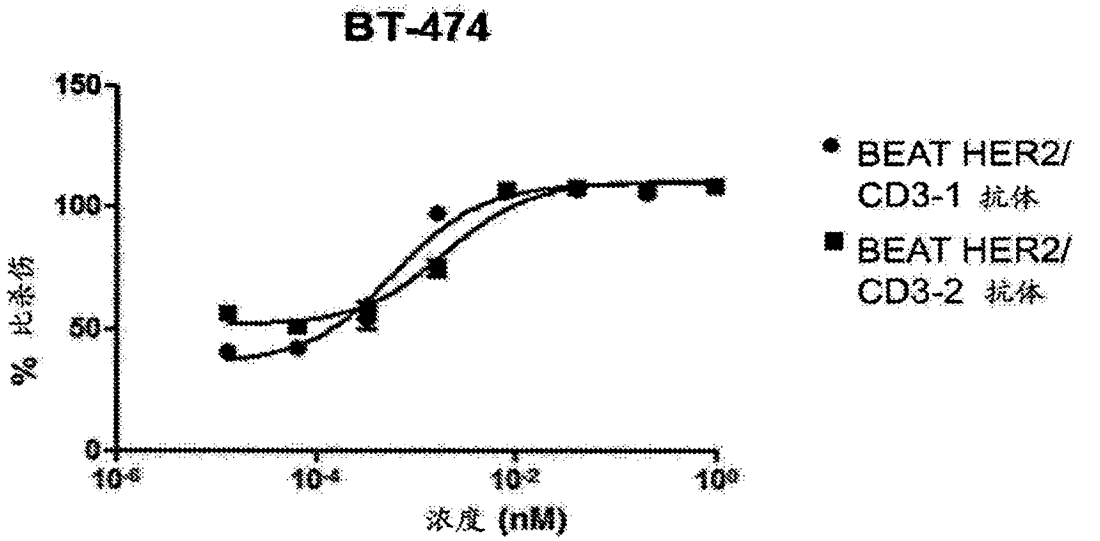

BT-474
150
100
50
0
% 比杀伤
10-6
10-4
10-2
100
浓度 (nM)
BEAT HER2/ CD3-1 抗体
BEAT HER2/ CD3-2 抗体

图17A

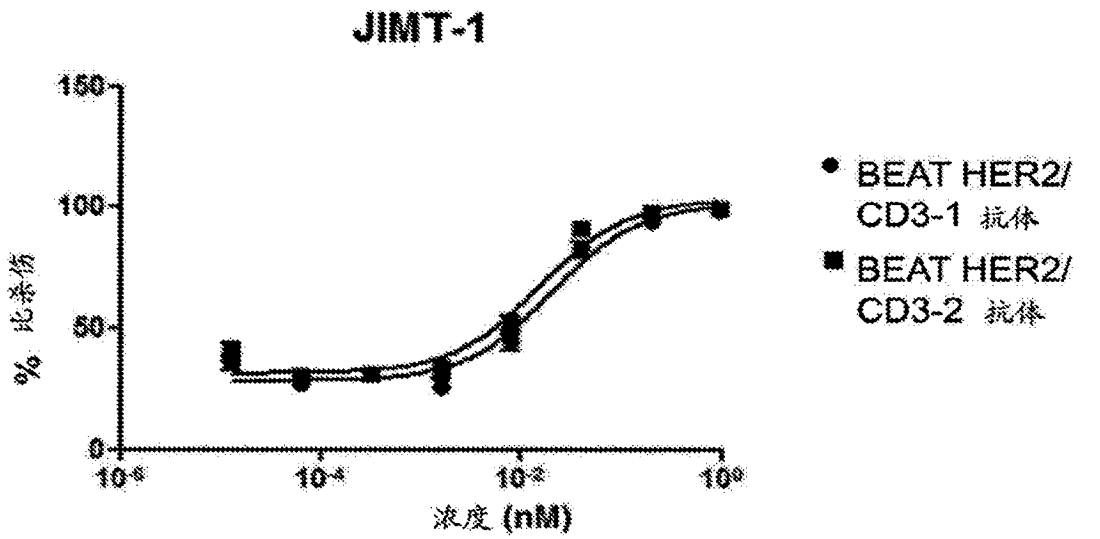

JIMT-1
150
100
50
0
% 比杀伤
10-6
10-4
10-2
100
浓度 (nM)
BEAT HER2/ CD3-1 抗体
BEAT HER2/ CD3-2 抗体

图17B

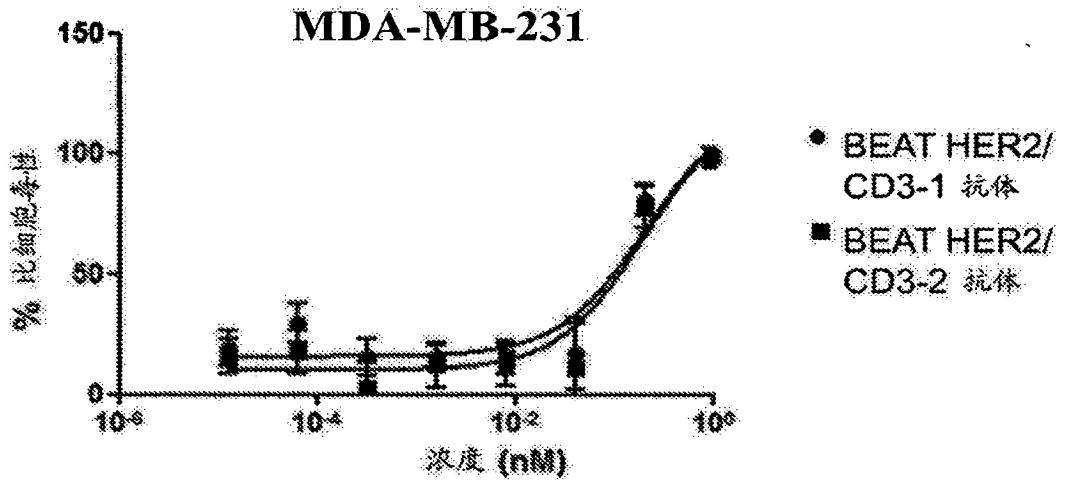

MDA-MB-231
150
100
50
0
% 比细胞毒性
10-6
10-4
10-2
100
浓度 (nM)
BEAT HER2/ CD3-1 抗体
BEAT HER2/ CD3-2 抗体

图17C

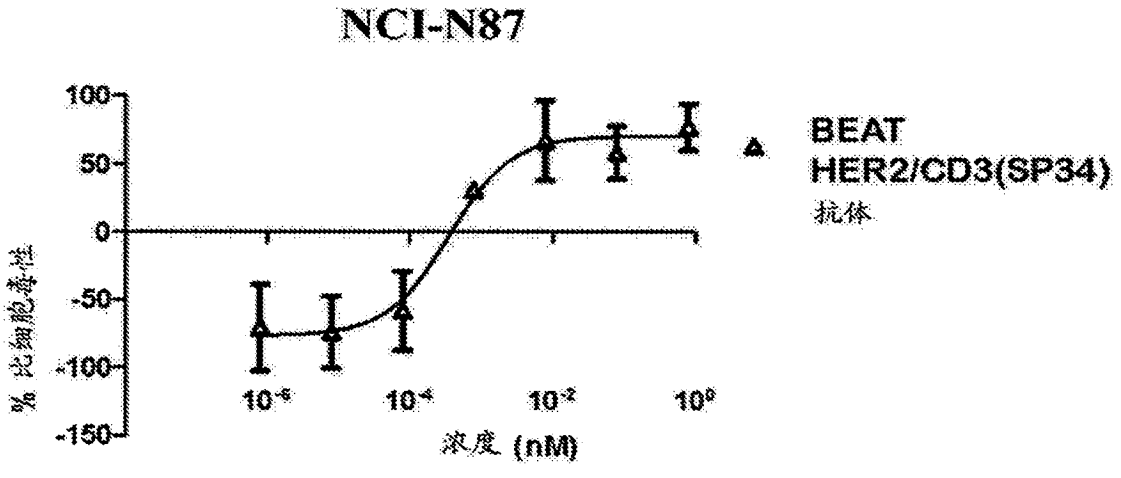

NCI-N87
100
50
0
-50
-100
-150
% 比细胞毒性
10-6
10-4
10-2
100
浓度 (nM)
BEAT HER2/CD3(SP34) 抗体

图17D

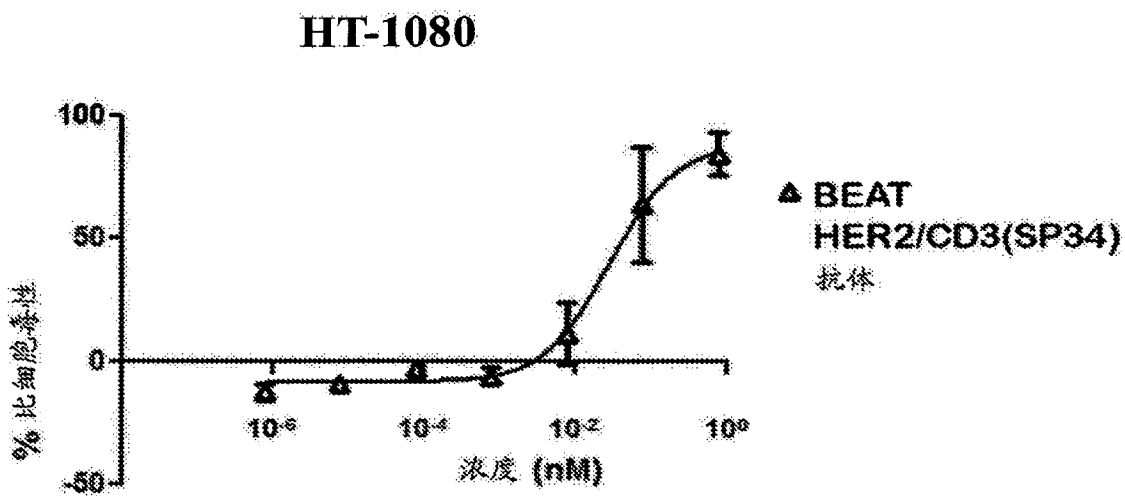

HT-1080
100
50
0
-50
% 比细胞毒性
10⁻⁶
10⁻⁴
10⁻²
10⁰
浓度 (nM)
BEAT
HER2/CD3(SP34)
抗体

图17E

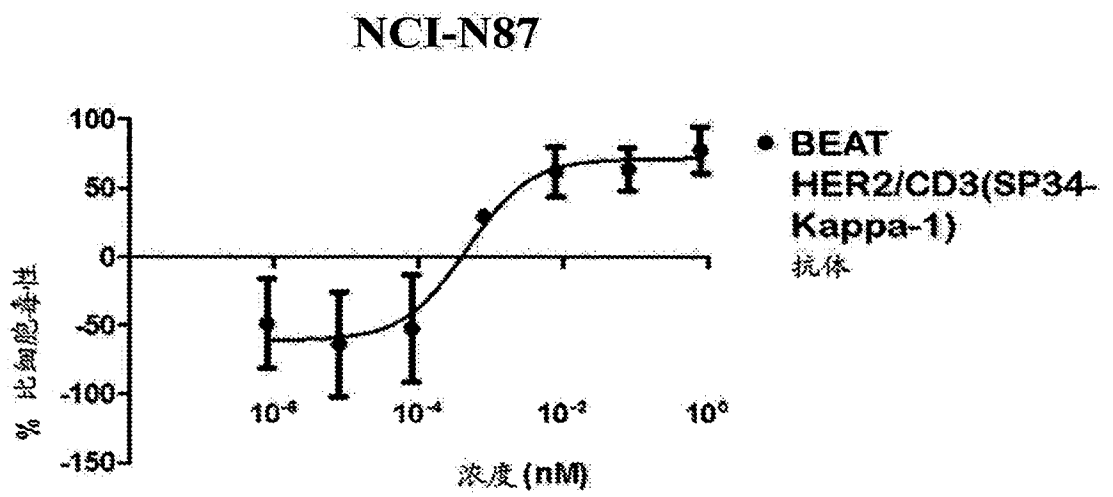

NCI-N87
100
50
0
-50
-100
-150
% 比细胞毒性
10⁻⁶
10⁻⁴
10⁻²
10⁰
浓度 (nM)
BEAT
HER2/CD3(SP34-
Kappa-1)
抗体

图17F

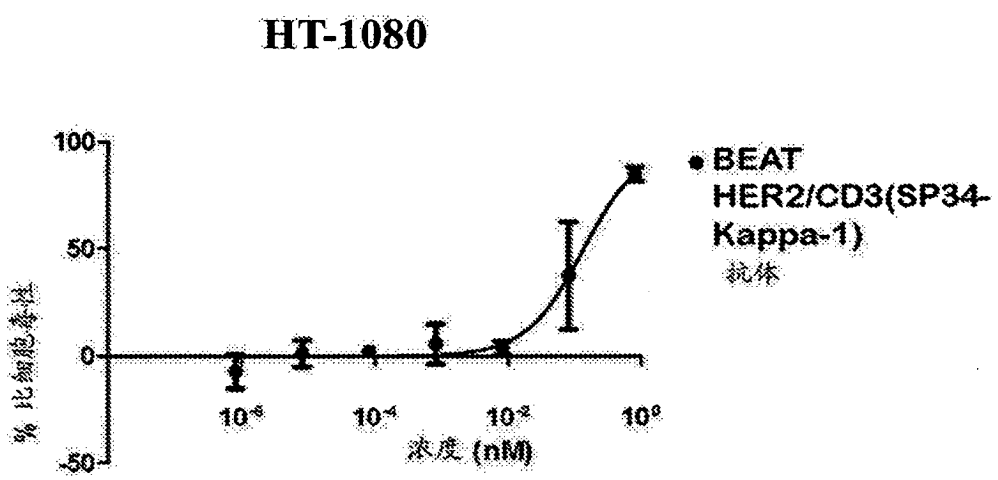

HT-1080
100
50
0
-50
% 比细胞毒性
10^{-6}
10^{-4}
10^{-2}
10^{0}
浓度 (nM)
BEAT HER2/CD3(SP34-Kappa-1) 抗体

图17G

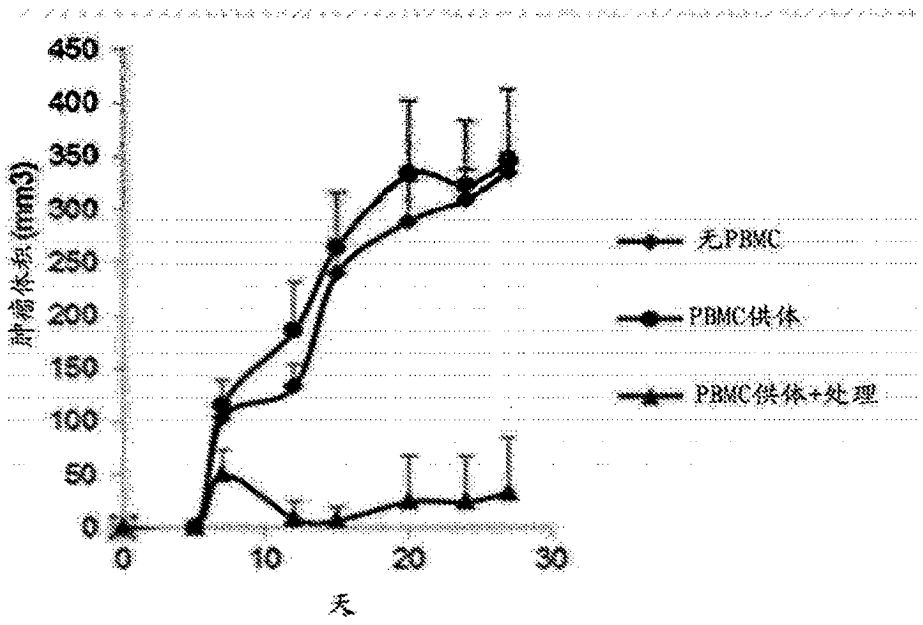

450
400
350
300
250
200
150
100
50
0
肿瘤体积 (mm3)
0
10
20
30
天
无PBMC
PBMC供体
PBMC供体+处理

图18A

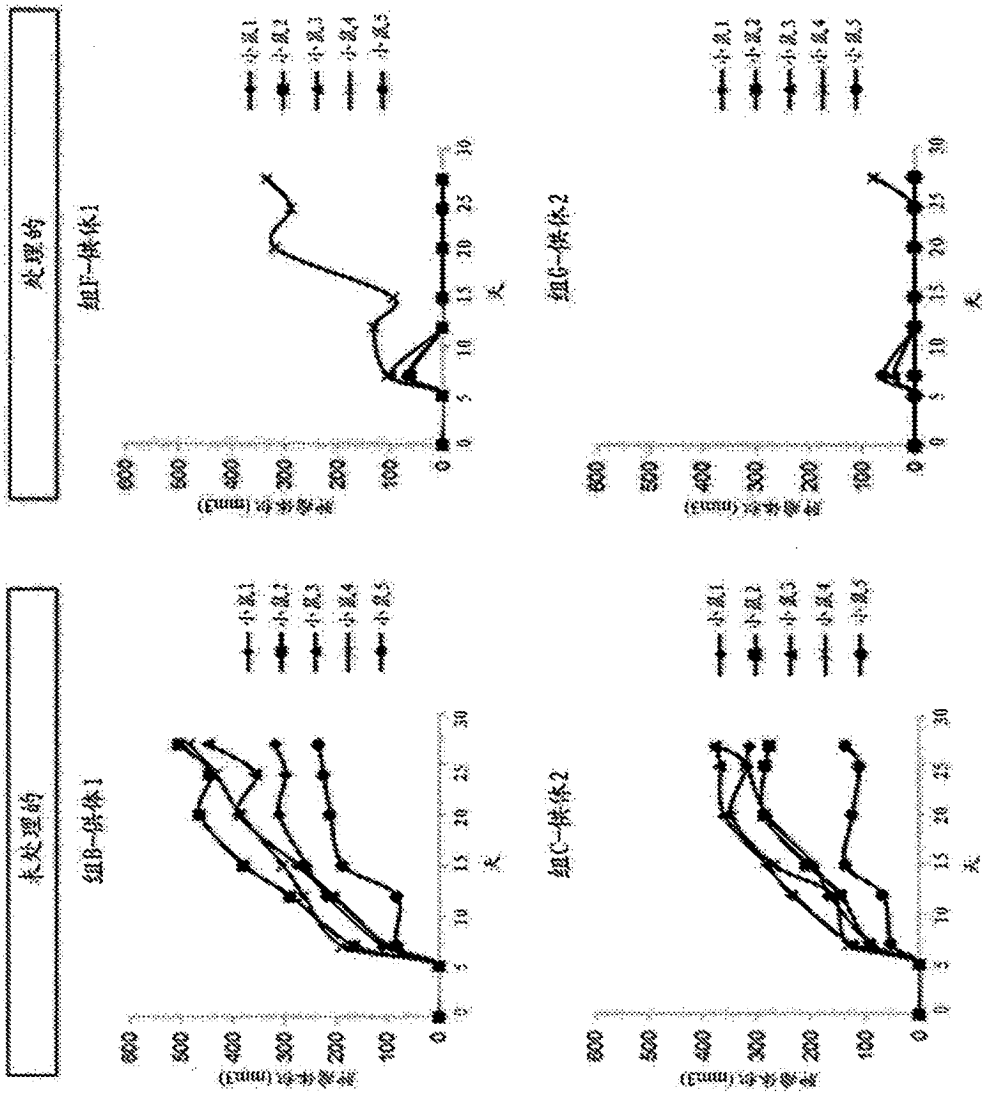

处理的
未处理的
组F—供体1
组G—供体2
组B—供体1
组C—供体2
小盒1
小盒2
小盒3
小盒4
小盒5
天
平均体积 (mm3)

图18B

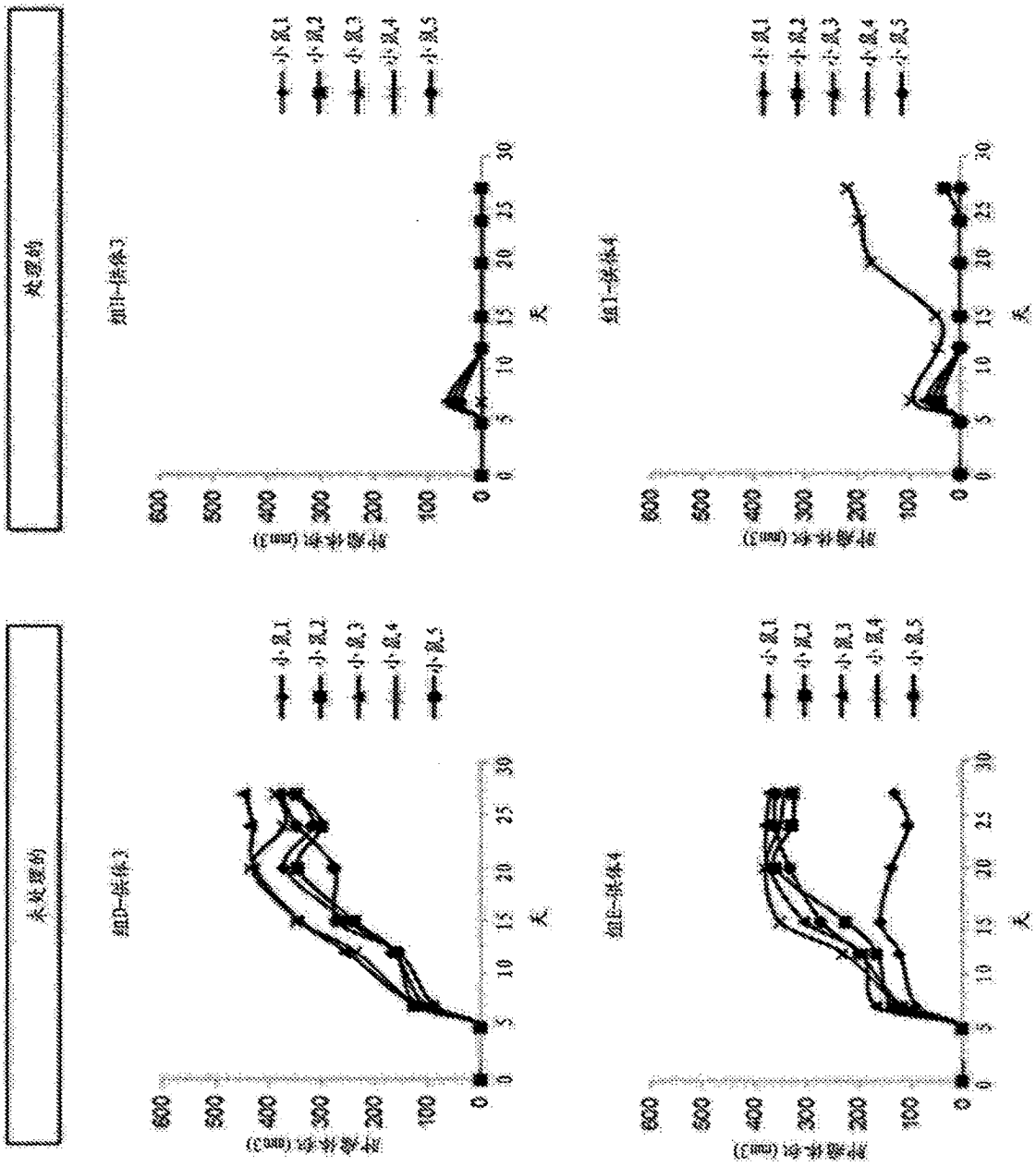

处理的
小鼠1
小鼠2
小鼠3
小鼠4
小鼠5
组H-株体3
组I-株体4
未处理的
组D-株体3
组E-株体4
天
0
5
10
15
20
25
30
100
200
300
400
500
600

图18C

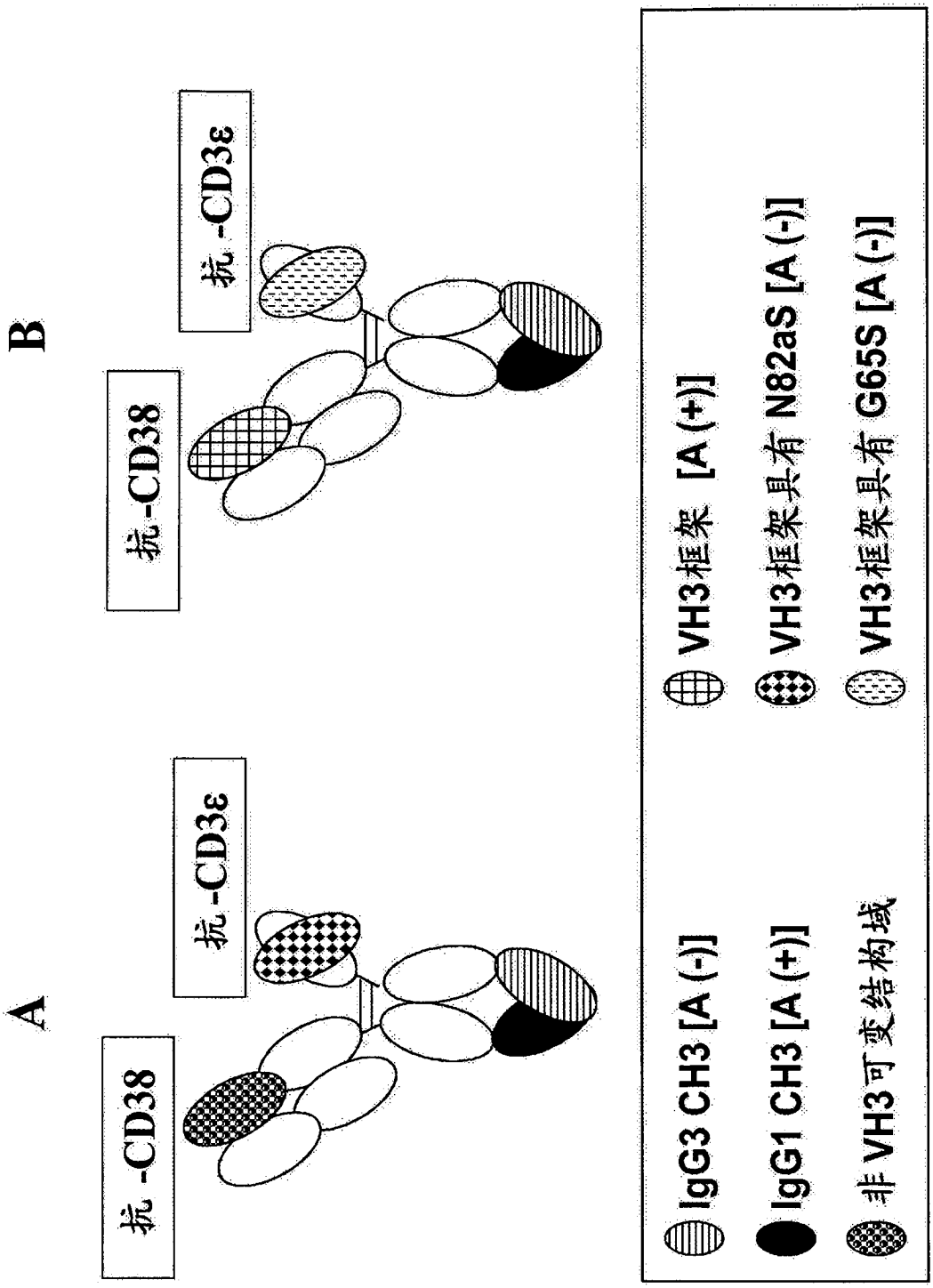

A
抗 -CD38
抗 -CD3ε
B
抗 -CD38
抗 -CD3ε
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
非 VH3可变结构域
VH3框架 [A (+)]
VH3框架具有 N82aS [A (-)]
VH3框架具有 G65S [A (-)]

图19

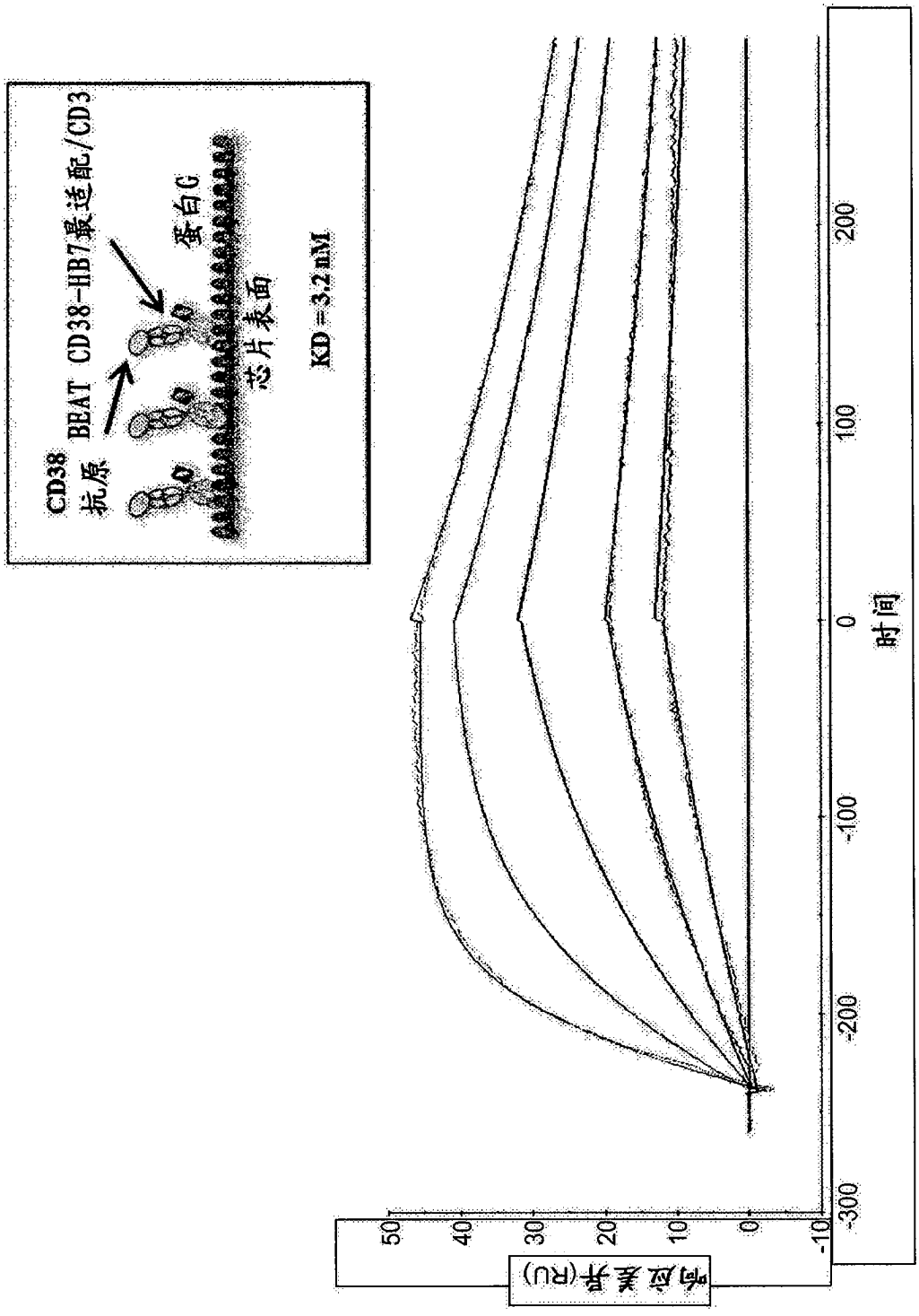
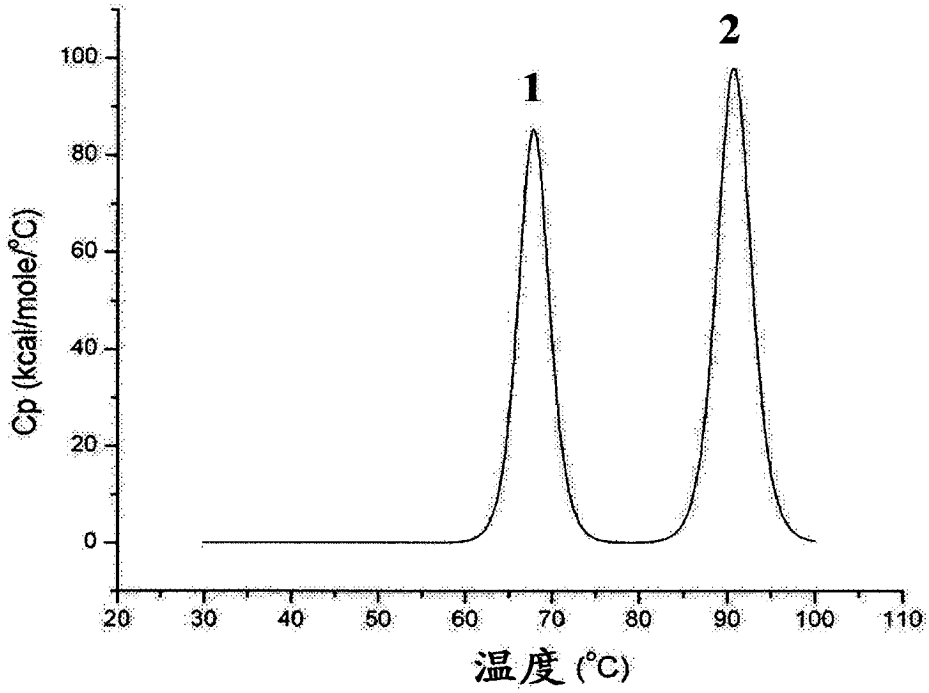

CD38
抗原
BEAT CD38-HB7最适配/CD3
蛋白G
芯片表面
KD = 3.2 nM
响应差异(RU)
50
40
30
20
10
0
-10
-300
-200
-100
0
100
200
时间

图20A



Tm(1): 67.9°C (scFv); Tm(2): 90.8°C (FAB)

图20B

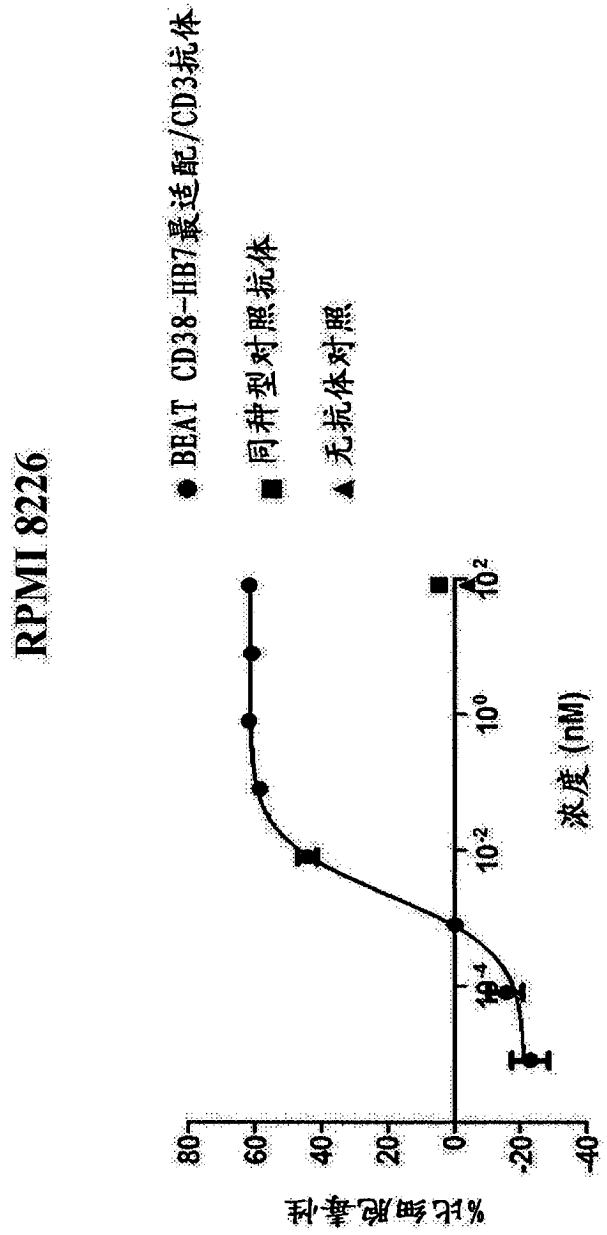

RPMI 8226
● BEAT CD38-HB7最适配/CD3抗体
■ 同种型对照抗体
▲ 无抗体对照
%比细胞毒性
80
60
40
20
0
-20
-40
10^{-4}
10^{-2}
10^{0}
10^{2}
浓度 (nM)

图21

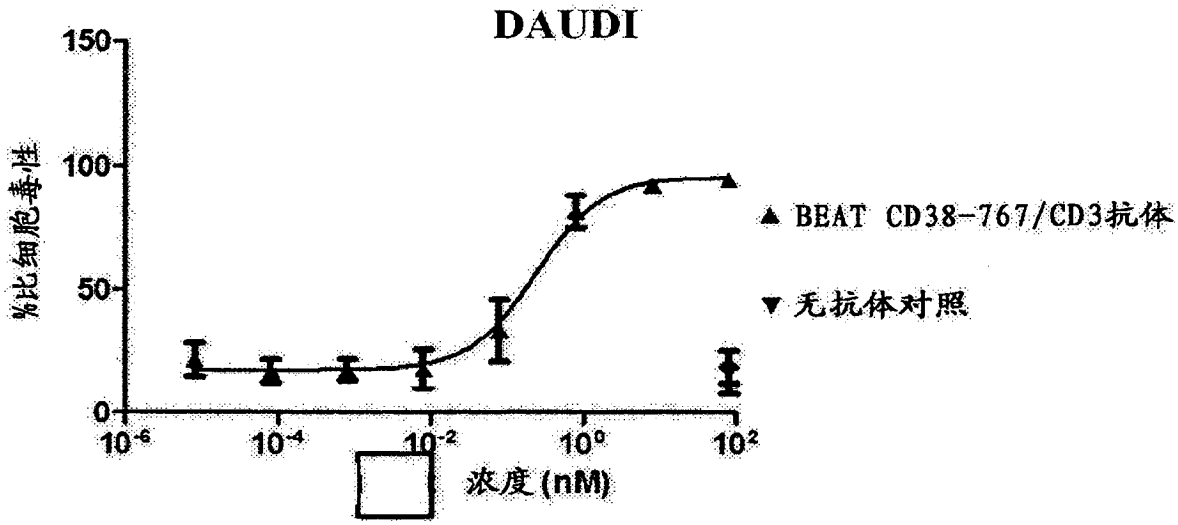

DAUDI
150
100
50
0
%比细胞毒性
10-6
10-4
10-2
100
102
浓度(nM)
▲ BEAT CD38-767/CD3抗体
▼ 无抗体对照

图22

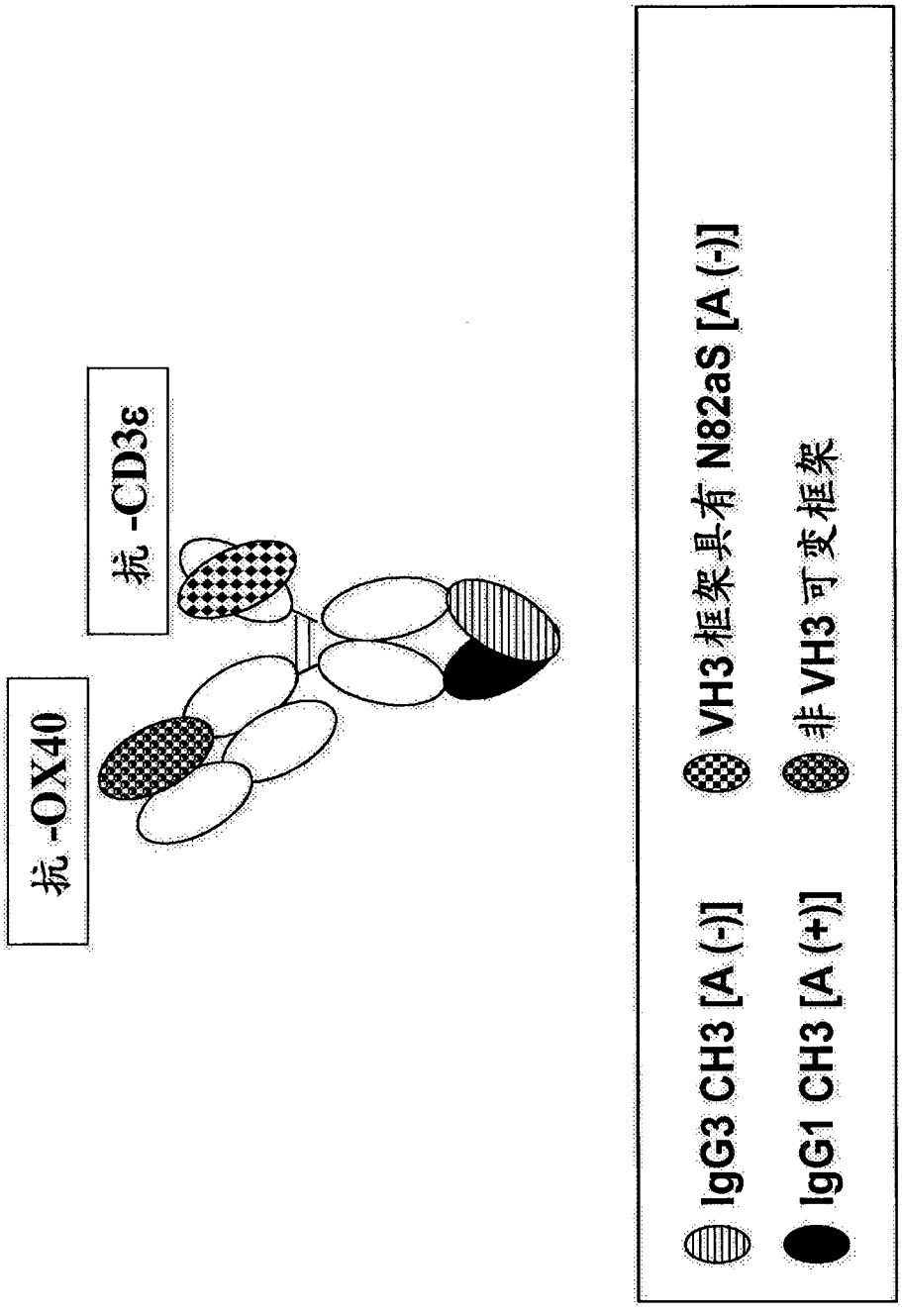


图23

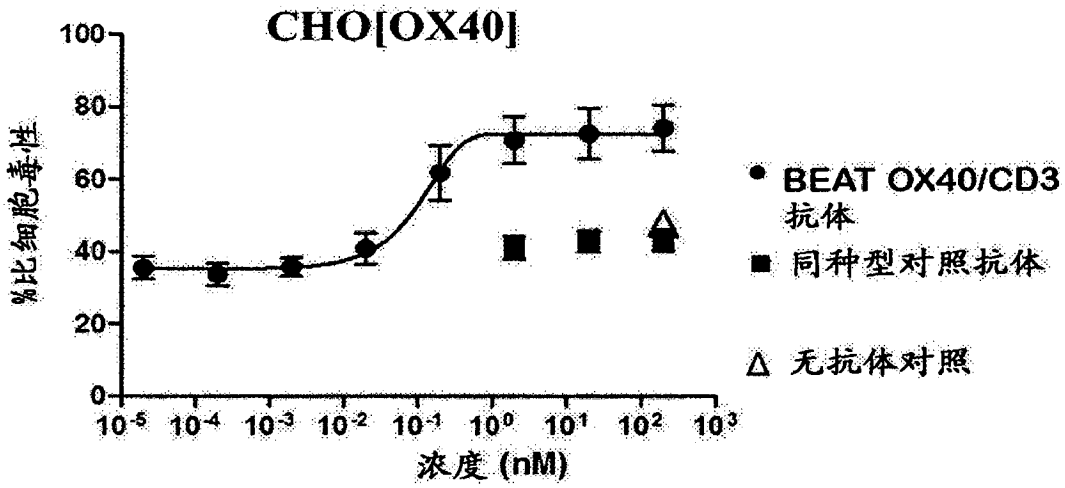

CHO[OX40]
%比细胞毒性
100
80
60
40
20
0
10-5 10-4 10-3 10-2 10-1 100 101 102 103
浓度 (nM)
BEAT OX40/CD3 抗体
同种型对照抗体
无抗体对照

图24

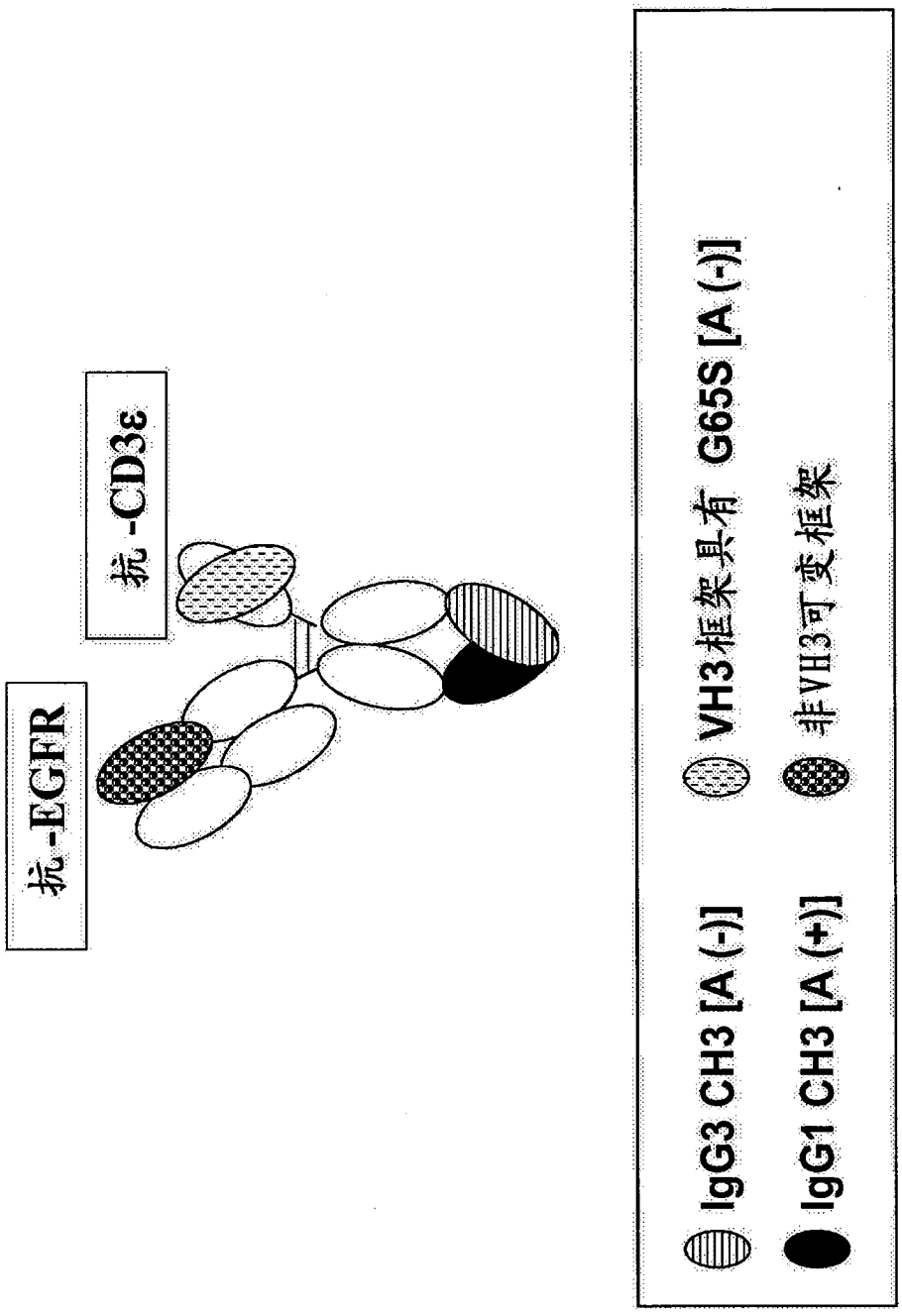


图25

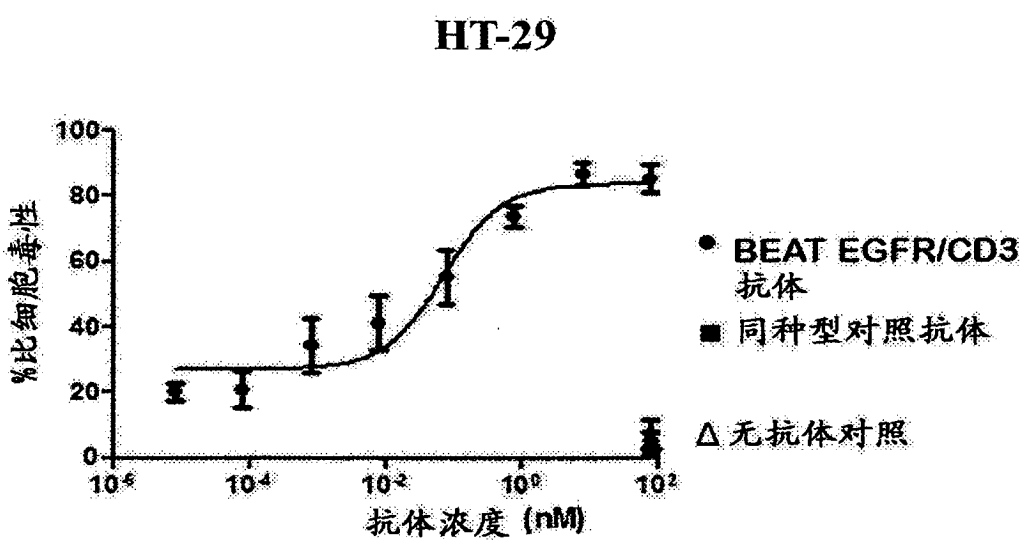

HT-29
%比细胞毒性
100
80
60
40
20
0
10^{-6}
10^{-4}
10^{-2}
10^{0}
10^{2}
抗体浓度 (nM)
BEAT EGFR/CD3 抗体
同种型对照抗体
无抗体对照

图26

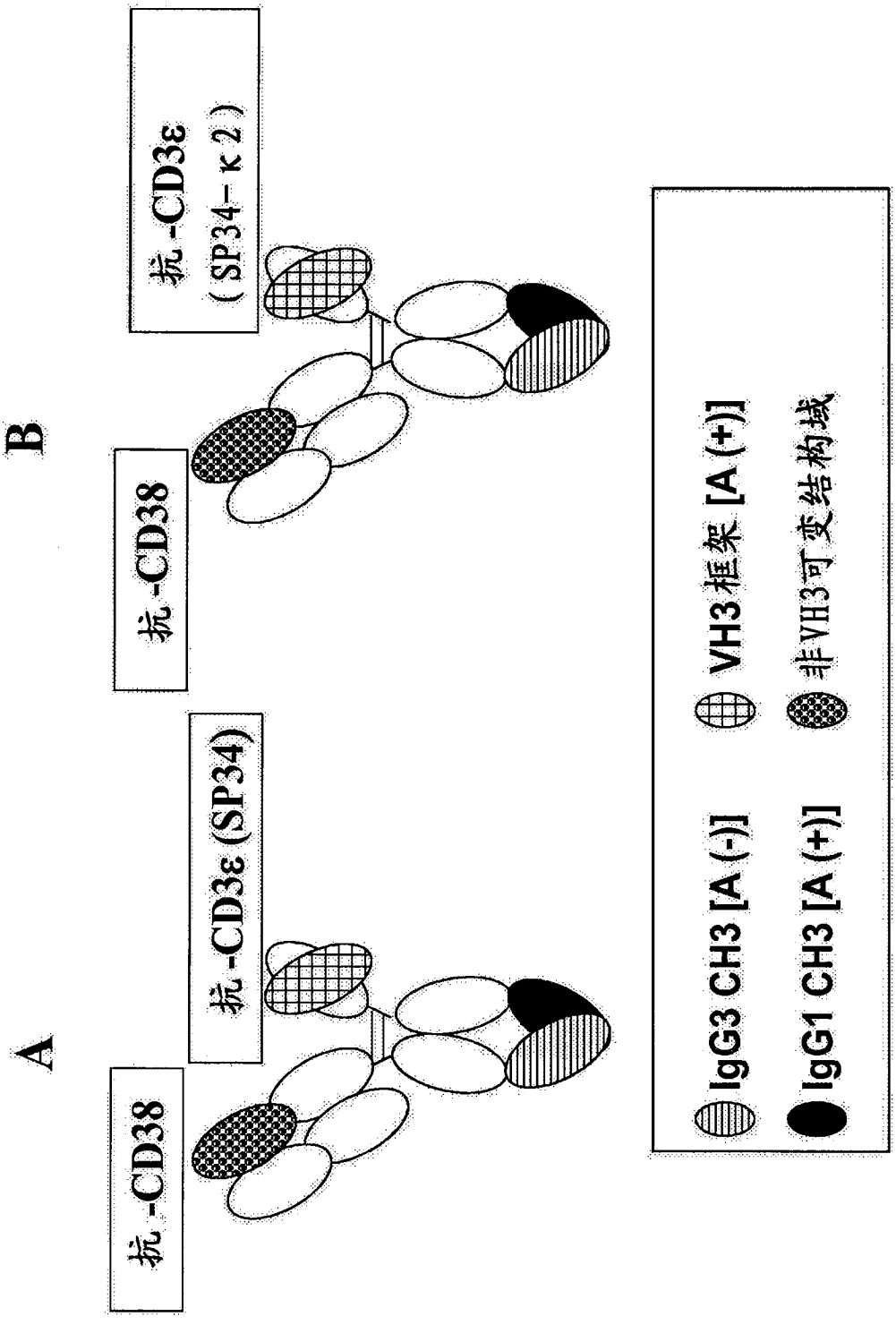

A
抗-CD38
抗-CD3ε (SP34)
B
抗-CD38
抗-CD3ε
(SP34-κ2)
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 框架 [A (+)]
非VH3可变结构域

图27

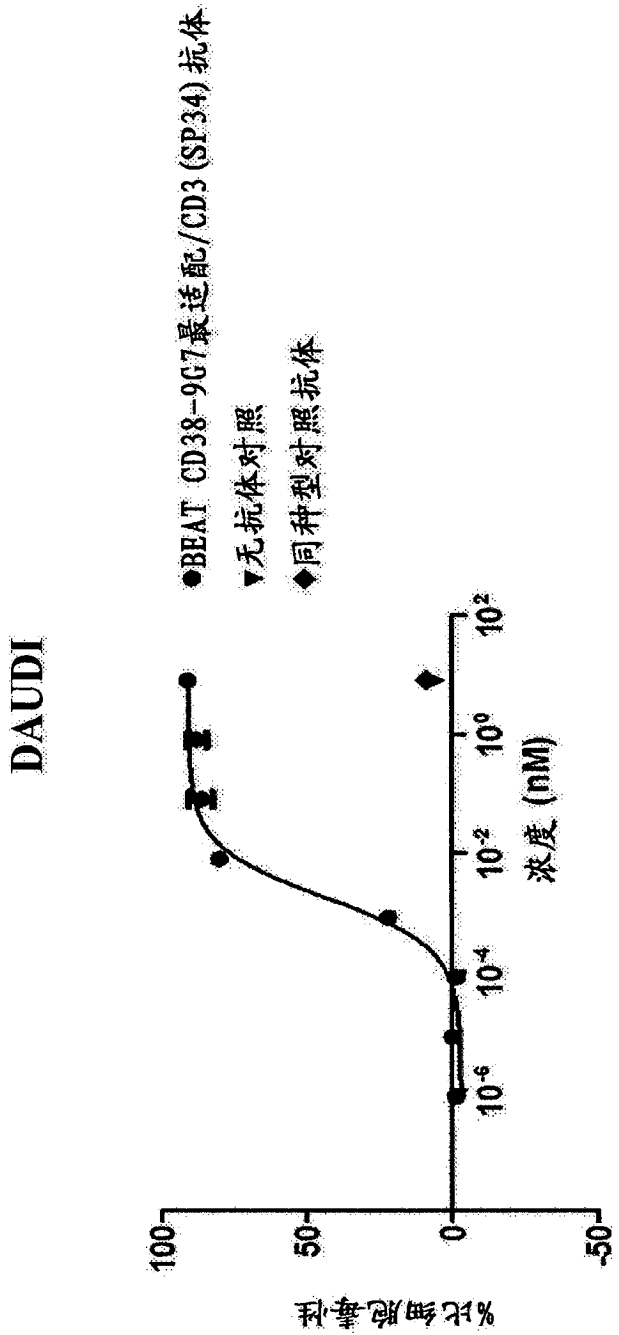

DAUDI
●BEAT CD38-9G7最适配/CD3(SP34)抗体
▼无抗体对照
◆同种型对照抗体
%比细胞毒性
100
50
0
-50
10^{-6}
10^{-4}
10^{-2}
10^{0}
10^{2}
浓度(nM)

图28

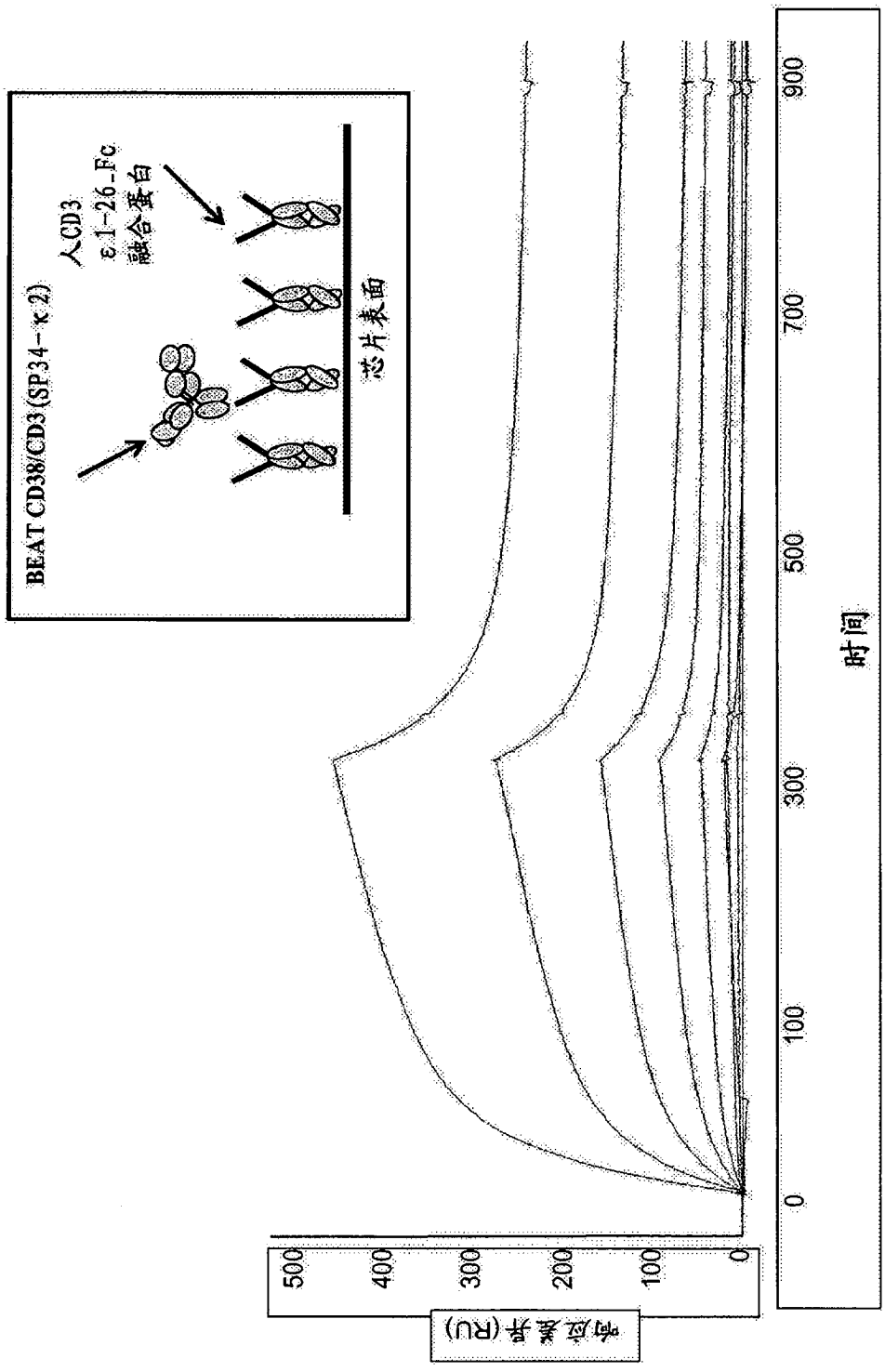

BEAT CD38/CD3 (SP34-κ2)
人CD3
ε.1-26_Fc
融合蛋白
芯片表面
响应差异(RU)
500
400
300
200
100
0
0
100
300
500
700
900
时间

图29

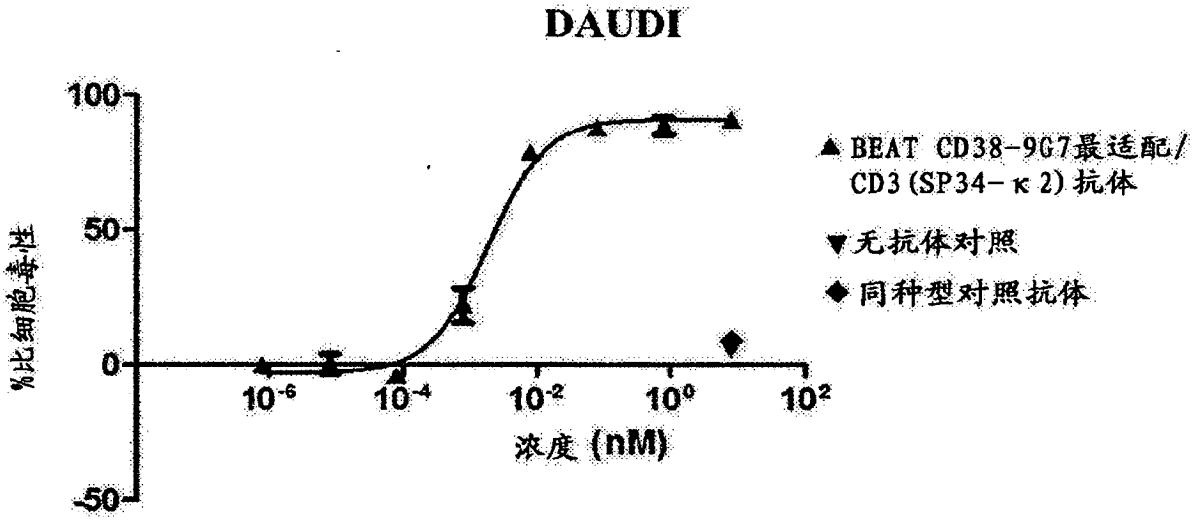

DAUDI
100
50
0
-50
%比细胞毒性
10-6
10-4
10-2
100
102
浓度 (nM)
▲ BEAT CD38-9G7最适配/ CD3 (SP34-κ2) 抗体
▼无抗体对照
◆ 同种型对照抗体

图30

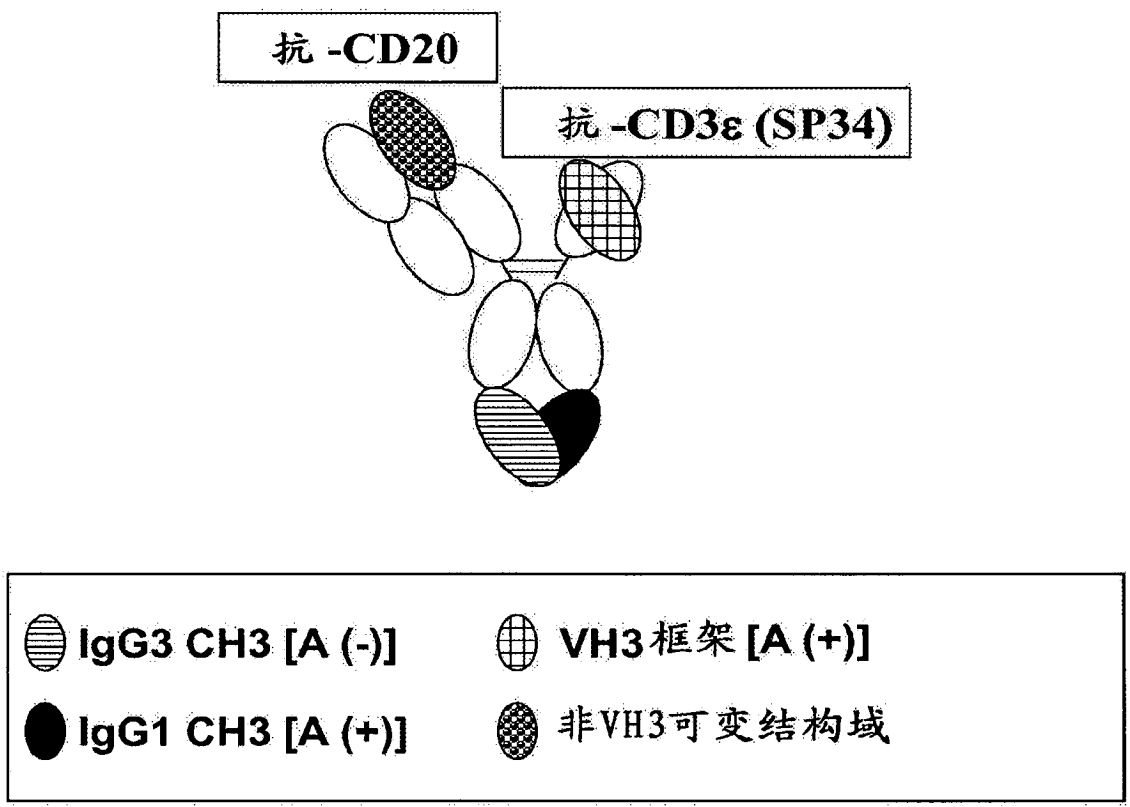

抗 -CD20
抗 -CD3ε (SP34)
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3框架 [A (+)]
非VH3可变结构域

图31

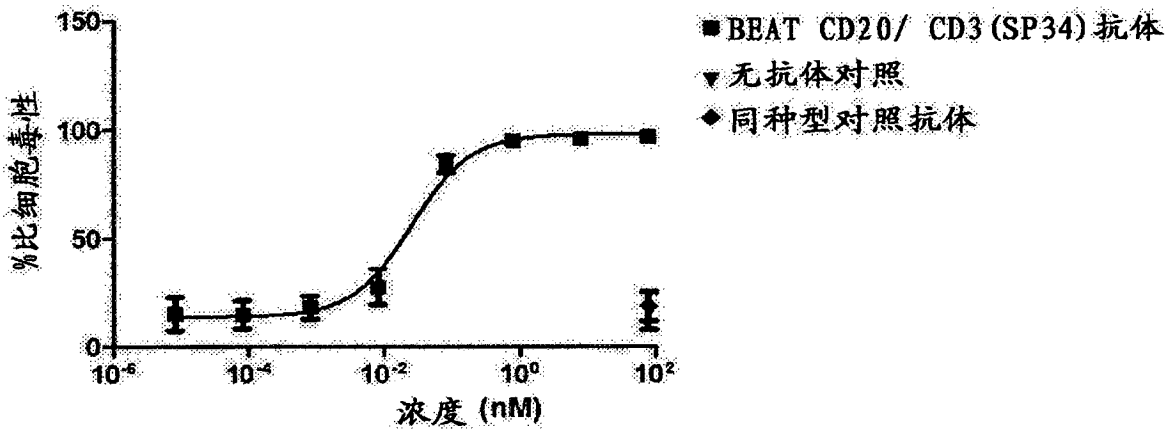

DAUDI
%比细胞毒性
150
100
50
0
10^{-6}
10^{-4}
10^{-2}
10^{0}
10^{2}
浓度 (nM)
■BEAT CD20/ CD3(SP34)抗体
▼无抗体对照
◆同种型对照抗体

图32

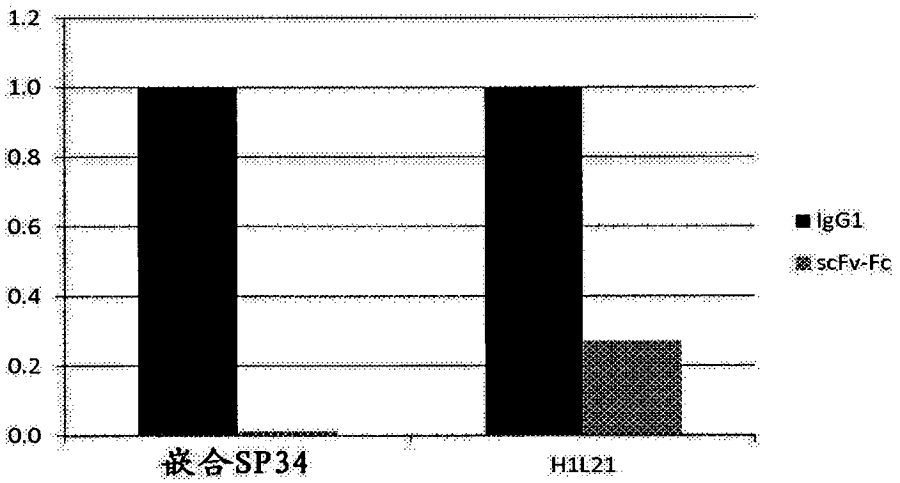

1.2
1.0
0.8
0.6
0.4
0.2
0.0
嵌合SP34
H1L21
■IgG1
scFv-Fc

图33

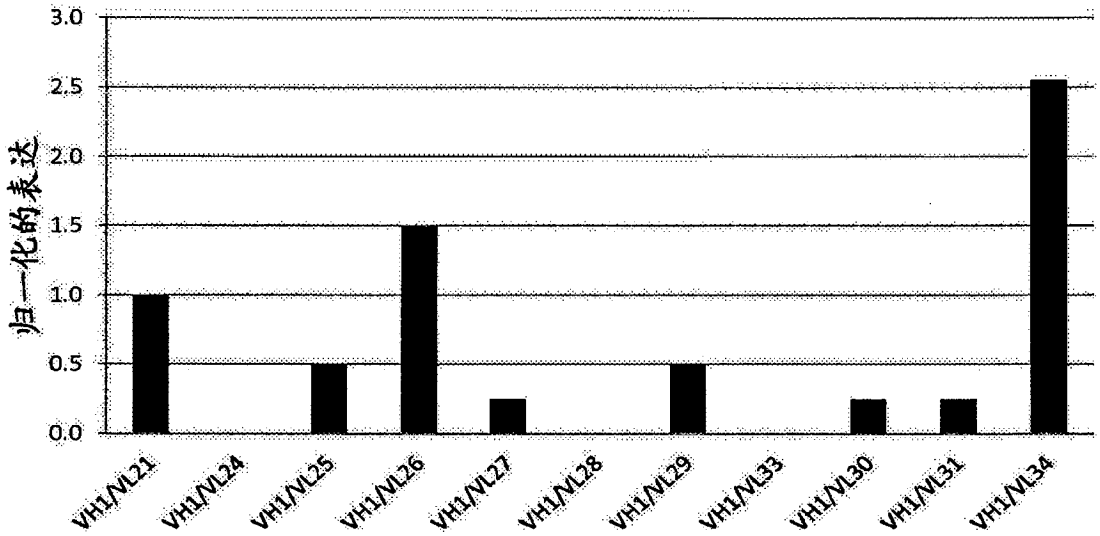

归一化的表达
3.0
2.5
2.0
1.5
1.0
0.5
0.0
VH1/VL21
VH1/VL24
VH1/VL25
VH1/VL26
VH1/VL27
VH1/VL28
VH1/VL29
VH1/VL33
VH1/VL30
VH1/VL31
VH1/VL34

图34

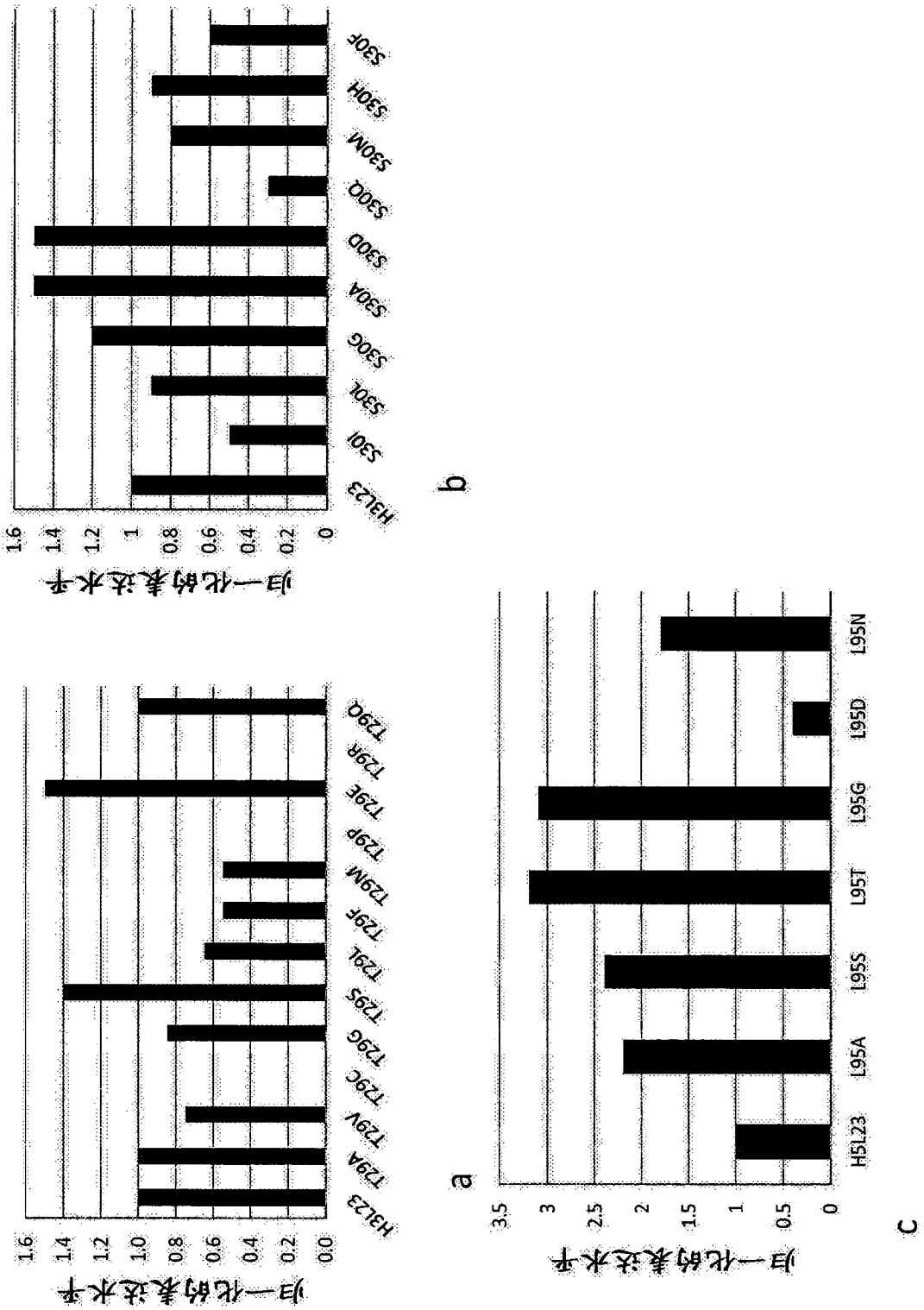

a
归一化的表达水平
3.5
3
2.5
2
1.5
1
0.5
0
H5L23
L95A
L95S
L95T
L95G
L95D
L95N
b
归一化的表达水平
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
H3L23
S30I
S30L
S30G
S30A
S30D
S30Q
S30M
S30H
S30F
c
归一化的表达水平
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
H3L23
T29A
T29V
T29C
T29G
T29S
T29L
T29F
T29M
T29P
T29E
T29R
T29Q

图35

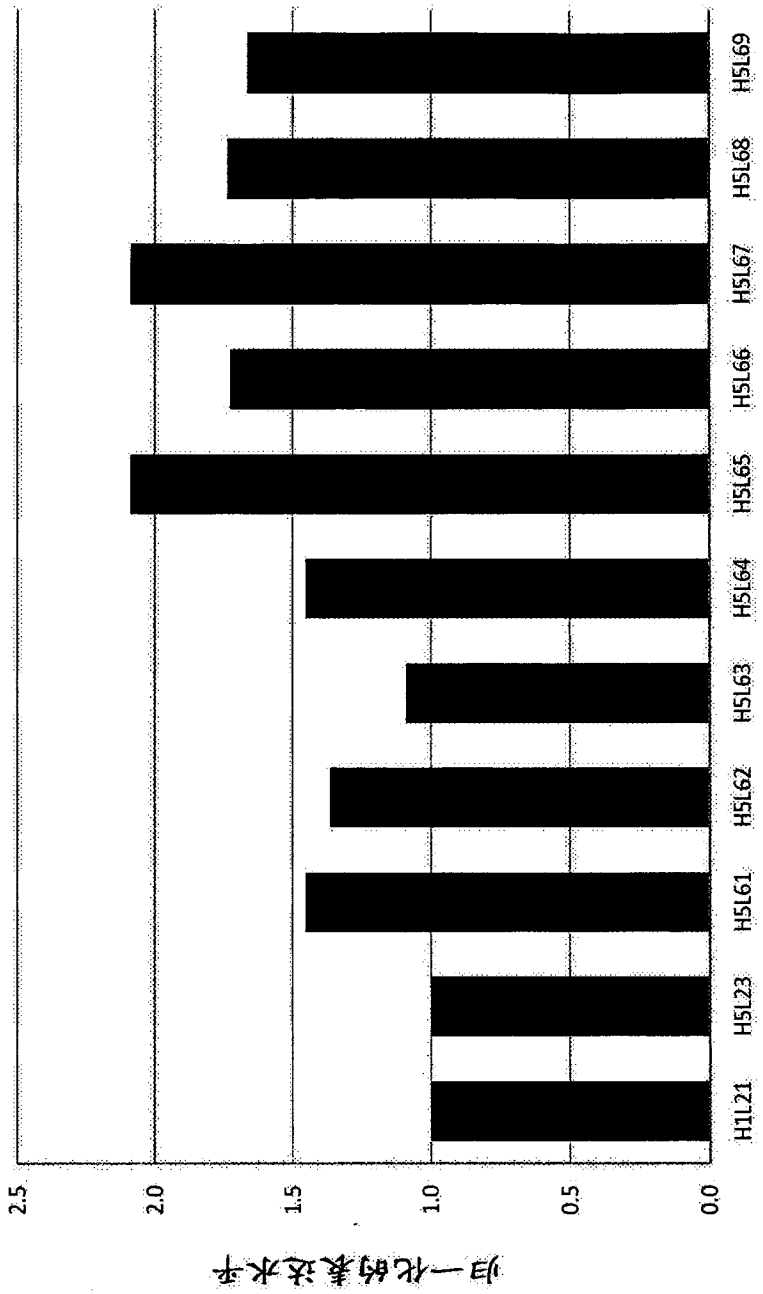
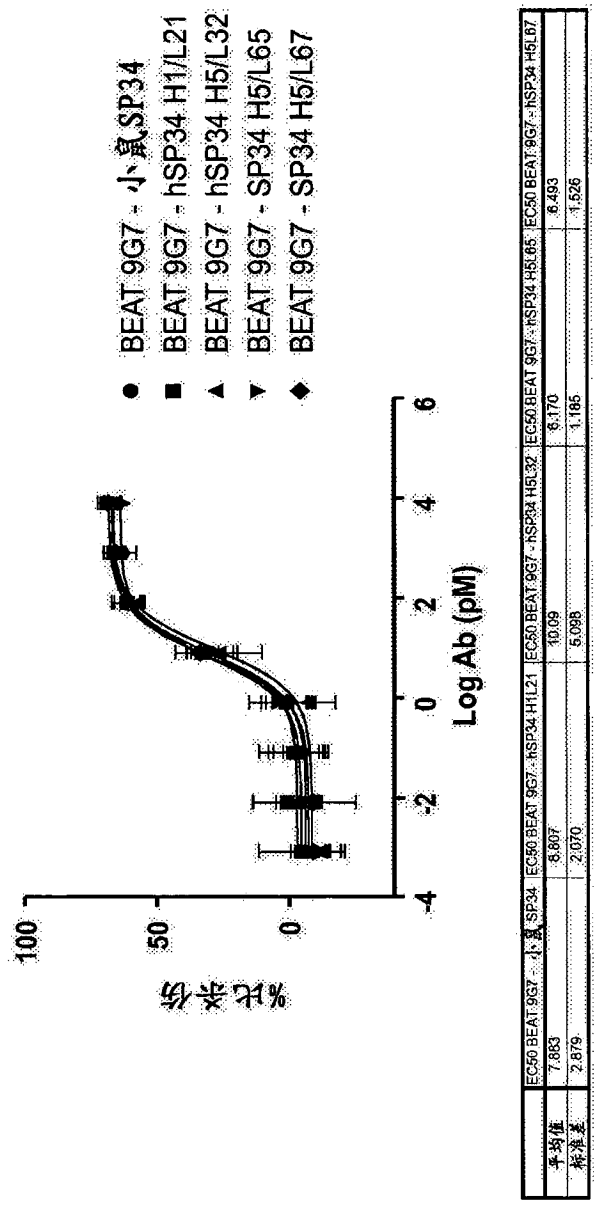
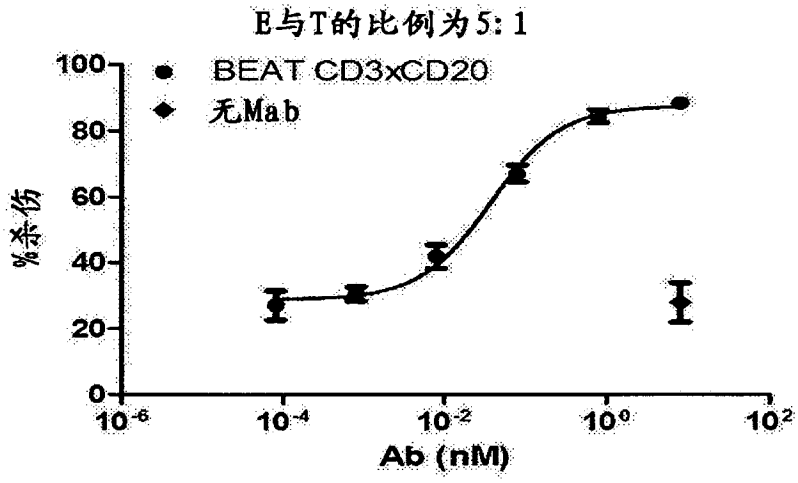

H1121
H5123
H5161
H5162
H5163
H5164
H5165
H5166
H5167
H5168
H5169
2.5
2.0
1.5
1.0
0.5
0.0
归一化的表达水平

图36



	EC50 BEAT 9G7 - 小鼠SP34	EC50 BEAT 9G7 - hSP34 H1/L21	EC50 BEAT 9G7 - hSP34 H5/L32	EC50 BEAT 9G7 - hSP34 H5/L65	EC50 BEAT 9G7 - hSP34 H5/L67
平均值	7.883	8.807	10.09	6.170	6.493
标准差	2.879	2.070	5.098	1.185	1.526

图37



BEAT CD3xCD20	EC50 (pM)
E:T的比例5:1	37.56

图38

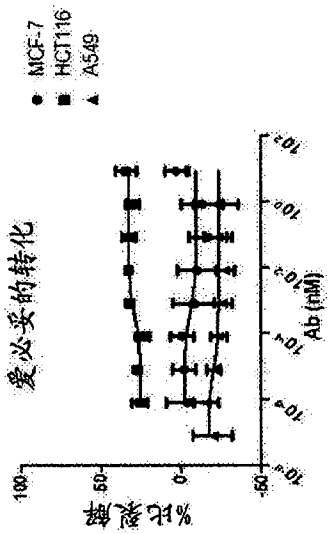


图39

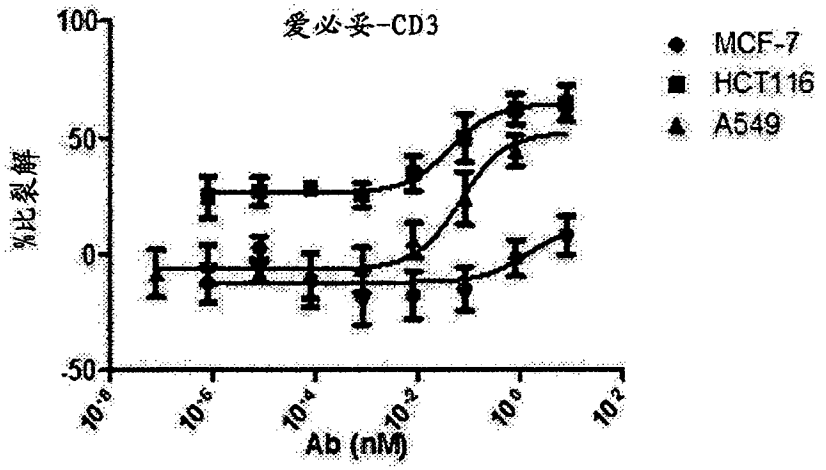

爱必妥-CD3
MCF-7
HCT116
A549
%比裂解
100
50
0
-50
Ab (nM)

图40

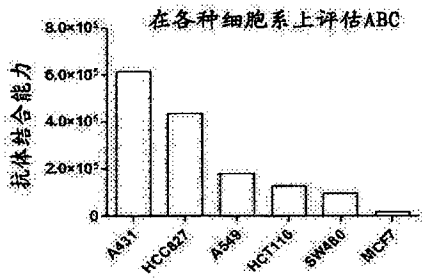

在各种细胞系上评估ABC
抗体结合能力
A431
HCC827
A549
HCT116
SW480
MCF7

图41

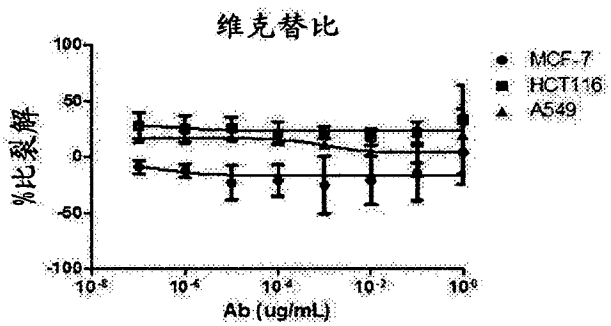
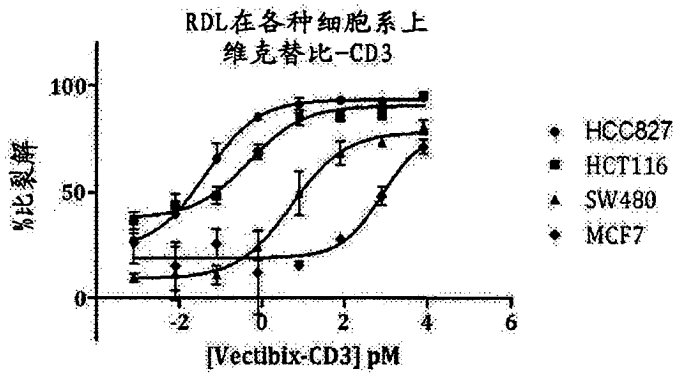

维克替比
MCF-7
HCT116
A549
%比裂解
100
50
0
-50
-100
Ab (ug/mL)

图42



	HCC827	HCT116	SW480	MCF7
EC50	0.04302	0.4863	5.260	795.8

图43

Abstract

The present invention describes novel hetero-dimeric immunoglobulins or fragments thereof which bind to CD3 and a disease associated antigen. These hetero-dimeric immunoglobulins have been engineered to promote hetero-dimer formation during expression and can be purified to a high degree using a Protein A differential purification technique.