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(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING A BISPECIFIC ANTIBODY SPECIFIC FOR EPCAM

(57) **Abrégé/Abstract:**

The present invention provides a pharmaceutical composition comprising a bispecific single chain antibody construct. Said bispecific single chain antibody construct is characterized to comprise or consist of at least two domains, whereby one of said at least two domains specifically binds to human EpCAM and comprises at least one CDR-H3 region comprising the amino acid sequence NXID antigen and a second domain binds to human CD3 antigen. The invention further provides a process for the production of the pharmaceutical composition of the invention, a method for the prevention, treatment or amelioration of a tumorous disease and the use of the disclosed bispecific single chain antibody construct and corresponding means in the prevention, treatment or amelioration of a tumorous disease.



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(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING A BISPECIFIC ANTIBODY FOR EPCAM

(57) Abstract: The present invention provides a pharmaceutical composition comprising a bispecific single chain antibody construct. Said bispecific single chain antibody construct is characterized to comprise or consist of at least two domains, whereby one of said at least two domains specifically binds to human EpCAM and comprises at least one CDR-H3 region comprising the amino acid sequence NXID antigen and a second domain binds to human CD3 antigen. The invention further provides a process for the production of the pharmaceutical composition of the invention, a method for the prevention, treatment or amelioration of a tumorous disease and the use of the disclosed bispecific single chain antibody construct and corresponding means in the prevention, treatment or amelioration of a tumorous disease.



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PHARMACEUTICAL COMPOSITION COMPRISING A BISPECIFIC ANTIBODY SPECIFIC FOR EPCAM

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The invention relates to a pharmaceutical composition comprising a bispecific single chain antibody construct. Said bispecific single chain antibody construct is characterized to comprise or consist of at least two domains, whereby one of said at least two domains specifically binds to human EpCAM antigen and comprises at least one CDR-H3 region comprising the amino acid sequence NXD and a second domain binds to human CD3 antigen. The invention further provides a process for the production of the pharmaceutical composition of the invention, a method for the prevention, treatment or amelioration of a tumorous disease and the use of the disclosed bispecific single chain antibody construct and corresponding means in the prevention, treatment or amelioration of a tumorous disease.

A variety of documents is cited throughout this specification. The disclosure content of said documents is herewith incorporated by reference.

Epithelial cell adhesion molecule (EpCAM, also called 17-1A antigen, KSA, EGP40, GA733-2, ks1-4 or esa) is a 40-kDa membrane-integrated glycoprotein of 314 amino acids with specific expression in certain epithelia and on many human carcinomas (reviewed in Balzar, J. Mol. Med. 1999, 77, 699-712). EpCAM was discovered and subsequently cloned through its recognition by the murine monoclonal antibody 17-1A/edrecolomab (Goettlinger, Int J Cancer. 1986; 38, 47-53 and Simon, Proc. Natl. Acad. Sci. USA. 1990; 87, 2755-2759). Monoclonal antibody 17-1A was generated by immunization of mice with human colon carcinoma cells (Koprowski, Somatic Cell Genet. 1979, 5, 957-971).

The EGF-like repeats of EpCAM were shown to mediate lateral and reciprocal interactions in homophilic cell adhesion (Balzar, Mol. Cell. Biol. 2001, 21, 2570-2580) and, for that reason, is predominantly located between epithelial cells (Litvinov, J Cell Biol. 1997, 139, 1337-1348, Balzar, J Mol Med. 1999, 77, 699-712 and Trebak, J Biol Chem. 2001, 276, 2299-2309). EpCAM serves to adhere epithelial cells in an oriented and highly ordered fashion (Litvinov, J Cell Biol. 1997, 139, 1337-1348). Data from experiments with transgenic mice and rats expressing human EpCAM on their epithelia suggest that EpCAM on normal tissue may however not be accessible to systemically administered antibody (McLaughlin, Cancer Immunol. Immunother., 1999, 48, 303-311). Upon malignant transformation of epithelial cells the rapidly growing tumor cells are abandoning the high cellular order of epithelia. Consequently, the surface distribution of EpCAM becomes less restricted and the molecule better exposed on tumor cells. Due to their epithelial cell origin, tumor cells from most carcinomas still express EpCAM on their surface.

In vivo, expression of EpCAM is related to increased epithelial proliferation and negatively correlates with cell differentiation (for review see Balzar, 1999, J. Mol. Med. 77, 699-712). Expression of EpCAM, as detected by immunohistochemistry using anti-EpCAM monoclonal antibodies, is essentially seen with all major carcinomas (reviewed in Balzar, J Mol Med. 1999, 77, 699-712). Best EpCAM expression was observed with non-small cell lung cancer (De Bree, Nucl Med Commun. 1994, 15, 613-27) and prostate cancer (Zhang, Clin Cancer Res. 1998, 4, 295-302) where 100% of tumor patient samples showed positive EpCAM staining. In these studies, EpCAM is also reported to homogeneously stained tumor tissues indicating that the antigen is expressed on a large proportion of cells of a given tumor. Because of its widespread expression, EpCAM is referred to as a "pan-carcinoma" antigen.

EpCAM has been shown in various studies to be beneficial in diagnosis and therapy of various carcinomas. Furthermore, in many cases, tumor cells were

observed to express EpCAM to a much higher degree than their parental epithelium or less aggressive forms of said cancers. For example, EpCAM expression was shown to be significantly higher on neoplastic tissue and in adenocarcinoma than on normal prostate epithelium (n=76; p<0.0001), suggesting that increased EpCAM expression represents an early event in the development of prostate cancer (Poczatek, J Urol., 1999, 162, 1462-1644). In addition, in the majority of both squamous and adenocarcinomas of the cervix a strong EpCAM expression correlates with an increased proliferation and the disappearance of markers for terminal differentiation (Litvinov, Am. J. Pathol. 1996, 148, 865-75). One example is breast cancer where overexpression of EpCAM on tumor cells is a predictor of survival (Gastl, Lancet. 2000, 356, 1981-1982). Furthermore, EpCAM has been described as a marker for the detection of disseminated tumor cells in patients suffering from squamous cell carcinoma of the head, neck and lung (Chaubal, Anticancer Res 1999, 19, 2237-2242, Piyathilake, Hum Pathol. 2000, 31, 482-487). Normal squamous epithelium, as found in epidermis, oral cavity, epiglottis, pharynx, larynx and esophagus did not significantly express EpCAM (Quak, Hybridoma, 1990, 9, 377-387).

In addition to the above-mentioned carcinomas, EpCAM has been shown to be expressed on the majority of primary, metastatic, and disseminated NSCLC (non small cell lung cancer cells) (Passlick, Int J Cancer, 2000, 87, 548-552), on gastric and gastro-oesophageal junction adenocarcinomas (Martin, J Clin Pathol 1999, 52, 701-4) and in cell lines derived from colorectal, pancreatic carcinomas and breast carcinomas (Szala, Proc Natl Acad Sci U S A 1990, 87, 3542-6, Packeisen, Hybridoma, 1999, 18, 37-40).

Clinical trials have shown that the use of antibodies directed against 17-1A (EpCAM) for treatment of patients with surgically completely resected colorectal carcinoma leads to a significant benefit concerning the overall survival and the frequency of distant metastasis (Riethmüller, Lancet, 1994, 343, 1177-1183). Murine monoclonal antibody against EpCAM was found to reduce the 5-year

mortality (Riethmüller, Lancet, 1994, 343, 1177-1183) and also the 7-year mortality (Riethmüller, Proceedings of the American Society of Clinical Oncology, 1996, 15, 444) of patients with minimal residual disease. Example of murine monoclonal antibody recognizing EpCAM is Edrecolomab (Panorex) (Koprowski, Somatic Cell Genet. 1979, 5, 957-971 and Herlyn, Cancer Res., 1980, 40, 717-721). However, the first administration of Panorex during adjuvant immunotherapy of colon cancer led to the development and exacerbation of Wegener's granulomatosis suggesting that mAb 17-1A should be applied cautiously in a patient with autoimmune disease (Franz, Onkologie, 2000, 23, 472-474). The limitations of Panorex are the rapid formation of human anti-mouse antibodies (HAMA), the limited ability to interact by its murine IgG2a Fc-portion with human immune effector mechanisms and the short half-life in circulation (Frodin, Cancer Res., 1990, 50, 4866-4871). Furthermore, the murine antibody caused immediate-type allergic reactions and anaphylaxis upon repeated injection in patients (Riethmüller, Lancet. 1994, 343, 1177-1183, Riethmüller, J Clin Oncol., 1998, 16, 1788-1794 and Mellstedt, Annals New York Academy of Sciences. 2000, 910, 254-261).

Humanized anti-EpCAM antibody called 3622W94 resulted in pancreatitis and increased serum levels of amylase, as being indicative for damage of pancreas epithelium, which were a dose-limiting toxicity of this high-affinity anti-EpCAM monoclonal antibody (LoBuglio, Proceedings of the American Society of Clinical Oncology (Abstract). 1997, 1562 and Khor, Proceedings of the American Society of Clinical Oncology (Abstract), 1997, 847).

Bispecific antibodies comprising a region directed against EpCAM and a region directed against CD3 have also been described. The authors of Möller & Reisfeld 1991 Cancer Immunol. Immunother. 33:210-216 describe the construction of two different bispecific antibodies by fusing a hybridoma producing monoclonal antibody against EpCAM with either of the two hybridomas OKT3 and 9.3. Furthermore, Kroesen, Cancer Research, 1995, 55:4409-4415 describe a

quadroma bispecific monoclonal antibodies against CD3 (BIS-1) and EpCAM.

Other examples of bispecific antibodies against EpCAM comprise the bispecific antibody, BiUII, (anti-CD3 (rat IgG2b) x anti-EpCAM (mouse IgG2a)) a complete Ig molecule which also binds and activates Fc-receptor positive accessory cells (like monocytes/macrophages, NK cells and dendritic cells) through its Fc-region (Zeidler, J. Immunol., 1999, 163:1247-1252) and an anti-EpCAMxanti-CD3 bispecific antibody in the arrangement $V_{L17-1A}-V_{H17-1A}-V_{Hanti-CD3}-V_{Lanti-CD3}$ (Mack, Proc. Natl. Acad. Sci., 1995, 92:7021-7025).

In addition, other formats of antibody constructs comprising EpCAM have been described; e.g. a bispecific diabody having the structure $V_{H anti-CD3}-V_{L anti-EpCAM}-V_{H anti-EpCAM}-V_{L anti-CD3}$ (Helfrich, Int. J. Cancer, 1998, 76:232-239) and a trispecific antibody having two different tumour antigen specificities (two antigen binding regions which bind two different antigens on a tumour cell) and which may have a further specificity for an antigen localized on an effector cell (DE 195 31 348).

There exist various descriptions in the prior art of using phage display technology to identify antibodies or fragments thereof, which specifically bind to the human EpCAM antigen (De Kruif JMB, 1995, 248:97-105, WO 99/25818). However, it has been extremely difficult to identify antibodies against EpCAM, which show cytotoxic activity sufficient for therapeutic applications in a bispecific format.

It is therefore an aim of the present invention to provide a bispecific single chain molecule with a binding domain specific for EpCAM with strong cytotoxic activity mediated by target specific activation of T cells.

Thus, the technical problem underlying the present invention was to provide means

and methods for the generation of well tolerated and convenient medicaments for the treatment and or amelioration of tumorous diseases.

5 The solution to said technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to a composition, preferably a pharmaceutical composition, comprising a bispecific single chain antibody construct, whereby said construct comprises or consists of at least two binding
10 domains, whereby one of said domains binds to human EpCAM antigen and a second domain binds to human CD3 antigen, wherein said binding domain specific for EpCAM comprises at least one CDR-H3 region comprising the amino acid sequence NXD preferably in position 102 to 104 of SEQ ID NOs: 80, 88 and 96, or preferably in position 106 to 108 of SEQ ID NOs: 84 and 92, wherein X is an
15 aromatic amino acid.

Preferably or alternatively, the present invention relates to a composition, preferably a pharmaceutical composition, comprising a bispecific single chain antibody construct, whereby said construct comprises or consists of at least two domains, whereby one of said at least two domains specifically binds to human
20 EpCAM antigen and a second domain binds to human CD3 antigen, wherein said binding domain specific for EpCAM comprises at least one CDR-H3 region of least 9 amino acid residues and wherein said binding domain specific for EpCAM has a K_D value of more than 5×10^{-9} M.

25 In accordance with this invention, the term "pharmaceutical composition" relates to a composition for administration to a patient, preferably a human patient. In a preferred embodiment, the pharmaceutical composition comprises a composition for parenteral, transdermal, intraluminal, intra-arterial, intrathecal or intravenous administration or for direct injection into the tumor. It is in particular envisaged that
30 said pharmaceutical composition is administered to a patient via infusion or injection. Administration of the suitable compositions may be effected by different

ways, e.g., by intravenous, subcutaneous, intraperitoneal, intramuscular, topical or intradermal administration. The pharmaceutical composition of the present invention may further comprise a pharmaceutically acceptable carrier. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions, etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. A preferred dosage for administration might be in the range of 0.24 μg to 48 mg, preferably 0.24 μg to 24 mg, more preferably 0.24 μg to 2.4 mg, even more preferably 0.24 μg to 1.2 mg and most preferably 0.24 μg to 240 μg units per kilogram of body weight per day. Particularly preferred dosages are recited herein below. Progress can be monitored by periodic assessment. Dosages will vary but a preferred dosage for intravenous administration of DNA is from approximately 10^6 to 10^{12} copies of the DNA molecule. The compositions of the invention may be administered locally or systematically. Administration will generally be parenteral, e.g., intravenous; DNA may also be administered directly to the target site, e.g., by biolistic delivery to an internal or external target site or by catheter to a site in an artery. In an preferred embodiment, the pharmaceutical composition is administered subcutaneously and in an even more preferred embodiment intravenously. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated

Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishes, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. In addition, the pharmaceutical composition of the present invention might comprise proteinaceous carriers, like, e.g., serum albumine or immunoglobuline, preferably of human origin. It is envisaged that the pharmaceutical composition of the invention might comprise, in addition to the proteinaceous bispecific single chain antibody constructs or nucleic acid molecules or vectors encoding the same (as described in this invention), further biologically active agents, depending on the intended use of the pharmaceutical composition. Such agents might be drugs acting on the gastro-intestinal system, drugs acting as cytostatica, drugs preventing hyperurikemia, agents such as T-cell co-stimulatory molecules or cytokines, drugs inhibiting immune reactions (e.g. corticosteroids) and/or drugs acting on the circulatory system, e.g. on the blood pressure, known in the art.

Possible indications for administration of the composition(s) of the invention are tumorous diseases especially epithelial cancers/carcinomas such as breast cancer, colon cancer, prostate cancer, head and neck cancer, skin cancer, cancers of the genito-urinary tract, e.g. ovarian cancer, endometrial cancer, cervix cancer and kidney cancer, lung cancer, gastric cancer, cancer of the small intestine, liver cancer, pancreas cancer, gall bladder cancer, cancers of the bile duct, esophagus cancer, cancer of the salivatory glands and cancer of the thyroid gland. The administration of the composition(s) of the invention is especially indicated for minimal residual disease preferably early solid tumor, advanced solid tumor or metastatic solid tumor, which is characterized by the local and non-local reoccurrence of the tumor caused by the survival of single cells.

The invention further envisages the co-administration protocols with other compounds, e.g. bispecific antibody constructs, targeted toxins or other compounds, which act via T cells. The clinical regimen for co-administration of the inventive compound(s) may encompass co-administration at the same time, before or after the administration of the other component.

A possible approach to demonstrate the efficacy/activity of the inventive constructs is an in vivo model like mouse. Suitable models may be transgenic and chimeric mouse models. Mouse models expressing human CD3 and human EpCAM, a chimeric mouse model expressing murine CD3 and into which tumour cells expressing human EpCAM can be transfected and chimeric mouse models comprising nude mice into which human tumours expressing EpCAM can be transplanted or tumour cells expressing human EpCAM can be injected and, additionally, human PBMCs are injected. The term "bispecific single chain antibody construct" relates to a construct comprising two antibody derived binding domains.

One of said binding domains consists of variable regions (or parts thereof) of an antibody, antibody fragment or derivative thereof, capable of specifically binding to/interacting with human EpCAM antigen (target molecule 1). The second binding domain consists of variable regions (or parts thereof) of an antibody, antibody fragment or derivative thereof, capable of specifically binding to/interacting with human CD3 antigen (target molecule 2). As will be detailed below, a part of a variable region may be at least one CDR ("Complementary determining region"), most preferably at least the CDR3 region. Said two domains/regions in the single chain antibody construct are preferably covalently connected to one another as a single chain. This connection can be effected either directly (domain 1 [specific for the CD3 antigen] – domain 2 [specific for the EpCAM antigen] or domain 1 [specific for the EpCAM antigen] – domain 2 [specific for the CD3 antigen]) or through an additional polypeptide linker sequence (domain1 – linker sequence – domain2). In the event that a linker is used, this linker is preferably of a length and sequence sufficient to ensure that each of the first and second domains can, independently from one another, retain their differential binding specificities. Most preferably and as documented in the appended examples, the "bispecific single chain antibody construct" to be employed in the pharmaceutical composition of the invention is a bispecific single chain Fv (scFv). Bispecific single chain molecules are known in the art and are described in WO 99/54440, Mack, J. Immunol. (1997), 158, 3965-3970, Mack, PNAS, (1995), 92, 7021-7025, Kufer, Cancer Immunol. Immunother., (1997), 45, 193-197, Löffler, Blood, (2000), 95, 6, 2098-2103 and Brühl, J.

Immunol., (2001), 166, 2420-2426 . A particularly preferred molecular format of the invention provides a polypeptide construct wherein the antibody-derived region comprises one V_H and one V_L region. The intramolecular orientation of the V_H -domain and the V_L -domain, which are linked to each other by a linker-domain, in the scFv format is not decisive for the recited bispecific single chain constructs. Thus, scFvs with both possible arrangements (V_H -domain – linker domain – V_L -domain; V_L -domain – linker domain – V_H -domain) are particular embodiments of the recited bispecific single chain construct.

The antibody construct may also comprise additional domains, e.g. for the isolation and/or preparation of recombinantly produced constructs.

A corresponding format for a bispecific single chain antibody construct is described in the appended example 1.

The term "single-chain" as used in accordance with the present invention means that said first and second domain of the bispecific single chain construct are covalently linked, preferably in the form of a co-linear amino acid sequence encodable by a single nucleic acid molecule.

The term "binding to/interacting with" as used in the context with the present invention defines a binding/interaction of at least two "antigen-interaction-sites" with each other. The term "antigen-interaction-site" defines, in accordance with the present invention, a motif of a polypeptide which shows the capacity of specific interaction with a specific antigen or a specific group of antigens. Said binding/interaction is also understood to define a "specific recognition". The term "specifically recognizing" means in accordance with this invention that the antibody molecule is capable of specifically interacting with and/or binding to at least two amino acids of each of the human target molecule as defined herein. Said term relates to the specificity of the antibody molecule, i.e. to its ability to discriminate between the specific regions of the human target molecule as defined herein. The specific interaction of the antigen-interaction-site with its specific antigen may result in an initiation of a signal, e.g. due to the induction of a change of the conformation of the antigen, an oligomerization of the antigen, etc. Further, said binding may be

exemplified by the specificity of a "key-lock-principle". Thus, specific motifs in the amino acid sequence of the antigen-interaction-site and the antigen bind to each other as a result of their primary, secondary or tertiary structure as well as the result of secondary modifications of said structure. The specific interaction of the antigen-interaction-site with its specific antigen may result as well in a simple binding of said site to the antigen.

The term "specific interaction" as used in accordance with the present invention means that the bispecific single chain construct does not or essentially does not cross-react with (poly)peptides of similar structures. Cross-reactivity of a panel of bispecific single chain construct under investigation may be tested, for example, by assessing binding of said panel of bispecific single chain construct under conventional conditions (see, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988 and Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1999) to the (poly)peptide of interest as well as to a number of more or less (structurally and/or functionally) closely related (poly)peptides. Only those antibodies that bind to the (poly)peptide/protein of interest but do not or do not essentially bind to any of the other (poly)peptides are considered specific for the (poly)peptide/protein of interest. Examples for the specific interaction of an antigen-interaction-site with a specific antigen comprise the specificity of a ligand for its receptor. Said definition particularly comprises the interaction of ligands which induce a signal upon binding to its specific receptor. Examples for corresponding ligands comprise cytokines which interact/bind with/to its specific cytokine-receptors. Also particularly comprised by said definition is the binding of an antigen-interaction-site to antigens like antigens of the selectin family, integrins and of the family of growth factors like EGF. An other example for said interaction, which is also particularly comprised by said definition, is the interaction of an antigenic determinant (epitope) with the antigenic binding site of an antibody.

The term "binding to/interacting with" may also relate to a conformational epitope, a structural epitope or a discontinuous epitope consisting of two regions of the human target molecules or parts thereof. In context of this invention, a

conformational epitope is defined by two or more discrete amino acid sequences separated in the primary sequence which come together on the surface of the molecule when the polypeptide folds to the native protein (Sela, (1969) Science 166, 1365 and Laver, (1990) Cell 61, 553-6).

- 5 The term "discontinuous epitope" means in context of the invention non-linear epitopes that are assembled from residues from distant portions of the polypeptide chain. These residues come together on the surface of the molecule when the polypeptide chain folds into a three-dimensional structure to constitute a conformational/structural epitope.
- 10 The constructs of the present invention are also envisaged to specifically bind to/interact with a conformational/structural epitope(s) composed of and/or comprising the two regions of the human CD3 complex described herein or parts thereof as disclosed herein below.

Accordingly, specificity can be determined experimentally by methods known in the art and methods as disclosed and described herein. Such methods comprise, but
15 are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-, EIA-tests and peptide scans.

The term "antibody fragment or derivative thereof" relates to single chain antibodies, or fragments thereof, synthetic antibodies, antibody fragments, such as
20 Fab, a F(ab₂)', Fv or scFv fragments etc., or a chemically modified derivative of any of these. Antibodies to be employed in accordance with the invention or their corresponding immunoglobulin chain(s) can be further modified using conventional techniques known in the art, for example, by using amino acid deletion(s), insertion(s), substitution(s), addition(s), and/or recombination(s) and/or any other
25 modification(s) (e.g. posttranslational and chemical modifications, such as glycosylation and phosphorylation) known in the art either alone or in combination. Methods for introducing such modifications in the DNA sequence underlying the amino acid sequence of an immunoglobulin chain are well known to the person skilled in the art; see, e.g., Sambrook (1989), loc. cit.

- 30 The term "(poly)peptide" as used herein describes a group of molecules which comprise the group of peptides, as well as the group of polypeptides. The group of

peptides is consisting of molecules with up to 30 amino acids, the group of polypeptides is consisting of molecules with more than 30 amino acids.

The term "antibody fragment or derivative thereof" particularly relates to (poly)peptide constructs comprising at least one CDR.

- 5 Fragments or derivatives of the recited antibody molecules define (poly)peptides which are parts of the above antibody molecules and/or which are modified by chemical/biochemical or molecular biological methods. Corresponding methods are known in the art and described inter alia in laboratory manuals (see Sambrook et al.; Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press, 10 2nd edition 1989 and 3rd edition 2001; Gerhardt et al.; Methods for General and Molecular Bacteriology; ASM Press, 1994; Lefkovits; Immunology Methods Manual: The Comprehensive Sourcebook of Techniques; Academic Press, 1997; Golemis; Protein-Protein Interactions: A Molecular Cloning Manual; Cold Spring Harbor Laboratory Press, 2002).

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Bispecific antibodies that specifically recognize the EpCAM antigen and the CD3 antigen are described in the prior art, e.g., in Mack (Proc. Natl. Acad. Sci., 1995, 92:7021-7025).

- 20 As mentioned above, the said variable domains comprised in the herein described bispecific single chain constructs are connected by additional linker sequences. The term "peptide linker" defines in accordance with the present invention an amino acid sequence by which the amino acid sequences of the first domain and the second domain of the defined construct are linked with each other. An essential 25 technical feature of such peptide linker is that said peptide linker does not comprise any polymerization activity. A particularly preferred peptide linker is characterized by the amino acid sequence Gly-Gly-Gly-Gly-Ser, i.e. (Gly)₄Ser, or polymers thereof, i.e. ((Gly)₄Ser)_x. The characteristics of said peptide linker, which comprise the absence of the promotion of secondary structures are known in the art and 30 described e.g. in Dall'Acqua et al. (Biochem. (1998) 37, 9266-9273), Cheadle et al. (Mol Immunol (1992) 29, 21-30) and Raag and Whitlow (FASEB (1995) 9(1), 73-

80). Also particularly preferred are peptide linkers which comprise less amino acid residues. An envisaged peptide linker with less than 5 amino acids can comprise 4, 3, 2 or one amino acids. A particularly preferred "single" amino acid in context of said "peptide linker" is Gly. Accordingly, said peptide linker may consist of the
5 single amino acid Gly. Furthermore, peptide linkers which also do not promote any secondary structures are preferred. The linkage of said domains to each other can be provided by, e.g. genetic engineering, as described in the examples. Methods for preparing fused and operatively linked bispecific single chain constructs and expressing them in mammalian cells or bacteria are well-known in the art (e.g. WO
10 99/54440, Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. 1989 and 1994 or Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001).

15 The bispecific single chain antibody constructs described herein above and below may be humanized or deimmunized antibody constructs. Methods for the humanization and/or deimmunization of (poly))peptides and, in particular, antibody constructs are known to the person skilled in the art.

20 Here it was surprisingly found that domains with specificity for the EpCAM antigen, comprising at least one CDR-H3 region comprising the amino acid sequence NXD (asparagine-X-aspartic acid) preferably in position 102 to 104 of SEQ ID NOs: 80, 88 and 96, or in position 106 to 108 of SEQ ID NOs: 84 and 92, wherein X is an aromatic amino acid are particularly useful in the specific format of a bispecific
25 single chain antibody construct. These bispecific single chain antibody constructs are particularly useful as pharmaceutical compositions since these constructs are advantageous over constructs which do not comprise said amino acids.

Furthermore, it was surprisingly found that domains with specificity for the EpCAM antigen, comprising at least one CDR-H3 region of at least 9 amino acid residues
30 and having a K_D value of more than 5×10^{-9} M are particularly useful in the specific format of a bispecific single chain antibody construct. These bispecific single chain

antibody construct are particularly useful as pharmaceutical compositions since these constructs are advantageous over constructs of less than 9 amino acid residues and wherein said binding domain specific for EpCAM has a K_D value of less than 5×10^{-9} M.

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The prior art constructs are characterized by less advantageous EC_{50} values and/or less efficient or complete purifications as shown in the appended examples. It was in particular surprising that the domain of the single chain constructs with specificity for the CD3 antigen to be employed in accordance with the invention are highly bioactive in N- as well as C-terminal position, wherein in particular arrangements in $V_{H(\text{anti-CD3})}$ - $V_{L(\text{anti-CD3})}$ are preferred. The constructs to be employed in the pharmaceutical composition of the invention are characterized by advantageous production and purification properties as well as by their high bioactivity, i.e. their desired cytotoxic activity. In particular, when the cytotoxic activity of the constructs of the invention were compared with cytotoxic activity of conventional M79xanti-CD3 and HD70xanti-CD3 constructs, the constructs of the invention showed clearly higher bioactivity (Figure 11B). The corresponding high bioactivity is reflected by low to very low EC_{50} values as determined in cytotoxicity tests. The lower the EC_{50} value of the molecule is, the higher cytotoxicity, i.e. the effectivity in the cell lysis, of the construct is higher. On the other hand, the higher the EC_{50} value, the less effective the molecule is in inducing cell lysis. The term " EC_{50} " corresponds, in context of this invention, to EC_{50} values as determined according to the methods known in the art and as illustrated in the appended examples: A standard dose-response curve is defined by four parameters: the baseline response (Bottom), the maximum response (Top), the slope, and the drug concentration that provokes a response halfway between baseline and maximum (EC_{50}). EC_{50} is defined as the concentration of a drug or molecule that provokes a response half way between the baseline (Bottom) and maximum response (Top). A lower K_D value of the constructs of the invention depicts higher binding affinity. E.g. a low K_D of 10^{-9} M shows high binding affinity of the binding construct. On the other hand a high K_D value of e.g. 10^{-6} M relates to lower binding affinity of the binding

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domain of the construct.

The percentage of cell lysis (i.e. cytotoxic activity) may be determined by, inter alia, release assays disclosed herein above, for example, ^{51}Cr release assays, LDH-release assays and the like. Most preferably, in context of this invention
5 fluorochrome release assays is employed as illustrated in the appended examples. Here, strong cytotoxic activity against EpCAM-positive cells (see CHO-EpCAM cells in appended example 3) of the bispecific single chain constructs described herein relates to a molecule comprising EC_{50} values preferably ≤ 500 pg/ml, more preferably ≤ 400 pg/ml, even more preferably ≤ 300 pg/ml, even more preferably $\leq$
10 250 pg/ml, most preferably ≤ 200 pg/ml ≤ 100 pg/ml, ≤ 50 pg/ml.

The bispecific constructs comprised in the pharmaceutical compositions of the present invention show a surprisingly high cytotoxic activity (preferably in the range of about 10 pg/ml to 170 pg/ml) compared to the prior art M79xanti-CD3 construct
15 (VL_{17-1A}- VH_{17-1A}- VH_{CD3}- VL_{CD3}; 8628 pg/ml). A skilled person is aware that EC_{50} values may vary depending to the bioactivity assay. Factors affecting EC_{50} value may comprise type of effector cells, activity of effector cells, type of target cells, E:T ratio, incubation time, incubation temperature and other external circumstances. Different EC_{50} values of same constructs in different experiments may be
20 compared with the EC_{50} values of controls. A construct having high cytotoxic activity according to the invention has at least 2.5 time lower EC_{50} value than the control (at least 2.5 times higher cytotoxicity than the control), preferably at least three times lower EC_{50} value and more preferably at least five times lower EC_{50} value.

25

Furthermore, the constructs of the invention bind EpCAM with a surprisingly high affinity measured by surface plasmon resonance (BIAcore[®]). The prior art EpCAM and CD3 binding construct M79xanti-CD3 has a K_D of 4×10^{-6} M and the constructs of the invention a K_D of $2,3 \times 10^{-7} - 2,5 \times 10^{-7}$ M.

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Preferably, the X in said NXD motif is W (tryptophan) or Y (tyrosine).

It is further envisaged that the pharmaceutical composition of the invention comprises a bispecific single chain antibody construct, wherein the CDR-H3 of the EpCAM specific domain comprises at least 9 amino acid residues, preferably at least 14 amino acids. Preferably the CDR-H3 comprises less than 18 amino acids, more preferably less than 15 amino acids. Thus, preferably the CDR-H3 comprises 9 to 17 amino acids, more preferably 9 to 15 amino acids and most preferably 10 or 14 amino acids.

Bispecific single chain antibody construct comprising a corresponding EpCAM specific domain have been surprisingly found to be advantageous in the format of the above described construct over other EpCAM specific domain known in the art. Such effect is demonstrated in appended examples 3, 4 and 5. The prior art EpCAM binding antibody M79 comprises eight amino acids in its CDR-H3 region and does not comprise the sequence NXD (Figure 11A).

The pharmaceutical composition according to the invention may also comprise constructs, wherein said binding domain specific for EpCAM has a K_D value of more than 5×10^{-9} M.

The pharmaceutical composition may additionally be characterized by the feature that said binding domain specific for the CD3 antigen has a K_D value of more than 10^{-7} M.

The K_D value is a physical value defining the tendency of a complex to dissociate. For the binding equilibrium $A+B \leftrightarrow AB$, the dissociation constant is given as the ratio of the two kinetic rate constants k_{off} and k_{on} : $[A][B] (k_{on})/[AB] (k_{off})$. The smaller the dissociation constant the tighter A and B bind to each other. In biological systems a good, specific binder has a dissociation constant in the range of 10^{-9} - 10^{-7} M. K_D can be measured with a number of methods known to the person skilled in the art, e.g. surface plasmon resonance (SPR, e.g. with BIAcore®), analytical ultracentrifugation, isothermal titration calorimetry, fluorescence anisotropy, fluorescence spectroscopy or by radiolabeled ligand binding assays.

The K_D s of the constructs of the invention have been measured using the surface

plasmon resonance (SPR) spectroscopy. The ligand is injected over the immobilized antigen chip surface and the change in optical density on the chip surface upon binding is measured. The change in optical density, monitored by a change in reflection angle, correlates directly to the amount of ligand binding to the chip surface - the biophysical phenomenon used is called surface plasmon resonance.

One of the interaction partners has to be immobilized on the surface of the sensor chip of the apparatus based on surface plasmon resonance (e.g. BIAcore®). The kinetics of association and dissociation of ligand with the immobilized antigen on the chip surface are observed in real time. The binding curves are fitted for kinetic rate constants k_{on} and k_{off} , resulting in an apparent equilibrium dissociation constant (K_D).

It is particularly preferred, that said binding domain specific for EpCAM has a K_D value in a range between 1×10^{-7} and 5×10^{-9} M and said binding domain specific for CD3 has a K_D value in a range between 1×10^{-6} and 5×10^{-9} M.

In a particularly preferred embodiment, the pharmaceutical composition may additionally be characterized by the feature that said binding domain specific for the CD3 antigen has a K_D value of $> (more\ than)\ 1 \times 10^{-7}$ M.

The constructs of the invention have the advantage that they may be used a number of times for killing tumour cells since the EpCAM binding part has an affinity with a K_D value of more than 5×10^{-9} M. If the affinity of a bispecific construct for binding an EpCAM-expressing tumour cell is too high, the construct binds one EpCAM expressing tumour cell and remains on its surface even when it has been killed and cannot continue to another tumour cell to be killed. A further advantage of the construct of the invention is that the binding domain specific for EpCAM binds with a high affinity (corresponds to lower K_D value), thus leading the circulating T-cells to the tumour cells marked with the bispecific construct. Therefore, the K_D of the binding domain specific for EpCAM of the bispecific construct is preferably in the range of 10^{-7} - 5×10^{-9} M and the K_D of the binding

domain specific for CD3 is preferably in the range of 10^{-6} - 5×10^{-9} M. In a preferred embodiment, the KD value of the EpCAM binding domain is lower than the KD value of the CD3 binding domain corresponding to a higher affinity of the EpCAM binding domain compared to the CD3 binding domain.

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Further it is envisaged that the pharmaceutical composition of the invention comprises a bispecific single chain antibody construct, wherein the CDR-H3 of the EpCAM specific domain comprises at least 9 amino acids, preferably at least 14 amino acids. Preferably the CDR-H3 comprises less than 18 amino acids, more preferably less than 15 amino acids. Thus, preferably the CDR-H3 comprises 9 to 17 amino acids, more preferably 9 to 15 amino acids and most preferably 10 or 14 amino acids.

In a preferred embodiment of the pharmaceutical composition of the invention the V_H chain of the domain specific for human EpCAM antigen is selected from the group consisting of:

- (a) an amino acid sequence as shown in any of SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92 and SEQ ID NO: 96;
- (b) an amino acid sequence encoded by a nucleic acid sequence as shown in SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91 and SEQ ID NO: 95;
- (c) an amino acid sequence encoded by a nucleic acid sequence hybridizing with the complementary strand of a nucleic acid sequence as defined in (b) under stringent hybridization conditions;
- (d) an amino acid sequence encoded by a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence of any one of (b) and (c).

The term "hybridizing" as used herein refers to polynucleotides/nucleic acid sequences which are capable of hybridizing to the polynucleotides encoding bispecific single chain constructs as defined herein or parts thereof. Therefore, said polynucleotides may be useful as probes in Northern or Southern Blot analysis of

RNA or DNA preparations, respectively, or can be used as oligonucleotide primers in PCR analysis dependent on their respective size. Preferably, said hybridizing polynucleotides comprise at least 10, more preferably at least 15 nucleotides in length while a hybridizing polynucleotide of the present invention to be used as a probe preferably comprises at least 100, more preferably at least 200, or most preferably at least 500 nucleotides in length.

It is well known in the art how to perform hybridization experiments with nucleic acid molecules, i.e. the person skilled in the art knows what hybridization conditions s/he has to use in accordance with the present invention. Such hybridization conditions are referred to in standard text books such as Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (2001) N.Y. Preferred in accordance with the present inventions are polynucleotides which are capable of hybridizing to the polynucleotides of the invention or parts thereof, under stringent hybridization conditions.

"Stringent hybridization conditions" refer, e.g. to an overnight incubation at 42°C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1 x SSC at about 65°C. Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH₂PO₄; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA; followed by washes at 50°C with 1 X SSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC). It is of note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to

suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

The recited nucleic acid molecules may be, e.g., DNA, cDNA, RNA or synthetically produced DNA or RNA or a recombinantly produced chimeric nucleic acid molecule comprising any of those polynucleotides either alone or in combination.

10 Preferably said pharmaceutical composition of the invention may comprise a bispecific single chain construct, wherein the V_L chain domains specific for human EpCAM antigen is selected from the group consisting of:

- (a) an amino acid sequence as shown in any of SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94 and SEQ ID NO: 98;
- 15 (b) an amino acid sequence encoded by a nucleic acid sequence as shown in SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93 and SEQ ID NO: 97 ;
- (c) an amino acid sequence encoded by a nucleic acid sequence hybridizing with the complementary strand of a nucleic acid sequence as defined in (b) under stringent hybridization conditions;
- 20 (d) an amino acid sequence encoded by a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence of any one of (b) and (c).

25 In a preferred embodiment of the pharmaceutical composition of this invention, the V_H and V_L regions of said human CD3 specific domain are derived from an CD3 specific antibody selected from the group consisting of X35-3, VIT3, BMA030 (BW264/56), CLB-T3/3, CRIS7, YTH12.5, F111-409, CLB-T3.4.2, TR-66, WT32, SPv-T3b, 11D8, XIII-141, XIII-46, XIII-87, 12F6, T3/RW2-8C8, T3/RW2-4B6, 30 OKT3D, M-T301, SMC2, WT31 and F101.01. These CD3-specific antibodies are well known in the art and, inter alia, described in Tunnaclyffe (1989), Int. Immunol.

1, 546-550. In a more preferred embodiment, said V_H and V_L regions of said CD3 specific domain are derived from OKT-3 (as defined and described above). Even more preferred (and as illustrated in the appended examples) said V_H and V_L regions are or are derived from an antibody/antibody derivative with specificity for the CD3 molecule described by Traunecker (1991), EMBO J. 10, 3655-3659. In accordance with this invention, said V_H and V_L regions are derived from antibodies/antibody derivatives and the like which are capable of specifically recognizing the human CD3- ϵ chain in the context of other TCR subunits, e.g. in mouse cells transgenic for human CD3- ϵ chain. These transgenic mouse cells express human CD3- ϵ chain in a native or near native conformation. Accordingly, the V_H and V_L regions derived from an CD3- ϵ chain specific antibody is most preferred in accordance with this invention and said (parental) antibodies should be capable of specifically binding epitopes reflecting the native or near native structure or a conformational epitope of human CD3 presented in context of the TCR complex. Such antibodies have been classified by Tunncliffe (1989) as "group II" antibodies. Further classifications in Tunncliffe (1989) comprise the definition of "group I" and "group III" antibodies directed against CD3. "Group I" antibodies, like UCHT1, recognize CD3- ϵ chain expressed as recombinant protein and as part of the TCR on the cell surface. Therefore, "group I" antibodies are highly specific for CD3- ϵ chain. In contrast, the herein preferred "group II antibodies" recognize CD3- ϵ chain only in the native TCR complex in association with other TCR subunits. Without being bound by theory, it is speculated in context of this invention that in "group II" antibodies, the TCR context is required for recognition of CD3- ϵ chain. CD3- γ chain and δ chain, being associated with ϵ chain, are also involved in binding of "group II antibodies". All three subunits express immunoreceptor-tyrosine activation motifs (ITAMs) which can be tyrosine phosphorylated by protein tyrosine-based kinases. For this reason group II antibodies induce T cell signaling via CD3- ϵ chain, γ chain and δ chain, leading to a stronger signal compared to group I antibodies selectively inducing T cell signaling via CD3- ϵ chain. Yet, since for therapeutic applications induction of a strong T cell signaling is desired, the V_H (anti-CD3) / V_L (anti-CD3)- regions (or parts thereof) to be employed in the bispecific single

chain constructs comprised in the inventive pharmaceutical composition, are preferably derived from antibodies directed against human CD3 and classified in "group II" by Tunnacliffe (1989), loc.cit.

- 5 In one embodiment the present invention relates to a pharmaceutical composition wherein said bispecific single chain antibody construct comprises an amino acid sequence selected from the group of:
- (a) an amino acid sequence as shown in any of SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 30, 36, 39, 42, 44, 46, 48, 50, 52, 54, 56, 58 and 60;
 - 10 (b) an amino acid sequence encoded by a nucleic acid sequence as shown in any of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 29, 35, 38, 41, 43, 45, 47, 49, 51, 53, 55, 57 and 59;
 - (c) an amino acid sequence encoded by a nucleic acid sequence hybridizing with the complementary strand of a nucleic acid sequence as defined in (b)
 - 15 under stringent hybridization conditions;
 - (d) an amino acid sequence encoded by a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence of any one of (b) and (c).

- 20 The present invention also provides for a pharmaceutical composition comprising a nucleic acid sequence encoding a bispecific single chain antibody construct as defined above.

Said nucleic acid molecule may be a natural nucleic acid molecule as well as a recombinant nucleic acid molecule. The nucleic acid molecule may, therefore, be
25 of natural origin, synthetic or semi-synthetic. It may comprise DNA, RNA as well as PNA (peptide nucleic acid) and it may be a hybrid thereof.

Thus, the present invention relates to a pharmaceutical composition comprising a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- 30 (a) a nucleotide sequence encoding the mature form of a protein comprising the amino acid sequence of the bispecific single chain antibody constructs

defined herein, preferably as given in SEQ ID Nos: 2, 4, 8, 10, 12, 14, 16, 18, 20, 30, 36, 39, 42, 44, 46, 48, 50, 52, 54, 56, 58 and 60;

(b) a nucleotide sequence comprising or consisting of the DNA sequence as given in SEQ ID Nos: 1, 3, 7, 9, 11, 13, 15, 17, 19, 29, 35, 38, 41, 43, 45, 47, 49, 51, 53, 55, 57 and 59;

(c) a nucleotide sequence hybridizing with the complementary strand of a nucleotide sequence as defined in (b) under stringent hybridization conditions;

(d) a nucleotide sequence encoding a protein derived from the protein encoded by a nucleotide sequence of (a) or (b) by way of substitution, deletion and/or addition of one or several amino acids of the amino acid sequence encoded by the nucleotide sequence of (a) or (b);

(e) a nucleotide sequence encoding a protein having an amino acid sequence at least 60 % identical to the amino acid sequence encoded by the nucleotide sequence of (a) or (b);

(f) a nucleotide sequence which is degenerate as a result of the genetic code to a nucleotide sequence of any one of (a) to (e);

The term "mature form of the protein" defines in context with the present invention a protein translated from its corresponding mRNA and optional subsequently modified.

The term "hybridizing" has been defined in the context of the present invention herein above.

It is evident to the person skilled in the art that regulatory sequences may be added to the nucleic acid molecule comprised in the pharmaceutical composition of the invention. For example, promoters, transcriptional enhancers and/or sequences which allow for induced expression of the polynucleotide of the invention may be employed. A suitable inducible system is for example tetracycline-regulated gene expression as described, e.g., by Gossen and Bujard (Proc. Natl. Acad. Sci. USA 89 (1992), 5547-5551) and Gossen et al. (Trends Biotech. 12 (1994), 58-62), or a dexamethasone-inducible gene expression system as described, e.g. by Crook (1989) EMBO J. 8, 513-519 .

Furthermore, it is envisaged for further purposes that nucleic acid molecules may contain, for example, thioester bonds and/or nucleotide analogues. Said modifications may be useful for the stabilization of the nucleic acid molecule against endo- and/or exonucleases in the cell. Said nucleic acid molecules may be transcribed by an appropriate vector containing a chimeric gene which allows for the transcription of said nucleic acid molecule in the cell. In this respect, it is also to be understood that such polynucleotide can be used for "gene targeting" or "gene therapeutic" approaches. In another embodiment said nucleic acid molecules are labeled. Methods for the detection of nucleic acids are well known in the art, e.g., Southern and Northern blotting, PCR or primer extension. This embodiment may be useful for screening methods for verifying successful introduction of the nucleic acid molecules described above during gene therapy approaches.

Said nucleic acid molecule(s) may be a recombinantly produced chimeric nucleic acid molecule comprising any of the aforementioned nucleic acid molecules either alone or in combination. Preferably, the nucleic acid molecule is part of a vector.

The present invention therefore also relates to a pharmaceutical composition comprising a vector comprising the nucleic acid molecule described in the present invention.

Many suitable vectors are known to those skilled in molecular biology, the choice of which would depend on the function desired and include plasmids, cosmids, viruses, bacteriophages and other vectors used conventionally in genetic engineering. Methods which are well known to those skilled in the art can be used to construct various plasmids and vectors; see, for example, the techniques described in Sambrook et al. (loc cit.) and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1989), (1994). Alternatively, the polynucleotides and vectors of the invention can be reconstituted into liposomes for delivery to target cells. As discussed in further details below, a cloning vector was used to isolate individual sequences of DNA. Relevant sequences can be transferred into expression vectors where expression of a

particular polypeptide is required. Typical cloning vectors include pBluescript SK, pGEM, pUC9, pBR322 and pGBT9. Typical expression vectors include pTRE, pCAL-n-EK, pESP-1, pOP13CAT.

Preferably said vector comprises a nucleic acid sequence which is a regulatory
5 sequence operably linked to said nucleic acid sequence encoding a bispecific single chain antibody constructs defined herein.

Such regulatory sequences (control elements) are known to the artisan and may include a promoter, a splice cassette, translation initiation codon, translation and insertion site for introducing an insert into the vector. Preferably, said nucleic acid
10 molecule is operatively linked to said expression control sequences allowing expression in eukaryotic or prokaryotic cells.

It is envisaged that said vector is an expression vector comprising the nucleic acid molecule encoding a bispecific single chain antibody constructs defined herein.

The term "regulatory sequence" refers to DNA sequences which are necessary to
15 effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, control sequences generally include promoters, ribosomal binding sites, and terminators. In eukaryotes generally control sequences include promoters, terminators and, in some instances, enhancers, transactivators or transcription
20 factors. The term "control sequence" is intended to include, at a minimum, all components the presence of which are necessary for expression, and may also include additional advantageous components.

The term "operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner.
25 A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. In case the control sequence is a promoter, it is obvious for a skilled person that double-stranded nucleic acid is preferably used.

Thus, the recited vector is preferably an expression vector. An "expression vector"
30 is a construct that can be used to transform a selected host and provides for expression of a coding sequence in the selected host. Expression vectors can for

instance be cloning vectors, binary vectors or integrating vectors. Expression comprises transcription of the nucleic acid molecule preferably into a translatable mRNA. Regulatory elements ensuring expression in prokaryotes and/or eukaryotic cells are well known to those skilled in the art. In the case of eukaryotic cells they
5 comprise normally promoters ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Possible regulatory elements permitting expression in prokaryotic host cells comprise, e.g., the P_L , *lac*, *trp* or *tac* promoter in *E. coli*, and examples of regulatory elements permitting expression in eukaryotic host cells are the *AOX1* or
10 *GAL1* promoter in yeast or the CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells.

Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as
15 the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. Furthermore, depending on the expression system used leader sequences capable of directing the polypeptide to a cellular compartment or secreting it into the medium may be added to the coding sequence of the recited nucleic acid sequence and are well known in the art; see also, e.g., the appended examples.

20 The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a portion thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired
25 characteristics, e.g., stabilization or simplified purification of expressed recombinant product; see supra. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pEF-Neo, pCDM8, pRc/CMV, pcDNA1, pcDNA3 (In-vitrogene), pEF-DHFR and pEF-ADA, (Raum et al. Cancer Immunol Immunother (2001) 50(3), 141-
30 150) or pSPORT1 (GIBCO BRL).

Preferably, the expression control sequences will be eukaryotic promoter systems

in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and as desired, the collection and purification of the polypeptide of the invention may follow; see, e.g., the appended examples.

An alternative expression system which could be used to express a cell cycle interacting protein is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The coding sequence of a recited nucleic acid molecule may be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of said coding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which the protein of the invention is expressed (Smith, J. Virol. 46 (1983), 584; Engelhard, Proc. Nat. Acad. Sci. USA 91 (1994), 3224-3227).

Additional regulatory elements may include transcriptional as well as translational enhancers. Advantageously, the above-described vectors of the invention comprises a selectable and/or scorable marker.

Selectable marker genes useful for the selection of transformed cells and, e.g., plant tissue and plants are well known to those skilled in the art and comprise, for example, antimetabolite resistance as the basis of selection for dhfr, which confers resistance to methotrexate (Reiss, Plant Physiol. (Life Sci. Adv.) 13 (1994), 143-149); npt, which confers resistance to the aminoglycosides neomycin, kanamycin and paromycin (Herrera-Estrella, EMBO J. 2 (1983), 987-995) and hygromycin (Marsh, Gene 32 (1984), 481-485). Additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman, Proc. Natl. Acad. Sci. USA 85 (1988), 8047); mannose-6-phosphate isomerase which allows cells to utilize mannose (WO 94/20627) and

ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.) or deaminase from *Aspergillus terreus* which confers resistance to
5 Blasticidin S (Tamura, Biosci. Biotechnol. Biochem. 59 (1995), 2336-2338).

Useful scorable markers are also known to those skilled in the art and are commercially available. Advantageously, said marker is a gene encoding luciferase (Giacomin, Pl. Sci. 116 (1996), 59-72; Scikantha, J. Bact. 178 (1996), 121), green fluorescent protein (Gerdes, FEBS Lett. 389 (1996), 44-47) or β -glucuronidase
10 (Jefferson, EMBO J. 6 (1987), 3901-3907). This embodiment is particularly useful for simple and rapid screening of cells, tissues and organisms containing a recited vector.

As described above, the recited nucleic acid molecule can be used alone or as part of a vector to express the encoded polypeptide in cells, for, e.g., gene therapy. The
15 nucleic acid molecules or vectors containing the DNA sequence(s) encoding any one of the above described bispecific single chain antibody constructs is introduced into the cells which in turn produce the polypeptide of interest. Gene therapy, which is based on introducing therapeutic genes into cells by ex-vivo or in-vivo techniques is one of the most important applications of gene transfer. Suitable
20 vectors, methods or gene-delivery systems for in-vitro or in-vivo gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, Nature Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813; Verma, Nature 389 (1994), 239; Isner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086;
25 Onodera, Blood 91 (1998), 30-36; Verma, Gene Ther. 5 (1998), 692-699; Nabel, Ann. N.Y. Acad. Sci. 811 (1997), 289-292; Verzeletti, Hum. Gene Ther. 9 (1998), 2243-51; Wang, Nature Medicine 2 (1996), 714-716; WO 94/29469; WO 97/00957, US 5,580,859; US 5,589,466; or Schaper, Current Opinion in Biotechnology 7 (1996), 635-640. The recited nucleic acid molecules and vectors may be designed
30 for direct introduction or for introduction via liposomes, or viral vectors (e.g., adenoviral, retroviral) into the cell. Preferably, said cell is a germ line cell,

embryonic cell, or egg cell or derived therefrom, most preferably said cell is a stem cell. An example for an embryonic stem cell can be, inter alia, a stem cell as described in, Nagy, Proc. Natl. Acad. Sci. USA 90 (1993), 8424-8428.

In accordance with the above, the present invention relates to methods to derive
5 vectors, particularly plasmids, cosmids, viruses and bacteriophages used conventionally in genetic engineering that comprise a nucleic acid molecule encoding the polypeptide sequence of a bispecific single chain antibody constructs defined herein. Preferably, said vector is an expression vector and/or a gene transfer or targeting vector. Expression vectors derived from viruses such as
10 retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the recited polynucleotides or vector into targeted cell populations. Methods which are well known to those skilled in the art can be used to construct recombinant vectors; see, for example, the techniques described in Sambrook et al. (loc cit.), Ausubel (1989, loc cit.) or other standard
15 text books. Alternatively, the recited nucleic acid molecules and vectors can be reconstituted into liposomes for delivery to target cells. The vectors containing the nucleic acid molecules of the invention can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas
20 calcium phosphate treatment or electroporation may be used for other cellular hosts; see Sambrook, supra.

The recited vector may be the pEF-DHFR, pEF-ADA or pEF-neo.

The vectors pEF-DHFR and pEF-ADA have been described in the art, e.g. in Mack et al. (PNAS (1995) 92, 7021-7025) and Raum et al. (Cancer Immunol Immunother
25 (2001) 50(3), 141-150).

It is further envisaged that the pharmaceutical composition of the invention comprises a host transformed or transfected with a vector defined herein above.

Said host may be produced by introducing said at least one of the above described
30 vector or at least one of the above described nucleic acid molecules into the host. The presence of said at least one vector or at least one nucleic acid molecule in

the host may mediate the expression of a gene encoding the above described bespecific single chain antibody constructs.

The described nucleic acid molecule or vector which is introduced in the host may either integrate into the genome of the host or it may be maintained
5 extrachromosomally.

The host can be any prokaryote or eukaryotic cell.

The term "prokaryote" is meant to include all bacteria which can be transformed or transfected with a DNA or RNA molecules for the expression of a protein of the invention. Prokaryotic hosts may include gram negative as well as gram positive
10 bacteria such as, for example, *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. The term "eukaryotic" is meant to include yeast, higher plant, insect and preferably mammalian cells. Depending upon the host employed in a recombinant production procedure, the protein encoded by the polynucleotide of the present invention may be glycosylated or may be non-glycosylated. Especially
15 preferred is the use of a plasmid or a virus containing the coding sequence of the polypeptide of the invention and genetically fused thereto an N-terminal FLAG-tag and/or C-terminal His-tag. Preferably, the length of said FLAG-tag is about 4 to 8 amino acids, most preferably 8 amino acids. An above described polynucleotide can be used to transform or transfect the host using any of the techniques
20 commonly known to those of ordinary skill in the art. Furthermore, methods for preparing fused, operably linked genes and expressing them in, e.g., mammalian cells and bacteria are well-known in the art (Sambrook, loc cit.).

Preferably, said the host is a bacteria, an insect, fungal, plant or animal cell.

It is particularly envisaged that the recited host may be a mammalian cell, more
25 preferably a human cell or human cell line.

Particularly preferred host cells comprise CHO cells, COS cells, myeloma cell lines like SP2/0 or NS/0.

The pharmaceutical composition of the invention may also comprise a
30 proteinaceous compound capable of providing an activation signal for immune effector cells useful for cell proliferation or cell stimulation.

The proteinaceous compound is not understood as an additional domain of the above defined bispecific single chain antibody construct, but at least one additional component of the pharmaceutical composition of the invention.

In the light of the present invention, said "proteinaceous compounds" providing an activation signal for immune effector cells" may be, e.g. a further activation signal for T cells (e.g. a further costimulatory molecule: molecules of the B7-family, Ox40 L, 4.1 BBL), or a further cytokine: interleukin (e.g. IL-2), or an NKG-2D engaging compound. Preferred formats of proteinaceous compounds comprise additional bispecific antibodies and fragments or derivatives thereof, e.g. bispecific scFv.

Proteinaceous compounds can comprise, but are not limited to scFv fragments specific for the T cell receptor or superantigens. Superantigens directly bind to certain subfamilies of T cell receptor variable regions in an MHC-independent manner thus mediating the primary T cell activation signal. The proteinaceous compound may also provide an activation signal for immune effector cell which is a non-T cell. Examples for immune effector cells which are non-T cells comprise, inter alia, NK cells.

An additional technical feature of the pharmaceutical composition of the invention is that said pharmaceutical composition is thermostable at $\geq 37^{\circ}\text{C}$.

20

An alternative embodiment of the invention relates to a process for the production of a pharmaceutical composition of the invention, said process comprising culturing a host defined herein above under conditions allowing the expression of the construct and recovering the produced bispecific single chain antibody construct from the culture.

25

The transformed hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. The polypeptide of the invention can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the, e.g., microbially expressed polypeptides of the invention may be by any conventional means such as, for example, preparative chromatographic separations and immunological

30

separations such as those involving the use of monoclonal or polyclonal antibodies directed, e.g., against a tag of the polypeptide of the invention or as described in the appended examples.

5 The conditions for the culturing of a host which allow the expression are known in the art and discussed herein above. The same holds true for procedures for the purification/recovery of said constructs.

10 A further alternative embodiment of the invention relates to the use of a bispecific single chain antibody construct as defined above, a nucleic acid sequence as defined above, a vector as defined above, a host as defined above and/or produced by a process as defined above for the preparation of a pharmaceutical composition for the prevention, treatment or amelioration of a tumorous disease.

In particular, the pharmaceutical composition of the present invention may be particularly useful in preventing, ameliorating and/or treating cancer.

15 Preferably said tumorous disease is epithelial cancer or a minimal residual cancer.

It is envisaged by the present invention that the above defined bispecific single chain antibody construct, nucleic acid molecules and vectors are administered either alone or in any combination using standard vectors and/or gene delivery
20 systems, and optionally together with a pharmaceutically acceptable carrier or excipient. Subsequent to administration, said nucleic acid molecules or vectors may be stably integrated into the genome of the subject.

On the other hand, viral vectors may be used which are specific for certain cells or tissues and persist in said cells. Suitable pharmaceutical carriers and excipients
25 are well known in the art. The pharmaceutical compositions prepared according to the invention can be used for the prevention or treatment or delaying the above identified diseases.

Furthermore, it is possible to use a pharmaceutical composition of the invention which comprises described nucleic acid molecules or vectors in gene therapy.
30 Suitable gene delivery systems may include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses,

adenoviruses, and adeno-associated viruses, among others. Delivery of nucleic acids to a specific site in the body for gene therapy may also be accomplished using a biolistic delivery system, such as that described by Williams (Proc. Natl. Acad. Sci. USA 88 (1991), 2726-2729). Further methods for the delivery of nucleic acids comprise particle-mediated gene transfer as, e.g., described in Verma, Gene Ther. 15 (1998), 692-699.

Furthermore the invention relates to a method for the prevention, treatment or amelioration of a tumorous disease comprising the step of administering to a subject in the need thereof an effective amount a bispecific single chain antibody construct as defined above, a nucleic acid sequence as defined above, a vector as defined as defined above, a host as defined above and/or produced in by a process as defined above.

Preferably said subject is a human.

The method for the prevention, treatment or amelioration of the invention may comprise the co-administration of an above defined proteinaceous compound capable of an activation signal for immune effector cells to the subject. The co-administration may be a simultaneous co-administration or a non-simultaneous co-administration.

It is particularly preferred for the use and the method of the invention that said tumorous disease is epithelial cancer, preferably adenocarcinomas, or a minimal residual cancer, preferably early solid tumor, advanced solid tumor or metastatic solid tumor.

Finally, the present invention relates to a kit comprising a bispecific single chain antibody construct as defined above, a nucleic acid sequence as defined above, a vector as defined above and/or a host as defined above. It is also envisaged that the kit of this invention comprises a pharmaceutical composition as described herein above, either alone or in combination with further medicaments to be administered to a patient in need of medical treatment or intervention.

34a

In one aspect, the present invention relates to a pharmaceutical composition comprising a bispecific single chain antibody construct and a pharmaceutically acceptable carrier or excipient, wherein said construct comprises or consists of two domains, wherein one of said domains binds to human EpCAM antigen and a
5 second domain binds to human CD3 antigen, wherein said bispecific single chain antibody construct comprises:

(a) an amino acid sequence as shown in any one of SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 30, 36, 39, 42, 44, 46, 48, 50, 52, 54, 56, 58 and 60;

10 (b) an amino acid sequence encoded by a nucleic acid sequence as shown in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 29, 35, 38, 41, 43, 45, 47, 49, 51, 53, 55, 57 and 59; or

(c) an amino acid sequence encoded by a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence of
15 (b).

In another aspect, the present invention relates to a pharmaceutical composition comprising a nucleic acid sequence encoding the bispecific single chain antibody construct as defined above, and a pharmaceutically acceptable carrier or excipient.

20

In another aspect, the present invention relates to pharmaceutical composition comprising a vector which comprises the nucleic acid sequence as defined above, and a pharmaceutically acceptable carrier or excipient.

25 In another aspect, the present invention relates to a pharmaceutical composition comprising a host cell transformed or transfected with the vector defined above.

In another aspect, the present invention relates to a process for producing a bispecific single chain antibody as defined above, said process comprising

30 (a) culturing the above mentioned host cell under conditions allowing the expression of the bispecific single chain antibody construct

34b

mentioned above; and

- (b) recovering the produced bispecific single chain antibody construct from the culture.

5 In another aspect, the present invention relates to the use of:

- (a) the bispecific single chain antibody construct as defined above;
- (b) the nucleic acid sequence as defined above;
- (c) the vector as defined above;
- (d) the host cell as defined above; or

10 (e) the bispecific single chain antibody produced by the process defined above,

for the prevention, treatment or amelioration of a tumorous disease in a subject, or for the preparation of a medicament for same.

15 In another aspect, the present invention relates to a product which is:

- (a) the bispecific single chain antibody construct as defined above;
- (b) the nucleic acid sequence as defined above;
- (c) the vector as defined above;
- (d) the host cell as defined above; or

20 (e) the bispecific single chain antibody produced by the process defined above,

for the prevention, treatment or amelioration of a tumorous disease in a subject.

In another aspect, the present invention relates to a kit comprising:

25 (a) the bispecific single chain antibody construct as defined above;

(b) the nucleic acid sequence as defined above;

(c) the vector as defined above;

(d) the host cell as defined above; or

(e) the bispecific single chain antibody produced by the process defined

30 above,

and a suitable container housing any one of (a) to (e).

The Figures show:

Figure 1:

DNA and amino acid sequence of the anti-CD3-anti-EpCAM constructs **A)** anti-
 5 CD3 VHVL stL x 3-1 VHVL (SEQ ID NO.:11,12), **B)** anti-CD3 VHVL aL x 4-7 VHVL
 (SEQ ID NO.:1,2), **C)** anti-CD3 VHVL aL Ser x 4-7 VHVL (SEQ ID NO.:7, 8), **D)**
 anti-CD3 VHVL stL x 4-7 VHVL (SEQ ID NO.:13,14), **E)** anti-CD3 VHVL stL x 4-7
 VLVH (SEQ ID NO.:15,16), **F)** anti-CD3 VHVL aL x 5-10 VHVL (SEQ ID NO.:3,4),
G) anti-CD3 VHVL aL Ser x 5-10 VHVL (SEQ ID NO.:9, 10), **H)** anti-CD3 VHVL stL
 0 x 5-10 VHVL (SEQ ID NO.:17,18), **I)** anti-CD3 VHVL stL x 5-10 VLVH (SEQ ID
 NO.:19,20), **J)** anti-CD3 VHVL aL x 3-1 VHVL (SEQ ID NO.:45, 46), **K)** anti-CD3
 VHVL aL Ser x 3-1 VHVL (SEQ ID NO.:47,48), **L)** anti-CD3 VHVL aL x 3-5 VHVL
 (SEQ ID NO.:49,50), **M)** anti-CD3 VHVL aL Ser x 3-5 VHVL (SEQ ID NO.:51,52),
N) anti-CD3 VHVL stL x 3-5 VHVL (SEQ ID NO.:53,54), **O)** anti-CD3 VHVL aL x 4-
 5 1 VHVL (SEQ ID NO.:55,56), **P)** anti-CD3 VHVL aL Ser x 4-1 VHVL (SEQ ID
 NO.:57,58) and **Q)** anti-CD3 VHVL stL x 4-1 VHVL (SEQ ID NO.:59,60).

Figure 2:

FACS analysis of the constructs **A)** anti-CD3 VHVL stL x 5-10 VHVL (SEQ ID
 10 NO.:18), **B)** anti-CD3 VHVL stL x 4-7 VHVL (SEQ ID NO.:14), **C)** anti-CD3 VHVL
 aL x 5-10 VHVL (SEQ ID NO.:4), **D)** anti-CD3 VHVL aL x 4-7 VHVL (SEQ ID
 NO.:2), **E)** anti-CD3 VHVL aL Ser x 5-10 VHVL (SEQ ID NO.:10), **F)** anti-CD3
 VHVL aL Ser x 4-7 VHVL (SEQ ID NO.:8), **G)** anti-CD3 VHVL stL x 3-1 VHVL
 (SEQ ID NO.:12), **H)** anti-CD3 VHVL stL x 5-10 VLVH (SEQ ID NO.:20) and **I)** anti-
 15 CD3 VHVL stL x 4-7 VLVH (SEQ ID NO.:16) in CD3 positive Jurkat and EpCAM-
 positive Kato III cells. A shift to the right shows binding. In Jurkat and KatoIII cells
 the dotted line indicates the shift of the negative control (only secondary antibody),
 dashed line shows the binding of an anti-EpCAM-anti-CD3 control antibody and the
 bold line shows the bispecific construct of interest.

Figure 3:

DNA and amino acid sequence of the anti-EpCAM-anti-CD3- constructs **A)** 4-7 VLVHx anti-CD3 VHVL (SEQ ID NO.:41,42), **B)** 3-5 VLVHx anti-CD3 VHVL (SEQ ID NO.:29,30), **C)** 3-1 VLVHx anti-CD3 VHVL (SEQ ID NO.:35,36), **D)** 4-1 VLVHx anti-CD3 VHVL (SEQ ID NO.:38,39) and **E)** 5-10 VLVHx anti-CD3 VHVL (SEQ ID NO.:43,44).

Figure 4: FACS analysis of the constructs **A)** 4-7 VLVHx anti-CD3 VHVL (SEQ ID NO.:42), **B)** 3-5 VLVHx anti-CD3 VHVL (SEQ ID NO.:30), **C)** 3-1 VLVHx anti-CD3 VHVL (SEQ ID NO.:36), **D)** 4-1 VLVHx anti-CD3 VHVL (SEQ ID NO.:39) and **E)** 5-10 VLVHx anti-CD3 VHVL (SEQ ID NO.: 44) constructs in CD3 positive Jurkat and EpCAM-positive Kato III cells. A shift to the right shows binding.

Figure 5:

A representative elution pattern of an EpCAM bispecific antibody containing protein fractions from a Zn-Chelating Fractogel® column at 280 nm. High adsorption at 280 nm from 50-450 ml retention time was due to non-bound protein in the column flow through. The arrow at the peak at 530.09 ml indicates the EpCAM bispecific construct containing protein fraction that was used for further purification.

Figure 6:

A representative protein elution pattern from a Sephadex® S200 gel filtration column at 280 nm. The protein peak at 82.66 ml containing bispecific antibodies against CD3 and EpCAM corresponds to a molecular weight of ca. 52 kD. Fractions were collected from 40-140 ml retention time.

Figure 7

A) Cation exchange chromatogram of 3-1 x anti-CD3 (SEQ ID NO.:36) shows the overall charge isoforms of the protein. Cation exchange chromatography was performed on a MiniS® (Amersham) column. After washing with 20 mM MES buffer pH 5.5, the protein was eluted with a gradient of elution buffer containing 1 M NaCl: 0-30% in 60 column volumes. The bispecific construct was eluted at 23,58 ml. Unspecific protein was eluted with 1 M NaCl starting at 50 ml.

B) Cation exchange chromatogram of 5-10 x anti-CD3 (SEQ ID NO.:44) shows the overall charge isoforms of the protein. Cation exchange chromatography was performed as in Figure 7A. The bispecific construct was eluted at a shoulder at 35,77 ml. Unspecific protein was eluted with 1 M NaCl starting at 50 ml.

Figure 8:

A) Representative SDS-PAGE analysis of EpCAM bispecific single chain antibody protein fractions. Lane **M**: Molecular weight marker Lane **1**: cell culture supernatant; lane **2**: IMAC flow through; lane **3**: IMAC wash; lane **4**: IMAC eluate; lane **5**: purified antibody against EpCAM and CD3 obtained from gel filtration.

B) Representative Western blot analysis of purified EpCAM bispecific single chain antibody protein fractions Lane **1**: cell culture supernatant; lane **2**: IMAC flow through; lane **3**: IMAC wash; lane **4**: IMAC eluate; lane **5**: purified antibody against EpCAM and CD3 obtained from gel filtration.

Figure 9:

Cytotoxicity assay of C-terminal EpCAM binders anti-CD3x3-1 (SEQ ID NO.:46), anti-CD3 x-5-10 (SEQ ID NO.:4), and anti-CD3 x4-7 (SEQ ID NO.:2). CB15 T cell clone and CHO-EpCAM cells were used in an E:T ratio of 5:1. CHO-EpCAM cells were stained with PKH26 dye and the cells were counted after bispecific single chain antibody incubation with FACS analysis.

Figure 10:

Cytotoxicity assay of N-terminal EpCAM binders 3-1xanti-CD3 (SEQ ID NO.:36), and 5-10xanti-CD3 (SEQ ID NO.:44) . CB15 T cell clone and CHO-EpCAM cells were used in an E:T ration of 5:1. CHO-EpCAM cells were stained with PKH26 dye and the cells were counted after bispecific single chain antibody incubation with FACS analysis.

Figure 11:

A) Sequence alignment of the CDR3 of the VH chains of EpCAM 3-1 (SEQ ID NO.:80), EpCAM 4-1 (SEQ ID NO.: 88), EpCAM 5-10 (SEQ ID NO.: 96), EpCAM 3-5 (SEQ ID NO.: 84), EpCAM 4-7 (SEQ ID NO.:92), compared with CDR3 of the
 5 VH chain of EpCAM M79, HD70 and 3B10. The NXD motif is depicted as bold.

B) Comparison of the cytotoxic activity of 3-1xanti-CD3 (SEQ ID NO.: 36), 5-10xanti-CD3 (SEQ ID NO.:44), anti-CD3x4-7 (SEQ ID NO.:2) and anti-CD3x5-10 (SEQ ID NO.:18) with M79Xanti-CD3 and HD70xanti-CD3 controls. PBMC cells and Kato III cells were used in a E:T ratio of 10:1. KatoIII cells were stained with
 10 propidium iodide and the cells were counted after bispecific single chain antibody incubation with FACS analysis.

The scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent
 15 with the description as a whole.

Example 1: Cloning and expression of the EpCAM constructs

A number of constructs comprising anti-CD3 and anti-EpCAM in various structures and domain arrangements were generated. Anti-EpCAM VH and VL variable
 20 domains of the antibodies 3-1 are shown in SEQ ID NO.:79, 80, 81, 82, 3-5 in SEQ ID NO.:83, 84, 85, 86, 4-1 in SEQ ID NO.:87, 88, 89, 90, 4-7 SEQ ID NO.:91, 92, 93, 94 and 5-10 in SEQ ID NO.:95, 96, 97, 98. The constructs are summarized in Table 1.

Table 1. anti-CD3-anti-EpCAM and anti-EpCAM-anti-CD3 constructs

SEQ ID NO.: Construct No.	Construct	Domain arrangement	Distinctive feature
anti-CD3xanti-EpCAM constructs			
SEQ ID NO.:1,2	anti-CD3x4-7	VH-VLXVH-VL	
SEQ ID NO.: 3, 4	anti-CD3x5-10	VH-VLXVH-VL	
SEQ ID NO.: 45,46	anti-CD3x3-1	VH-VLXVH-VL	
SEQ ID NO.: 49,50	anti-CD3x3-5	VH-VLXVH-VL	
SEQ ID NO.: 55,56	anti-CD3x4-1	VH-VLXVH-VL	
SEQ ID NO.: 7,8	anti-CD3x 4-7Cys-Ser	VH-VLXVH-VL	Cys-Ser mutation
SEQ ID NO.: 9,10	anti-CD3x 5-10Cys-Ser	VH-VLXVH-VL	Cys-Ser mutation
SEQ ID NO.: 47,48	anti-CD3x3-1	VH-VLXVH-VL	Cys-Ser mutation
SEQ ID NO.: 51,52	anti-CD3x3-5	VH-VLXVH-VL	Cys-Ser mutation
SEQ ID NO.: 57,58	anti-CD3x4-1	VH-VLXVH-VL	Cys-Ser mutation
SEQ ID NO.: 11,12	anti-CD3x3-1	VH-VLXVH-VL	(G ₄ S) ₃ -linker
SEQ ID NO.: 13,14	anti-CD3x4-7	VH-VLXVH-VL	(G ₄ S) ₃ -linker
SEQ ID NO.: 15,16	anti-CD3x4-7	VH-VLXVL-VH	(G ₄ S) ₃ -linker
SEQ ID NO.: 17,18 1	anti-CD3x5-10	VH-VLXVH-VL	(G ₄ S) ₃ -linker
SEQ ID NO.: 19,20	anti-CD3x5-10	VH-VLXVL-VH	(G ₄ S) ₃ -linker
SEQ ID NO.: 53,54	anti-CD3x3-5	VH-VLXVH-VL	(G ₄ S) ₃ -linker
SEQ ID NO.: 59, 60	anti-CD3x4-1	VH-VLXVH-VL	(G ₄ S) ₃ -linker
anti-EpCAM- anti-CD3 constructs			
SEQ ID NO.: 29,30	3-5xanti-CD3	VL-VHxVH-VL	
SEQ ID NO.: 35,36	3-1xanti-CD3	VL-VHxVH-VL	
SEQ ID NO.: 38,39	4-1xanti-CD3	VL-VHxVH-VL	
SEQ ID NO.: 41,42	4-7xanti-CD3	VL-VHxVH-VL	
SEQ ID NO.: 43,44	5-10xanti- CD3	VL-VHxVH-VL	

1.1 Cloning of C-terminal EpCAM-binders

1.1.1 Preparation of anti-CD3 PCR products

a) Anti-CD3 constructs with original 18 amino acid linker (SEQ ID NOs.:1, 2, 3 and 4)

5 The N-terminal original anti-CD3 containing the 18 amino acid linker (SEQ ID NO.:70) was obtained by PCR using the CD19xCD3 construct (Löffler A et al., Blood 2000 95:2098-103) as template and the following primers (CD3 VH *BsrGI*: AGGTGTACACTCCGATATCAAACCTGCAGCAG (SEQ ID NO.:5), CD3 VL *BspEI*: AATCCGGATTTCAGCTCCAGCTTGG (SEQ ID NO.:6)).

b) Anti-CD3 constructs with original 18 amino acid linker and Cys to Ser mutation in CDRH3 (SEQ ID Nos. 7,8, 9 and 10)

The N-terminal original anti-CD3 containing the 18 amino acid linker (Seq ID NO.:70) and the Cys to Ser mutation was obtained by PCR using a CD19xanti-CD3 (C→S mutation) construct as template and the primers CD3 VH *BsrGI* and
 15 CD3 VL *BspEI* (Seq ID Nos. 5 and 6). The CDRH3 sequence with the Cys-Ser mutation is shown in SEQ ID NO.:78.

c) Anti-CD3-anti-EpCAM constructs with (G₄S)₃ linker (Seq ID Nos. 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20)

The N-terminal anti-CD3 containing the 15 amino acid standard (G₄S)₃ linker (SEQ
 20 ID NO.:99) was obtained by PCR using the CD19xCD3 (Löffler A et al., Blood 2000 95:2098-103) as template. The anti-CD3 VH region and the anti-CD3 VL region were separately amplified by the following primers (CD3 VH: CD3 VH *BsrGI* AGGTGTACACTCCGATATCAAACCTGCAGCAG (SEQ ID NO.:5), 3'CD3 VH GS15 GGAGCCGCCGCCGCGCCAGAACCAACCACCTGAGGAGACTGTGA
 25 GAGTGGTGCCTTG (SEQ ID NO.:21); CD3 VL: 5'CD3 VL GS15 GGCGGCGGCGGCTCCGGTGGTGGTGGTTCTGACATTCAGC TGACCCAGTCTCC (SEQ ID NO.:22), CD3 VL *BspEI* AATCCGGATTTCAGCTCCAGCTTGG (SEQ ID NO.:6)). Overlapping complementary sequences introduced into the PCR products were used to form the
 30 coding sequence of a 15-amino acid (G₄S)₃ (single-letter amino acid code) (SEQ ID

NO.:99) linker during the subsequent fusion PCR. This amplification step was performed with the primer pair CD3 VH *BsrGI* (SEQ ID NO.:5) and CD3 VL *BspEI* (SEQ ID NO.:6).

1.1.2 Cloning of the anti-CD3xanti EpCAM constructs in $VH_{\text{anti-CD3}}-VL_{\text{anti-CD3}} \times$

5 $VH_{\text{anti-EpCAM}}-VL_{\text{anti-EpCAM}}$ orientation (SEQ ID NO.:1,2, SEQ ID NO.:3,4, SEQ ID NO.:7,8, SEQ ID NO.:9,10, SEQ ID NO.:11,12, SEQ ID NO.:13,14 and SEQ ID NO.:17,18)

The N-terminal original anti-CD3 containing the 18 amino acid linker (SEQ ID NO.:70) or the N-terminal original anti-CD3 containing the 15 amino acid standard (G₄S)₃ linker (SEQ ID NO.:99) was cleaved with the restriction enzymes *BsrGI* and *BspEI* and subsequently cloned into the bluescript KS vector (Stratagene, La Jolla, CA), containing the amino acid sequence of an eukaryotic secretory signal (leader peptide) as a *EcoRI/BsrGI*-Fragment. After cleavage of this construct with *EcoRI* and *BspEI* the resulting DNA fragment comprising the respective anti-CD3 scFv
15 with the leader peptide was cloned into a *EcoRI/BspEI* cleaved plasmid containing the c-terminal EpCAM binders 3-1 (SEQ ID NO.:79-82), 4-7 (SEQ ID NO.:91-94), or 5-10 (SEQ ID NO.:95-98) in pEFDHFR. pEFDHFR was described in Mack et al. Proc. Natl. Acad. Sci. USA 92 (1995) 7021-7025).

1.1.3. Cloning of the anti-CD3xanti EpCAM constructs in $VH_{\text{anti-CD3}}-VL_{\text{anti-CD3}} \times$

20 $VL_{\text{anti-EpCAM}}-VH_{\text{anti-EpCAM}}$ orientation (SEQ ID Nos.: 15, 16, 19 and 20)

The C-terminal anti-EpCAM antibody 4-7 (SEQ ID NO.:91-94) in VLVH orientation containing the 15 amino acid standard linker (SEQ ID NO.:99) was obtained by PCR. The 4-7 VH region and the 4-7 VL region were separately amplified by the following primers (4-7 VL: 4-7 VL *BspEI* FOR
25 CTGAAATCCGGAGGTGGTGGATCCGAGCTCGTGATGACCCAGACTCC (SEQ ID NO.:100), 4-7 VL GS15 REV GGAGCCGCCGCCGCGCCAGAACCACCA CCACCTTTGATCTCAAGCTTGGTCCCC (SEQ ID NO.:101); 4-7 VH: 4-7 VH GS15 FOR
GGCGGCGGCGGCTCCGGTGGTGGTGGTTCTGAGGTGCAGCTGCTCGAGCA
30 G (SEQ ID NO.:23), 4-7 VH *SaI* REV TTTTAAGTCGACCTAATGATGATGAT-

GATGATGTGAGGAGACGGTGACCGTGG (SEQ ID NO.:24)). Overlapping complementary sequences introduced into the PCR products were used to form the coding sequence of a 15-amino acid (G₄S)₃ (single-letter amino acid code) linker (SEQ ID NO.:99) during the subsequent fusion PCR. This amplification step was performed with the primer pair 4-7 VL *Bsp*El FOR and 4-7 VH *Sa*II REV (SEQ ID NO.100, SEQ ID NO.:24).

The C-terminal anti-EpCAM antibody 5-10 (SEQ ID NO.:95-98) in VLVH orientation containing the 15 amino acid standard linker (SEQ ID NO.:99) was obtained by PCR. The 5-10 VH region and the 5-10 VL region were separately amplified by the

following primers (5-10 VL: 5-10 VL *Bsp*El FOR
CTGAAATCCGGAGGTGGTGGATCCGAGCTCGTGATGACACAGTCTCCAT
(SEQ ID NO.:25), 5-10 VL GS15 REV

GGAGCCGCCGCCGCGCCAGAACCACCACCTTTGATCTCAAGCTTGGTCCCA
G (SEQ ID NO.: 26); 5-10 VH: 5-10 VH GS15 FOR

GGCGGCGGCGGCTCCGGTGGTGGTGGTTCTGAGGTGCAGCTGCTCGAGC
(SEQ ID NO.:27), 5-10 VH *Sa*II REV

TTTTAAGTCGACCTAATGATGATGATGATGTGAGGAGACGGTGACCGTG
G (SEQ ID NO.:28)). Overlapping complementary sequences introduced into the

PCR products were used to form the coding sequence of a 15-amino acid (G₄S)₃ linker (SEQ ID NO.:99) during the subsequent fusion PCR. This amplification step was performed with the primer pair 5-10 VL *Bsp*El FOR and 5-10 VH *Sa*II REV (SEQ ID NO.:25, SEQ ID NO.:28).

These PCR products (5-10 VLVH and 4-7 VLVH) were cleaved with *Bsp*El and *Sa*II and ligated in the *Bsp*El/*Sa*II cleaved anti-CD3 VHVL stLx5-10 VHVL (SEQ ID NO.:17,18) or anti-CD3 VHVL stL x 4-7 (SEQ ID NO.:13, 14) VHVL in pEFDHFR replacing the 5-10 VHVL DNA fragment.

1.1.4. Expression and binding of the anti-CD3-EpCAM constructs

After confirmation of the sequence coding for the bispecific single chain by sequencing the plasmid was transfected into DHFR deficient CHO cells for eukaryotic expression. Eukaryotic protein expression in DHFR deficient CHO cells

was performed as described in Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566). The transfected cells were then expanded and 1 litre of supernatant produced. Expression and binding of the bispecific single chain molecules were confirmed by FACS analyses. For that purpose the EpCAM positive human gastric cancer cell line Kato III (obtained from American Type Culture Collection (ATCC) Manassas, VA 20108 USA, ATCC number: HTB-103) was used. Binding of the anti-CD3 part was demonstrated on Jurkat cells (ATCC TIB 152).

Cells were cultured according to the recommendations of the supplier and ca. 200000 cells were incubated with 10µg/ml of the construct in 50µl PBS with 2%FCS. The binding of the construct was detected with an anti-His antibody (Penta-His Antibody, BSA free, obtained from Quiagen GmbH, Hilden, FRG) at 2µg/ml in 50µl PBS with 2%FCS. As a second step reagent a R-Phycoerythrin-conjugated affinity purified F(ab')₂ fragment, goat anti-mouse IgG, Fc-gamma fragment specific antibody, diluted 1:100 in 50µl PBS with 2% FCS (obtained from Dianova, Hamburg, FRG) was used. The samples were measured on a FACSscan (BD biosciences, Heidelberg, FRG). All the constructs comprising anti-CD3 and anti-EpCAM showed stronger binding affinity to CD3 and to EpCAM than the prior art anti-EpCAM (M79)xanti-CD3 bispecific antibody (**Figure 2**).

1.2 N-terminal EpCAM binders

1.2.1 Cloning of the anti-EpCAMxanti-CD3 constructs

Cloning of the construct 3-5xanti-CD3 (SEQ ID NOs.29, 30):

The C-terminal 3-5 in VH-VL orientation was obtained by PCR for the construction of 3-5 xanti-CD3 (SEQ ID NO.:29) molecule. Fragments I and II were amplified by PCR using primer pairs me 81 (SEQ ID NO.:31) /me 90 (SEQ ID NO.:34) and me 83 (SEQ ID NO.:32) /me 84 (SEQ ID NO.:33), respectively. Hot Start PCR was done using the Expand High Fidelity System of Roche Diagnostics. 20 cycles (94°C/30 sec; 60°C/1min;72°C/1min) were used for amplification followed by one cycle of 3 min at 72°C.

PCR fragments I and II were subjected to electrophoresis on a 1.5% agarose gel. Fragments were mixed (1 ng of each) and used as a template for the next PCR

reaction performed with primer pair me 81 (SEQ ID NO.:31) and me 84 (SEQ ID NO.:33) for amplification of fragment III. PCR was performed as described above. Fragment III was purified on an agarose gel and digested with BssHII and BspEI (Biolabs), purified and subsequently cloned into the corresponding sites of the pEF-dHFR-signal peptide (77/78)- anti-CD3 cloning vector, which facilitates cloning of anti-target variable regions in front of the anti-CD3 region. The vector has a unique BssHII site just after the signal peptide followed by BspEI site, linker (G₄S) and anti-CD3 region. The cloned region was verified by restriction digests and by DNA-sequencing.

10 Sequences of the Primers used:

Me 81: 5'- GGA TGC GCG CGA GCT CGT GAT GAC CCA GAC TCCA CTC TCC -3' (SEQ ID NO.:31)

Me 83: 5'- GGT TCT GGC GGC GGC GGC TCC GGT GGT GGT GGT TCT GAG GTG CAG CTG CTC GA CAG TCT G -3' (SEQ ID NO.:32)

15 Me 84: 5'- GTG CTC CGG AGG AGA CGG TGA CCG TGG TCC CTT GGC CCC AG -3' (SEQ ID NO.:33)

Me 90: 5'- CCG GAG CCG CCG CCG CCA GAA CCA CCA CCA CCT TTG ATC TCA AGC TTG GTC CC -3' (SEQ ID NO.:34)

Cloning of the construct 3-1xanti-CD3 (SEQ ID NO.:35, 36):

20 The C-terminal 3-1 in VH-VL orientation was obtained by PCR for the construction of 3-1 xanti-CD3 (SEQ ID NO.:35) molecule. Fragments I and II were amplified by PCR using primer pairs me 91a (SEQ ID NO.:37) /me 90 (SEQ ID NO.:34) and me 83 (SEQ ID NO.:32) /me 84 (SEQ ID NO.:33) , respectively. PCR was performed as above.

25 Agarose gel fragments comprising PCR fragments I and II were used as a template for the next PCR reaction performed with primer pair me 91a (SEQ ID NO.:37) and me 84 (SEQ ID NO.:33) for amplification of fragment III. PCR was performed as described above except, annealing was performed at 68°C instead of at 60°C. Fragment III was purified on an agarose gel and digested with BsrGI and BspEI

(Biolabs), purified and subsequently cloned into the corresponding sites of the pEF-dHFR-M79 X anti-CD3 cloning vector. The cloned region was verified by restriction digests and by DNA-sequencing.

Me 91a: 5'- GGA TTG TAC A CTCC GA GCT CGT CAT GAC CCA GTC TCC ATC
5 TTA TCT TGC TGC -3' (SEQ ID NO.:37)

Cloning of the construct 4-1xanti-CD3 (SEQ ID NO.:38, 39) :

The C-terminal 4-1 in VH-VL orientation was obtained by PCR for the construction of 4-1 xanti-CD3 (SEQ ID NO.:38, 39) molecule. Fragments I and II were amplified by PCR using primer pairs me 92a (SEQ ID NO.:40) /me 90 (SEQ ID NO.:34) and
10 me 83 (SEQ ID NO.:32) /me 84 (SEQ ID NO.:33), respectively. PCR was performed as above in annealing temperature of 60°C.

Agarose gel fragments comprising PCR fragments I and II were used as a template for the next PCR reaction performed with primer pair me 92a (SEQ ID NO.:40) and me 84 (SEQ ID NO.:33) for amplification of fragment III. PCR was performed as
15 described above except, annealing was performed at 68°C instead of at 60°C. Fragment III was purified on an agarose gel and digested with BsrGI and BspEI (Biolabs), purified and subsequently cloned into the corresponding sites of the pEF-dHFR-M79 X anti-CD3 is cloning vector. The cloned region was verified by restriction digests and by DNA-sequencing.

20 Me 92a: 5'- GGA TTG TAC A CTCC GA GCT CGT GAT GAC ACA GTCTCC ATC CTC C -3' (SEQ ID NO.:40)

Cloning of the construct 4-7xanti-CD3 (SEQ ID NO.:41,42)

The C-terminal 4-7 in VH-VL orientation was obtained by PCR for the construction of 4-7 xanti-CD3 (SEQ ID NO.:41, 42) molecule. Fragments I and II were amplified
25 by PCR using primer pairs me 81 (SEQ ID NO.:31) /me 90 (SEQ ID NO.:34) and me 83 (SEQ ID NO.:32) /me 84 SEQ ID NO.:33), respectively. PCR was performed as above with an annealing temperature of 60°C.

Agarose gel fragments comprising PCR fragments I and II were used as a template for the next PCR reaction performed with primer pair me 81 (SEQ ID NO.:31) and

me 84 (SEQ ID NO.:33) for amplification of fragment III. PCR was performed as described above. Fragment III was purified on an agarose gel and digested with BssHII and BspEI (Biolabs), purified and subsequently cloned into the corresponding sites of the pEF-dhfr-signal peptide (77/78)– anti-CD3 cloning vector. The cloned region was verified by restriction digests and by DNA-sequencing.

Cloning of the construct 5-10xanti-CD3 (SEQ ID NO.:43, 44) :

The C-terminal 5-10 in VH-VL orientation was obtained by PCR for the construction of 5-10xanti-CD3 (SEQ ID NO.:43, 44) molecule. Fragments I and II were amplified by PCR using primer pairs me 92a (SEQ ID NO.:40) /me 90 (SEQ ID NO.:34) and me 83 (SEQ ID NO.:32) /me 84 (SEQ ID NO.:33), respectively. PCR was performed as above with an annealing temperature of 60°C.

Agarose gel fragments comprising PCR fragments I and II were used as a template for PCR with primer pair me 92a (SEQ ID NO.:40) and me 84 (SEQ ID NO.:33) for amplification of fragment III. PCR was performed as described above except, annealing was performed at 68°C instead of at 60°C. Fragment III was purified on an agarose gel and digested with BsrGI and BspEI (Biolabs), purified and subsequently cloned into the corresponding sites of the pEF-dhfr-M79 X anti-CD3 cloning vector. The cloned region was verified by restriction digests and by DNA-sequencing.

1.2.2 Expression of anti-EpCAMxanti-CD3 bispecific molecules

CHO-cells lacking DHFR gene were maintained in alpha MEM medium (Life Technologies, cat.no: 32561) supplemented with 10% fetal Calf Serum (Life Technologies, heat inactivated at 65°C for 30 minutes) and with HT (Hypoxanthin and Thymidine; Life Technologies, cat. no: 41065-012). The cells were transfected with pEF-dHFR-3-1xanti-CD3 (SEQ ID NO.:35, 36), pEF-dHFR-3-5xanti-CD3 (SEQ ID NO.:29, 30), pEF-dHFR-4-1xanti-CD3 (SEQ ID NO.:38, 39), pEF-dHFR-4-7xanti-CD3 (SEQ ID NO.:41, 42) and pEF-dHFR-5-10xanti-CD3 (SEQ ID NO.:43, 44) using Lipofectamine 2000 kit (Invitrogen; cat. no:11668-019) according to the instructions provided by the Manufacturer. After 48 hrs, the cells were subjected to

selection by transferring the transfected cells into the selection medium (alpha MEM medium (cat. no:32561) containing heat inactivated 10% dialysed fetal Calf Serum (Life Technologies). After 2-3 weeks of selection, the cells were grown for 8 to 9 days (in 500 ml of selection medium) for production of bispecific molecules in 2
5 litre Tissue culture Roller Bottles (Falcon (cat. no: 353068;Becton Dickinson Labware). The tissue culture medium was centrifuged at 4°C for 10 minutes at 300g (1300rpm) to remove the cells and cell debris. The supernatant containing the secreted bispecific molecules was stored at -20°C until further analysis.

1.2.3 Binding assays of bispecific anti EpCAMxanti CD3 variants

10 In order to analyze the binding strength of the bispecific anti-EpCAMxanti-CD3 single chain constructs of the invention, the following binding assay was carried out.

250000 Jurkat cells (for CD3 binding) and Kato cells (for EpCAM binding) were independently incubated with crude supernatants (50µl) containing bispecific
15 construct for 45 min. at 4°C. Thereafter, the cells were washed twice in FACS buffer (phosphate-buffered saline containing 1% fetal calf serum (FCS) and 0.05% sodium azide) and incubated with mouse anti-His antibody (Dianova,DIA910) for 60 min. at 4°C. Washing steps were performed as above.

The cells were finally incubated either with goat anti-mouse-FITC-conjugated
20 antibody (BD 550003) or with anti-mouse-PE conjugated antibody (IgG) (Sigma, P8547). After washing steps, 10,000 events were analysed using FACS Calibur (B&D). All the EpCAM constructs showed strong binding (Figure 4).

Example 2. Purification of the EpCAM constructs

25 In order to purify the bispecific single chain constructs comprising anti-EpCAM and anti-CD3 the CHO-EpCAM cells were grown in roller bottles with HiClone® CHO modified DMEM medium (HiQ) for 7 days before harvest. The cells were removed by centrifugation and the supernatant containing the expressed protein was stored at -20°C.

Äkta FPLC System® (Pharmacia) and Unicorn Software® were used for chromatography. All chemicals were of research grade and purchased from Sigma (Deisenhofen) or Merck (Darmstadt).

IMAC was performed, using a Fractogel® column (Pharmacia) that was loaded
5 with ZnCl_2 according to the manufacturers protocol. The column was equilibrated
with buffer A2 (20 mM NaPP pH 7.5, 0.4 M NaCl) and the cell culture supernatant
(500ml) was applied to the column (10 ml) with a flow rate of 3 ml/min. The column
was washed with buffer A2 to remove unbound sample. Bound protein was eluted
using a 2-step gradient of buffer B2 (20 mM NaPP pH 7.5, 0.4 M NaCl, 0.5 M
10 Imidazol). In Step 1 20% buffer B2 in 10 column volumes was used and in Step2
100% buffer B2 in 10 column volumes was used. Eluted protein fractions from the
100% step were pooled for further purification.

Gel filtration chromatography was performed on a Sephadex S200 HiPrep®
column (Pharmacia) equilibrated with PBS (Gibco). Eluted protein samples (flow
15 rate 1ml/min) were subjected to SDS-Page and Western Blot for detection.

The column was previously calibrated for molecular weight determination
(molecular weight marker kit, Sigma MW GF-200).

Protein concentrations were determined using protein assay dye (MicroBCA,
Pierce) and IgG (Biorad) as standard protein. The yields of the protein are shown
20 in Table 2. All constructs were producible.

Table 2. Yields of the single-chain bispecific constructs comprising anti-EpCAM and anti-CD3

Construct	Yield [µg purified protein per liter culture]
4-1 x anti-CD3 (SEQ ID NO.:39)	172.5
3-5 x anti-CD3 (SEQ ID NO.:30)	265
4-7 x anti-CD3 (SEQ ID NO.:42)	37
anti-CD3 x 4-7. (SEQ ID NO.:2)	112.5
anti-CD3 Cys-Ser x 4-7 (SEQ ID NO.:8)	140
3-1 x anti-CD3 (SEQ ID NO.:36)	265
5-10 x anti-CD3 (SEQ ID NO.:44)	400
anti-CD3 x 5-10 (SEQ ID NO.:4)	195

A further high resolution cation exchange chromatography was performed on a MiniS® column (Amersham), equilibrated with 20mM MES buffer pH 5.5. The sample was diluted 1:3 with the same buffer before loading to the column. Bound protein was eluted with a gradient of equilibration buffer containing 1M NaCl: 0-30% in 60 column volumes. Remaining protein was eluted in 3 column volumes of 1M NaCl (**Figure 7**).

The EpCAM bispecific single chain construct proteins were isolated in a two-step purification process including immobilized metal affinity chromatography (IMAC) (**Figure 5**) and gel filtration (**Figure 6**). The main product had a molecular weight of 52 kDa under native conditions as determined by gelfiltration in PBS.

Purified bispecific protein was analyzed in SDS PAGE under reducing conditions performed with precast 4-12% Bis Tris gels (Invitrogen). Sample preparation and application were according to the manufacturers protocol. The molecular weight was determined with MultiMark® protein standard (Invitrogen). The gel was stained with colloidal Coomassie (Invitrogen protocol). The purity of the isolated protein was shown to be >95% (**Figure 8A**). Western Blot was performed with an Optitran BA-S83® membrane and the Invitrogen Blot Module according to the manufacturers protocol. The antibodies used were Penta His (Qiagen) and Goat-anti-Mouse-Ig labeled with alkaline phosphatase (AP) (Sigma), the chromogenic

substrate solution was BCIP/NBT liquid (Sigma). The EpCAM bispecific protein could be specifically detected by Western Blot (**Figure 8B**). The main signal corresponds to the main band in the SDS PAGE at 52 kD corresponding to the purified bispecific molecule.

5

Example 3. Cytotoxicity assays of the constructs comprising anti-CD3 and anti-EpCAM

In order to test the bioactivity of the constructs comprising anti-EpCAM and anti-CD3 a FACS based cytotoxicity test was performed.

10 For the cytotoxicity test, CHO cells from the American Type Cell Culture Collection (ATCC, Manassas, USA) were transfected with epithelial cell adhesion molecule (EpCAM). A cell clone derived from this transfection, referred to as CHO-EpCAM cells, was used for the experiments. CHO-EpCAM (1.5×10^7) cells were washed free of serum two times with PBS and incubated with PKH26 dye (Sigma-Aldrich
15 Co.) according to the manufacturers instructions. After staining cells were washed two times with RPMI/10% FCS.

Cells were counted and mixed with CB15 effector cells. The CD4-positive T cell clone CB15 was provided by Dr. Fickenscher, University of Erlangen/Nuernberg, Germany. Cells were cultured as recommended by the suppliers. The resulting cell
20 suspension contained 400.000 target and 2×10^6 effector cells per ml. 50 μ l of the mixture was used per well in a 96 well round bottom plate.

Antibodies were diluted in RPMI/10% FCS to the required concentration and 50 μ l of this solution was added to the cell suspension. A standard reaction was incubated for 16 h at 37°C / 5% CO₂. Propidium iodide was added to a final
25 concentration of 1 μ g/ml. After 10 min of incubation at room temperature cells were analysed by FACS. PKH26 fluorescence was used for positive identification of target cells. Cytotoxicity was measured as ratio of PI positive over all target cells. Sigmoidal dose response curves typically had R² values >0.97 as determined by Prism Software (GraphPad Software Inc., San Diego, USA) (**Figure 9 and 10**).

EC₅₀ values calculated by the analysis program were used for comparison of bioactivity. All the constructs of the invention show at least 50 times better cytotoxicity (maximum EC₅₀-value 169 pg/ml) than the prior art construct M79xanti-CD3 (8628 pg/ml).

5

Example 4. Determination of the binding affinity by BIAcore™ 2000 of the constructs comprising anti-EpCAM and anti-CD3 to EpCAM

In order to show the superior binding affinity of the constructs of the invention, the KD values of the constructs and of the prior art anti-EpCAM construct (M79)xanti-
10 CD3 were determined.

Kinetic binding experiments were performed using surface plasmon resonance on the BIAcore™ 2000, Biacore AB (Uppsala, Sweden) with a flow rate of 5 µL/min and HBS-EP (0.01 M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20) as running buffer at 25 °C. The extracellular domain of the EpCAM
15 antigen (residues 17-265) was immobilized onto flow cells 2-4 on a CM5 sensor chip. The chip surface was activated injecting 80 µL of 0.1 M sodium-hydroxysuccinimid, 0.4 M N-ethyl-N'(3—dimethylaminepropyl)-carbodiimid (NHS/EDC). The antigen was coupled by manual injection of 60 µg/mL EpCAM in 0.01 M sodium-acetate, pH 4.7. Different densities of antigen were immobilized on
20 flow cells 2-4 adjusting the amount of manual injection times. Flow cell 1 was left empty while flow cell 2 was coated with the highest density of EpCAM (4100 RU). Flow cell 3 was coated with ¼ of the amount of antigen immobilized on flow cell 2 (974 RU) and flow cell 4 was coated with lowest density of Ep-CAM antigen (265 RU). The activated surface of the sensor chip was blocked injecting 85 µL of 1 M
25 ethanolamine and the chip was left to equilibrate over night at a constant flow of 5 µL/min of HBS-EP.

Binding kinetics of the bispecific constructs were measured injecting 10 µL of protein solution at concentrations ranging from 4 µM-0.07 µM and monitoring the dissociation for 100 sec. Protein was buffered in HBS-EP. The data were fitted
30 using BIAevaluation™ software determining the rate constant for dissociation and

association kinetics with a 1:1 Langmuir binding equation (1, 2). Where A is the concentration of injected analyte and B[0] is Rmax.

$$dB/dt = -(ka * [A] * [B] - kd * [AB]) \quad (1)$$

$$dAB/dt = -(ka * [A] * [B] - kd * [AB]) \quad (2)$$

- 5 Kinetic binding curves were determined in four concentrations of each bispecific construct analysed. The independent fitting of the raw data resulted in dissociation and association rate constants that were used to calculate the equilibrium dissociation constant (KD). The calculated KD values were unbiased for concentration indicating reliable data analysis. The average of the independently
10 determined dissociation constants as well as the standard deviation are summarized in table 3.

The analysed bispecific constructs bind to the Ep-CAM antigen immobilized on the chip surface within a well defined affinity range. The standard deviation for the calculated average dissociation constant is as expected.

15

Table 3: Dissociation constants for the bispecific constructs binding to EpCAM.

	KD (M)
M79 x anti-CD3 (control)	4,0x10 ⁻⁶
4-1 x anti-CD3 (SEQ ID NO.:39)	2,5x10 ⁻⁷
3-5 x anti-CD3 (SEQ ID NO.:30)	2,3 x10 ⁻⁷

- The prior art anti-EpCAM x anti-CD3 construct M79xCD3 had a KD of 4,0x10⁻⁶ M
20 while surprisingly the constructs of the invention have a KD in the range of 2,3 x10⁻⁷-2,5 x10⁻⁷ M. Thus, the constructs of the invention have more than 15 times stronger binding affinity than the prior art construct.

Example 5. Comparison of the cytotoxic activity of the constructs of the invention with prior art constructs

In order to compare the bioactivity of constructs having the NXD motif with
5 conventional M79xCD3 and HD70xCD3 constructs the following cytotoxic assay was carried out.

Katolli cells (ATCC HTB-103) were used as target cells and grown in RPMI supplemented with 10% fetal calf serum at 37°C in a 5% CO₂ humidified incubator. Subconfluent cultures were treated with 0.25% trypsin, counted in a Neubauer
10 chamber slide and checked for vitality by trypan-blue exclusion. Only cultures with >95% vitality were used for cytotoxicity assays. Target cells were stained with PKH26 fluorescent membrane dye according to the manufacturers manual (Sigma-Aldrich GmbH, Germany, PKH26-GL). Cell number was adjusted to 8×10^5 cells/ml in RPMI complete medium.

15

Human peripheral blood mononuclear cells (PBMCs) were used as effector cells and isolated from healthy donors using ficoll density gradient centrifugation with subsequent 100 x g centrifugation to remove thrombocytes. The pellet was resuspended in 10 vol. erythrocyte lysing buffer and incubated at room
20 temperature for 10 min. Lysing reaction was stopped by addition PBS. PBMCs were resuspended in RPMI 1640 complete medium and cell number adjusted to 8×10^6 cells/ml.

Equal volumes of target and effector cell suspension were mixed and 50 µl of this
25 suspension transferred to each well of a 96 well round bottom plate, 50 µl of EpCAM bispecific antibody serial dilution or RPMI complete medium as a negative control was added. Plates were incubated for 16 to 20 hrs at 37°C, 5% CO₂ in a humidified incubator. 50 µl propidium iodide was added to a final concentration of 1 µg/ml and incubated 15 min at room temperature. Samples were analysed by flow
30 cytometry (FACSCalibur, Becton Dickinson). 2×10^4 events were counted.

Target cells were identified by their PKH26 fluorescent label and cytotoxicity within this population was subsequently determined. Viable cells were separated from dead cells by propidium iodide staining and the percentage of dead target cells was used as a measure for cytotoxicity. Mean values were plotted against the concentration of the bispecific antibody on a logarithmic scale, resulting in a dose response curve (Figure 11B). The corresponding EC₅₀ values were obtained after nonlinear fitting of data with the GraphPad Prism software.

The cytotoxic activity of constructs having the NXD motif (SEQ ID NO.:36, 44, 2 and 18) was compared with conventional constructs M79xanti-CD3 and HD70-xanti-CD3 (Fig. 11B). A sequence alignment of the CDR3 regions of the VH chains of 3-1, 5-10, 4-7, 3-5 and 4-1 with M79, HD70 and 3B10 is shown in Figure 11A. Only 3-1, 5-10, 4-7, 3-5 and 4-1 have the NXD motif and furthermore, the lengths of the CDR3 regions differ. As can be seen from Figure 11A, 3-1, 4-1 and 5-10 have a CDR-H3 region of 10 amino acids, 3-5 and 4-7 have 14 amino acids whereas the prior art M79 has 8 amino acids, 3B10 has 6 amino acids and HD70 has 18 amino acids.

SEQ ID NO.: 36, 44, 2 and 18 showed a clearly better bioactivity compared to the conventional M79 and HD70 constructs (2250 pg/ml and less compared to 71460 and 11327 pg/ml of the prior art constructs, respectively) demonstrating the advantageous effects of the constructs of the invention.

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85           90           95

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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	Val	Glu	Gly	Gly	Ser	Gly	Gly	Ser	Gly			
		115					120					125						
Gly	Ser	Gly	Gly	Ser	Gly	Gly	Val	Asp	Asp	Ile	Gln	Leu	Thr	Gln	Ser			
	130					135					140							
Pro	Ala	Ile	Met	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys			
145					150					155					160			
Arg	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Ser			
				165					170					175				
Gly	Thr	Ser	Pro	Lys	Arg	Trp	Ile	Tyr	Asp	Thr	Ser	Lys	Val	Ala	Ser			
			180					185					190					
Gly	Val	Pro	Tyr	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser			
		195					200					205						
Leu	Thr	Ile	Ser	Ser	Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys			
	210					215					220							
Gln	Gln	Trp	Ser	Ser	Asn	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu			
225					230					235					240			
Glu	Leu	Lys	Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	Gln	Leu	Leu	Glu	Gln			
				245					250					255				
Ser	Gly	Ala	Glu	Leu	Ala	Arg	Pro	Gly	Ala	Ser	Val	Lys	Leu	Ser	Cys			
			260					265					270					
Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Asn	Tyr	Gly	Leu	Ser	Trp	Val	Lys			
		275					280					285						
Gln	Arg	Pro	Gly	Gln	Val	Leu	Glu	Trp	Ile	Gly	Glu	Val	Tyr	Pro	Arg			
	290					295					300							
Ile	Gly	Asn	Ala	Tyr	Tyr	Asn	Glu	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu			
305					310					315					320			

Thr Ala Asp Lys Ser Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu
 325 330 335

Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr
 340 345 350

Asp Thr Asn Tyr Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr
 355 360 365

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 370 375 380

Gly Gly Gly Ser Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu Pro
 385 390 395 400

Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
 405 410 415

Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys
 420 425 430

Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe
 435 440 445

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
 450 455 460

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe
 465 470 475 480

Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys
 485 490 495

Leu Glu Ile Lys His His His His His His
 500 505

<210> 3
 <211> 1512
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL aL x 5-10 VHVL

<400> 3

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cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcagtc	360
gaaggtggaa gtggaggttc tgggtggaagt ggaggttcag gtggagtcga cgacattcag	420
ctgacccagt ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc	480
agagccagtt caagtgtaag ttacatgaac tggtagcagc agaagtcagg cacctcccc	540
aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc	600
agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgctgcc	660
acttattact gccaacagtg gagtagtaac ccgctcacgt tcggtgctgg gaccaagctg	720
gagctgaaat ccggaggtgg tggatccgag gtgcagctgc tcgagcagtc tggagctgag	780
ctggtaaggc ctgggacttc agtgaagata tcttgcaagg cttctggata cgccttcact	840
aactactggc taggttgggt aaagcagagg cctggacatg gacttgagtg gattggagat	900
atthtccctg gaagtggtaa tatccactac aatgagaagt tcaagggcaa agccacactg	960
actgcagaca aatcttcgag cacagcctat atgcagctca gtagcctgac atthgaggac	1020
tctgctgtct atthctgtgc aagactgagg aactgggacg agcctatgga ctactggggc	1080
caagggacca cggtcaccgt ctctcaggt ggtggtggtt ctggcggcgg cggctccggt	1140
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gagaagggtca ctatgagctg caagtccagt cagagtctgt taaacagtgg aaatcaaaag	1260
aactacttga cctggtacca gcagaaacca gggcagcctc ctaaactgtt gatctactgg	1320
gcatccacta gggaaatctgg ggtccctgat cgcttcacag gcagtggatc tggaacagat	1380
ttcactctca ccatcagcag tgtgcaggct gaagacctgg cagtttatta ctgtcagaat	1440
gattatagtt atccgctcac gttcggtgct gggaccaagc ttgagatcaa acatcatcac	1500
catcatcatt ag	1512

<210> 4

<211> 503

<212> PRT

<213> artificial sequence

<220>

<223> CD3 VHVL aL x 5-10 VHVL

<400> 4

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr 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 Val Glu Gly Gly Ser Gly Gly Ser Gly
115 120 125

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser
130 135 140

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
165 170 175

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser
180 185 190

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser
195 200 205

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
 210 215 220

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
 225 230 235 240

Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln
 245 250 255

Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Ile Ser Cys
 260 265 270

Lys Ala Ser Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys
 275 280 285

Gln Arg Pro Gly His Gly Leu Glu Trp Ile Gly Asp Ile Phe Pro Gly
 290 295 300

Ser Gly Asn Ile His Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu
 305 310 315 320

Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu
 325 330 335

Thr Phe Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp
 340 345 350

Asp Glu Pro Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser
 355 360 365

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 370 375 380

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Thr Val Thr Ala Gly
 385 390 395 400

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
 405 410 415

Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln
 420 425 430

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
 435 440 445

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 450 455 460

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
 465 470 475 480

Asp Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile
 485 490 495

Lys His His His His His His
 500

<210> 5
 <211> 31
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VH BsrGI primer

<400> 5
 aggtgtacac tccgatatca aactgcagca g 31

<210> 6
 <211> 25
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VL BspEI primer

<400> 6
 aatccggatt tcagctccag cttgg 25

<210> 7
 <211> 1521
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL aL Ser x 4-7 VHVL

<400> 7
 gatatacaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg 60

tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120

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cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac      180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac      240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat      300
gatgatcatt actcccttga ctactggggc caaggcacca ctctcacagt ctccctcagtc      360
gaaggtggaa gtggaggttc tgggtggaagt ggaggttcag gtggagtcga cgacattcag      420
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agagccagtt caagtgtaag ttacatgaac tggtagcagc agaagtcagg cacctcccc      540
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agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgctgcc      660
acttattact gccaacagtg gagtagtaac ccgctcacgt tcggtgctgg gaccaagctg      720
gagctgaaat ccggaggtgg tggatccgag gtgcagctgc tcgagcagtc tggagctgag      780
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aactatgggt taagctgggt gaagcagagg cctggacagg tccttgagtg gattggagag      900
gtttatccta gaattggtaa tgcttactac aatgagaagt tcaagggcaa ggccacactg      960
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tctgcggtct atttctgtgc aagacgggga tcctacgata ctaactacga ctggtacttc     1080
gatgtctggg gccaaaggac cacggtcacc gtctcctcag gtggtggtgg ttctggcggc     1140
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gtcagtcttg gagatcaagc ctccatctct tgcagatcta gtcagagcct tgtacacagt     1260
aatggaaaca cctatttaca ttggtacctg cagaagccag gccagtctcc aaagctcctg     1320
atctacaaag tttccaaccg attttctggg gtcccagaca ggttcagtgg cagtggatca     1380
gggacagatt tcacactcaa gatcagcaga gtggaggctg aggatctggg agttttatttc     1440
tgctctcaaa gtacacatgt tccgtacacg ttcggagggg ggaccaagct tgagatcaaa     1500
catcatcacc atcatcatta g                                             1521

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<210> 8
<211> 506
<212> PRT
<213> artificial sequence

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<220>
<223> CD3 VHVL aL Ser x 4-7 VHVL

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<400> 8

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr 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 Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly
115 120 125

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser
130 135 140

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
165 170 175

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser
180 185 190

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser
195 200 205

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
210 215 220

Gln	Gln	Trp	Ser	Ser	Asn	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	225	230	235	240
Glu	Leu	Lys	Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	Gln	Leu	Leu	Glu	Gln	245	250	255	
Ser	Gly	Ala	Glu	Leu	Ala	Arg	Pro	Gly	Ala	Ser	Val	Lys	Leu	Ser	Cys	260	265	270	
Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Asn	Tyr	Gly	Leu	Ser	Trp	Val	Lys	275	280	285	
Gln	Arg	Pro	Gly	Gln	Val	Leu	Glu	Trp	Ile	Gly	Glu	Val	Tyr	Pro	Arg	290	295	300	
Ile	Gly	Asn	Ala	Tyr	Tyr	Asn	Glu	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	305	310	315	320
Thr	Ala	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Ser	Met	Glu	Leu	Arg	Ser	Leu	325	330	335	
Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg	Arg	Gly	Ser	Tyr	340	345	350	
Asp	Thr	Asn	Tyr	Asp	Trp	Tyr	Phe	Asp	Val	Trp	Gly	Gln	Gly	Thr	Thr	355	360	365	
Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	370	375	380	
Gly	Gly	Gly	Ser	Glu	Leu	Val	Met	Thr	Gln	Thr	Pro	Leu	Ser	Leu	Pro	385	390	395	400
Val	Ser	Leu	Gly	Asp	Gln	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	405	410	415	
Leu	Val	His	Ser	Asn	Gly	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	420	425	430	
Pro	Gly	Gln	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	435	440	445	

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
 450 455 460

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe
 465 470 475 480

Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys
 485 490 495

Leu Glu Ile Lys His His His His His His
 500 505

<210> 9
 <211> 1512
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL aL Ser x 5-10 VHVL

<400> 9
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 tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120
 cctggacagg gtctggaatg gattggatac attaatecta gccgtgggta tactaattac 180
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240
 atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
 gatgatcatt actcccttga ctactggggc caaggcacca ctctcacagt ctctcagtc 360
 gaaggtggaa gtggaggttc tgggtggaagt ggaggttcag gtggagtcga cgacattcag 420
 ctgacccagt ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc 480
 agagccagtt caagtgtaag ttacatgaac tggtagcagc agaagtcagg cacctcccc 540
 aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc 600
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 ctggtaaggc ctgggacttc agtgaagata tcctgcaagg cttctggata cgccttcact 840
 aactactggc taggttgggt aaagcagagg cctggacatg gacttgagtg gattggagat 900
 attttccctg gaagtggtaa tatccactac aatgagaagt tcaagggcaa agccacactg 960

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caagggacca cggtcaccgt ctcttcaggt ggtggtgggt ctggcggcgg cggctccggt 1140
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ttcactctca ccatcagcag tgtgcaggct gaagacctgg cagtttatta ctgtcagaat 1440
gattatagtt atccgctcac gttcgggtgct gggaccaagc ttgagatcaa acatcatcac 1500
catcatcatt ag 1512

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<210> 10
<211> 503
<212> PRT
<213> artificial sequence

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<220>
<223> CD3 VHVL aL Ser x 5-10 VHVL

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<400> 10

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```

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1           5           10           15

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Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr
          20           25           30

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```

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

```

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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 Ser Leu Asp Tyr Trp Gly Gln Gly

```

100	105	110
Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly		
115	120	125
Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser		
130	135	140
Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys		
145	150	155
Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser		
	165	170
Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser		
	180	185
Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser		
	195	200
Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys		
	210	215
Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu		
225	230	235
Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln		
	245	250
Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Ile Ser Cys		
	260	265
Lys Ala Ser Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys		
	275	280
Gln Arg Pro Gly His Gly Leu Glu Trp Ile Gly Asp Ile Phe Pro Gly		
	290	295
Ser Gly Asn Ile His Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu		
305	310	315
Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu		
	325	330
		335

Thr Phe Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp
 340 345 350

Asp Glu Pro Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser
 355 360 365

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 370 375 380

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Thr Val Thr Ala Gly
 385 390 395 400

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
 405 410 415

Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln
 420 425 430

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
 435 440 445

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 450 455 460

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
 465 470 475 480

Asp Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile
 485 490 495

Lys His His His His His His
 500

<210> 11
 <211> 1485
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL stL x 3-1 VHVL

<400> 11
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60

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tcttgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120
cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac 180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctcctcaggt 360
gggtgggtggt ctggcggcgg cggctccggg ggtgggtggt ctgacattca gctgaccag 420
tctccagcaa tcatgtctgc atctccaggg gagaagggtca ccatgacctg cagagccagt 480
tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaaagatgg 540
atztatgaca catccaaagt ggcttctgga gtcccttata gcttcagtgg cagtgggtct 600
gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac 660
tgccaacagt ggagtagtaa cccgctcacg ttcgggtgctg ggaccaagct ggagctgaaa 720
tccggagggtg gtggatccga ggtgcagctg ctcgagcagt ctggagctga gctgggtgaaa 780
cctggggcct cagtgaagat atcctgcaag gcttctggat acgccttcac taactactgg 840
ctagggttggg taaagcagag gcctggacat ggacttgagt ggattggaga tcttttcct 900
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tcaaggttca gtggcagtgg atctggtaca gatttcactc tcaccatcag tagcctggag 1380
cctgaagatt ttgcaatgta ttactgtcaa cagcataatg aatatccgta cacgttcgga 1440
ggggggacca agcttgagat caaacatcat caccatcacc attag 1485

```

```

<210> 12
<211> 494
<212> PRT
<213> artificial sequence

```

```

<220>
<223> CD3 VHVL stL x 3-1 VHVL

```

<400> 12

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr 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 Gly Gly Gly Gly Ser Gly Gly Gly Gly
 115 120 125

Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile
 130 135 140

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg 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 Val Ala Ser Gly Val Pro
 180 185 190

Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
 195 200 205

Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
 210 215 220

Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
 225 230 235 240

Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala
 245 250 255

Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser
 260 265 270

Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro
 275 280 285

Gly His Gly Leu Glu Trp Ile Gly Asp Leu Phe Pro Gly Ser Gly Asn
 290 295 300

Thr His Tyr Asn Glu Arg Phe Arg Gly Lys Ala Thr Leu Thr Ala Asp
 305 310 315 320

Lys Ser Ser Ser Thr Ala Phe Met Gln Leu Ser Ser Leu Thr Ser Glu
 325 330 335

Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp Asp Glu Ala
 340 345 350

Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly
 355 360 365

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Val
 370 375 380

Met Thr Gln Ser Pro Ser Tyr Leu Ala Ala Ser Pro Gly Glu Thr Ile
 385 390 395 400

Thr Ile Asn Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr Leu Ala Trp
 405 410 415

Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile Tyr Ser Gly
 420 425 430

Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser
 435 440 445

Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe
 450 455 460

Ala Met Tyr Tyr Cys Gln Gln His Asn Glu Tyr Pro Tyr Thr Phe Gly
 465 470 475 480

Gly Gly Thr Lys Leu Glu Ile Lys His His His His His His
 485 490

<210> 13
 <211> 1512
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL stL x 4-7 VHVL

<400> 13
 gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg 60
 tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120
 cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac 180
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240
 atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
 gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctcctcaggt 360
 ggtggtgggt ctggcggcgg cggctccggt ggtggtgggt ctgacattca gctgacctag 420
 tctccagcaa tcatgtctgc atctccaggg gagaagggtca ccatgacctg cagagccagt 480
 tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaaagatgg 540
 atttatgaca catccaaagt ggcttctgga gtcccttata gcttcagtgg cagtgggtct 600
 gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac 660
 tgccaacagt ggagtagtaa cccgctcacg ttcggtgctg ggaccaagct ggagctgaaa 720
 tccggagggtg gtggatccga ggtgcagctg ctcgagcagt ctggagctga gctggcgagg 780
 cctggggcctt cagtgaagct gtcttgcaag gcttctggct acaccttcac aaactatggt 840
 ttaagctggg tgaagcagag gcctggacag gtcttgagt ggattggaga ggtttatcct 900
 agaattggta atgcttacta caatgagaag ttcaagggca aggccacact gactgcagac 960
 aaatcctcca gcacagcgtc catggagctc cgcagcctga cctctgagga ctctgcggtc 1020
 tatttctgtg caagacgggg atcctacgat actaactacg actggtactt cgatgtctgg 1080

```

ggccaagggga ccacgggtcac cgtctcctca ggtggtggtg gttctggcgg cggcggctcc 1140
ggtggtggtg gttctgagct cgtgatgacc cagactccac tctccctgcc tgtcagtctt 1200
ggagatcaag cctccatctc ttgcagatct agtcagagcc ttgtacacag taatggaaac 1260
acctatttac attggtacct gcagaagcca ggccagtctc caaagctcct gatctacaaa 1320
gtttccaacc gattttcttg ggtcccagac aggttcagtg gcagtggatc agggacagat 1380
ttcacactca agatcagcag agtggaggct gaggatctgg gagtttattt ctgctctcaa 1440
agtacacatg ttccgtacac gttcggaggg gggaccaagc ttgagatcaa acatcatcac 1500
catcatcatt ag 1512

```

```

<210> 14
<211> 503
<212> PRT
<213> artificial sequence

```

```

<220>
<223> CD3 VHVL stL x 4-7 VHVL

```

```

<400> 14

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```

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1             5             10             15

```

```

Ser Val Lys Met Ser Cys Lys Thr 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 Gly Gly Gly Gly Ser Gly Gly Gly Gly
          115             120             125

```

Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile
 130 135 140

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg 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 Val Ala Ser Gly Val Pro
 180 185 190

Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
 195 200 205

Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
 210 215 220

Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
 225 230 235 240

Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala
 245 250 255

Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser
 260 265 270

Gly Tyr Thr Phe Thr Asn Tyr Gly Leu Ser Trp Val Lys Gln Arg Pro
 275 280 285

Gly Gln Val Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn
 290 295 300

Ala Tyr Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp
 305 310 315 320

Lys Ser Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu Thr Ser Glu
 325 330 335

Asp Ser Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr Asp Thr Asn
 340 345 350

Tyr Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val
 355 360 365

Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
 370 375 380

Ser Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu
 385 390 395 400

Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His
 405 410 415

Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln
 420 425 430

Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val
 435 440 445

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
 450 455 460

Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln
 465 470 475 480

Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
 485 490 495

Lys His His His His His His
 500

<210> 15
 <211> 1512
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL stL x 4-7 VLVH

<400> 15
 gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg 60
 tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120
 cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac 180
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240

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atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctcctcaggt 360
ggtaggtggt ctggcggcgg cggctccggt ggtggtaggt ctgacattca gctgacctag 420
tctccagcaa tcatgtctgc atctccaggg gagaagggtc ccatgacctg cagagccagt 480
tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaaagatgg 540
atztatgaca catccaaagt ggcttctgga gtcccttata gcttcagtgg cagtgggtct 600
gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac 660
tgccaacagt ggagtagtaa cccgctcacg ttccggtgctg ggaccaagct ggagctgaaa 720
tccggagggtg gtggatccga gctcgtgatg acccagactc cactctccct gcctgtcagt 780
cttgagatc aagcctccat ctcttgaga tctagtcaga gccttgtaga cagtaatgga 840
aacacctatt tacattggta cctgcagaag ccaggccagt ctccaaagct cctgatctac 900
aaagtttcca accgattttc tgggggtcca gacagggtca gtggcagtgg atcagggaca 960
gatttcacac tcaagatcag cagagtggag gctgaggatc tgggagttta tttctgctct 1020
caaagtacac atgttccgta cacgttcgga ggggggacca agcttgagat caaagggtgg 1080
ggtaggttctg gcggcggcgg ctccggtggt ggtggttctg aggtgcagct gctcgagcag 1140
tctggagctg agctggcgag gcctggggct tcagtgaagc tgcctgcaa ggcttctggc 1200
tacaccttca caaactatgg tttaagctgg gtgaagcaga ggcttgaga ggtccttgag 1260
tggtattggag aggtttatcc tagaattggt aatgcttact acaatgagaa gttcaagggc 1320
aaggccacac tgactgcaga caaatcctcc agcacagcgt ccatggagct ccgcagcctg 1380
acctctgagg actctgcggt ctatttctgt gcaagacggg gatcctacga tactaactac 1440
gactggtact tcgatgtctg gggccaaggg accacggtca ccgtctctc acatcatcac 1500
catcatcatt ag 1512

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```

<210> 16
<211> 503
<212> PRT
<213> artificial sequence

```

```

<220>
<223> CD3 VHVL stL x 4-7 VLVH

```

```

<400> 16

```

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala

1		5		10		15											
Ser	Val	Lys	Met	Ser	Cys	Lys	Thr	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	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly		
		115					120					125					
Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ala	Ile		
	130					135					140						
Met	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys	Arg	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	Val	Ala	Ser	Gly	Val	Pro		
			180					185					190				
Tyr	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile		
	195						200					205					
Ser	Ser	Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp		
	210					215					220						
Ser	Ser	Asn	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys		
225					230					235					240		

Ser Gly Gly Gly Gly Ser Glu Leu Val Met Thr Gln Thr Pro Leu Ser
245 250 255

Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser
260 265 270

Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu
275 280 285

Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn
290 295 300

Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
305 310 315 320

Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val
325 330 335

Tyr Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly
340 345 350

Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
355 360 365

Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu
370 375 380

Leu Ala Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly
385 390 395 400

Tyr Thr Phe Thr Asn Tyr Gly Leu Ser Trp Val Lys Gln Arg Pro Gly
405 410 415

Gln Val Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn Ala
420 425 430

Tyr Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys
435 440 445

Ser Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu Thr Ser Glu Asp
450 455 460

Ser Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr Asp Thr Asn Tyr
 465 470 475 480

Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser
 485 490 495

Ser His His His His His His
 500

<210> 17
 <211> 1503
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL stL x 5-10 VHVL

<400> 17
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 cctggacagg gtctggaatg gattggatac attaatccta gccgtgggtta tactaattac 180
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240
 atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
 gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcaggt 360
 ggtggtgggt ctggcggcgg cggctccggt ggtggtgggt ctgacattca gctgaccag 420
 tctccagcaa tcatgtctgc atctccaggg gagaaggcca ccatgacctg cagagccagt 480
 tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaaagatgg 540
 atttatgaca catccaaagt ggcttctgga gtcccttata gcttcagtgg cagtgggtct 600
 gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac 660
 tgccaacagt ggagtagtaa cccgctcacg ttcggtgctg ggaccaagct ggagctgaaa 720
 tccggaggtg gtggatccga ggtgcagctg ctcgagcagt ctggagctga gctggtaagg 780
 cctgggactt cagtgaagat atcctgcaag gcttctggat acgccttcac taactactgg 840
 ctaggttggg taaagcagag gcctggacat ggacttgagt ggattggaga tattttccct 900
 ggaagtggta atatccacta caatgagaag ttcaagggca aagccacact gactgcagac 960
 aaatcttcga gcacagccta tatgcagctc agtagcctga catttgagga ctctgctgtc 1020
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acggtcaccg tctcctcagg tgggtgggtggt tctggcggcg gcggctccgg tgggtgggtggt 1140
tctgagctcg tgatgacaca gtctccatcc tccctgactg tgacagcagg agagaaggtc 1200
actatgagct gcaagtccag tcagagtctg ttaaacagtg gaaatcaaaa gaactacttg 1260
acctgggtacc agcagaaacc agggcagcct cctaaactgt tgatctactg ggcattccact 1320
agggaatctg gggtccttga tcgcttcaca ggcagtggat ctggaacaga ttctactctc 1380
accatcagca gtgtgcaggc tgaagacctg gcagtttatt actgtcagaa tgattatagt 1440
tatccgctca cgttcgggtgc tgggaccaag cttgagatca aacatcatca ccatcatcat 1500
tag 1503

<210> 18
<211> 500
<212> PRT
<213> artificial sequence

<220>
<223> CD3 VHVL stL x 5-10 VHVL

<400> 18

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr 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 Gly Gly Gly Gly Ser Gly Gly Gly Gly

115		120		125											
Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ala	Ile
130					135						140				
Met	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys	Arg	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	Val	Ala	Ser	Gly	Val	Pro
			180					185					190		
Tyr	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile
	195						200					205			
Ser	Ser	Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp
210						215					220				
Ser	Ser	Asn	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys
225					230					235					240
Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	Gln	Leu	Leu	Glu	Gln	Ser	Gly	Ala
				245					250					255	
Glu	Leu	Val	Arg	Pro	Gly	Thr	Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser
			260					265					270		
Gly	Tyr	Ala	Phe	Thr	Asn	Tyr	Trp	Leu	Gly	Trp	Val	Lys	Gln	Arg	Pro
	275						280					285			
Gly	His	Gly	Leu	Glu	Trp	Ile	Gly	Asp	Ile	Phe	Pro	Gly	Ser	Gly	Asn
290						295					300				
Ile	His	Tyr	Asn	Glu	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp
305					310					315					320
Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln	Leu	Ser	Ser	Leu	Thr	Phe	Glu
				325					330					335	
Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg	Leu	Arg	Asn	Trp	Asp	Glu	Pro
			340					345					350		

Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly
 355 360 365

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser Glu Leu Val
 370 375 380

Met Thr Gln Ser Pro Ser Ser Leu Thr Val Thr Ala Gly Glu Lys Val
 385 390 395 400

Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser Gly Asn Gln
 405 410 415

Lys Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys
 420 425 430

Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg
 435 440 445

Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser
 450 455 460

Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp Tyr Ser
 465 470 475 480

Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys His His
 485 490 495

His His His His
 500

<210> 19
 <211> 1503
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL stL x 5-10 VLVH

<400> 19
 gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg 60
 tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120
 cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac 180

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aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcaggt 360
ggtggtggtt ctggcggcgg cggctccggt ggtggtggtt ctgacattca gctgaccag 420
tctccagcaa tcatgtctgc atctccaggg gagaagggtca ccatgacctg cagagccagt 480
tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaaagatgg 540
atztatgaca catccaaagt ggcttctgga gtcccttata gcttcagtgg cagtgggtct 600
gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac 660
tgccaacagt ggagtagtaa cccgctcacg ttcggtgctg ggaccaagct ggagctgaaa 720
tccggaggtg gtggatccga gctcgtgatg acacagtctc catcctccct gactgtgaca 780
gcaggagaga aggtcactat gagctgcaag tccagtcaga gtctgttaaa cagtggaaat 840
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tactgggcat ccactaggga atctggggtc cctgatcgct tcacaggcag tggatctgga 960
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cagaatgatt atagttatcc gctcacgttc ggtgctggga ccaagcttga gatcaaaggt 1080
ggtggtggtt ctggcggcgg cggctccggt ggtggtggtt ctgaggtgca gctgctcgag 1140
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ctgacatttg aggactctgc tgtctatttc tgtgcaagac tgaggaactg ggacgagcct 1440
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tag 1503

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<210> 20
<211> 500
<212> PRT
<213> artificial sequence

<220>
<223> CD3 VHVL stL x 5-10 VLVH

<400> 20

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Ser	Val	Lys	Met	Ser	Cys	Lys	Thr	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	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	115	120	125	
Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ala	Ile	130	135	140	
Met	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys	Arg	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	Val	Ala	Ser	Gly	Val	Pro	180	185	190	
Tyr	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	195	200	205	
Ser	Ser	Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	210	215	220	
Ser	Ser	Asn	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys				

225					230						235					240
Ser	Gly	Gly	Gly	Gly	Ser	Glu	Leu	Val	Met	Thr	Gln	Ser	Pro	Ser	Ser	
				245					250					255		
Leu	Thr	Val	Thr	Ala	Gly	Glu	Lys	Val	Thr	Met	Ser	Cys	Lys	Ser	Ser	
			260					265					270			
Gln	Ser	Leu	Leu	Asn	Ser	Gly	Asn	Gln	Lys	Asn	Tyr	Leu	Thr	Trp	Tyr	
		275					280					285				
Gln	Gln	Lys	Pro	Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	
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Thr	Arg	Glu	Ser	Gly	Val	Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Gly	
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Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Val	Gln	Ala	Glu	Asp	Leu	Ala	
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Val	Tyr	Tyr	Cys	Gln	Asn	Asp	Tyr	Ser	Tyr	Pro	Leu	Thr	Phe	Gly	Ala	
			340					345					350			
Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	
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Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	Gln	Leu	Leu	Glu	Gln	Ser	Gly	Ala	
	370					375					380					
Glu	Leu	Val	Arg	Pro	Gly	Thr	Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	
385					390					395					400	
Gly	Tyr	Ala	Phe	Thr	Asn	Tyr	Trp	Leu	Gly	Trp	Val	Lys	Gln	Arg	Pro	
				405					410					415		
Gly	His	Gly	Leu	Glu	Trp	Ile	Gly	Asp	Ile	Phe	Pro	Gly	Ser	Gly	Asn	
			420					425					430			
Ile	His	Tyr	Asn	Glu	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	
		435					440					445				
Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln	Leu	Ser	Ser	Leu	Thr	Phe	Glu	
	450					455					460					

Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp Asp Glu Pro
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Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser His His
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His His His His
 500

<210> 21
 <211> 57
 <212> DNA
 <213> artificial sequence

<220>
 <223> 3' CD3 VH GS15 primer

<400> 21
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<210> 22
 <211> 53
 <212> DNA
 <213> artificial sequence

<220>
 <223> 5' CD3 VL GS15 primer

<400> 22
 ggcggcgggcg gctccggtgg tgggtggttct gacattcagc tgaccagtc tcc 53

<210> 23
 <211> 51
 <212> DNA
 <213> artificial sequence

<220>
 <223> 4-7 VH GS15 FOR

<400> 23
 ggcggcgggcg gctccggtgg tgggtggttct gaggtgcagc tgctcgagca g 51

<210> 24
 <211> 53
 <212> DNA
 <213> artificial sequence

<220>
 <223> 4-7 VH SalI REV primer

<400> 24
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<210> 25
 <211> 49
 <212> DNA
 <213> artificial sequence

<220>
 <223> 5-10 VLBspEI38 primer

<400> 25
 ctgaaatccg gaggtggtgg atccgagctc gtgatgacac agtctccat 49

<210> 26
 <211> 53
 <212> DNA
 <213> artificial sequence

<220>
 <223> 5-10 VLGS15REV primer

<400> 26
 ggagccgccg ccgccagaac caccaccacc ttgatctca agcttggtcc cag 53

<210> 27
 <211> 49
 <212> DNA
 <213> artificial sequence

<220>
 <223> 5-10 VH GS15 FOR primer

<400> 27
 ggcggcggcg gctccggtgg tgggtggttct gaggtgcagc tgctcgagc 49

<210> 28
 <211> 53
 <212> DNA
 <213> artificial sequence

<220>
 <223> 5-10 VHSalIREV primer

<400> 28
 ttttaagtcg acctaatagat gatgatgatg atgtgaggag acggtgaccg tgg 53

<210> 29
 <211> 1581
 <212> DNA
 <213> artificial sequence

<220>

<223> 3-5 (VL-VH) xanti-CD3

<400> 29

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cgcgagctcg tgatgacca gactccactc tccctgcctg tcagtcttgg agatcaagcc	120
tccatctctt gcagatctag tcagagcctt gtacacagta atggaaacac ctattttacat	180
tggtacctgc agaagccagg ccagtctcca aagctcctga tctacaaagt ttccaaccga	240
ttttctgggg tcccagacag gttcagtggc agtggatcag ggacagattt cacactcaag	300
atcagcagag tggaggctga ggatctggga gtttatttct gctctcaaag tacacatggt	360
ccgtacacgt tcggaggggg gaccaagctt gagatcaaag gtggtggtgg ttctggcggc	420
ggcggctccg gtggtggtgg ttctgaggtg cagctgctcg agcagtctgg agctgagctg	480
gtaaggcctg ggacttcagt gaagctgtcc tgcaaggctt ctggctacac cttcacaagc	540
tatggtttaa gctgggtgaa gcagagaact ggacagggcc ttgagtggat tggagaggtt	600
tatcctagaa ttggtaatgc ttactacaat gagaagttca agggcaaggc cacactgact	660
gcagacaaat cctccagcac agcgtccatg gagctccgca gcctgacatc tgaggactct	720
gcggtctatt tctgtgcaag acggggatcc tacggtagta actacgactg gtacttcgat	780
gtctggggcc aagggaccac ggtcaccgtc tcctccggag gtggtggatc cgatatcaaa	840
ctgcagcagt caggggctga actggcaaga cctggggcct cagtgaagat gtcctgcaag	900
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tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaaagatgg	1380
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gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac	1500
tgccaacagt ggagtagtaa cccgctcacg ttcggtgctg ggaccaagct ggagctgaaa	1560

catcatcacc atcatcatta g

1581

<210> 30
 <211> 526
 <212> PRT
 <213> artificial sequence

<220>
 <223> 3-5 (VL-VH)xanti-CD3

<400> 30

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Val His Ser Ala Arg Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu
 20 25 30

Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln
 35 40 45

Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln
 50 55 60

Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg
 65 70 75 80

Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
 85 90 95

Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr
 100 105 110

Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr
 115 120 125

Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 130 135 140

Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu
 145 150 155 160

Val Arg Pro Gly Thr Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr
 165 170 175

Thr Phe Thr Ser Tyr Gly Leu Ser Trp Val Lys Gln Arg Thr Gly Gln
 180 185 190

Gly Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn Ala Tyr
 195 200 205

Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
 210 215 220

Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser
 225 230 235 240

Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr Gly Ser Asn Tyr Asp
 245 250 255

Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
 260 265 270

Gly Gly Gly Gly Ser Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu
 275 280 285

Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr
 290 295 300

Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln
 305 310 315 320

Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn
 325 330 335

Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser
 340 345 350

Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser
 355 360 365

Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp
 370 375 380

Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly
 385 390 395 400

Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile

405

410

415

Gln Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys
 420 425 430

Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp
 435 440 445

Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr
 450 455 460

Ser Lys Val Ala Ser Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser
 465 470 475 480

Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala
 485 490 495

Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly
 500 505 510

Ala Gly Thr Lys Leu Glu Leu Lys His His His His His His
 515 520 525

<210> 31
 <211> 40
 <212> DNA
 <213> artificial sequence

<220>
 <223> Me81 primer

<400> 31
 ggatgcgcgc gagctcgtga tgacccagac tccactctcc

40

<210> 32
 <211> 60
 <212> DNA
 <213> artificial sequence

<220>
 <223> Me83 primer

<400> 32
 gggttctggcg gcggcggctc cggtggtggt gggttctgagg tgcagctgct cgacagtctg

60

<210> 33
 <211> 41

<212> DNA
 <213> artificial sequence

<220>
 <223> Me84 primer

<400> 33
 gtgctccgga ggagacggtg accgtggtcc cttggcccca g 41

<210> 34
 <211> 53
 <212> DNA
 <213> artificial sequence

<220>
 <223> Me90 primer

<400> 34
 ccggagccgc cgccgccaga accaccacca cttttgatct caagcttggt ccc 53

<210> 35
 <211> 1548
 <212> DNA
 <213> artificial sequence

<220>
 <223> 3-1 (VLVH) xanti-CD3

<400> 35
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 ctcgtcatga ccagtcctcc atcttatctt gctgcatctc ctggagaaac cattactatt 120
 aattgcaggg caagtaagag cattagcaaa tatttagcct ggtatcaaga gaaacctggg 180
 aaaactaata agcttcttat ctactctgga tccactttgc aatctggaat tccatcaagg 240
 ttcagtggca gtggatctgg tacagatttc actctcacca tcagtagcct ggagcctgaa 300
 gattttgcaa tgtattactg tcaacagcat aatgaatata cgtacacgtt cggagggggg 360
 accaagcttg agatcaaagg tggtggtggt tctggcggcg gcggctccgg tggtggtggt 420
 tctgaggtgc agctgctcga gcagtctgga gctgagctgg tgaaacctgg ggcctcagtg 480
 aagatatcct gcaaggcttc tggatacgcc ttcactaact actggctagg ttgggtaaag 540
 cagaggcctg gacatggact tgagtggatt ggagatcttt tccctggaag tggtaatact 600
 cactacaatg agaggttcag gggcaaagcc acactgactg cagacaaatc ctcgagcaca 660
 gcctttatgc agctcagtag cctgacatct gaggactctg ctgtctatct ctgtgcaaga 720
 ttgaggaact gggacgaggc tatggactac tggggccaag ggaccacggt caccgtctcc 780

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ggggcctcag tgaagatgtc ctgcaagact tctggctaca cctttactag gtacacgatg      900
cactgggtaa aacagaggcc tggacagggt ctggaatgga ttggatacat taatcctagc      960
cgtgggttata ctaattacaa tcagaagttc aaggacaagg ccacattgac tacagacaaa     1020
tcctccagca cagcctacat gcaactgagc agcctgacat ctgaggactc tgcagtctat     1080
tactgtgcaa gatattatga tgatcattac tgccttgact actggggcca aggcaccact     1140
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aaggtcacca tgacctgcag agccagttca agtgtaagtt acatgaactg gtaccagcag     1320
aagtcaggca cctcccccaa aagatggatt tatgacacat ccaaagtggc ttctggagtc     1380
ccttatcgct tcagtggcag tgggtctggg acctcatact ctctcacaat cagcagcatg     1440
gaggctgaag atgctgccac ttattactgc caacagtgga gtagtaacc gctcacgttc     1500
ggtgctggga ccaagctgga gctgaaacat catcaccatc atcattag                    1548

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<210> 36
<211> 515
<212> PRT
<213> artificial sequence

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<220>
<223> 3-1(VLVH)xanti-CD3

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<400> 36

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Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1              5              10              15

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Val His Ser Glu Leu Val Met Thr Gln Ser Pro Ser Tyr Leu Ala Ala
20              25              30

```

```

Ser Pro Gly Glu Thr Ile Thr Ile Asn Cys Arg Ala Ser Lys Ser Ile
35              40              45

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Ser Lys Tyr Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys
50              55              60

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Leu Leu Ile Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg
65              70              75              80

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Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	85	90	95	
Leu	Glu	Pro	Glu	Asp	Phe	Ala	Met	Tyr	Tyr	Cys	Gln	Gln	His	Asn	Glu	100	105	110	
Tyr	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly	Gly	115	120	125	
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	Gln	130	135	140	
Leu	Leu	Glu	Gln	Ser	Gly	Ala	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser	Val	145	150	155	160
Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Thr	Asn	Tyr	Trp	Leu	165	170	175	
Gly	Trp	Val	Lys	Gln	Arg	Pro	Gly	His	Gly	Leu	Glu	Trp	Ile	Gly	Asp	180	185	190	
Leu	Phe	Pro	Gly	Ser	Gly	Asn	Thr	His	Tyr	Asn	Glu	Arg	Phe	Arg	Gly	195	200	205	
Lys	Ala	Thr	Leu	Thr	Ala	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Phe	Met	Gln	210	215	220	
Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg	225	230	235	240
Leu	Arg	Asn	Trp	Asp	Glu	Ala	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	245	250	255	
Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Lys	Leu	Gln	Gln	260	265	270	
Ser	Gly	Ala	Glu	Leu	Ala	Arg	Pro	Gly	Ala	Ser	Val	Lys	Met	Ser	Cys	275	280	285	
Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Arg	Tyr	Thr	Met	His	Trp	Val	Lys	290	295	300	
Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Tyr	Ile	Asn	Pro	Ser				

305		310		315		320
Arg Gly Tyr Thr	Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu					
	325			330		335
Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu						
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Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp						
	355			360		365
His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser						
	370			375		380
Ser Val Glu Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly						
385		390		395		400
Gly Val Asp Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile Met Ser Ala						
	405			410		415
Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val						
	420			425		430
Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg						
	435			440		445
Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly Val Pro Tyr Arg Phe						
	450			455		460
Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met						
465		470		475		480
Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn						
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Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys His His His						
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His His His						
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<210>	37					
<211>	52					

<212> DNA
 <213> artificial sequence

<220>
 <223> Me91a primer

<400> 37
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<210> 38
 <211> 1566
 <212> DNA
 <213> artificial sequence

<220>
 <223> 4-1 (VLVH) xanti-CD3

<400> 38
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 agctgcaagt ccagtcagag tctgttaaac agtggaaatc aaaagaacta cttggcctgg 180
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 ggatacatta atcctagccg tggttatact aattacaatc agaagttcaa ggacaaggcc 1020
 acattgacta cagacaaatc ctccagcaca gcctacatgc aactgagcag cctgacatct 1080
 gaggactctg cagtctatta ctgtgcaaga tattatgatg atcattactg ccttgactac 1140

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tctgcatctc caggggagaa ggtcaccatg acctgcagag ccagttcaag tgtaagttac 1320
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aaagtggctt ctggagtccc ttatcgcttc agtggcagtg ggtctgggac ctcatactct 1440
ctcacaatca gcagcatgga ggctgaagat gctgccactt attactgcca acagtggagt 1500
agtaaccgcg tcacgttcgg tgctgggacc aagctggagc tgaaacatca tcaccatcat 1560
cattag 1566

```

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<210> 39
<211> 521
<212> PRT
<213> artificial sequence

```

```

<220>
<223> 4-1 (VLVH) xanti-CD3

```

```

<400> 39

```

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1           5           10           15

```

```

Val His Ser Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val
          20           25           30

```

```

Ser Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu
          35           40           45

```

```

Leu Asn Ser Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
          50           55           60

```

```

Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu
65           70           75           80

```

```

Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe
          85           90           95

```

```

Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr
          100          105          110

```

```

Cys Gln Asn Asp Tyr Ser Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys
          115          120          125

```

Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
 130 135 140

Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu Val
 145 150 155 160

Arg Pro Gly Thr Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala
 165 170 175

Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly
 180 185 190

Leu Glu Trp Val Gly Asp Ile Phe Pro Gly Ser Gly Asn Ala His Tyr
 195 200 205

Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser
 210 215 220

Tyr Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala
 225 230 235 240

Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp Asp Glu Ala Met Asp Tyr
 245 250 255

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser
 260 265 270

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
 275 280 285

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr
 290 295 300

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 305 310 315 320

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
 325 330 335

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr
 340 345 350

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 355 360 365

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
 370 375 380

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly
 385 390 395 400

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser
 405 410 415

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
 420 425 430

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
 435 440 445

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser
 450 455 460

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser
 465 470 475 480

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
 485 490 495

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
 500 505 510

Glu Leu Lys His His His His His His
 515 520

<210> 40
 <211> 44
 <212> DNA
 <213> artificial sequence

<220>
 <223> Me92a primer

<400> 40
 ggattgtaca ctccgagctc gtgatgacac agtctccatc ctcc

44

<210> 41
 <211> 1581
 <212> DNA
 <213> artificial sequence

<220>
 <223> 4-7 (VL-VH)xanti CD3

<400> 41
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 cgcgagctcg tgatgaccca gactccactc tccctgcctg tcagtcttgg agatcaagcc 120
 tccatctctt gcagatctag tcagagcctt gtacacagta atggaaacac ctattttacat 180
 tggtagctgc agaagccagg ccagtctcca aagctcctga tctacaaagt ttccaaccga 240
 ttttctgggg tcccagacag gttcagtggc agtggatcag ggacagattt cacactcaag 300
 atcagcagag tggaggctga ggatctggga gtttatttct gctctcaaag tacacatggt 360
 ccgtacacgt tcggaggggg gaccaagctt gagatcaaag gtgggtgggtg ttctggcggc 420
 ggcggctccg gtgggtgggtg ttctgaggtg cagctgctcg agcagtctgg agctgagctg 480
 gcgaggcctg gggcttcagt gaagctgtcc tgcaaggctt ctggctacac cttcacaaac 540
 tatggtttaa gctgggtgaa gcagaggcct ggacaggctc ttgagtggat tggagagggt 600
 taccctagaa ttggtaatgc ttactacaat gagaagttca agggcaaggc cacactgact 660
 gcagacaaat cctccagcac agcgtccatg gagctccgca gcctgacctc tgaggactct 720
 gcggtctatt tctgtgcaag acgggggatcc tacgatacta actacgactg gtacttcgat 780
 gtctggggcc aagggaccac ggtcacctgc tcctccggag gtgggtggatc cgatatcaaa 840
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 tactgccttg actactgggg ccaaggcacc actctcacag tctcctcagt cgaagggtga 1200
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 tctccagcaa tcatgtctgc atctccaggg gagaagggtca ccatgacctg cagagccagt 1320
 tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaaagatgg 1380
 atttatgaca catccaaagt ggcttctgga gtcccttata gcttcagtgg cagtgggtct 1440

gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac 1500
 tgccaacagt ggagtagtaa cccgctcacg ttcgggtgctg ggaccaagct ggagctgaaa 1560
 catcatcacc atcatcatta g 1581

<210> 42
 <211> 526
 <212> PRT
 <213> artificial sequence

<220>
 <223> 4-7(VL-VH)xanti CD3

<400> 42

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15

Val His Ser Ala Arg Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu
 20 25 30

Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln
 35 40 45

Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln
 50 55 60

Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg
 65 70 75 80

Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
 85 90 95

Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr
 100 105 110

Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr
 115 120 125

Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 130 135 140

Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu
 145 150 155 160

Ala	Arg	Pro	Gly	Ala	Ser	Val	Lys	Leu	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	165	170	175
Thr	Phe	Thr	Asn	Tyr	Gly	Leu	Ser	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	180	185	190
Val	Leu	Glu	Trp	Ile	Gly	Glu	Val	Tyr	Pro	Arg	Ile	Gly	Asn	Ala	Tyr	195	200	205
Tyr	Asn	Glu	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Lys	Ser	210	215	220
Ser	Ser	Thr	Ala	Ser	Met	Glu	Leu	Arg	Ser	Leu	Thr	Ser	Glu	Asp	Ser	225	230	235
Ala	Val	Tyr	Phe	Cys	Ala	Arg	Arg	Gly	Ser	Tyr	Asp	Thr	Asn	Tyr	Asp	245	250	255
Trp	Tyr	Phe	Asp	Val	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	260	265	270
Gly	Gly	Gly	Gly	Ser	Asp	Ile	Lys	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	275	280	285
Ala	Arg	Pro	Gly	Ala	Ser	Val	Lys	Met	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	290	295	300
Thr	Phe	Thr	Arg	Tyr	Thr	Met	His	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	305	310	315
Gly	Leu	Glu	Trp	Ile	Gly	Tyr	Ile	Asn	Pro	Ser	Arg	Gly	Tyr	Thr	Asn	325	330	335
Tyr	Asn	Gln	Lys	Phe	Lys	Asp	Lys	Ala	Thr	Leu	Thr	Thr	Asp	Lys	Ser	340	345	350
Ser	Ser	Thr	Ala	Tyr	Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	355	360	365
Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Tyr	Tyr	Asp	Asp	His	Tyr	Cys	Leu	Asp	370	375	380
Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Val	Glu	Gly	Gly			


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ggctccggtg gtggtggttc tgaggtgcag ctgctcgagc agtctggagc tgagctggta 480
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tggctaggtt gggtaaagca gaggcctgga catggacttg agtggattgg agatattttc 600
cctggaagtg gtaatatcca ctacaatgag aagttcaagg gcaaagccac actgactgca 660
gacaaatctt cgagcacagc ctatatgcag ctcagtagcc tgacatttga ggactctgct 720
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accacgggtca cegtctcctc cggaggtggg ggatccgata tcaaactgca gcagtcaggg 840
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cattag 1566

```

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<210> 44
<211> 521
<212> PRT
<213> artificial sequence

```

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<220>
<223> 5-10 (VLVH) xanti-CD3

```

```

<400> 44

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```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1           5           10           15

```

```

Val His Ser Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Thr Val

```

	20		25		30											
Thr	Ala	Gly	Glu	Lys	Val	Thr	Met	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu	
	35						40					45				
Leu	Asn	Ser	Gly	Asn	Gln	Lys	Asn	Tyr	Leu	Thr	Trp	Tyr	Gln	Gln	Lys	
	50					55					60					
Pro	Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Thr	Arg	Glu	
65					70					75					80	
Ser	Gly	Val	Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	
				85					90					95		
Thr	Leu	Thr	Ile	Ser	Ser	Val	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	
			100					105					110			
Cys	Gln	Asn	Asp	Tyr	Ser	Tyr	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	
	115						120					125				
Leu	Glu	Ile	Lys	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	
	130					135					140					
Gly	Gly	Ser	Glu	Val	Gln	Leu	Leu	Glu	Gln	Ser	Gly	Ala	Glu	Leu	Val	
145					150					155					160	
Arg	Pro	Gly	Thr	Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	
				165					170					175		
Phe	Thr	Asn	Tyr	Trp	Leu	Gly	Trp	Val	Lys	Gln	Arg	Pro	Gly	His	Gly	
			180					185					190			
Leu	Glu	Trp	Ile	Gly	Asp	Ile	Phe	Pro	Gly	Ser	Gly	Asn	Ile	His	Tyr	
	195						200					205				
Asn	Glu	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Lys	Ser	Ser	
	210					215					220					
Ser	Thr	Ala	Tyr	Met	Gln	Leu	Ser	Ser	Leu	Thr	Phe	Glu	Asp	Ser	Ala	
225					230					235					240	
Val	Tyr	Phe	Cys	Ala	Arg	Leu	Arg	Asn	Trp	Asp	Glu	Pro	Met	Asp	Tyr	
				245					250					255		

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser
 260 265 270

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
 275 280 285

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr
 290 295 300

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 305 310 315 320

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
 325 330 335

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr
 340 345 350

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 355 360 365

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
 370 375 380

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly
 385 390 395 400

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser
 405 410 415

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
 420 425 430

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
 435 440 445

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser
 450 455 460

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser
 465 470 475 480

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
 485 490 495

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
 500 505 510

Glu Leu Lys His His His His His His
 515 520

<210> 45
 <211> 1494
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL aL x 3-1 VHVL

<400> 45
 gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg 60
 tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120
 cctggacagg gtctggaatg gattggatac attaatecta gccgtgggta tactaattac 180
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240
 atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
 gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcagtc 360
 gaaggtggaa gtggagggtc tgggtggaagt ggagggttcag gtggagtcga cgacattcag 420
 ctgacccagt ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc 480
 agagccagtt caagtgtaag ttacatgaac tggtagcagc agaagtcagg cacctcccc 540
 aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc 600
 agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgctgcc 660
 acttattact gccaacagtg gagtagtaac ccgctcacgt tcggtgctgg gaccaagctg 720
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 ctggtgaaac ctggggcctc agtgaagata tcctgcaagg cttctggata cgccttcact 840
 aactactggc taggttgggt aaagcagagg cctggacatg gacttgagtg gattggagat 900
 cttttccctg gaagtggtaa tactcactac aatgagaggt tcaggggcaa agccacactg 960
 actgcagaca aatcctcgag cacagccttt atgcagctca gtagcctgac atctgaggac 1020
 tctgctgtct atttctgtgc aagattgagg aactgggacg aggctatgga ctactggggc 1080

caagggacca cggtcaccgt ctcctcaggt ggtggtggtt ctggcggcgg cggctccggt 1140
 ggtggtggtt ctgagctcgt catgacccag tctccatctt atcttgctgc atctcctgga 1200
 gaaaccatta ctattaattg cagggcaagt aagagcatta gcaaataattt agcctgggtat 1260
 caagagaaaac ctgggaaaac taataagctt cttatctact ctggatccac tttgcaatct 1320
 ggaattccat caaggttcag tggcagtgga tctggtacag atttcactct caccatcagt 1380
 agcctggagc ctgaagattt tgcaatgtat tactgtcaac agcataatga atatccgtac 1440
 acgttcggag gggggaccaa gcttgagatc aaacatcatc accatcatca ttag 1494

<210> 46
 <211> 497
 <212> PRT
 <213> artificial sequence

<220>
 <223> CD3 VHVL aL x 3-1 VHVL

<400> 46

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr 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 Val Glu Gly Gly Ser Gly Gly Ser Gly
 115 120 125

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser
 130 135 140

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
 145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
 165 170 175

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser
 180 185 190

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser
 195 200 205

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
 210 215 220

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
 225 230 235 240

Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln
 245 250 255

Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys
 260 265 270

Lys Ala Ser Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys
 275 280 285

Gln Arg Pro Gly His Gly Leu Glu Trp Ile Gly Asp Leu Phe Pro Gly
 290 295 300

Ser Gly Asn Thr His Tyr Asn Glu Arg Phe Arg Gly Lys Ala Thr Leu
 305 310 315 320

Thr Ala Asp Lys Ser Ser Ser Thr Ala Phe Met Gln Leu Ser Ser Leu
 325 330 335

Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp
 340 345 350

Asp Glu Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser
 355 360 365

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 370 375 380

Glu Leu Val Met Thr Gln Ser Pro Ser Tyr Leu Ala Ala Ser Pro Gly
 385 390 395 400

Glu Thr Ile Thr Ile Asn Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
 405 410 415

Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile
 420 425 430

Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly
 435 440 445

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
 450 455 460

Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln His Asn Glu Tyr Pro Tyr
 465 470 475 480

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys His His His His His
 485 490 495

His

<210> 47
 <211> 1494
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL aL Ser x 3-1 VHVL

<400> 47
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 cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac 180
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240

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atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
gatgatcatt actcccttga ctactggggc caaggcacca ctctcacagt ctccctcagtc 360
gaaggtggaa gtggaggttc tgggtggaagt ggaggttcag gtggagtcga cgacattcag 420
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aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc 600
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acttattact gccaacagtg gagtagtaac ccgctcacgt tcggtgctgg gaccaagctg 720
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ctggtgaaac ctggggcctc agtgaagata tcctgcaagg cttctggata cgccttcact 840
aactactggc taggttgggt aaagcagagg cctggacatg gacttgagtg gattggagat 900
cttttcctg gaagtggtaa tactcactac aatgagaggt tcaggggcaa agccacactg 960
actgcagaca aatcctcgag cacagccttt atgcagctca gtagcctgac atctgaggac 1020
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agcctggagc ctgaagattt tgcaatgtat tactgtcaac agcataatga atatccgtac 1440
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<210> 48

<211> 497

<212> PRT

<213> artificial sequence

<220>

<223> CD3 VHVL aL Ser x 3-1 VHVL

<400> 48

```

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1           5           10           15

```

Ser Val Lys Met Ser Cys Lys Thr 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 Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly
115 120 125

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser
130 135 140

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
165 170 175

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser
180 185 190

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser
195 200 205

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
210 215 220

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
225 230 235 240

Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln

245	250	255
Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys		
260	265	270
Lys Ala Ser Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys		
275	280	285
Gln Arg Pro Gly His Gly Leu Glu Trp Ile Gly Asp Leu Phe Pro Gly		
290	295	300
Ser Gly Asn Thr His Tyr Asn Glu Arg Phe Arg Gly Lys Ala Thr Leu		
305	310	315
Thr Ala Asp Lys Ser Ser Ser Thr Ala Phe Met Gln Leu Ser Ser Leu		
325	330	335
Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp		
340	345	350
Asp Glu Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser		
355	360	365
Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser		
370	375	380
Glu Leu Val Met Thr Gln Ser Pro Ser Tyr Leu Ala Ala Ser Pro Gly		
385	390	395
Glu Thr Ile Thr Ile Asn Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr		
405	410	415
Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile		
420	425	430
Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly		
435	440	445
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro		
450	455	460
Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln His Asn Glu Tyr Pro Tyr		
465	470	475
		480

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys His His His His His
 485 490 495

His

<210> 49
 <211> 1521
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL aL x 3-5 VHVL

<400> 49
 gatatacaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg 60
 tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120
 cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac 180
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240
 atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
 gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcagtc 360
 gaaggtggaa gtggagggtc tgggtggaagt ggagggttcag gtggagtcga cgacattcag 420
 ctgacccagt ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc 480
 agagccagtt caagtgtaag ttacatgaac tggtaccagc agaagtcagg cacctcccc 540
 aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc 600
 agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgctgcc 660
 acttattact gccaacagtg gagtagtaac ccgctcacgt tcgggtgctgg gaccaagctg 720
 gagctgaaat ccggagggtg tggatccgag gtgcagctgc tcgagcagtc tggagctgag 780
 ctggtaaggc ctgggacttc agtgaagctg tcctgcaagg cttctggcta caccttcaca 840
 agctatgggt taagctgggt gaagcagaga actggacagg gccttgagtg gattggagag 900
 gtttatccta gaattggtaa tgcttactac aatgagaagt tcaagggcaa ggccacactg 960
 actgcagaca aatcctccag cacagcgtcc atggagctcc gcagcctgac atctgaggac 1020
 tctgcggtct atttctgtgc aagacgggga tcctacggta gtaactacga ctgggtacttc 1080
 gatgtctggg gccaaaggac cacggtcacc gtctcctcag gtgggtgggtg ttctggcggc 1140

```

ggcggctccg gtggtggtgg ttctgagctc gtgatgaccc agactccact ctccctgcct 1200
gtcagtcttg gagatcaagc ctccatctct tgcagatcta gtcagagcct tgtacacagt 1260
aatggaaaca cctatttaca ttggtacctg cagaagccag gccagtctcc aaagctcctg 1320
atctacaaag tttccaaccg attttctggg gtcccagaca ggttcagtgg cagtggatca 1380
gggacagatt tcacactcaa gatcagcaga gtggaggctg aggatctggg agtttatttc 1440
tgctctcaaa gtacacatgt tccgtacacg ttcggagggg ggaccaagct tgagatcaaa 1500
catcatcacc atcatcatta g 1521

```

```

<210> 50
<211> 506
<212> PRT
<213> artificial sequence

```

```

<220>
<223> CD3 VHVL aL x 3-5 VHVL

```

```

<400> 50

```

```

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1           5           10           15

```

```

Ser Val Lys Met Ser Cys Lys Thr 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 Val Glu Gly Gly Ser Gly Gly Ser Gly
          115          120          125

```

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser
 130 135 140

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
 145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
 165 170 175

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser
 180 185 190

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser
 195 200 205

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
 210 215 220

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
 225 230 235 240

Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln
 245 250 255

Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Leu Ser Cys
 260 265 270

Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Gly Leu Ser Trp Val Lys
 275 280 285

Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg
 290 295 300

Ile Gly Asn Ala Tyr Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu
 305 310 315 320

Thr Ala Asp Lys Ser Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu
 325 330 335

Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr
 340 345 350

Gly Ser Asn Tyr Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr

355

360

365

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 370 375 380

Gly Gly Gly Ser Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu Pro
 385 390 395 400

Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
 405 410 415

Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys
 420 425 430

Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe
 435 440 445

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
 450 455 460

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe
 465 470 475 480

Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys
 485 490 495

Leu Glu Ile Lys His His His His His His
 500 505

<210> 51
 <211> 1521
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL aL Ser x 3-5 VHVL

<400> 51
 gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg 60
 tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120
 cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac 180
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240
 atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300

```

gatgatcatt actcccttga ctactggggc caaggcacca ctctcacagt ctccctcagtc 360
gaaggtggaa gtggagggttc tgggtggaagt ggagggttcag gtggagtcga cgacattcag 420
ctgacccagt ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc 480
agagccagtt caagtgtaag ttacatgaac tgggtaccagc agaagtcagg cacctcccc 540
aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc 600
agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgctgcc 660
acttattact gccaacagtg gagtagtaac ccgctcacgt tcgggtgctgg gaccaagctg 720
gagctgaaat ccggagggtg tggatccgag gtgcagctgc tcgagcagtc tggagctgag 780
ctggtaaggc ctgggacttc agtgaagctg tcctgcaagg cttctggcta caccttcaca 840
agctatgggt taagctgggt gaagcagaga actggacagg gccttgagtg gattggagag 900
gtttatccta gaattggtaa tgcttactac aatgagaagt tcaagggcaa ggccacactg 960
actgcagaca aatcctccag cacagcgtcc atggagctcc gcagcctgac atctgaggac 1020
tctgcggtct atttctgtgc aagacgggga tcctacggta gtaactacga ctggtacttc 1080
gatgtctggg gccaaaggac cacggtcacc gtctcctcag gtgggtgggtg ttctggcggc 1140
ggcggctccg gtgggtgggtg ttctgagctc gtgatgacct agactccact ctccctgcct 1200
gtcagtcttg gagatcaagc ctccatctct tgcagatcta gtcagagcct tgtacacagt 1260
aatggaaaca cctatttaca ttggtacctg cagaagccag gccagtctcc aaagctcctg 1320
atctacaaag tttccaaccg attttctggg gtcccagaca ggttcagtgg cagtggatca 1380
gggacagatt tcacactcaa gatcagcaga gtggaggctg aggatctggg agttttatttc 1440
tgctctcaaa gtacacatgt tccgtacacg ttcggagggg ggaccaagct tgagatcaaa 1500
catcatcacc atcatcatta g 1521

```

<210> 52

<211> 506

<212> PRT

<213> artificial sequence

<220>

<223> CD3 VHVL aL Ser x 3-5 VHVL

<400> 52

```

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1           5           10           15

```

Ser Val Lys Met Ser Cys Lys Thr 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 Ser Leu Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly
 115 120 125

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser
 130 135 140

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
 145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
 165 170 175

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser
 180 185 190

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser
 195 200 205

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
 210 215 220

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
 225 230 235 240

Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln
245 250 255

Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Leu Ser Cys
260 265 270

Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Gly Leu Ser Trp Val Lys
275 280 285

Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg
290 295 300

Ile Gly Asn Ala Tyr Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu
305 310 315 320

Thr Ala Asp Lys Ser Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu
325 330 335

Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr
340 345 350

Gly Ser Asn Tyr Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr
355 360 365

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
370 375 380

Gly Gly Gly Ser Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu Pro
385 390 395 400

Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
405 410 415

Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys
420 425 430

Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe
435 440 445

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
450 455 460

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe

465		470		475		480									
Cys	Ser	Gln	Ser	Thr	His	Val	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys
				485				490						495	

Leu	Glu	Ile	Lys	His	His	His	His	His	His
			500					505	

<210> 53
 <211> 1512
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL stL x 3-5 VHVL

<400> 53	
gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg	60
tcttgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg	120
cctggacagg gtctggaatg gattggatac attaatccta gccgtgggtta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcaggt	360
ggtggtggtt ctggcggcgg cggctccggt ggtggtggtt ctgacattca gctgacccag	420
tctccagcaa tcatgtctgc atctccaggg gagaaggtea ccatgacctg cagagccagt	480
tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaaagatgg	540
atztatgaca catccaaagt ggcttctgga gtcccttata gcttcagtgg cagtgggtct	600
gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac	660
tgccaacagt ggagtagtaa cccgctcacg ttcggtgctg ggaccaagct ggagctgaaa	720
tccggagggtg gtggatccga ggtgcagctg ctcgagcagt ctggagctga gctggtaagg	780
cctgggactt cagtgaagct gtcctgcaag gcttctgggt acaccttcac aagctatggt	840
ttaagctggg tgaagcagag aactggacag ggccttgagt ggattggaga ggtttatcct	900
agaattggta atgcttacta caatgagaag ttcaagggca aggccacact gactgcagac	960
aaatcctcca gcacagcgtc catggagctc cgcagcctga catctgagga ctctgcggtc	1020
tatttctgtg caagacgggg atcctacggt agtaactacg actggtactt cgatgtctgg	1080
ggccaaggga ccacgggtcac cgtctcctca ggtggtggtg gttctggcgg cggcggctcc	1140

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ggtggtggtg gttctgagct cgtgatgacc cagactccac tctccctgcc tgtcagtctt 1200
ggagatcaag cctccatctc ttgcagatct agtcagagcc ttgtacacag taatggaaac 1260
acctattttac attggtacct gcagaagcca ggccagtctc caaagctcct gatctacaaa 1320
gtttccaacc gattttctgg ggtcccagac aggttcagtg gcagtggatc agggacagat 1380
ttcacactca agatcagcag agtggaggct gaggatctgg gagtttattt ctgctctcaa 1440
agtacacatg ttccgtacac gttcggaggg gggaccaagc ttgagatcaa acatcatcac 1500
catcatcatt ag 1512

```

```

<210> 54
<211> 503
<212> PRT
<213> artificial sequence

```

```

<220>
<223> CD3 VHVL stL x 3-5 VHVL

```

```

<400> 54

```

```

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1           5           10           15

```

```

Ser Val Lys Met Ser Cys Lys Thr 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 Gly Gly Gly Gly Ser Gly Gly Gly Gly
          115           120           125

```

Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile
 130 135 140

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg 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 Val Ala Ser Gly Val Pro
 180 185 190

Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
 195 200 205

Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
 210 215 220

Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
 225 230 235 240

Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala
 245 250 255

Glu Leu Val Arg Pro Gly Thr Ser Val Lys Leu Ser Cys Lys Ala Ser
 260 265 270

Gly Tyr Thr Phe Thr Ser Tyr Gly Leu Ser Trp Val Lys Gln Arg Thr
 275 280 285

Gly Gln Gly Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn
 290 295 300

Ala Tyr Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp
 305 310 315 320

Lys Ser Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu Thr Ser Glu
 325 330 335

Asp Ser Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr Gly Ser Asn
 340 345 350

Tyr Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val
 355 360 365

Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
 370 375 380

Ser Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu
 385 390 395 400

Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His
 405 410 415

Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln
 420 425 430

Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val
 435 440 445

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
 450 455 460

Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln
 465 470 475 480

Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
 485 490 495

Lys His His His His His His
 500

<210> 55
 <211> 1512
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL aL x 4-1 VHVL

<400> 55
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 tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120
 cctggacagg gtctggaatg gattggatac attaatccta gccgtgggtta tactaattac 180
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240

```

atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctccctcagtc 360
gaaggtggaa gtggaggttc tgggtggaagt ggaggttcag gtggagtcga cgacattcag 420
ctgacccagt ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc 480
agagccagtt caagtgtaag ttacatgaac tgggtaccagc agaagtcagg cacctcccc 540
aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc 600
agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgctgcc 660
acttattact gccaacagtg gagtagtaac ccgctcacgt tcggtgctgg gaccaagctg 720
gagctgaaat ccggaggtgg tggatccgag gtgcagctgc tcgagcagtc tggagctgag 780
ctggtaaggc ctgggacttc agtgaagata tcctgcaagg cttctggata cgccttcact 840
aactactggc taggttgggt taagcagagg cctggacatg gacttgaatg ggttggagat 900
atcttcctg gaagtggtaa tgctcactac aatgagaagt tcaagggcaa agccacactg 960
actgcagaca agtcctcgta cacagcctat atgcagctca gtagcctgac atctgaggac 1020
tctgctgtct atctctgtgc aagattgcgg aactgggacg aggctatgga ctactggggc 1080
caagggacca cggtcaccgt ctccctcaggt ggtggtggtt ctggcggcgg cggctccggt 1140
ggtggtggtt ctgagctcgt gatgacacag tctccatcct ccctgagtgt gtcagcagga 1200
gagaaggtca ctatgagctg caagtccagt cagagtctgt taaacagtgg aaatcaaaag 1260
aactacttgg cctggtacca gcagaaacca gggcagcctc ctaaactggt gatctacggg 1320
gcatccacta gggaatctgg ggtccctgat cgcttcacag gcagtggatc tggaacagat 1380
ttcactctca ccatcagcag tgtgcaggct gaagacctgg cagtttatta ctgtcagaat 1440
gattatagtt atccgtacac gttcggaggg gggaccaagc ttgagatcaa acatcatcac 1500
catcatcatt ag 1512

```

```

<210> 56
<211> 503
<212> PRT
<213> artificial sequence

```

```

<220>
<223> CD3 VHVL aL x 4-1 VHVL

```

```

<400> 56

```

```

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1           5           10           15

```

Ser	Val	Lys	Met	Ser	Cys	Lys	Thr	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	Val	Glu	Gly	Gly	Ser	Gly	Gly	Ser	Gly			
		115					120					125						
Gly	Ser	Gly	Gly	Ser	Gly	Gly	Val	Asp	Asp	Ile	Gln	Leu	Thr	Gln	Ser			
	130					135					140							
Pro	Ala	Ile	Met	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys			
145					150					155					160			
Arg	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Ser			
				165					170					175				
Gly	Thr	Ser	Pro	Lys	Arg	Trp	Ile	Tyr	Asp	Thr	Ser	Lys	Val	Ala	Ser			
			180					185					190					
Gly	Val	Pro	Tyr	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser			
		195					200					205						
Leu	Thr	Ile	Ser	Ser	Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys			
	210					215					220							
Gln	Gln	Trp	Ser	Ser	Asn	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu			
225					230					235					240			

Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln
 245 250 255

Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Ile Ser Cys
 260 265 270

Lys Ala Ser Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys
 275 280 285

Gln Arg Pro Gly His Gly Leu Glu Trp Val Gly Asp Ile Phe Pro Gly
 290 295 300

Ser Gly Asn Ala His Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu
 305 310 315 320

Thr Ala Asp Lys Ser Ser Tyr Thr Ala Tyr Met Gln Leu Ser Ser Leu
 325 330 335

Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp
 340 345 350

Asp Glu Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser
 355 360 365

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 370 375 380

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly
 385 390 395 400

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
 405 410 415

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
 420 425 430

Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val
 435 440 445

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 450 455 460

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
 465 470 475 480

Asp Tyr Ser Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
 485 490 495

Lys His His His His His His
 500

<210> 57

<211> 1512

<212> DNA

<213> artificial sequence

<220>

<223> CD3 VHVL aL Ser x 4-1 VHVL

<400> 57

gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg	60
tcttgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg	120
cctggacagg gtctggaatg gattggatac attaatecta gccgtgggta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actcccttga ctactggggc caaggcacca ctctcacagt ctctcagtc	360
gaaggtggaa gtggagggtc tgggtggaagt ggagggtcag gtggagtcga cgacattcag	420
ctgacccagt ctccagcaat catgtctgca tctccagggg agaagggtcac catgacctgc	480
agagccagtt caagtgtaag ttacatgaac tgggtaccagc agaagtcagg cacctcccc	540
aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc	600
agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgctgcc	660
acttattact gccaacagtg gagtagtaac ccgctcacgt tcggtgctgg gaccaagctg	720
gagctgaaat ccggagggtg tggatccgag gtgcagctgc tcgagcagtc tggagctgag	780
ctggtaaggc ctgggacttc agtgaagata tcttgcaagg cttctggata cgccttcact	840
aactactggc taggttgggt taagcagagg cctggacatg gacttgaatg ggttggagat	900
attttcctg gaagtggtaa tgctcactac aatgagaagt tcaagggcaa agccacactg	960
actgcagaca agtcctcgta cacagcctat atgcagctca gtagcctgac atctgaggac	1020
tctgctgtct atttctgtgc aagattgcgg aactgggacg aggctatgga ctactggggc	1080

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caagggacca cggtcaccgt ctccctcaggt ggtggtgggt ctggcggcgg cggctccggt 1140
ggtggtgggt ctgagctcgt gatgacacag tctccatcct ccctgagtgt gtcagcagga 1200
gagaagggtca ctatgagctg caagtccagt cagagtctgt taaacagtgg aaatcaaaag 1260
aactacttgg cctggtacca gcagaaacca gggcagcctc ctaaactgtt gatctacggg 1320
gcatccacta ggggaatctgg ggtccctgat cgcttcacag gcagtggatc tggaacagat 1380
ttcactctca ccatcagcag tgtgcaggct gaagacctgg cagtttatta ctgtcagaat 1440
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catcatcatt ag 1512

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<210> 58
<211> 503
<212> PRT
<213> artificial sequence

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<220>
<223> CD3 VHVL aL Ser x 4-1 VHVL

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```

<400> 58

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Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1           5           10           15

```

```

Ser Val Lys Met Ser Cys Lys Thr 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 Ser Leu Asp Tyr Trp Gly Gln Gly
100           105           110

```

```

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly
115           120           125

```

Gly	Ser	Gly	Gly	Ser	Gly	Gly	Val	Asp	Asp	Ile	Gln	Leu	Thr	Gln	Ser	130	135	140	
Pro	Ala	Ile	Met	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys	145	150	155	160
Arg	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Ser	165	170	175	
Gly	Thr	Ser	Pro	Lys	Arg	Trp	Ile	Tyr	Asp	Thr	Ser	Lys	Val	Ala	Ser	180	185	190	
Gly	Val	Pro	Tyr	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	195	200	205	
Leu	Thr	Ile	Ser	Ser	Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	210	215	220	
Gln	Gln	Trp	Ser	Ser	Asn	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	225	230	235	240
Glu	Leu	Lys	Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	Gln	Leu	Leu	Glu	Gln	245	250	255	
Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Thr	Ser	Val	Lys	Ile	Ser	Cys	260	265	270	
Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Thr	Asn	Tyr	Trp	Leu	Gly	Trp	Val	Lys	275	280	285	
Gln	Arg	Pro	Gly	His	Gly	Leu	Glu	Trp	Val	Gly	Asp	Ile	Phe	Pro	Gly	290	295	300	
Ser	Gly	Asn	Ala	His	Tyr	Asn	Glu	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	305	310	315	320
Thr	Ala	Asp	Lys	Ser	Ser	Tyr	Thr	Ala	Tyr	Met	Gln	Leu	Ser	Ser	Leu	325	330	335	
Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg	Leu	Arg	Asn	Trp	340	345	350	

Asp Glu Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser
 355 360 365

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 370 375 380

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly
 385 390 395 400

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
 405 410 415

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
 420 425 430

Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val
 435 440 445

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 450 455 460

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
 465 470 475 480

Asp Tyr Ser Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
 485 490 495

Lys His His His His His His
 500

<210> 59
 <211> 1503
 <212> DNA
 <213> artificial sequence

<220>
 <223> CD3 VHVL stL x 4-1 VHVL

<400> 59
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 cctggacagg gtctggaatg gattggatac attaatccta gccgtgggtta tactaattac 180
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240

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atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctcttcaggt 360
ggtggtggtt ctggcggcgg cggctccggt ggtggtggtt ctgacattca gctgacccag 420
tctccagcaa tcatgtctgc atctccaggg gagaaggcca ccatgacctg cagagccagt 480
tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctccc caaaagatgg 540
atztatgaca catccaaagt ggcttctgga gtcccttata gcttcagtgg cagtgggtct 600
gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac 660
tgccaacagt ggagtagtaa cccgctcacg ttcggtgctg ggaccaagct ggagctgaaa 720
tccggagggtg gtggatccga ggtgcagctg ctcgagcagt ctggagctga gctggtaagg 780
cctgggactt cagtgaagat atcctgcaag gcttctggat acgccttcac taactactgg 840
ctagggtggg ttaagcagag gcctggacat ggacttgaat gggttggaga tattttccct 900
ggaagtggta atgctcacta caatgagaag ttcaagggca aagccacact gactgcagac 960
aagtcctcgt acacagccta tatgcagctc agtagcctga catctgagga ctctgctgtc 1020
tattttctgtg caagattgcg gaactgggac gaggctatgg actactgggg ccaagggacc 1080
acggtcaccg tctcctcagg tgggtggtggt tctggcggcg gcggctccgg tgggtggtggt 1140
tctgagctcg tgatgacaca gtctccatcc tcctgagtg tgtcagcagg agagaaggtc 1200
actatgagct gcaagtccag tcagagtctg ttaaacagtg gaaatcaaaa gaactacttg 1260
gcctgggtacc agcagaaacc agggcagcct cctaaactgt tgatctacgg ggcattccact 1320
agggaatctg gggtccttga tcgcttcaca ggcagtggat ctggaacaga tttcactctc 1380
accatcagca gtgtgcaggc tgaagacctg gcagtttatt actgtcagaa tgattatagt 1440
tatccgtaca cgttcggagg ggggaccaag cttgagatca aacatcatca ccatcatcat 1500
tag 1503

```

```

<210> 60
<211> 500
<212> PRT
<213> artificial sequence

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```

<220>
<223> CD3 VHVL stL x 4-1 VHVL

```

```

<400> 60

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Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala

1		5		10		15										
Ser	Val	Lys	Met	Ser	Cys	Lys	Thr	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	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	
		115					120					125				
Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ala	Ile	
	130					135					140					
Met	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys	Arg	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	Val	Ala	Ser	Gly	Val	Pro	
			180					185					190			
Tyr	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	
		195					200					205				
Ser	Ser	Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	
		210				215					220					
Ser	Ser	Asn	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys	
225					230					235					240	

Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala
 245 250 255

Glu Leu Val Arg Pro Gly Thr Ser Val Lys Ile Ser Cys Lys Ala Ser
 260 265 270

Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro
 275 280 285

Gly His Gly Leu Glu Trp Val Gly Asp Ile Phe Pro Gly Ser Gly Asn
 290 295 300

Ala His Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp
 305 310 315 320

Lys Ser Ser Tyr Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu
 325 330 335

Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp Asp Glu Ala
 340 345 350

Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly
 355 360 365

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Val
 370 375 380

Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly Glu Lys Val
 385 390 395 400

Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser Gly Asn Gln
 405 410 415

Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys
 420 425 430

Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg
 435 440 445

Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser
 450 455 460

Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp Tyr Ser
 465 470 475 480

Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys His His
 485 490 495

His His His His
 500

<210> 61
 <211> 10
 <212> PRT
 <213> artificial sequence

<220>
 <223> CDRH3 M1 mutant

<400> 61

His Tyr Asp Asp His Tyr Cys Leu Asp Tyr
 1 5 10

<210> 62
 <211> 10
 <212> PRT
 <213> artificial sequence

<220>
 <223> CDRH3 M4 mutant

<400> 62

Tyr Ser Asp Asp His Tyr Cys Leu Asp Tyr
 1 5 10

<210> 63
 <211> 10
 <212> PRT
 <213> artificial sequence

<220>
 <223> CDRH3 M7 mutant

<400> 63

Tyr Tyr Asp Ala His Tyr Cys Leu Asp Tyr
 1 5 10

<210> 64
 <211> 10

<212> PRT
 <213> artificial sequence

<220>
 <223> CDRH3 M9 mutant

<400> 64

Tyr	Tyr	Asp	Asp	Gln	Tyr	Cys	Leu	Asp	Tyr
1				5					10

<210> 65
 <211> 10
 <212> PRT
 <213> artificial sequence

<220>
 <223> CDRH3 M10 mutant

<400> 65

Tyr	Tyr	Asp	Asp	Pro	Tyr	Cys	Leu	Asp	Tyr
1				5					10

<210> 66
 <211> 10
 <212> PRT
 <213> artificial sequence

<220>
 <223> CDRH3 M11 mutant

<400> 66

Tyr	Phe	Asn	Asp	His	Tyr	Cys	Leu	Asp	Tyr
1				5					10

<210> 67
 <211> 10
 <212> PRT
 <213> artificial sequence

<220>
 <223> CDRH3 M13 mutant

<400> 67

Tyr	Tyr	Asn	Asp	Gln	Tyr	Cys	Leu	Asp	Tyr
1				5					10

<210> 68
 <211> 10
 <212> PRT

<213> artificial sequence

<220>

<223> CDRH3 M20 mutant

<400> 68

Tyr	His	Asp	Asp	Pro	Tyr	Cys	Leu	Asp	Tyr
1				5					10

<210> 69

<211> 10

<212> PRT

<213> artificial sequence

<220>

<223> CDRH3 M76 mutant

<400> 69

Tyr	Tyr	Asp	Asp	Asn	Tyr	Cys	Leu	Asp	Tyr
1				5					10

<210> 70

<211> 18

<212> PRT

<213> artificial sequence

<220>

<223> original linker

<400> 70

Val	Glu	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly
1				5				10						15	

Val Asp

<210> 71

<211> 357

<212> DNA

<213> artificial sequence

<220>

<223> anti-CD3 VH

<400> 71

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tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120

cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac 180

aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240
 atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
 gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctcctca 357

<210> 72
 <211> 119
 <212> PRT
 <213> artificial sequence

<220>
 <223> anti-CD3VH

<400> 72

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr 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> 73
 <211> 318
 <212> DNA
 <213> artificial sequence

<220>
 <223> anti-CD3 VL

<400> 73
gacattcagc tgacccagtc tccagcaatc atgtctgcat ctccagggga gaaggtcacc 60
atgacctgca gagccagttc aagtgttaagt tacatgaact ggtaccagca gaagtcaggc 120
acctccccca aaagatggat ttatgacaca tccaaagtgg cttctggagt cccttatcgc 180
ttcagtggca gtgggtctgg gacctcatatc tctctcacia tcagcagcat ggaggctgaa 240
gatgctgccca cttattactg ccaacagtgg agtagtaacc cgctcacgtt cgggtgctggg 300
accaagctgg agctgaaa 318

<210> 74
<211> 106
<212> PRT
<213> artificial sequence

<220>
<223> anti-CD3 VL

<400> 74

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile Met 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

Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
35 40 45

Asp Thr Ser Lys Val Ala Ser Gly Val Pro Tyr Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
85 90 95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

<210> 75
<211> 10
<212> PRT
<213> artificial sequence

<220>

<223> vH CDR1 anti-CD3

<400> 75

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His
 1 5 10

<210> 76

<211> 357

<212> DNA

<213> artificial sequence

<220>

<223> vH anti-CD3 cys->ser

<400> 76

gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg 60
 tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120
 cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac 180
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240
 atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300
 gatgatcatt actcccttga ctactggggc caaggcacca ctctcacagt ctccctca 357

<210> 77

<211> 119

<212> PRT

<213> artificial sequence

<220>

<223> vH anti-CD3 cys->ser

<400> 77

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr 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 Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

<210> 78
<211> 10
<212> PRT
<213> artificial sequence

<220>
<223> VH CDR3 anti-CD3 cys->ser

<400> 78

Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr
1 5 10

<210> 79
<211> 360
<212> DNA
<213> artificial sequence

<220>
<223> EpCAM 3-1 VH

<400> 79
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aggcctggac atggacttga gtggattgga gatcttttcc ctggaagtgg taatactcac 180
tacaatgaga ggttcagggg caaagccaca ctgactgcag acaaatcctc gagcacagcc 240
tttatgcagc tcagtagcct gacatctgag gactctgctg tctatctctg tgcaagattg 300
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<210> 80
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35 40 45

Ile Gly Asp Leu Phe Pro Gly Ser Gly Asn Thr His Tyr Asn Glu Arg
50 55 60

Phe Arg Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala
65 70 75 80

Phe Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe
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Cys Ala Arg Leu Arg Asn Trp Asp Glu Ala Met Asp Tyr Trp Gly Gln
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gggaaaacta ataagcttct tatctactct ggatccactt tgcaatctgg aattccatca 180

aggttcagtg gcagtggtac tggtacagat ttcactctca ccatcagtag cctggagcct 240

gaagattttg caatgtatta ctgtcaacag cataatgaat atccgtacac gttcggaggg 300

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Glu Thr Ile Thr Ile Asn Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
 20 25 30

Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile
 35 40 45

Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
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 35 40 45

Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn Ala Tyr Tyr Asn Glu Lys
 50 55 60

Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala
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Ser Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe
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35 40 45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
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Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp
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Val Gly Asp Ile Phe Pro Gly Ser Gly Asn Ala His Tyr Asn Glu Lys
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Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Tyr Thr Ala
65              70              75              80

Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe
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Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val
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Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
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 35 40 45

Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn Ala Tyr Tyr Asn Glu Lys

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Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala
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Ser Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe
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Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
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 35 40 45

Ile Gly Asp Ile Phe Pro Gly Ser Gly Asn Ile His Tyr Asn Glu Lys
 50 55 60

Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala
 65 70 75 80

Tyr Met Gln Leu Ser Ser Leu Thr Phe Glu Asp Ser Ala Val Tyr Phe
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Cys Ala Arg Leu Arg Asn Trp Asp Glu Pro Met Asp Tyr Trp Gly Gln
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			20					25					30		

Gly	Asn	Gln	Lys	Asn	Tyr	Leu	Thr	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln
		35					40					45			

Pro	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Thr	Arg	Glu	Ser	Gly	Val
	50					55					60				

Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr
65					70					75					80

Ile	Ser	Ser	Val	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Gln	Asn
				85					90					95	

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100

100/100

Claims

1. A pharmaceutical composition comprising a bispecific single chain antibody construct and a pharmaceutically acceptable carrier or excipient, wherein said construct comprises or consists of two domains, wherein one of said domains binds to human EpCAM antigen and a second domain binds to human CD3 antigen, wherein said bispecific single chain antibody construct comprises:
 - (a) an amino acid sequence as shown in any one of SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 30, 36, 39, 42, 44, 46, 48, 50, 52, 54, 56, 58 and 60;
 - (b) an amino acid sequence encoded by a nucleic acid sequence as shown in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 29, 35, 38, 41, 43, 45, 47, 49, 51, 53, 55, 57 and 59; or
 - (c) an amino acid sequence encoded by a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence of (b).

2. The pharmaceutical composition of claim 1, wherein said binding domain specific for EpCAM has a K_D value of more than 5×10^{-9} M.

3. The pharmaceutical composition of claim 1 or 2, wherein said binding domain specific for EpCAM has a K_D value in a range between 1×10^{-7} and 5×10^{-9} M and said binding domain specific for CD3 has a K_D value in a range between 1×10^{-6} and 5×10^{-9} M.

4. The pharmaceutical composition of any one of claims 1 to 3, wherein said binding domain specific for CD3 has a K_D value of more than 10^{-7} M.

5. A pharmaceutical composition comprising a nucleic acid sequence encoding the bispecific single chain antibody construct as defined in any one of claims 1 to 4, and a pharmaceutically acceptable carrier or excipient.

6. A pharmaceutical composition comprising a vector which comprises the nucleic acid sequence as defined in claim 5, and a pharmaceutically acceptable carrier or excipient.
7. The pharmaceutical composition of claim 6, wherein said vector further comprises a regulatory sequence which is operably linked to said nucleic acid sequence defined in claim 5.
8. The pharmaceutical composition of claim 6 or 7, wherein said vector is an expression vector.
9. A pharmaceutical composition comprising a host cell transformed or transfected with the vector defined in any one of claims 6 to 8.
10. The pharmaceutical composition of any one of claims 1 to 9, further comprising a proteinaceous compound providing an activation signal for immune effector cells.
11. The pharmaceutical composition of any one of claims 1 to 10, wherein the pharmaceutical composition is thermostable at $\geq 37^{\circ}\text{C}$.
12. A process for producing a bispecific single chain antibody as defined in any one of claims 1 to 4, said process comprising
 - (a) culturing the host cell defined in claim 9 under conditions allowing the expression of the bispecific single chain antibody construct as defined in any one of claims 1 to 4; and
 - (b) recovering the produced bispecific single chain antibody construct from the culture.
13. Use of:
 - (a) the bispecific single chain antibody construct as defined in any one of claims 1 to 4;
 - (b) the nucleic acid sequence as defined in claim 5;

- (c) the vector as defined in any one of claims 6 to 8;
- (d) the host cell as defined in claim 9; or
- (e) the bispecific single chain antibody produced by the process of claim 12,

for the prevention, treatment or amelioration of a tumorous disease in a subject, or for the preparation of a medicament for same.

14. The use of claim 13, wherein said subject is a human.

15. The use of claim 13 or 14, wherein said tumorous disease is epithelial cancer or a minimal residual cancer.

16. A product which is:

- (a) the bispecific single chain antibody construct as defined in any one of claims 1 to 4;
- (b) the nucleic acid sequence as defined in claim 5;
- (c) the vector as defined in any one of claims 6 to 8;
- (d) the host cell as defined in claim 9; or
- (e) the bispecific single chain antibody produced by the process of claim 12,

for the prevention, treatment or amelioration of a tumorous disease in a subject.

17. The product of claim 16, wherein said subject is a human.

18. The product of claim 16 or 17, wherein said tumorous disease is epithelial cancer or a minimal residual cancer.

19. A kit for use in the prevention, treatment or amelioration of a tumorous disease, said kit comprising:

- (a) the bispecific single chain antibody construct as defined in any one of claims 1 to 4;
- (b) the nucleic acid sequence as defined in claim 5;
- (c) the vector as defined in any one of claims 6 to 8; or

- (d) the host cell as defined in claim 9; or
- (e) the bispecific single chain antibody produced by the process of claim 12,

and a suitable container housing any one of (a) to (e).

Figure 1

A) anti-CD3 VHVL stL x 3-1 VHVL (SEQ ID NO: 11)

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Figure 1 A) continued

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(SEQ ID NO: 12)

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Figure 1

B) anti-CD3 VHVL aL x 4-7 VHVL (SEQ ID NO:1)

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GTCCTCCTCAGGTGGTGGTTCTGGCGGGCGGCTCCGGTGGTGGTTCTGAGCTCGTGATGACCCAGAC
TCCACTCTCCCTGCTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTGTGTACACA
GTAATGGAACACCTATTACATTTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAG

Figure 1 B) continued

CTCCTGATCTACAAGTTTCCAACCGATTCTCTGGGTTCCAGACAGGTTCAAGTGGCAGTGGATCAGGACAGAG
TTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGAGTTTATTTCTGCTCTCAAAGTACACATGTTC
CGTACACGTTTCGGAGGGGACCAAGCTTGAGATCAAACATCATCACCATCATCATTAG

(SEQ ID NO: 2)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQFKDKATLTDDK
SSSTAYMQLSSLTSEDSAVYYCARYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGVDIQLTQSPAIM
SASPGEKVMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSEAEADAATY
YCQQWSSNPLTFGAGTKLELKS GGGSEVQLLEQSGAEELARPGASVKLSCKASGYTFFTNYGLSWVKQRPQGVLE
WIGEVYPRIGNAYYNEKFKGKATLTADKSSSTASMELRSLTSEDSAVYFCARRGSYDTNYDWFVWGQGT'TVT
VSSGGGSGGGSGGGSELVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLNHWYLOKPGQSPKLLIYKV
SNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDLGVYFCQSQSTHVPYTFGGGTKLEIKHHHHHH

Figure 1

C) anti-CD3 VHVL aL Ser x 4-7 VHVL (SEQ ID NO: 7)

GATATCAAAC TGAGCAGT CAGGGGCT GAAC TGGCAAGACCT GGGGCT CAGTGAAGAT GTCTCTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCAC TGGTAAACAGAGGCTCTGGACAGGCTCTGGAATGGATTGGAT
ACATTAAATCCTAGCCGTGTTATACTAATAATCAGAAGTTC AAGGACAAGGCCACATTGACTACAGACAAA
TCCTCCAGCACAGCC TACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA
TTATGATGATCATTA CTCCCTTGACTACTGGGCCAAGGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAA
GTGGAGGTTCTGGTGGAAGTGGAAGTTCAAGTGGAGTCTGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG
TCTGCATCTCCAGGGAGAAAGTCA CCAATGACCTGCAGAGCCAGTTC AAGTGTAAAGTTACATGAAC TGGTACCA
GCAGAAGTCAAGCACCTCCCCCAAAGATGGATTATGACACATCCAAAGTGGCTTCTGGAGTCCCCTTATCGCT
TCAGTGGCAGTGGTCTGGGACCTCATACTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGGTCTGGACCAAGCTGGAGCTGAAATCCGGAGGTGG
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGCAGGCCCTGGGGCTTCAGTGAAGCTGTCTCT
GCAAGGCTTCTGGCTACACCTTCACAAACTATGTTTAAGCTGGTGAAGCAGAGGCCCTGGACAGGTCTCTTGAG
TGGATTGGAGAGGTTTATCCTAGAAATTGGTAATGCTTACTACAATGAGAAGTTC AAGGGCAAGGCCACACTGAC
TGCAGACAAATCCTCCAGCACAGCGTCCATGGAGCTCCGACGCTGACCTCTGAGGACTCTGCGGTCTATTCTT
GTGCAAGACGGGATCCTACGATACTA ACTACGACTGGTACTTCGATGTCTGGGGCCAAGGGACCA CGGTCAACC
GTCTCCTCAGGTGGTGGTTC TGGCGCGGCTCCGGTGGTGGTCTGAGCTCGTGA TGACCCAGAC
TCCACTCTCCCTGCTCAGTCTTGAGATCAAGCCTCCATCTCTTG CAGATCTAGTCAGAGCCTTGTA CACA
GTAA TGGAACACCTATTACATTTGGTACCTGCAGAACCCAGGCCAGTCTCCAAGCTCCTGATCTACAAGTTT
TCCAACCGATTTC TGGGGTCCCAGACAGGTTCA GTGGCATCAGGGACAG

Figure 1 C) continued

ATTTCACACTCAAGATCAGCAGAGTGAGGCTGAGGATCTGGGAGTTTATTCTGCTCTCAAAGTACACATGTT
CCGTACACGTTTCGGAGGGGGACCAAGCTTGAGATCAAACATCATCACCATCATCATTAG

(SEQ ID NO: 8)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGLEWIGYINPSRGYTNYNQKFKDKATLTTDK
SSSTAYMQLSLTSEDSAVYYCARYYDDHYSLDYWGQTTLTVSSVEGGSGGSGGVDDIQLTQSPAIM
SASPGEKVMTTCRASSSVS YMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGTSYSLTISSEAEADAATY
YCQQWSSNPLTFGAGTKLELKS GGGSEVQLLEQSGAELARPGASVKLSCKASGYTFNTNYGLSWVKQRPQGVLE
WIGEVYPRIGNAYYNEKFKGKATLTADKSSSTASMEI RSLTSEDSAVYFCARRGSYDNTNYDWYFDVWGQGT'TVT
VSSGGGSGGGSGGSELVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTY LHWY LQKPGQSPKLLIYKV
SNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSSQSTHVPYTFGGGTKLEIKHHHHHHH

Figure 1

D) anti-CD3 VHVL stL x 4-7 VHVL (SEQ ID NO: 13)

GATATCAAAC TGACAGTCA GGGGCTGA ACTGGCAAG ACCCTGGGG CCTCAGTGA AGATGT CCTGCAAG ACTTC
TGGCTACAC CTTACTAG GTACACGA TGCACTGG GTAAACAG AGGCCTGG ACAGGGTCTG GAATGGATTGGAT
ACATTAA TCCTAGCC GTGTTATA CTAATTACA ATCAGAAG TTCAAGGA CAAAGGCC ACATTGACTAC AGACAAA
TCCTCCAG CACAGCC TACATGCA ACTGAGC AGCCTGAC ATCTGAG GACTCTG CAGTCTAT TACTGTGCA AGATA
TTATGATG ATCATTA CTGCTTGA CTA CTGGGCCA AGCACTCTCAC AGTCTCCTCAG GTGGTGGTGT
CTGGCGG CGCTCCG GTGGTGTCTG ACATTCA GCTGACCC AGTCTCCAG CAAATCAT GTCTGCA TCT
CCAGGGAG AAGGTCAC CCA TGACCTGC AGAGCC AGTTCA AGTGTAA GTTACATG AA CTGGTACC AGCAGAA GTC
AGGCACCT CCCCCA AAAGATGG ATTTATG ACACATC CAAAGT GGCTTCTG GAGTCC CTTATCG CTTCAGTGG CA
GTGGGTCT GGGACCTC ATACTCTC TACAATC AGCAGCA TGAGGCTG AAGATG CTGCCACTT ATTACTGCCAA
CAGTGGAG TAGTAAC CCGCTCA CGTTCGG TGCTGG ACCCAAG CTGGAG CTGAAATC CGGAGGTGG TGGATCCGA
GGTGCAG CTGCTCG AGCAGTCTG GAGCTG AGCTGG GCTTCA GTGAAG CTGTCC TGCAAGGCTT
CTGGCTAC ACCCTTCA CAAACTA TGCTTA AGCTGG GTGAAG CAGAGG CCTGGAC AGGTCTT GAGTGGATTGGA
GAGGTTAT CCTAGAA ATTGGTA ATGCTTACT ACAATG AGAAGTTC AAGGGCA AGGCCACA CTGACTGCAGACAA
ATCCTCCAG CACAGCGTCC ATGGAG CTCCGCA GCTGACC TCTGAG GACTCTG CGGTCTAT TTTCTGTGCAAGAC
GGGATCCTAC GATACTA ACTACG ACTGGTACTTC GATGTCTG GGGCCA AGGACCAC GGTCA CCGTCTCCTCA
GGTGGTGGT GTCTGG CGGCGGCTCC GGTGGTGTCTG AGCTCG TGATGACCCAG ACTCCACTCTC
CCTGCC TGTCA GTCTTGG AGATCA AGCCCTCC ATCTCTG CAGATCTAG TCAGAG CCTTGTAC ACAGTAATGGAA
ACACCTATT TACA TTGGTACC TGAGA AGCCAG GCTCTCCA AAGCTCCTG ATCTACAA AGTTTCCA ACCGA
TTTCTGGGGT CCCAGACAG GTTCAGTGG CAGTGGATCAG GGACAGATTT CACAC

Figure 1 D) continued

TCAAGATCAGCAGAGTGAGGCTGAGGATCTGGAGTTTATTCTGCTCTCAAAGTACACATGTTCCGTACACG
TTCGAGGGGGACCAAGCTTGAGATCAAAACATCATCACCATCATCATTAG

(SEQ ID NO: 14)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLTDDK
SSSTAYMQLSLTSEDSAVYICARYYDDHYCLDYWGQGTTLTVSSGGGGGGGGSDIQLTQSPAIMSAS
PGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSTSYSLTSSMEAEADAATYYCQ
QWSSNPLTFGAGTKLELKSGGGGSEVQLLEQSGAELARPGASVKLSCKASGYTFTNYGLSWVKQRPQGVLEWIG
EVYPRIGNAYYNEKFKGKATLTADKSSSTASMELRSLTSEDSAVYFCARRGSDTNVDWYFDVWGQGTTVTVSS
GGGSGGGSGGGSELVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLNHWYLNQKPGQSPKLLIYKVSNR
FSGVPDRFSGSGGTDFTLKISRVEAEDLGVYFCSSQSTHVPYTFGGGTKLEIKHHHHH

Figure 1

E) anti-CD3 VHVL stL x 4-7 VLVH (SEQ ID NO: 15)

GATATCAAACTGCAGCAGTCAGGGGCTGAACCTGGCAAGACCTGGGGCCTCAGTGAAGATGTCTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT
ACATTAATCCTAGCCGTGTTATACTAATTAACAATCAGAAGTTCAGGACAAGGCCACATTGACTACAGACAAA
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA
TTATGATGATCATTAATGACTGCTTGAATACTGGGCCAAGGCACCACTCTCACAGTCTCCTCAGGTGGTGGTGGTT
CTGGCGCGCGCTCCGGTGGTGGTCTGACATTCAGCTGACCCAGTCTCCAGCAATCATGTCTGCATCT
CCAGGGAGAAAGTCAACCATGACCTGCAGAGCCAGTTCAAGTGAAGTTACATGAACCTGGTACCAGCAGAAAGTC
AGGCACCTCCCCAAAGATGGATTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCTTCAGTGGCA
GTGGTCTGGGACCTCATACTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA
CAGTGGAGTAGTAACCGCTCACGTTCCGGTGGTGGACCAAGCTGGAGCTGAAATCCGGAGGTGGTGGATCCGA
GCTCGTATGACCCAGACTCCACTCTCCCTGTCTGAGATCAAGCCTCCATCTCTTGACAGATCTA
GTCAGAGCCTTGTAACACAGTAATGGAAACACCTATTACATTTGTAACCTGCAGAAGCCAGGCCAGTCTCCAAG
CTCCTGATCTACAAAGTTTCCAAACCGATTTCTGGGTCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGA
TTTCACACTCAAGATCAGCAGAGTGGAGCTGAGGATCTGGAGTTTATTCTGCTCTCAAAGTACACATGTTT
CGTACACGTTCCGAGGGGACCAAGCTTGAGATCAAGTGGTGGTCTGGCGGGCGGCTCCGGTGGT
GGTGGTCTGAGGTGACGCTGCTCGAGCAGTCTGGAGCTGAGCTGGCGAGGCCCTGGGGCTTCAGTGAAGCTGTC
CTGCAAGGCTTCTGGCTACACCTTCACAAACTATGGTTTAAGCTGGTGAAGCAGAGGCCCTGGACAGGTCCCTTG
AGTGGATTGGAGAGGTTTATCCTAGAAATTGGTAATGCTTACTACAATGAGAAGTTCAAGGGCAAGGCCACACTG
ACTGCAGACAAATCCTCCAGCACAGCGTCCATGGAGCTCCGCAGCCTGACCTCTG

Figure 1 E) continued

AGGACTCTGCGGTCATTTCTGTGCAAGACGGGATCCTACGATACTAACTACGACTGGTACTTCGATGTCCTGG
GGCCAAGGACCAACGGTCACCGTCTCCTCACATCATCACCATCATCATAG

(SEQ ID NO: 16)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDK
SSSTAYMQLSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSGGGGGGGGSDIQLTQSPAIMSAS
PGEKVMTTCRASSVSVMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGTSYSLTISSEAEADAATYYCQ
QWSSNPLTFGAGTKLELKSGGGGSELVMTQTPLSLPVSIGDQASISCRSSQSLVHSNGNTYLHWY LQKPGQSPK
LLIYKVSNRFSGVPDRFSGSGGTDFTLKISRVEAEDLGVFCSQSTHVPYTFGGGTKLEIKGGGGSGGGSGG
GGSEVQLLEQSGAELARPGASVKLSCKASGYTFITNYGLSWVKQRPQGVLEWIGEVYPRIGNAYYNEKFKGKATL
TADKSSSTASMELRSLTSEDSAVYFCARRGSYDITNYDWYFDVWGQGTITVTVSSHHHHHH

Figure 1

F) anti-CD3 VHVL aL x 5-10 VHVL (SEQ ID NO: 3)

GATATCAAACCTGCAGCAGTCAGGGGCTGAACCTGGCAAGACCTGGGCCCTCAGTGAAGATGTCCCTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGGCCCTGGACAGGCTCTGGAATGGATTGGAT
ACATTAATCCTAGCCGTGGTTATACTAATTAACAATCAGAAAGTTCAAGGACAAGGCCACATTTGACTACAGACAAA
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA
TTATGATGATCATTTACTGCCTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAA
GTGGAGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG
TCTGCACTCCAGGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAGTTACATGAACCTGGTACCA
GCAGAAGTCAGGCACCTCCCCCAAAGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCT
TCAGTGGCAGTGGGTCTGGGACCTCATACTCTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGTGCTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGG
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCCCTGGGACTTCAGTGAAGATATCCT
GCAAGGCTTCTGGATACGCCCTTCACTAACTACTGGCTAGGTTGGGTAAAGCAGAGGCCCTGGACATGGACTTGAG
TGGAATTGGAGATATTTCCCTGGAAGTGGTAATATCCACTACAATGAGAAAGTTCAAGGGCAAGCCACACTGAC
TGACAGACAAATCTTCGAGCACAGCCTATATGCAGCTCAGTAGCCTGACATTTGAGGACTCTGCTGTCTATTCT
GTGCAAGACTGAGGAACCTGGGACGAGCCTATGGACTACTGGGCCAAGGGACCAAGGTCAACCGTCTCCTCAGGT
GGTGGTGGTCTGGCGGGCGGGCTCCGGTGGTGGTCTGAGCTCGTGATGACACAGTCTCCATCCCTCCCT
GACTGTGACAGCAGGAGAGAAGGTCACTATGAGCTGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGAATCAAA
AGAACTACTTGACCTGGTACCAGCAGAAACCAGGGCAGCCTCCTAAACTGTTGATCTACTGGGCATCCACTAGG
GAATCTGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGAACAGATTTCACTC

Figure 1 F) continued

TCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAGAATGATTATAGTTATCCGCTCACG
TTCGGTGCTGGGACCAAGCTTGAGATCAAAACATCATCACCATCATTTAG

(SEQ ID NO: 4)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLLTDDK
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGSGSGGVDIQLTQSPAIM
SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSEAEADAATY
YCQQWSSNPLTFGAGTKLELKS GGGSEVQLLEQSGAELVRPGTSVKISCKASGYAFNFWL GWVKQRP GHGLE
WIGDIFPGSGNIHYNEKFKGKATLTADKSSSTAYMQLSSLTFEDSAVYFCARLRNWDPEMDYWGQGT'TVTVSSG
GGSGGGGGGSELVMTQSPSSLTVTAGEKVTMSCKSSQSLNSGNQKNYLTWYQQKPGQPPKLLIYWASTR
ESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPLTFGAGTKLEIKHHHHHH

Figure 1

G) anti-CD3 VHVL aL Ser x 5-10 VHVL (SEQ ID NO: 9)

GATATCAAAC TGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCTTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCAC TGGGTAAACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT
ACATTAAATCCTAGCCGTGGTTATACTAATTA CAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA
TTATGATGATCATTA TCTCCCTTGACTACTGGGGCCAAGGCACCTCTCACAGTCTCCTCAGTCGAAGGTGGAA
GTGGAGGTTCTGGTGGAAGTGGAGGTTCAAGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG
TCTGCA TCTCCAGGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAAGTTACATGAAC TGGTACCA
GCAGAAGTCAGGCACCTCCCCCAAAGATGGATTATGACACATCCAAAGTGGCTTCTGGAGTCCCCTTATCGCT
TCAGTGGCAGTGGGTC TGGGACCTCATACTCTCACAA TCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGTTGCTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGG
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCTGGGACTTCAGTGAAGATATCCT
GCAAGGCTTCTGGATACGCCCTTCACTAACTACTGGCTAGGTTGGGTAAAGCAGAGGCCCTGGACATGGACTTGAG
TGGATTGGAGATATTTTCCCTGGAAGTGGTAATATCCACTACAAATGAGAAAGTTCAAGGGCAAAGCCACACTGAC
TGCAGACAAATCTTCGAGCACAGCC TATATGCAGCTCAGTAGCCTGACATTTGAGGACTCTGCTGTATTTCT
GTGCAAGACTGAGGAAC TGGGACGAGCCTATGGACTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGT
GGTGGTGGTTC TGGCGGGCGGCTCCGGTGGTGGTCTGAGCTCGTGATGACACAGTCTCCATCCTCCCT
GACTGTGACAGCAGGAGAGAAGTCACTATGAGCTGCAAGTCCAGTCCAGTCTGTTAAACAGTGGAAATCAAA
AGAACTACTTGACCTGGTACCAGCAGAAAC CAGGGCAGCCTCCTAAACTGTTGATC

Figure 1 G) continued

TACTGGGCATCCACTAGGGAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGAACAGATTTCACCTCT
CACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAGAATGATTATAGTTATCCGCTCACGCT
TCGGTGCTGGGACCAAGCTTGAGATCAAAACATCATCACCATCATCATTAG

(SEQ ID NO: 10)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDK
SSSTAYMQLSLTSEDSAVYYCARYYDDHYSLDYWGGTTLTVSSVEGGSGGSGGVDIQLTQSPAIM
SASPGEKVTMTCRASSVSVMNWWYQQKSGTSPKRWIYDTSKVASGVPRFSGSGTSYSLTISSEAEADAATY
YCQQWSSNPLTFGAGTKLELKSGGGSEVQLLEQSGAELVRPGTSVKISCKASGYAFTNYWLGWVKQRPQGHGLE
WIGDIFPGSGNIHYNEKFKGKATLTADKSSSTAYMQLSLTFEDSAVYFCARLRNWDPEPMDYWQGT'TVTVSSG
GGSGGGSGGSELVMTQSPSSLTVTAGEKVTMSCKSSQSLNSGNQKNYLTWYQQKPGQPPKLLIYWASTR
ESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPLTFGAGTKLEIKHHHHHHH

Figure 1

H) anti-CD3 VHVL stL x 5-10 VHVL (SEQ ID NO: 17)

GATATCAAAC TG CAGCAGT CAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGCCTGGACAGGGTCTGGAATGGATTGGAT
ACATTAATCCTAGCCGTGGTTATACTAATAATACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA
TCCTCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCACTCTATTACTGTGCAAGATA
TTATGATGATCATTA CTG CCTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGGTGGTGGTGGTT
CTGGCGGGCGGCTCCGGTGGTGGTTCTGACATTCAGCTGACCCAGTCTCCAGCAATCATGTCTGCATCT
CCAGGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTC AAGTGTAAAGTTACATGAAC TGGTACCAGCAGAAGTC
AGGCACCTCCCCCAAAGATGGATTATGACACATCCAAAGTGGCTTCTGGAGTCCCCTTATCGCTTCAGTGGCA
GTGGGTCTGGGACCTCATACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA
CAGTGGAGTAGTAACCCGCTCACGTTCCGGTGCTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGGTGGATCCGA
GGTGAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCCCTGGGACTTCAGTGAAGATATCCTGCAAGGCTT
CTGGATACGCCCTTCACTAACTACTGGCTAGGTTGGGTA AAGCAGAGGCCCTGGACATGGACTTGAGTGGATTGGA
GATATTTCCCTGGAAAGTGGTAATATCCACTACAATGAGAAGTTCAAGGGCA AAGCCACACTGACTGCAGACAA
ATCTTCGAGCACAGCCTATATGCAGCTCAGTAGCCTGACATTTGAGGACTCTGCTGTCTATTCTGTGCAAGAC
TGAGGAACTGGGACGAGCCTATGGACTACTGGGGCCAAGGCCACCGTCA CCGTCTCCTCAGGTGGTGGTGGT
TCTGGCGGGCGGCTCCGGTGGTGGTTCTGAGCTCGTGATGACACAGTCTCCATCCCTCCCTGACTGTGAC
AGCAGGAGAGAAGTCACTATGAGCTGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGAAATCAA AAGAACTACT
TGACCTGGTACCAGCAGAAAC CAGGGCAGCCTCCCTAAACTGTTGATCTACTGGGCATCCACTAGGGAATCTGGG
GTCCCTGATCGCTTCACAGGCAGTGGATCTGGAACAGATTTCACTCTCACCATCA

Figure 1 H) continued

GCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGT CAGAA TGATTATAGTTATCCGCTCACGTTTCGGTGCT
GGGACCAAGCTTGAGATCAAACATCATCACCATCATCATTAG

(SEQ ID NO: 18)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLTTDK
SSSTAYMQLSLTSSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSGGGGGGGGSDIQLTQSPAIMSAS
PGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGGTSYSLTISSEAEADAATYYCQ
QWSSNP LTFGAGTKLELKSGGGGSEVQLLEQSGAELVRPGTSVKISCKASGYAF TNYWLGWVKQRP GHGLEWIG
DIFPGSGNIHYNEKFKGKATLTADKSSSTAYMQLSSLTFEDSAVYFCARLRNWDPEPMDYWGQGT TTVTVSSGGGG
SGGGSGGGGSELVMTQSPSSLTVTAGEKVTMSCKSSQSLLSNGNQKNYLLTWYQQKPGQPPKLLIYWASTRESG
VPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPLTFFGAGTKLEIKHHHHHH

Figure 1

D) anti-CD3 VHVL stL x 5-10 VL VH (SEQ ID NO: 19)

GATATCAAACCTGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACAGAGGCCCTGGACAGGGTCTGGAAATGGATTGGAT
ACATTAAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAGGACAAGGCCACATTGACTACAGACAAA
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA
TTATGATGATCATTTACTGCCCTTGACTACTGGGCCAAGCACCACTCTCACAGTCTCCTCAGGTGGTGGTGGTT
CTGGCGCGGCTCCGGTGGTGGTTCTGACATTCAGCTGACCCAGTCTCCAGCAATCATGTCTGCATCT
CCAGGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAAGTGTAACTGAACTGGTACCAGCAGAAGTC
AGGCACCTCCCCAAAGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCTTCAGTGGCA
GTGGGTCTGGGACCTCATACTCTCTACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA
CAGTGGAGTAGTAACCCGCTCACGTTCCGGTGGTGGACCAAGCTGGAGCTGAAATCCGGAGGTGGTGGATCCGA
GCTCGTGATGACACAGTCTCCATCCCTGACTGTGACAGCAGGAGAGAGGTCACTATGAGCTGCAAGTCCA
GTCAGAGTCTGTAAACAGTGGAAATCAAAGAACTACTTGACCTGGTACCAGCAGAAACCAGGGCAGCCTCCT
AAACTGTTGATCTACTGGGCATCCACTAGGGAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGAAC
AGATTTCACTCTCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAGAATGATTATAGTT
ATCCGCTCACGTTCCGGTGGTGGACCAAGCTTGAGATCAAAGGTGGTGGTCTGGCGCGCGGCTCCGGT
GGTGGTGGTCTGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGGTAAGGCCCTGGGACTTCAGTGAAGAT
ATCCTGCAAGGCTTCTGGATACGCCCTTCACCTAACTACTGGCTAGGTTGGGTAAAGCAGAGGCCCTGGACATGGAC
TTGAGTGGATTGGAGATATTTCCCTGGAAAGTGGTAATATCCACTACAATGAGAAAGTTCAAGGCCAAAGCCACA
CTGACTGCAGACAAATCTTCGAGCACAGCCTATATGCAGCTCAGTAGCCTGACAT

Figure 1 I) continued

TTGAGGACTCTGCTGTCTATTCTGTGCAAGACTGAGGAAGCTGGGACGAGCCCTATGGACTACTGGGGCCAAGGG
ACCACGGTCACCGTCTCCTCACATCATCACCATCATCATTAG

(SEQ ID NO: 20)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQGLEWIGYINPSRGYTNYNQKFKDKATLTIDK
SSSTAYMQLSLTSFEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSGGSGGGSGGGSDIQLTQSPAIMASAS
PGEKVMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTSSMEAEADAATYYCQ
QWSSNPLTFGAGTKLELKS GGGGSELVMTQSPSSLTVTAGEKVTMSCKSSQSLNSGNQKNYLTWYQQKPGQPP
KLLIYWASTRESGVDPDRFTGSGSGTDFTLTSSVQAEDLAVYYCQNDYSYPLTFGAGTKLEIKGGGSGGGGSG
GGSEVQLLEQSGAELVRPGTSVKISCKASGYAFITNYWLGWVKQRP GHGLEWIGDIFPGSGNIHYNEKFKGKAT
LTADKSSSTAYMQLSLTFEDSAVYFCARLRNWDEPMDYWGQGT'TVTVSSHHHHHH

Figure 1

J) anti-CD3 VHVL aL x 3-1 VHVL (SEQ ID NO: 45)

GATATCAAACCTGCAGCAGTCAGGGGCTGAACCTGGCAAGACCTGGGGCCTCAGTGAAGATGTCTTGCAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT
ACATTAAATCCTAGCCGTGTTACTAATAATTACAATCAGAAGTTCAGGACAAGGCCACATTGACTACAGACAAA
TCCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA
TTATGATGATCATTTACTGCCCTTGACTACTGGGCCAAGGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAA
GTGGAGGTTCTGGTGGAAGTGGAGGTTCAAGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG
TCTGCACTCTCCAGGGAGAAAGTCAACCTGCAGAGCCAGTTCAAGTGTAAGTTACATGAACCTGGTACCA
GCAGAACTCAGGCACCTCCCCAAAGATGGATTATGACACATCCAAGTGGCTTCTGGAGTCCCCTTATCGCT
TCAGTGGCAGTGGTCTGGGACCTCATACTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCTGGTGGACCAAGCTGGAGCTGAAATCCGGAGGTGG
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTGAACCTGGGGCCTCAGTGAAGATAATCCT
GCAAGGCTTCTGGATACGCCCTTCACTAACTAGGTGGGTAAAGCAGAGGCCCTGGACATGGACTTGAG
TGGATTGGAGATCTTTCCCTGGAAGTGGTAATACTCACTACAATGAGAGGTTCAAGGGCAAAGCCACACTGAC
TGCAGACAAATCCTCGAGCACAGCCTTTATGCAGCTCAGTAGCCTGACATCTGAGGACTCTGCTGTCTATTCT
GTGCAAGATTGAGGAACCTGGGACGAGGCTATGGACTACTGGGCCAAGGACCAAGGTACCCGTCTCCTCAGGT
GGTGGTGGTCTGGCGCGCGGCTCCGGTGGTGGTCTGAGCTCGTCATGACCCAGTCTCCATCTTATCT
TGCTGCATCTCCTGGAGAAACCATTAATAATGCAGGGCAAGTAAGAGCATTAGCAAAATATTAGCCTGGT
ATCAAGAGAAACCTGGGAAAACATAAAGCTTCTTATCTACTCTGGATCCACTTTGCAATCTGGAAATTCATCA
AGGTTCAAGTGGCAGTGGATCTGGTACAGATTTCACTCTCACCATCAGTAGCCTGG

Figure 1 J) continued

AGCCTGAAGATTTTGCAATGTATTACTGTCAACAGCATAAATGAATATCCGTACACGTTCCGAGGGGGACCAAG
CTTGAGATCAAACATCATCACCATCATCATTAG

(SEQ ID NO: 46)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQFKDKATLTTDK
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGVDDIQLTQSPAIM
SASPGEKVTMTCRASSSVSVMNYYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGTSYSLTSSMEAFDAATY
YCQQWSSNPLTFGAGTKLELKSGGGGSEVQLLEQSGAELVKPGASVKISCKASGYAF'N'YWLGLGWVKQRP'GHGLE
WIGDLFP'GSGNTHYNERFRGKATLTADKSSSTA'FMQLSSLTSEDSAVYFCARLRNWD'EA'MDYWGQGT'V'TVSSG
GGSGGGSGGGSELVMTQSPSYLAASPGETITINCRASKSISKYLA'WYQ'EKPGKTNKLLIYSGSTLQSGIPS
RFSGSGSGTDFTLTISSELEPEDFAMYYCQQHNEYPYTFGGG'TKLEIKHHHHHHH

Figure 1

K) anti-CD3 VHVL aL Ser x 3-1 VHVL (SEQ ID NO: 47)

GATATCAAACCTGCAGCAGTCAGGGCTGAACCTGGGCGCTCAGTGAAGATGTCCTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGCGCTTGACAGGGTCTGGAATGGATTGGAT
ACATTAAATCCTAGCCGTGGTTATACTAATTAACAATCAGAACTTCAAGGACAAGCCACATTGACTACAGACAAA
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA
TTATGATGATCATTAATCCCTTGACTACTGGGCCAAGGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAA
GTGGAGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG
TCTGCACTCCAGGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAAGTTACATGAAC TGGTACCA
GCAGAACTCAGGCACCTCCCCCAAAGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCT
TCAGTGGCAGTGGGTCTGGGACCTCATACTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGGTCTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGG
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTGAACCTGGGCGCTCAGTGAAGATATCCT
GCAAGGCTTCTGGATACGCCCTTCACTAACTACTGGCTAGGTTGGGTAAAGCAGAGGCTTGACATGGACTTGAG
TGGATTGGAGATCTTTCCCTGGAAGTGGTAATACTCACTACAATGAGAGGTTCAAGGCAAGCCACACTGAC
TGCAGACAAATCCTCGAGCACAGCCTTTATGCAGCTCAGTAGCCTGACATCTGAGGACTCTGCTGTCTATTCT
GTGCAAGATTGAGGAACCTGGGACGAGGCTATGGACTACTGGGCCAAGGACCAAGGTCACCGTCTCCTCAGGT
GGTGGTGGTCTGGCGGCGGCGCTCCGGTGGTGGTCTGAGCTCGTCATGACCCAGTCTCCATCTTATCT
TGCTGCATCTCCTGGAGAAACCATTAATAATTGCAGGGCAAGTAAGAGCATTAGCAAAATATTAGCCTGGT
ATCAAGAGAAACCTGGGAAAACCTAATAAGCTTCTTATCTACTCTGGATCCACTTTG

Figure 1 K) continued

CAATCTGGAATTCCATCAAGGTTCAGTGGCAGTGGATCTGGTACAGATTTCACCTCTCACCATCAGTAGCCTGGA
GCCTGAAGATTTTGCAATGTATTACTGTCAACAGCATATAATGAATATCCGTACACGTTTCGGAGGGGGACCAAGC
TTGAGATCAAACATCATCACCATCATCATTAG

(SEQ ID NO: 48)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLTTDK
SSSTAYMQLSLTSEDSAVYYCARYYDDHYSLDYWGGQTTLTVSSVEGGSGGSGGVDDIQLTQSPAIM
SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGTSYSLTISSEAEADAATY
YCQQWSSNPLTFGAGTKLELKSGGGGSEVQLLEQSGAELVKPGASVKISCKASGYAFTNYWLGWVKQRPQGHGLE
WIGDLFPGSGNTHYNERFRGKATLTADKSSSTAQMQLSSTSEDSAVYFCARLRNWDEAMDYWGQGTTVTVSSG
GGSGGGGGGSELVMTQSPSYLAASPGETTTINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQSGIPS
RFSGSGSGTDEFTLTISSELEPEDEFAMYYCQQHNEYPTYTFGGGTKEIKHHHHHH

Figure 1

L) anti-CD3 VHVL aL x 3-5 VHVL (SEQ ID NO: 49)

GATATCAAACCTGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCTTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT
ACATTAAATCCTAGCCGTGGTTATACTAATTACAATCAGAAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA
TTATGATGATCATTTACTGCCCTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAA
GTGGAGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG
TCTGCACTCTCAGGGGAGAAGTCAACATGACCTGCAGAGCCAGTTCAAGTGTAAAGTTACATGAACCTGGTACCA
GCAGAAGTCAGGCACCTCCCCAAAGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCT
TCAGTGGCAGTGGTCTGGGACCTCATACTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGGTGCTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGG
TGGAATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGCCCTGGGACTTCAGTGAAGCTGTCCCT
GCAAGGCTTCTGGCTACACCTTCACAAGCTATGGTTAAAGCTGGGTGAAGCAGAGAACTGGACAGGGCCTTGAG
TGGATTGGAGAGGTTTATCCTAGAAATGGTAATGCTTACTACAATGAGAAAGTTCAAGGGCAAGGCCACACTGAC
TGCAGACAAATCCTCCAGCACAGCGTCCATGGAGCTCCGCAGCCTGACATCTGAGGACTCTGCCGTCTATTCT
GTGCAAGACGGGATCCTACGGTAGTAACGACTGGTACTTCGATGTCTGGGGCCAAGGGACCAAGGTCAACC
GTCCTCTCAGGTGGTGGTCTGGCGGGCGGCTCCGGTGGTGGTCTGAGCTCGTGATGACCCAGAC
TCCACTCTCCCTGCCCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTGTAACA
GTAATGGAACAACCTATTTACATTTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTT
TCCAACCGATTTTCTGGGTCCCAGACAGGTTTCAGTGGCAGTGGATCAGGGACAG

Figure 1 L) continued

ATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTCTGCTCTCAAAGTACACATGTT
CCGTACACGTTCCGAGGGGGACCAAGCTTGAGATCAAACATCATCACCATCATCTTAG

(SEQ ID NO: 50)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLTTDK
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGVDDIQLTQSPAIM
SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGGTSYSLTISSMFAEDAATY
YCQQWSSNPLTFGAGTKLELKSGGGGSEVQLLEQSGAELVRPGTSVKLSCKASGYTFTSYGLSWVKQRTGQGLE
WIGEVYPRIGNAYYNEKFKGKATLTADKSSSTASMEILRSLTSEDSAVYFCARRGSYGSNYDWYFDVWGQGTTVT
VSSGGGSGGGSGGGSELVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLRKPGQSPKLLIYKV
SNRFSGVPRDPSGSGSGTDFTLKISRVEAEDLGVYFCSSQSTHVPYTFGGGTKLEIKHHHHHH

Figure 1

M) anti-CD3 VHVL aL Ser x 3-5 VHVL (SEQ ID NO: 51)

GATATCAAAC TGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCTTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCACTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAAATGGATTGGAT
ACATTAAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA
TCCTCCAGCACAGCCCTACATGCAACTGAGCAGCCCTGACATCTGAGGACTCTGCACTCTATTACTGTGCAAGATA
TTATGATGATCATTTACTCCCTTGACTACTGGGGCCAAGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAA
GTGGAGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG
TCTGCACTCCAGGGGAGAAGTCAACATGACCTGCAGAGCCAGTTCAAGTGTAAAGTTACATGAAC TGGTACCA
GCAGAAGTCAGGCACCTCCCCAAAGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCCTTATCGCT
TCAGTGGCAGTGGGTCTGGGACCTCATACTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGGTGTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGG
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCCCTGGGACTTCAGTGAAGCTGTCCCT
GCAAGGCTTCTGGCTACACCTTCACAAGCTATGGTTTAAGCTGGGTGAAGCAGAGA AACTGGACAGGGCCTTGAG
TGGATTGGAGAGGTTTATCCTAGAAATTGGTAATGCTTACTACAATGAGAAGTTCAAGGGCAAGGCCACACTGAC
TGCAGACAAATCCTCCAGCACAGCGTCCATGGAGCTCCGCAGCCTGACATCTGAGGACTCTGCGGTCTATTCT
GTGCAAGACGGGATCCTACGGTAGTAACGACTGGTACTTCGATGTCTGGGGCCAAGGACCAAGGTCAACC
GTCCTCCTCAGGTGGTGGTCTGGCGGGCGGCTCCGGTGGTGGTCTGAGCTCGTGATGACCCAGAC
TCCACTCTCCCTGCCCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTGTACACA
GTAATGGAACAACCTATTTACATTTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTT
TCCAAACCGATTTTCTGGGTCCAGACACAGGTTCAAGTGGCAGTGGATCAGGGACAG

Figure 1 M) continued

ATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTCTGCTCTCAAAGTACACATGTT
CCGTACACGTTTCGGAGGGGGACCAAGCTTGAGATCAAACATCATCATCATTAG

(SEQ ID NO: 52)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLTTDK
SSSTAYMQLSSLTSEDSAVYYCARYDDHYSLDYWGQGTTLTVSSVEGGSGGSGGVDIQLTQSPAIM
SASPGGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSTSYSLTSSMEAEADAATY
YCQQWSSNPLTFGAGTKLELKS GGGSEVQLLEQSGAELVRPGTSVKLSCKASGYFTSYGLSWVKQRTGQGLE
WIGEVYPRIGNAYYNEKFKGKATLTADKSSSTASMELRSLTSEDSAVYFCARRGSYGSNYDWYFDVWGQGT'TVT
VSSGGGSGGGGSGGGSELVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLVHWYLRQKPGQSPKLLIYKV
SNRFSGVPPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSSQSTHVPYTFGGGTKLEIKHHHHHH

Figure 1

N) anti-CD3 VHVL stL x 3-5 VHVL (SEQ ID NO: 53)

GATATCAAAC TGACAGT CAGGGCT GAAC TGGCAAGACCTGGGGCCTCAGTGAAGATGTCTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCAC TGGTA AAAACAGAGGCTCTGGACAGGCTCTGGAATGGATTGGAT
ACATTAAATCCTAGCCGTGTTATACTAATTACAATCAGAAGTTC AAGGACAAGGCCACAT TGACTACAGACAAA
TCCTCCAGCACAGCCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA
TTATGATGATCATTA CTGCTTGACTACTGGGCCAAGGCACCACTCTCACAGTCTCCTCAGGTGGTGGTGT
CTGGCGGCGGCTCCGGTGGTGGTCTGACATTCAGCTGACCCAGTCTCCAGCAATCATGTCTGCATCT
CCAGGGAGAAGTCA CCAATGACCTGCAGAGCCAGTTCAAGTGTAAGTACATGAAC TGGTACCAGCAGAAAGTC
AGCACCTCCCCCAAAGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCTTCAGTGGCA
GTGGTCTGGGACCTCATACTCTCTACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA
CAGTGGAGTAGTAACCGCTCACGTTCCGGTCTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGGTGGATCCGA
GGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGCC TGGACTTCAGTGAAGCTGTCTGCAAGGCTT
CTGGCTACACCTTCACAAAGCTATGGTTTAAGCTGGTGAAGCAGAGA ACTGGACAGGGCCTTGAGTGGATTGGA
GAGGTTATCCTAGAAATTGGTAATGCTTACTACAATGAGAAGTTCAAGGCCAAGGCCACACTGACTGCAGACAA
ATCCTCCAGCACAGCGTCCATGGAGCTCCGCAGCTCTGAGGACTCTGCGGTCTATTCTGTGCAAGAC
GGGATCCTACGGTAGTAAC TACGACTGGTACTTCGATGTCTGGGCCAAGGACCAAGGTCA CCGTCTCCTCA
GGTGGTGGTGGTCTGGCGGCGGCTCCGGTGGTGGTCTGAGCTCGTGATGACCCAGACTCCACTCTC
CCTGCCCTGTCA GTCTTGAGATCAAGCCTCCATCTCTGCAGATCTAGTCAGAGCCTTGTA CACAGTAATGGAA
ACACCTATTACATTGGTACCCTGCAGAAAGCCAGGCCAGTCTCCAAGCTCCTGATCTACAAAGTTTCCAACCGA
TTTCTGGGGTCCCAGACAGGTT CAGTGGCAGTGGATCAGGGACAGATTTCACAC

Figure 1 N) continued

TCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTCTGCTCTCAAAGTACACATGTTCCGTACACG
TTCGGAGGGGACCAAGCTTGAGATCAAACATCATCACCATCATCATTAG

(SEQ ID NO: 54)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLTTDK
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSGGGGGGGGSDIQLTQSPAIMAS
PGEKVTMTCRASSVSVMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTSSMEAEADAATYYCQ
QWSSNPLTFAGATKLELKSGGGGSEVQLLEQSGAELVRPGTSVKLSCKASGYTFTSYGLSWVKQRTGQGLEWIG
EVYPRIGNAYYNEKFKGKATLTADKSSSTASMELRSLTSEDSAVYFCARRGSYGSNYDWYFDVWGQGT'TVTVSS
GGGGSGGGSGGSELVMTQTPLSLPVSIGDQASISCRSSQSLVHSNGNTYLNHWYLNQKPGQSPKLLIYKVSNR
FSGVPDRFSGSGGTDFTLKISRVEAEDLGVYFCSSQSTHVPYTFGGGTGLEIKHHHHHH

O) anti-CD3 VHVL aL x 4-1 VHVL (SEQ ID NO: 55)

GATATCAAACTGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCTCTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCACTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT
ACATTAAATCCTAGCCGTGTTATACTAATTACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA
TTATGATGATCATTA CTGCC TTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAA
GTGGAGGTTCTGGTGAAGTGGAGGTTCAAGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG
TCTGCACTCCAGGGGAGAAGTCACCATGACCTGCAGAGCCAGTTC AAGTGT AAGTTACATGAAC TGGTACCA
GCAGAAGTCAGGCACCTCCCCAAAGATGGATTTATGACACATCCA AAGTGGCTTCTGGAGTCCCTTATCGCT
TCAGTGGCAGTGGTCTGGGACCTCATACTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGGTCTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGG
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCTGGGACTTCAGTGAAGATATCCT
GCAAGGCTTCTGGATACGCCCTTCACCTAACTACTGGCTAGGTTGGGTTAAGCAGAGGCCCTGGACATGGACTTGAA
TGGGTGGAGATATTTCCCTGGAAGTGGTAATGCTCACTACAATGAGAAGTTC AAGGCCAAGCCACACTGAC
TGCAGACAAGTCCCTCGTACACAGCCCTATAATGCAGCTCAGTAGCCTGACATCTGAGGACTCTGCTGTCTATTCT
GTGCAAGATTGCGGA ACTGGGACGAGGCTATGGACTACTGGGCCAAGGCACCGTCA CCGTCTCCTCAGGT
GGTGGTGGTTCTGGCGCGGCTCCGGTGGTGGTCTGAGCTCGTGA TGACACAGTCTCCATCCTCCCT
GAGTGTTCAGCAGGAGAAGTCACTATGAGCTGCAAGTCCAGTCA GAGTCTGTTAACAGTGGAAATCAAA
AGAACTACTTGGCCTGGTACCAGCAGA AACAGGCCTCCTAAACTGTTGATCTACGGGCATCCACTAGG
GAA TCTGGGCTCCCTGATCGCTTCACAGGCAGTGGATCTGGAACAGATTTCACTC

Figure 1 O) continued

TCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAGAATGATTATAGTTATCCGTACACG
TTCGGAGGGGACCAAGCTTGAGATCAAAACATCATCACCATCATCATTAG

(SEQ ID NO: 56)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLTIDK
SSSTAYMQLSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGSGGSGGVDIQLTQSPAIM
SASPGEKVTMTCRASSVSVMNYYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGTSYSLTSSMEAEADAATY
YCQQWSSNPLTFGAGTKLELKS GGGSEVQLLEQSGAELVRPGTSVKISCKASGYAFITNYWLGWVKQRPQGHGLE
WVGDIFFPGSGNAHYNEKFKGKATLTADKSSYTAYMQLSLTSEDSAVYFCARLRNWDAMDYWGQGTITVTVSSG
GGSGGGGGGSELVMTQSPSSLSVSAGEKVTMSCKSSQSLNSGNQKNYLAWYQQKPGQPPKLLIYGASTR
ESGVPDRFTGSGSGTDFITLTISSVQAEDLAVYYCQNDYSYPYTFGGGTKEIKHHHHHH

Figure 1

P) anti-CD3 VHVL aL Ser x 4-1 VHVL (SEQ ID NO: 57)

GATATCAAACCTGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCTTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAAATGGATTGGAT
ACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCACTCTATTACTGTGCAAGATA
TTATGATGATCATTACTCCCTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAA
GTGGAGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG
TCTGCACTCCAGGGGAGAAGGTCAACATGACCTGCAGAGCCAGTTCAAGTGTAAAGTTACATGAACCTGGTACCA
GCAGAAGTCAGGCACCTCCCCAAAAGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCT
TCAGTGGCAGTGGGTCTGGGACCTCATACTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGGTGTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGG
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCCCTGGGACTTCAGTGAAGATATCCT
GCAAGGCTTCTGGATACGCCCTTCACTAACTACTGGCTAGGTTGGGTTAAGCAGAGGCCCTGGACATGGACTTGAA
TGGGTTGGAGATATTTCCCTGGAAGTGGTAATGCTCACTACAATGAGAAGTTCAAGGGCAAGCCACACTGAC
TGCAGACAAGTCCCTCGTACACAGCCTATATGCAGCTCAGTAGCCTGACATCTGAGGACTCTGCTGTCTATTCT
GTGCAAGATTGCCGAACCTGGACGAGGCTATGGACTACTGGGGCCAAGGGACCAAGGTCAAGGTCTCCTCAGGT
GGTGGTGGTCTGGCGCGCGGCTCCGGTGGTGGTCTGAGCTCGTGATGACACAGTCTCCATCCTCCCT
GAGTGTGTACGACGAGAGAAGGTCACTATGAGCTGCAAGTCCAGTCAGAGTCTGTAAACAGTGGAAATCAAA
AGAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGCCTCCTAAACTGTTGATCTACGGGGCATCCACTAGG
GAATCTGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGAACAGATTTCACTC

Figure 1 P) continued

TCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGT CAGAATGATTATAGTTATCCGTACACG
TTCGGAGGGGACCAAGCTTGAGATCAAACATCATCACCATCATCATTAG

(SEQ ID NO: 58)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKD KATLTTDK
SSSTAYMQLSLTSSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSSVEGGSGGSGGVDDIQLTQSPAIM
SASPGEKVMTTCRASSSVS YMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGGTSYSLTISMEAE DAATY
YCQQWSSNPLTFGAGTKLELKS GGGSEVQLLEQSGAELVRPGTSVKISCKASGYAF TNYWLGWVKQRP GHGLE
WVGDI FPGSGNAHYNEKFKGKATLTADKSSYTA YMQLSLTSSEDSAVYFCARLRNWDEAMDYWGQGT TVTVSSG
GGSGGGSGGGSELVMTQSPSSL SVSAGEKVTMSCKSSQSLINSGNQKNYLAWYQQKPGQPKLLIYGASTR
ESGVPDRFTGSGSGTDEFTLTISSVQAE DLAVYYCQNDYSYPYTFGGGTKLEIKHHHHHHH

Figure 1

Q) anti-CD3 VHVL stL x 4-1 VHVL (SEQ ID NO: 59)

GATATCAAACCTGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCTGCAAGACTTC
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT
ACATTAAATCCTAGCCGTGGTTATACTAATTACAATCAGAAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA
TCCTCCAGCACAGCCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA
TTATGATGATCATTTACTGCCCTTGACTACTGGGGCCAAGGCACCTCTCACAGTCTCCTCAGGTGGTGGTGGTT
CTGGCGCGGGCTCCGGTGGTGGTTCTGACATTCAGCTGACCCAGTCTCCAGCAATCATGTCTGCATCT
CCAGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAGTTACATGAACCTGGTACCAGCAAGATC
AGGCACCTCCCCCAAAGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCCGTTTCAAGTGGCA
GTGGTCTGGGACCTCATACTCTCTACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA
CAGTGGAGTAGTAACCCGCTCACGTTCCGGTCTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGGTGGATCCGA
GGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCTGGGACTTCAGTGAAGATATCCTGCAAGGCTT
CTGGATACGCCCTTCACTAATACTGGCTAGGTTGGTTAAGCAGAGGCCCTGGACATGGACTTGAATGGGTTGGA
GATAATTTCCCTGGAAGTGGTAATGCTCACTACAATGAGAAAGTTCAAGGGCAAAGCCACACTGACTGCAGACAA
GTCCCTCGTACACAGCCCTATATGCAGCTCAGTAGCCTGACATCTGAGGACTCTGCTGTCTATTCTGTGCAAGAT
TGCGGAACTGGGACGAGGCTATGGACTACTGGGCCAAGGACCACGGTCACCGTCTCCTCAGGTGGTGGTGGT
TCTGGCGCGGCTCCGGTGGTGGTTCTGAGCTCGTGATGACACAGTCTCCATCCTCCCTGAGTGTGTCTC
AGCAGGAGAGAAGTCACTATGAGCTGCAAGTCCAGTCAGAGTCTGTAAACAGTGGAAATCAAAGAACTACT
TGGCCTGGTACCAGCAGAAACCAGGGCAGCCTCCTAAACTGTTGATCTACGGGGCATCCACTAGGGAATCTGGG
GTCCCTGATCGCTTCACAGGCAGTGGATCTGGAACAGATTTCACTCTCACCATCA

Figure 1 Q) continued

GCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAGAAATGATTATAGTTATCCGTACACGTTTCGGAGGG
GGACCAAGCTTGAGATCAAAACATCATCACCATCATTAG

(SEQ ID NO: 60)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHNVKQRPQGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDK
SSSTAYMQLSLTSÉDSAVYYCARYYDDHYCLDYWGQGTTLTVSSGGGGGGGGSDIQLTQSPAIMAS
PGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPRFSGSGTSYSLTISSEAEADAATYYCQ
QWSSNPLTFGAGTKLELKSGGGGSEVQLLEQSGAEIVRPGETSVKISCKASGYAFTNRYWLGWVKQRPCHGLEWVG
DIFPGSGNAHYNEKFKGKATLTADKSSYTA YMQLSLTSÉDSAVYFCARLRNWDEAMDYWGQGT'TVTVSSGGGG
SGGGSGGGSELVMTQSPSSLVSAGEKVTMSCKSSQSLNSGNQKNYLAWYQQKPGQPPKLLIYGASTRESG
VPDRFTGSGSGTDFTLTISSVQAEFLAVYYCQNDYSYPYTFGGGTKLEIKHHHHHH

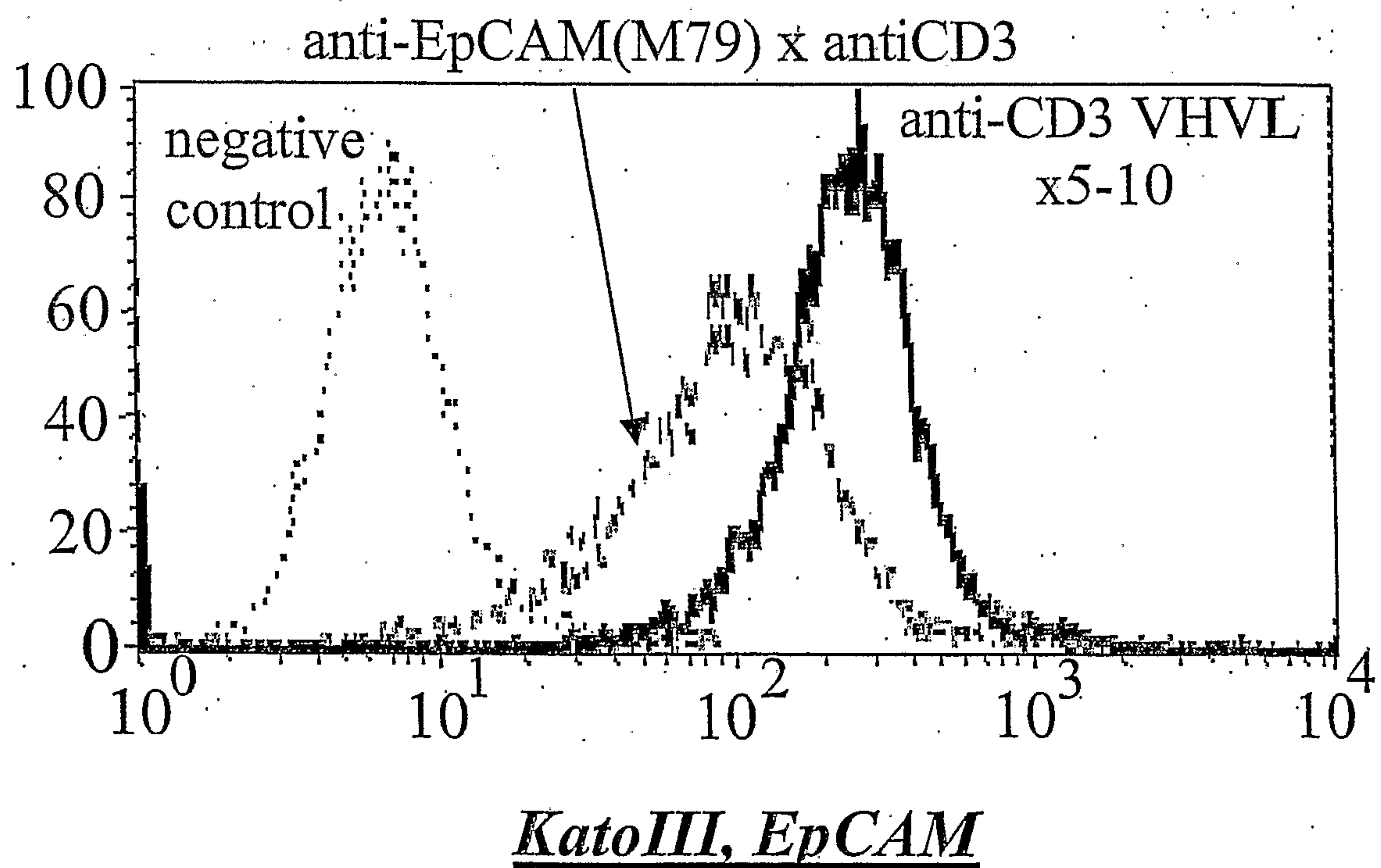
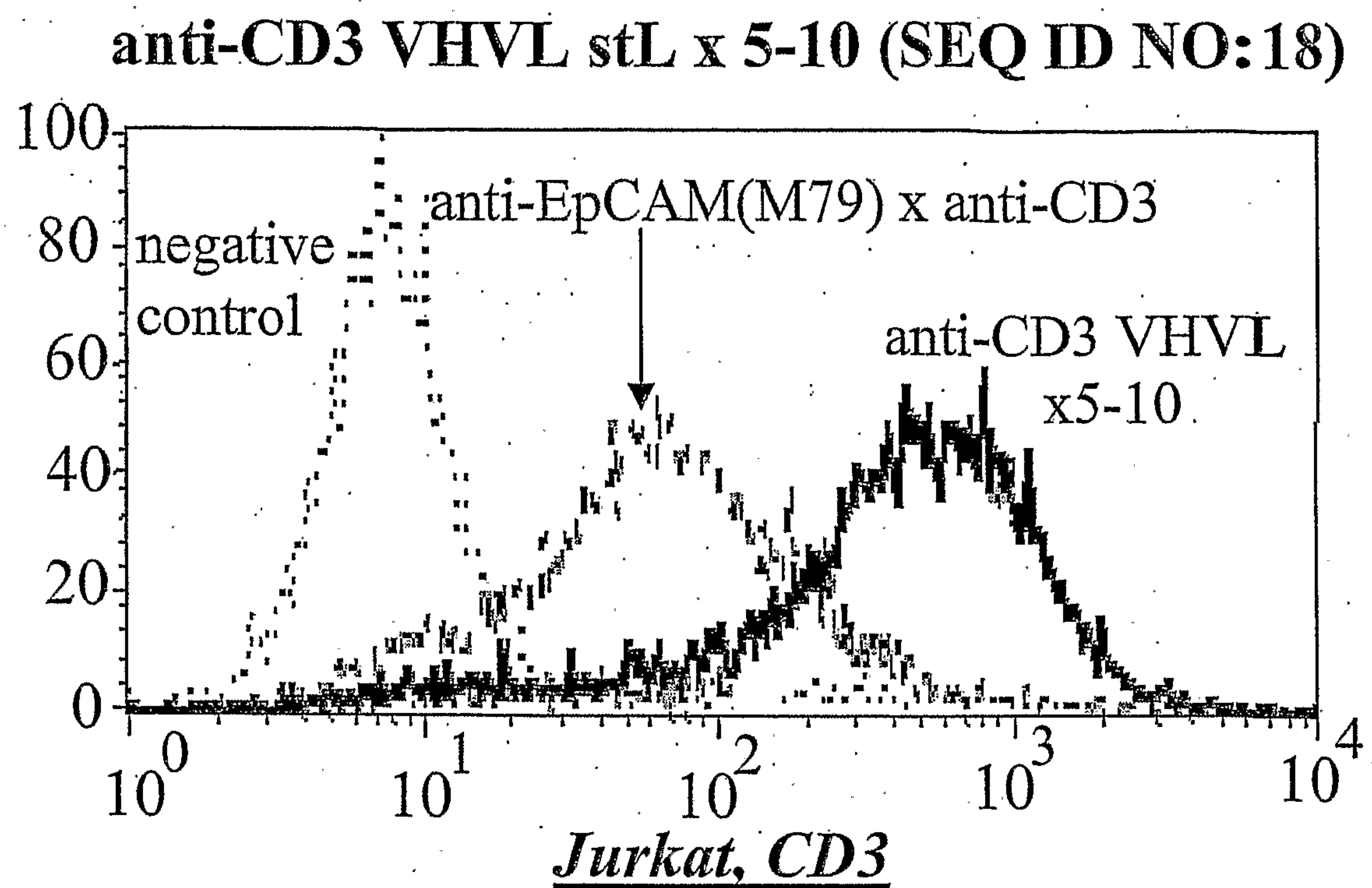
Figure 2 A

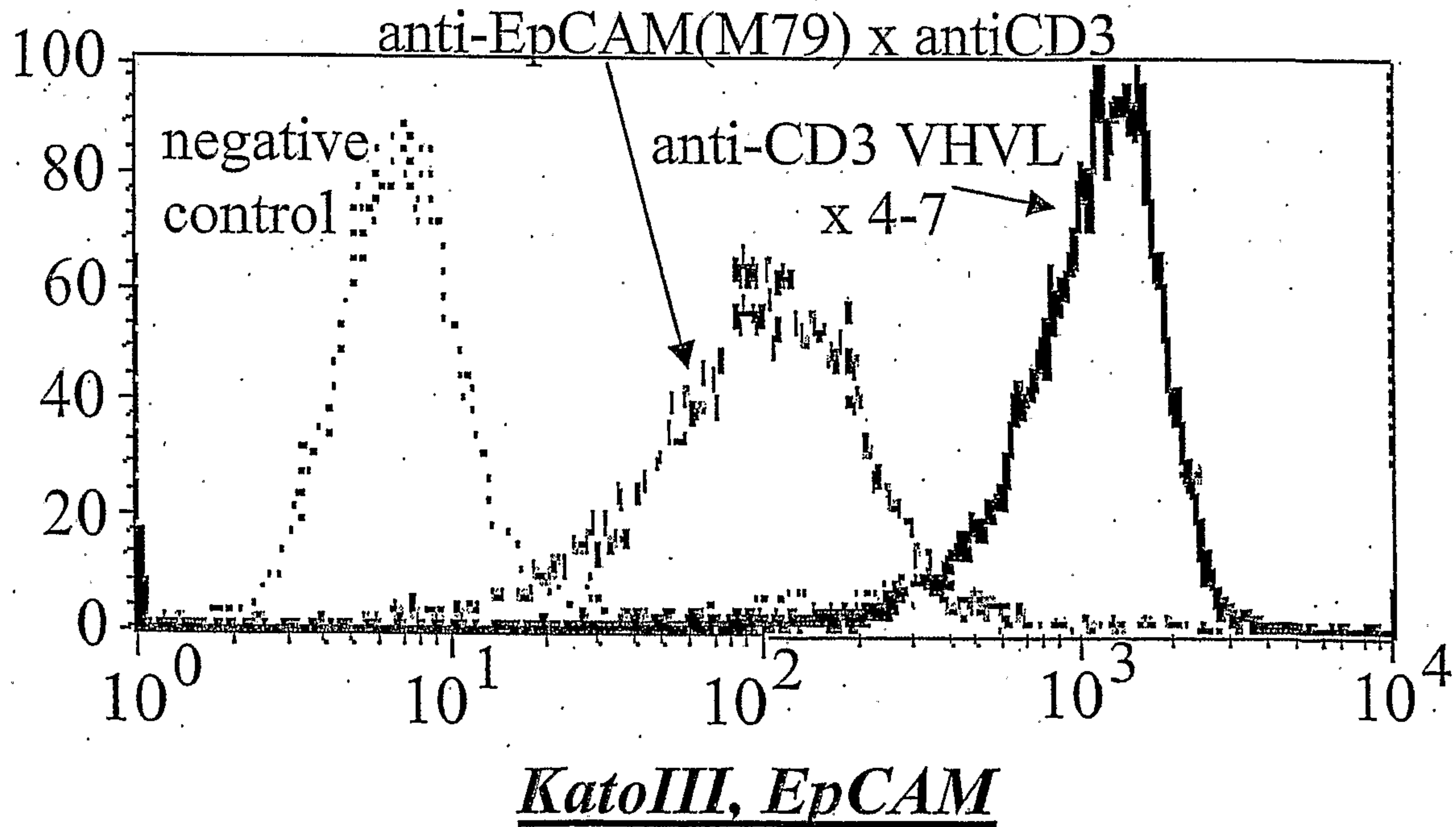
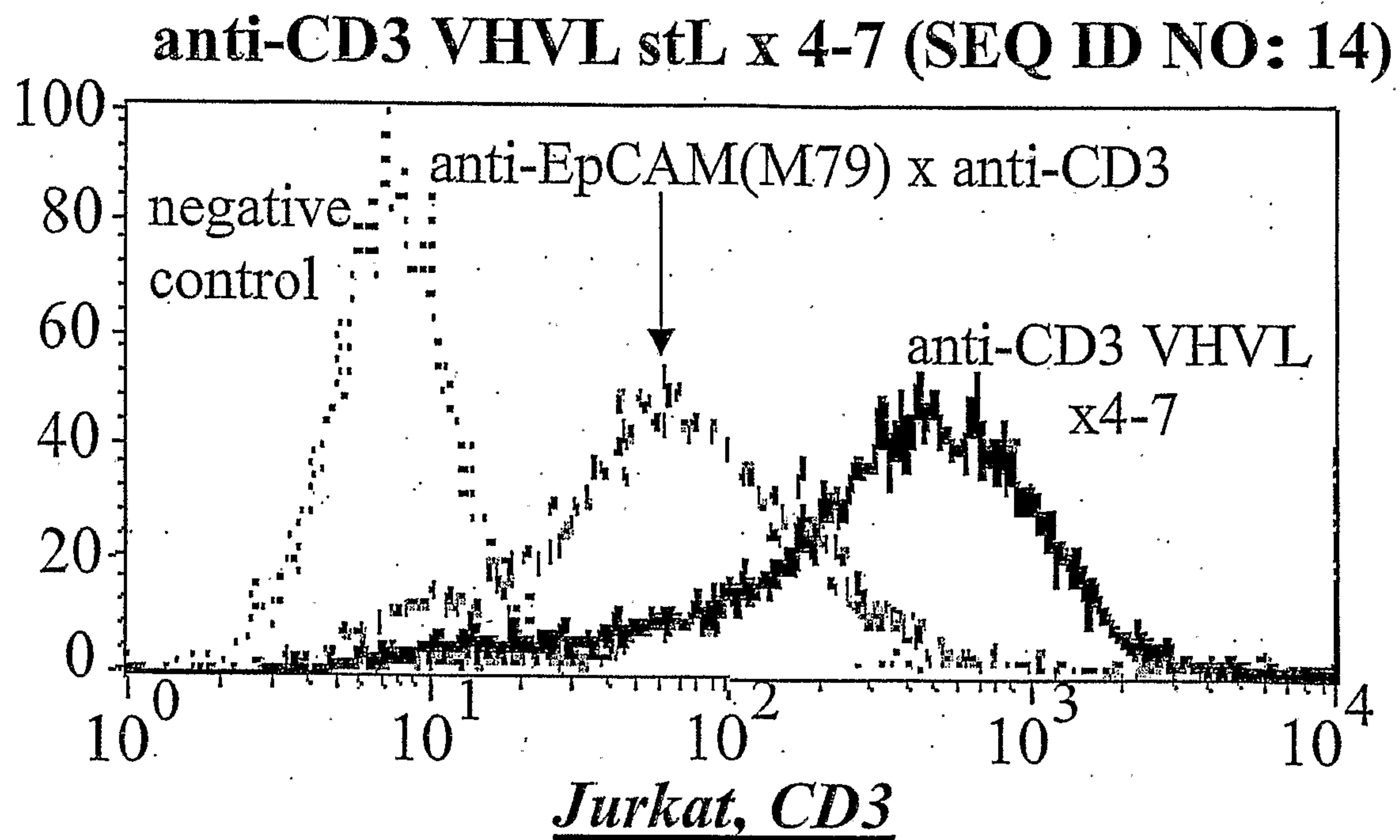
Figure 2 B

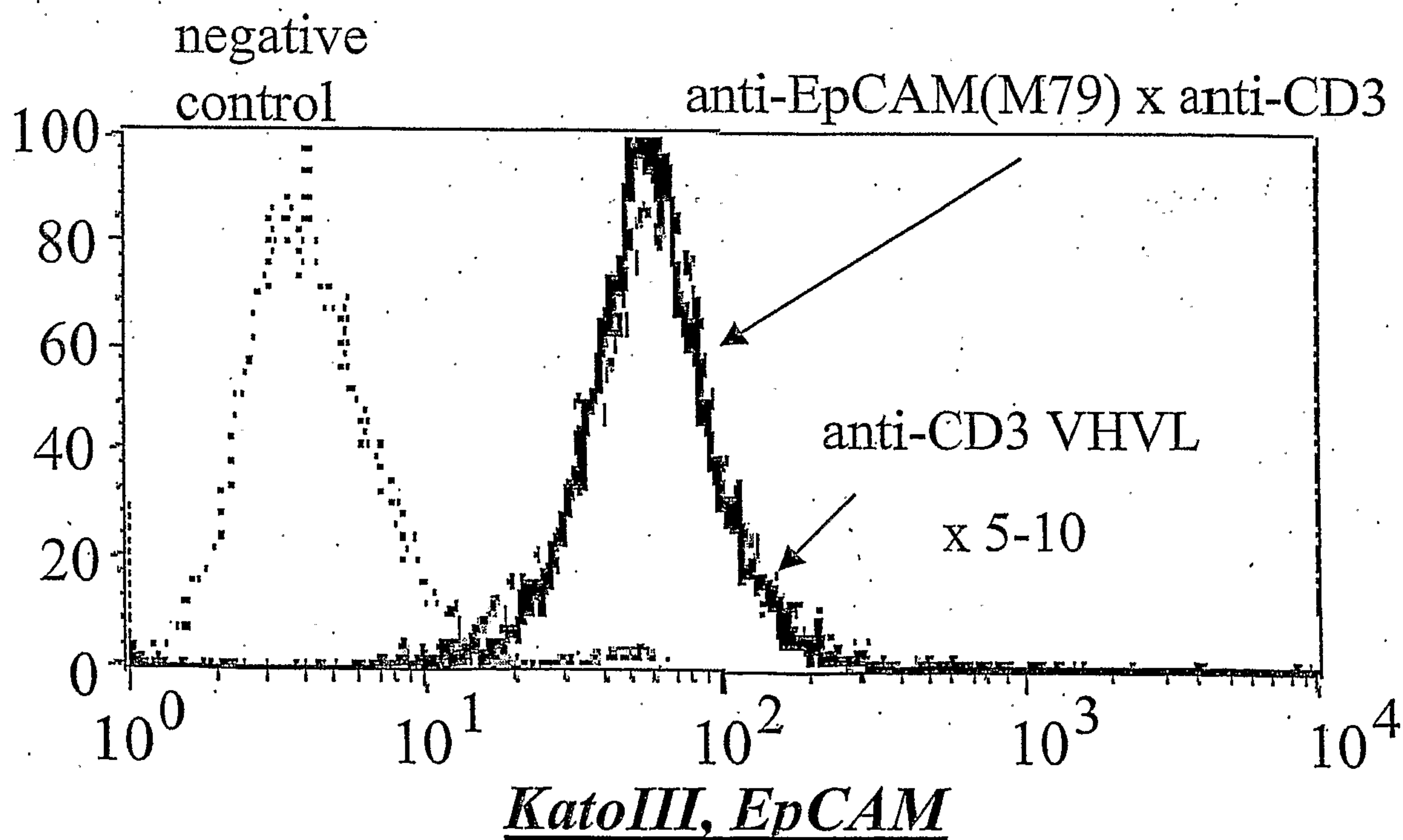
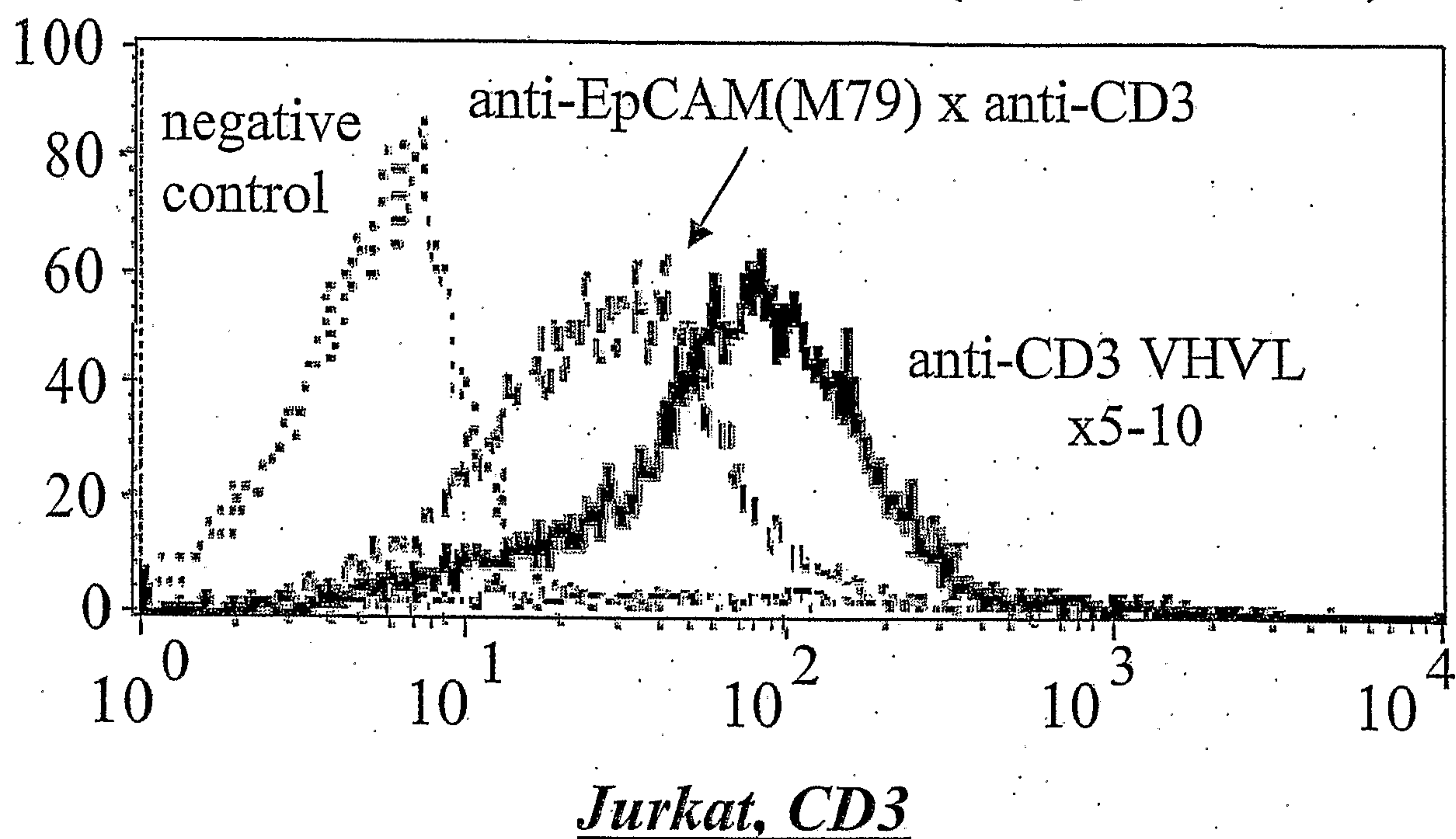
Figure 2C**anti-CD3 VHVL aL x 5-10 (SEQ ID NO: 4)**

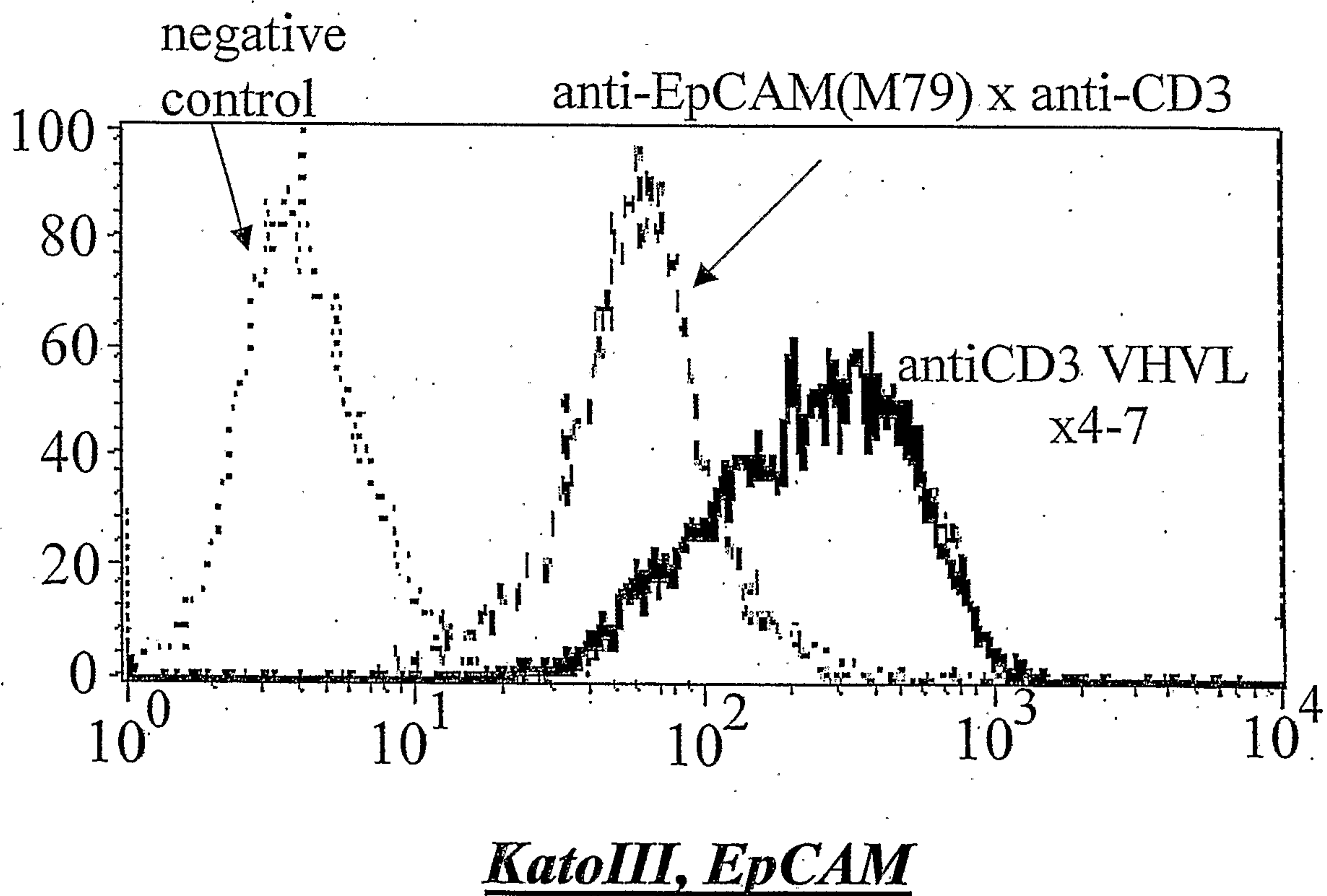
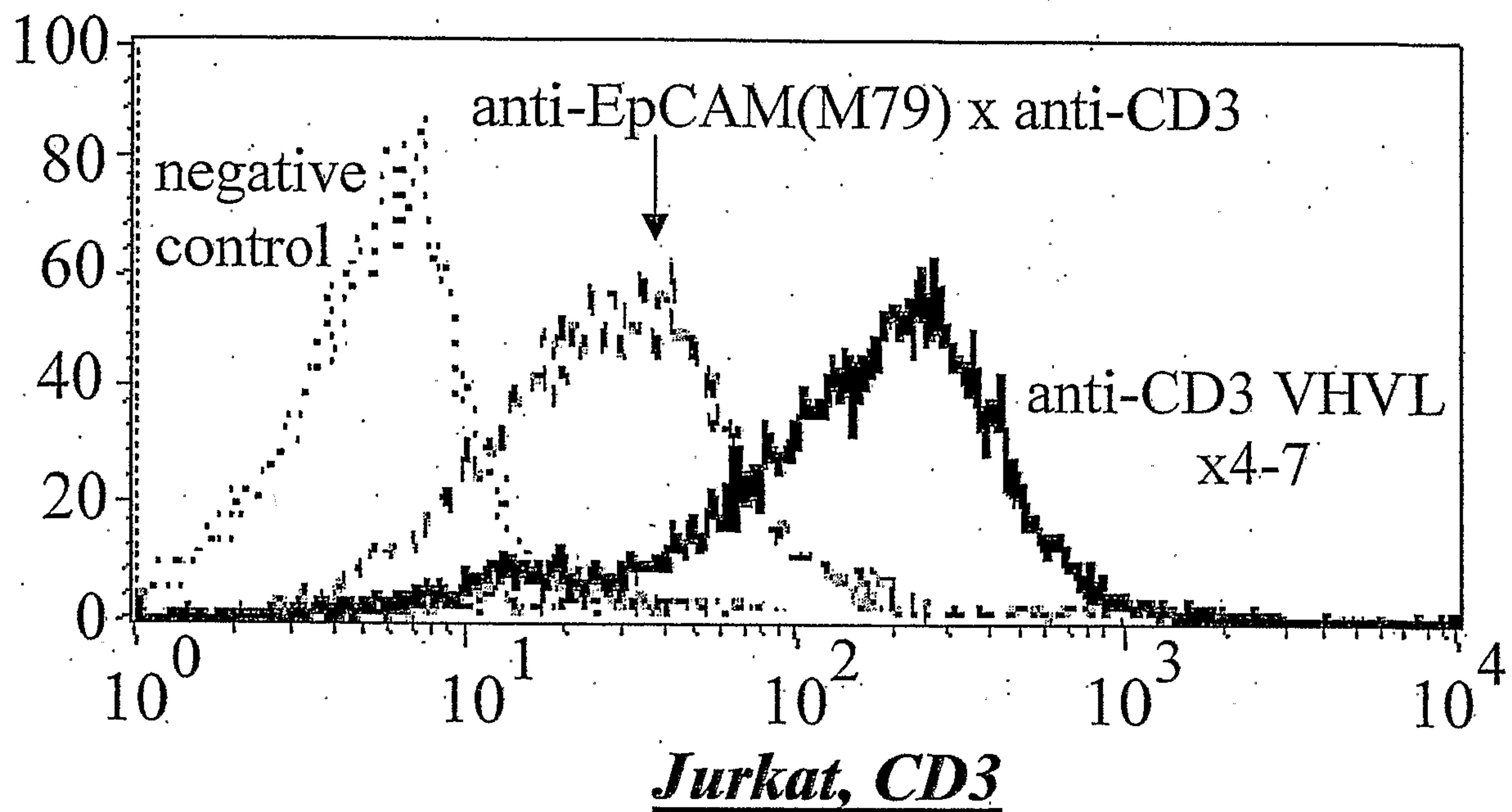
Figure 2D**anti-CD3 VHVL aL x 4-7 (SEQ ID NO: 2)**

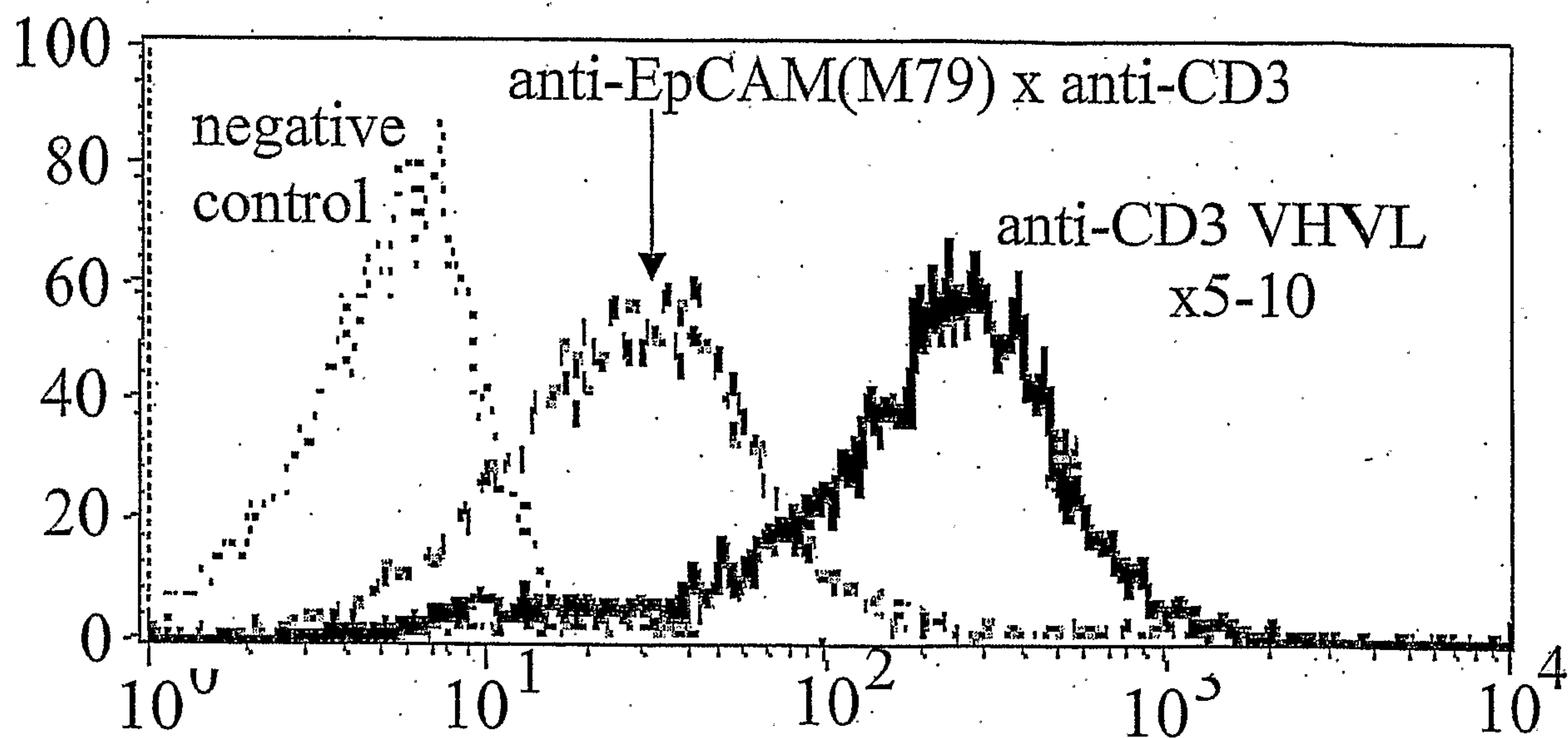
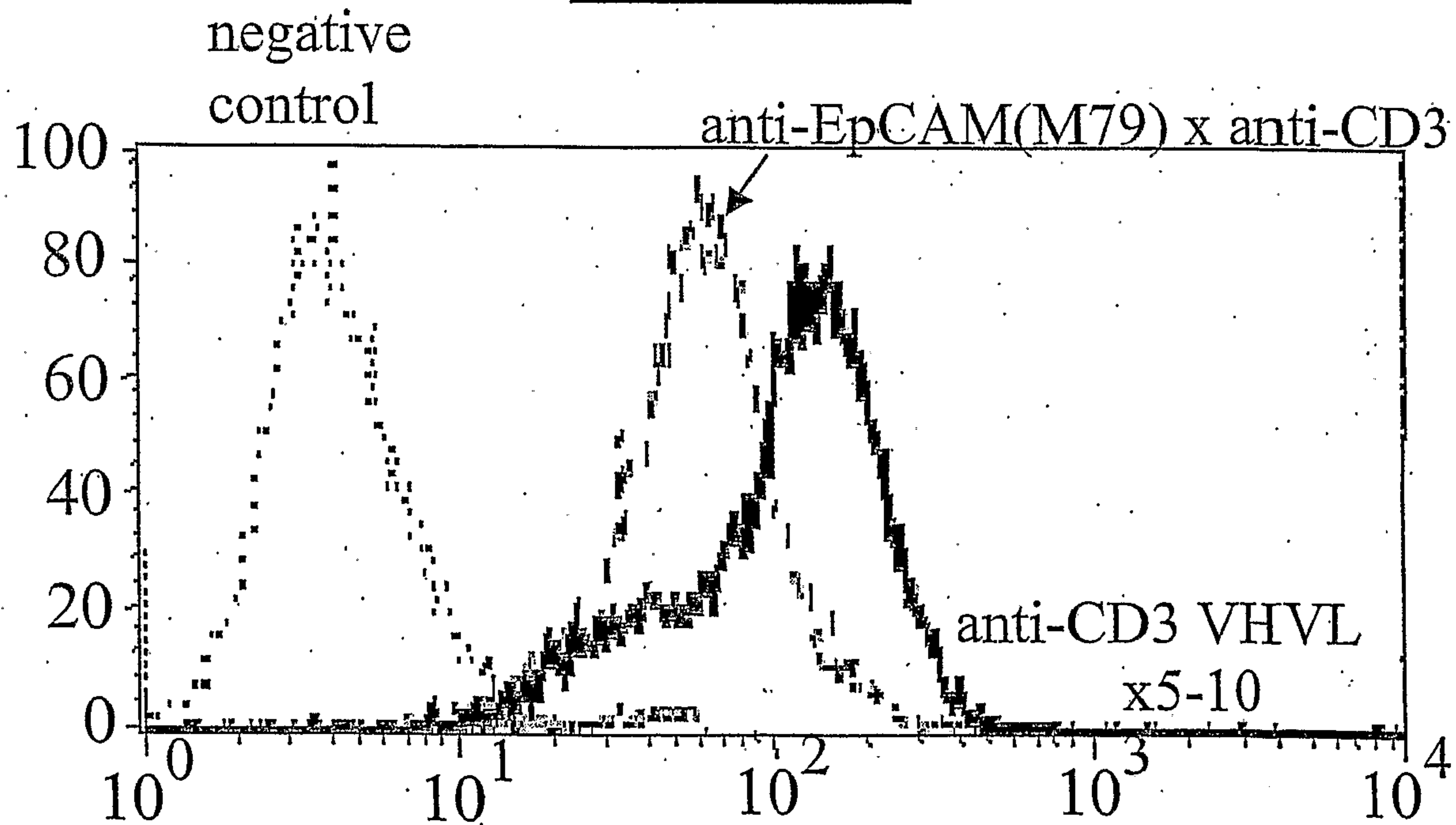
Figure 2E**anti-CD3VHVL aL Ser x 5-10 (SEQ ID NO: 10)****Jurkat, CD3****KatoIII, EpCAM**

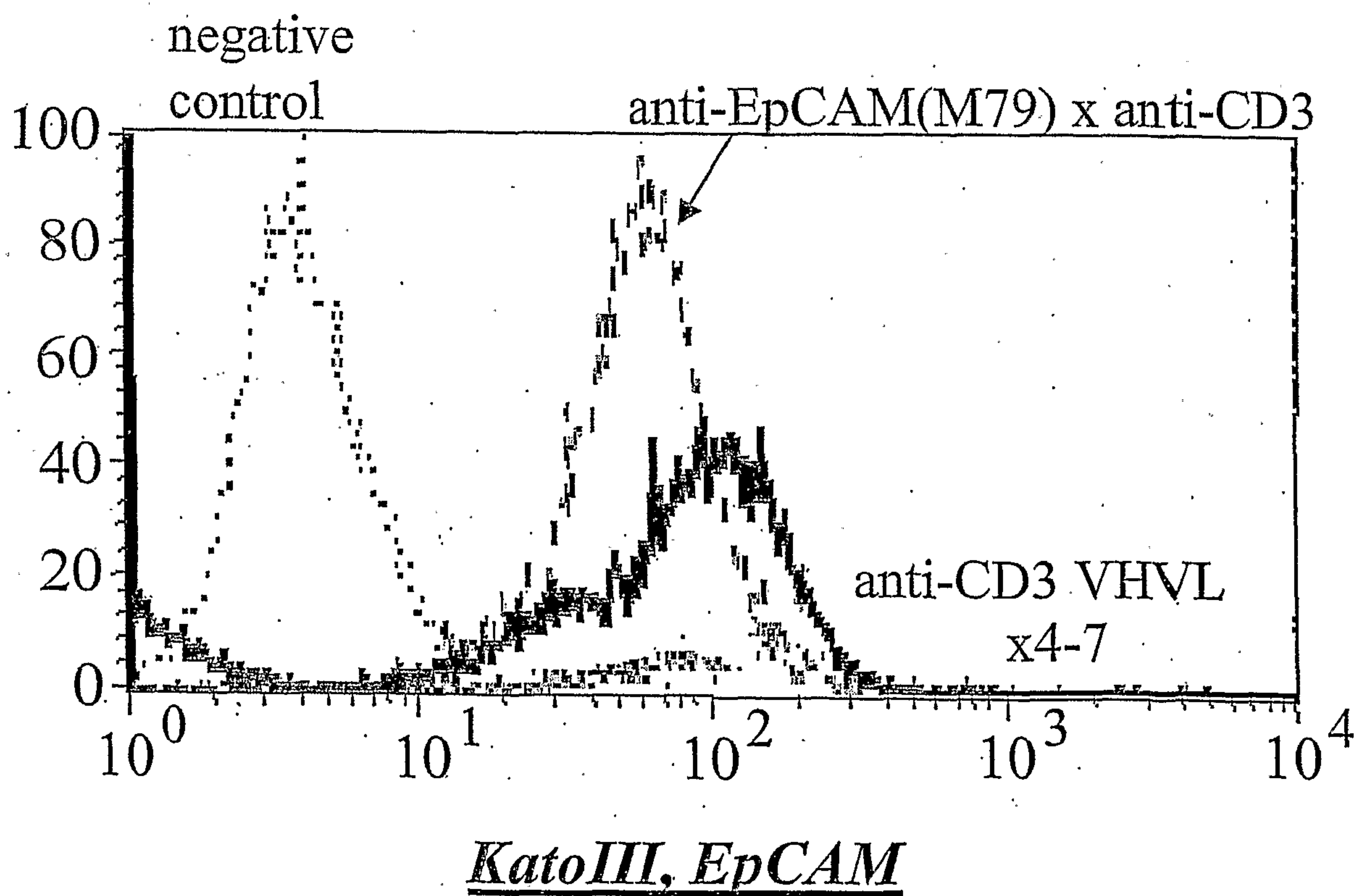
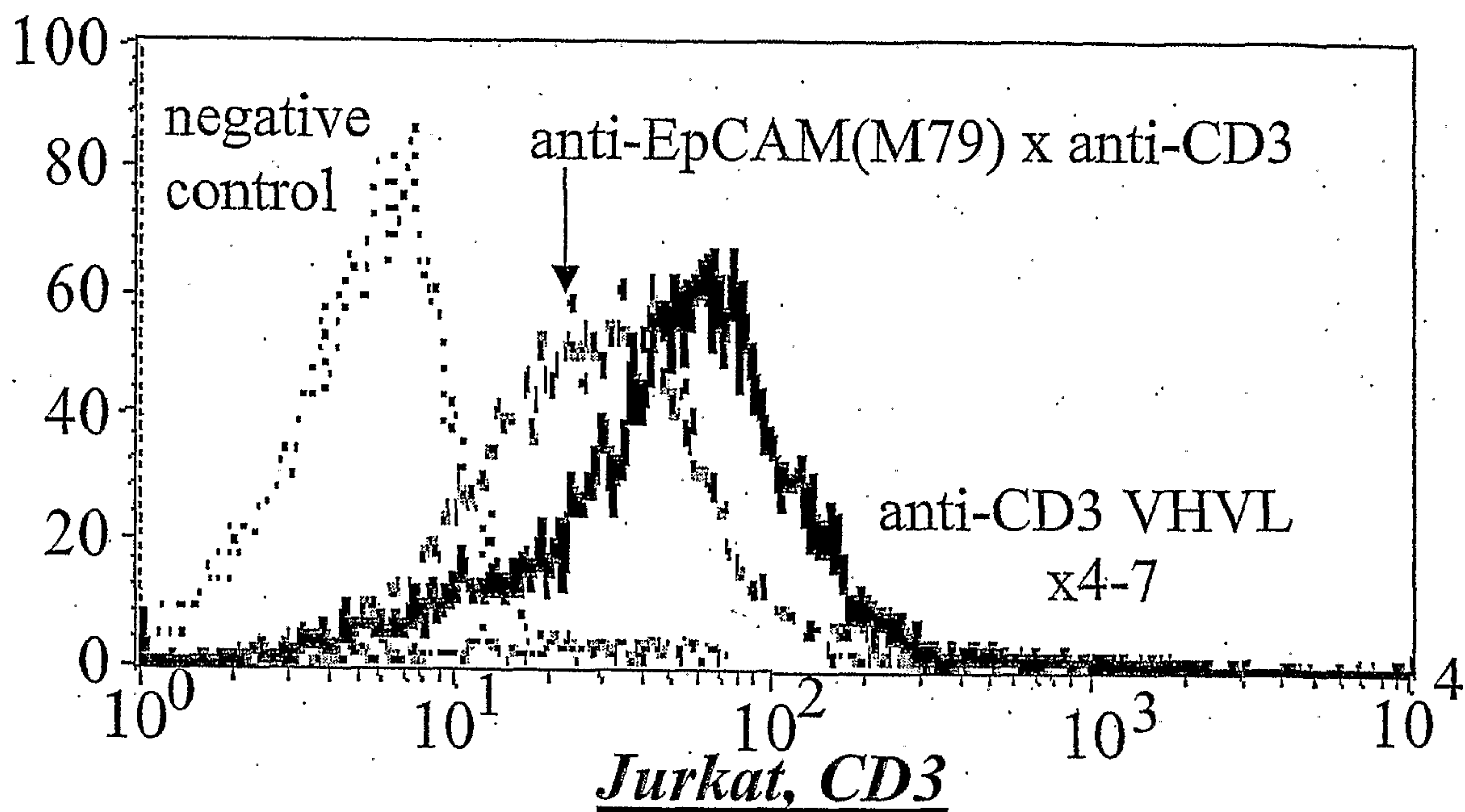
Figure 2F**anti-CD3 VHVL aL Ser x 4-7 (SEQ ID NO: 8)**

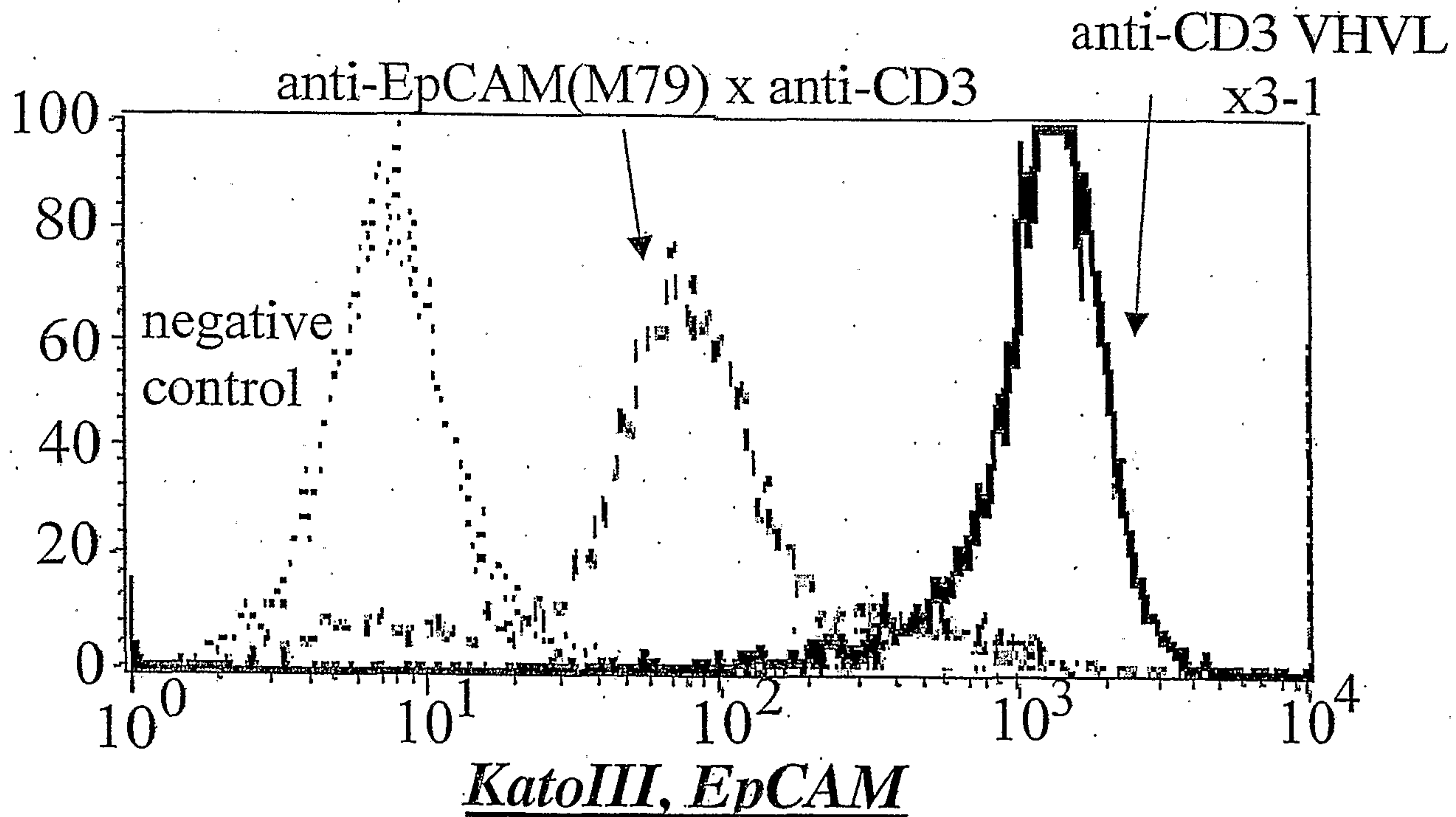
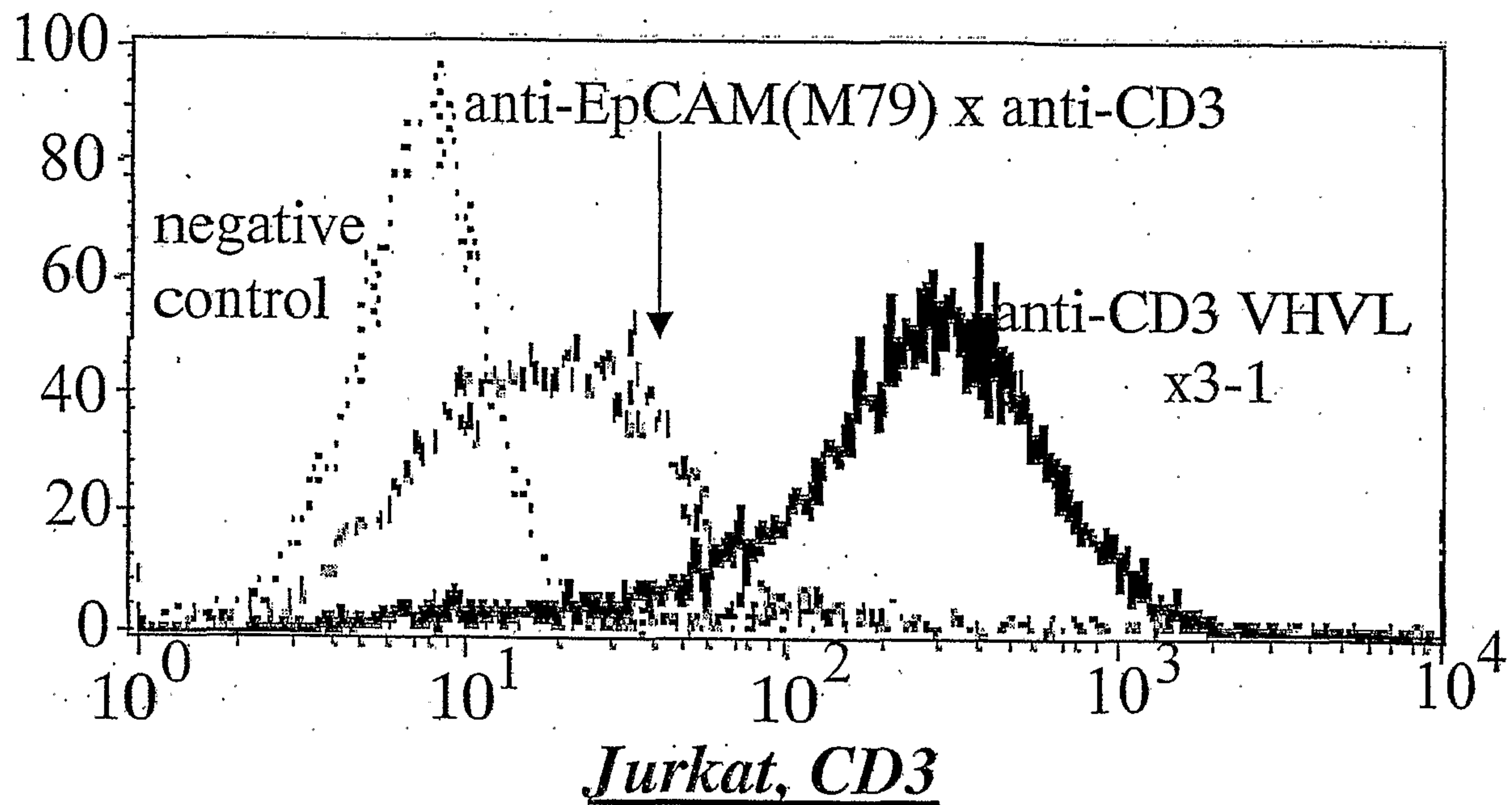
Figure 2G**anti-CD3 VHVL stL x 3-1 (SEQ ID NO: 12)**

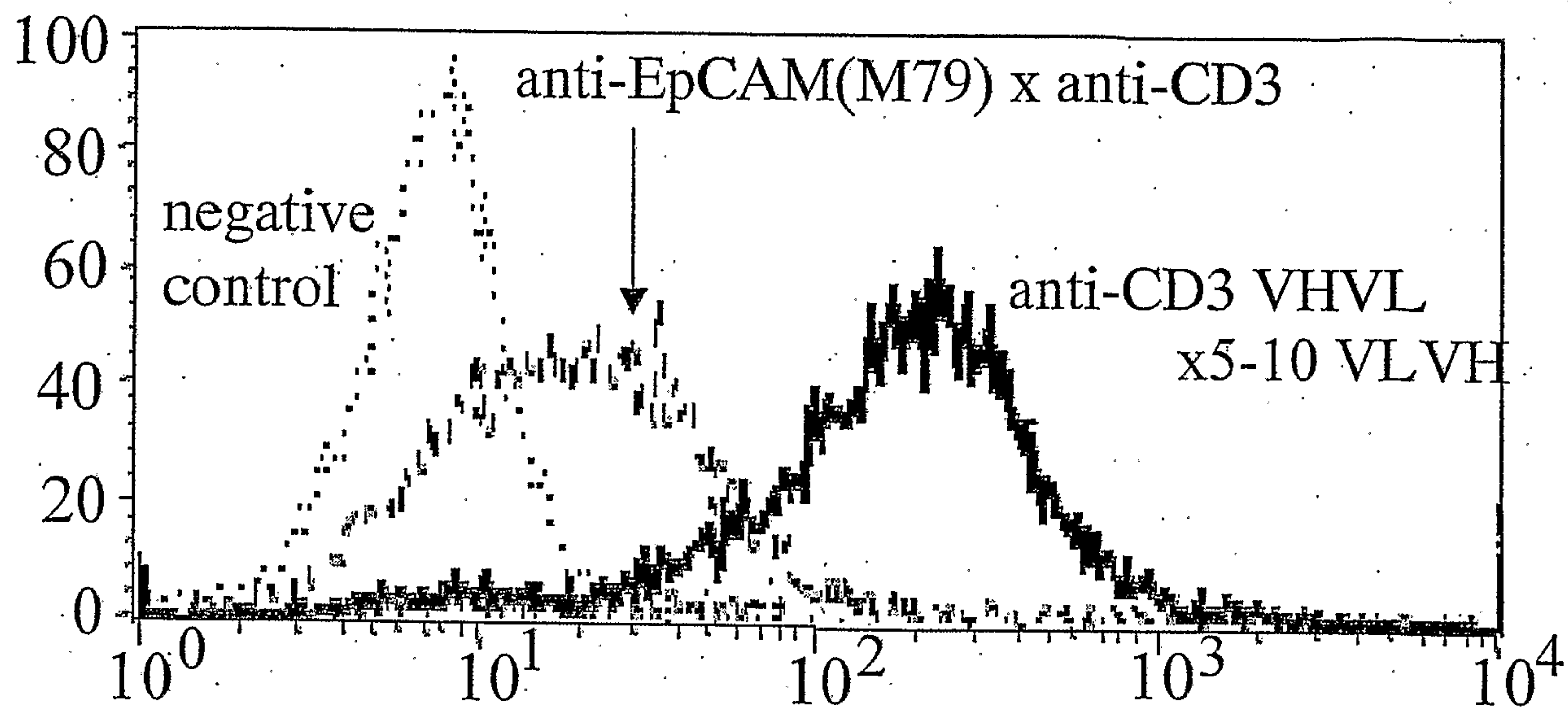
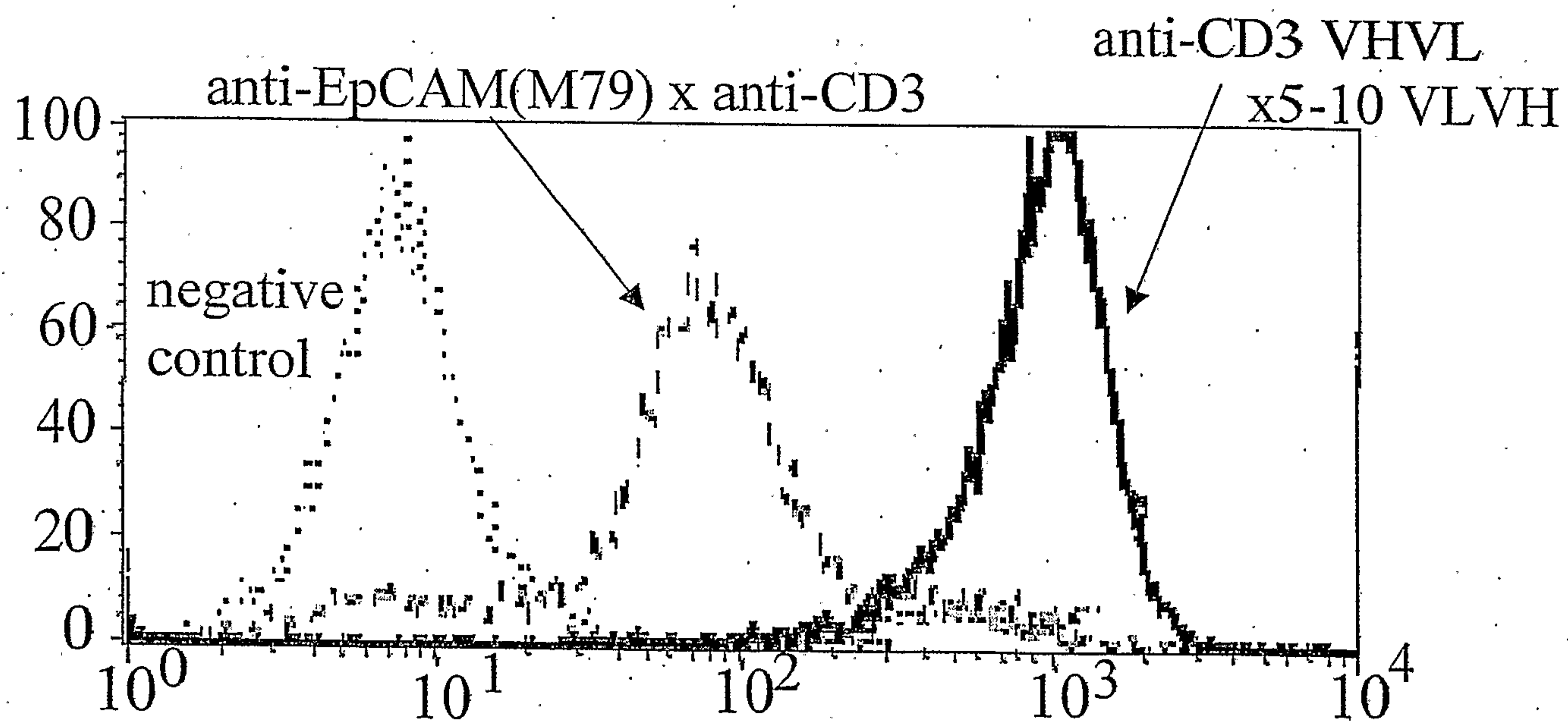
Figure 2H**anti-CD3 VHVL stL x 5-10 VLVH (SEQ ID NO: 20)***Jurkat, CD3**KatoIII, EpCAM*

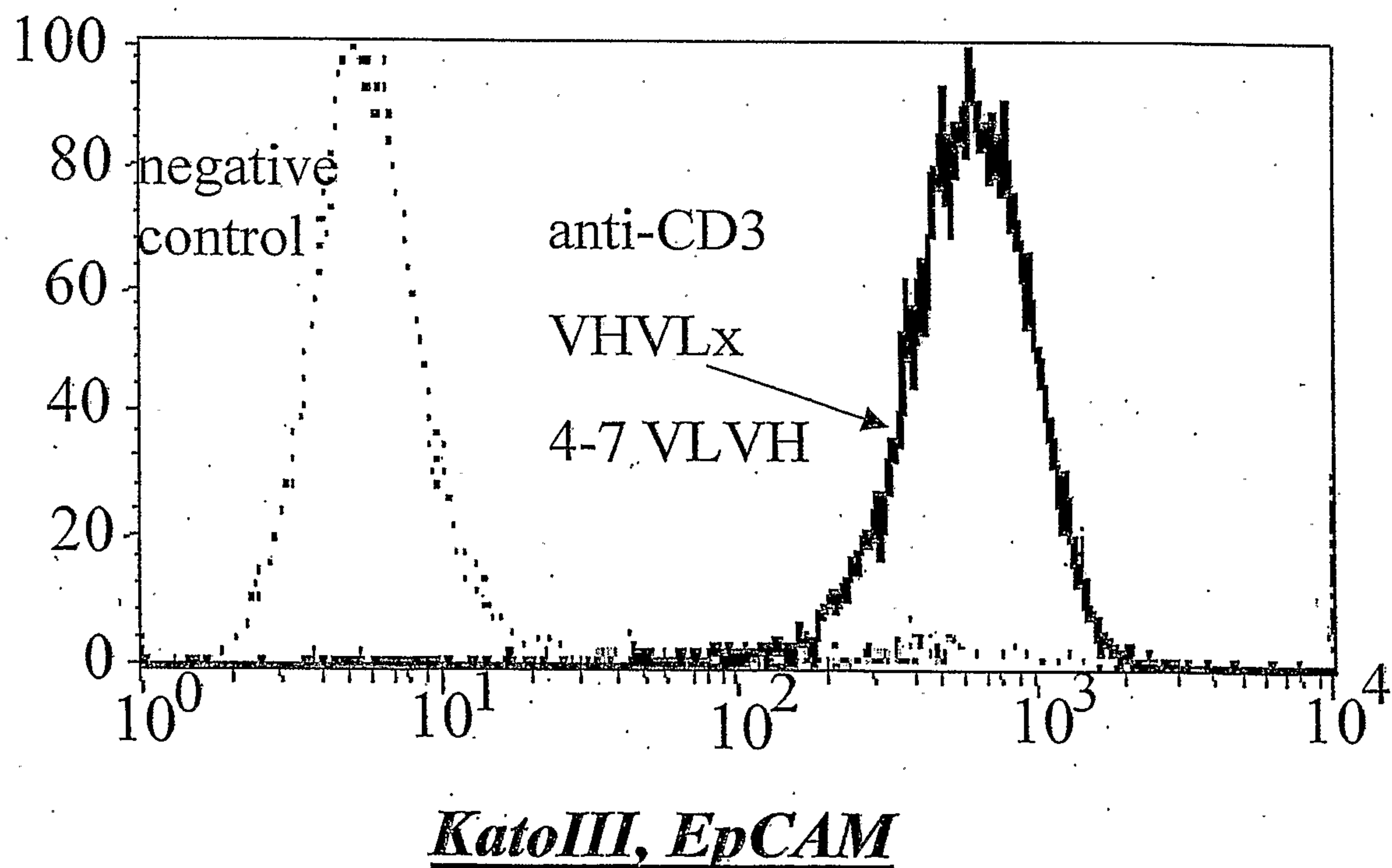
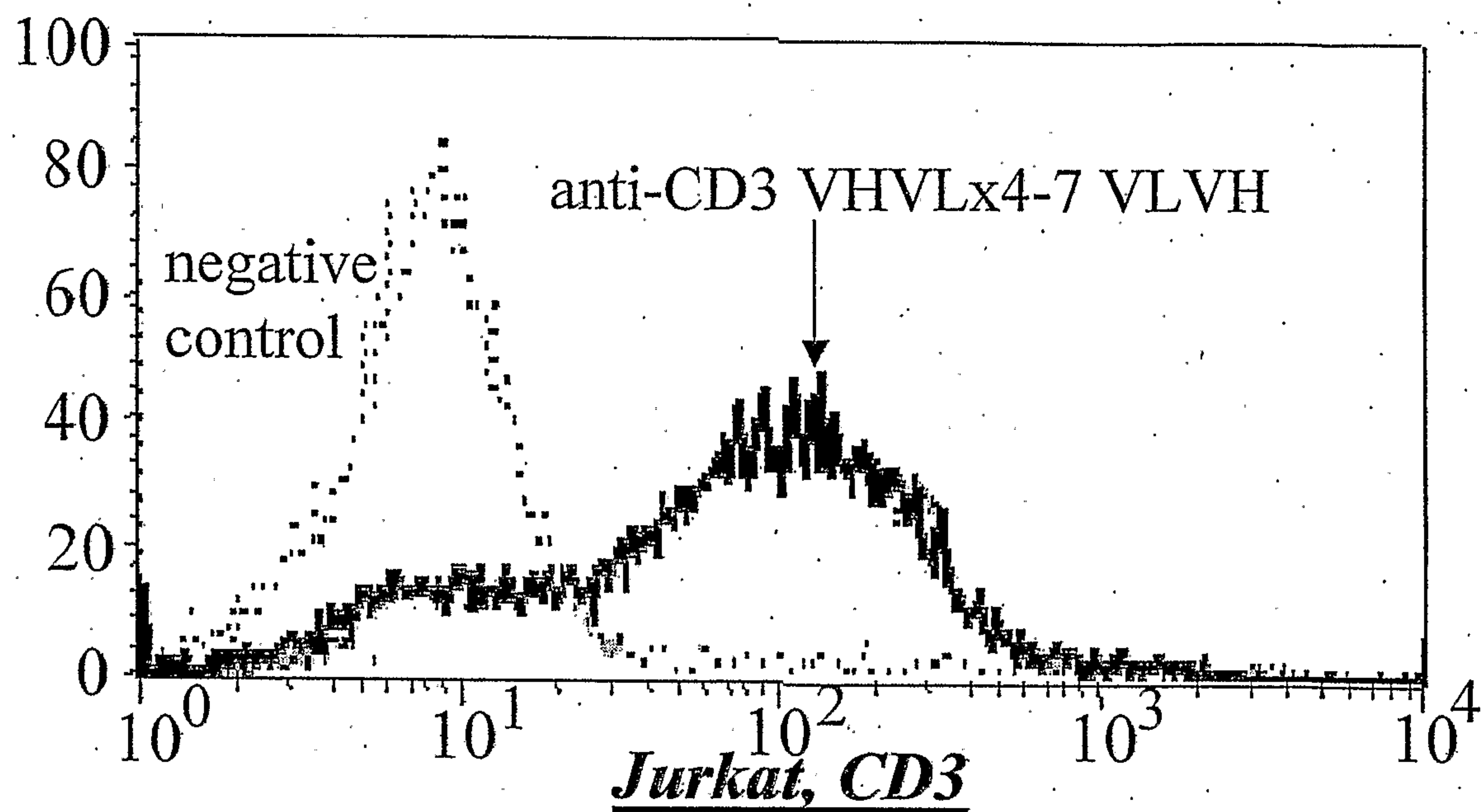
Figure 2I**anti-CD3 VHVL stL x 4-7 VLVH (SEQ ID NO: 16)**

Figure 3A

4-7 (vLvH) x anti-CD3 (SEQ ID NO: 42)

1 MGWSCIIILFL VATAATGVHSA RELVMTQTPL SLPVSLGDQA SISCRSSQSL
51 VHSNGNTYLH WYLQKPGQSP KLLIYKVSNR FSGVPDRFSG SGSGTDFTLK
101 ISRVEAEDLG VYFCSQSTHV PYTFGGGTKL EIKGGGSGG GSGGGGSEV
151 QLEQSGAEL ARPGASVKLS CKASGYTFN YGLSWVKQRP GQVLEWIGEV
201 YPRIGNAYN EKFKGKATLT ADKSSSTASM ELRSLTSEDS AVYFCARRGS
251 YDTNYDWYFD VWGQGTTVTV SSGGGGSDIK LQQSGAELAR PGASVKMSCK
301 TSGYTFTRYT MHWVKQRPQG GLEWIGYINP SRGYTNYNQK FKDKATLTTD
351 KSSSTAYMQL SSLTSEDSAV YYCARYYDDH YCLDYWGQGT TLTVSSVEGG
401 SGGSGSGGS GGVDIDIQLTQ SPAIMSASPG EKVTMTCRAS SSVSYMNWYQ
451 QKSGTSPKRW IYDTSKVASG VPYRFGSGS GTSYSLTISS MEAEADAATYY
501 CQQWSSNPILT FGAGTKLELK HHHHHH*

Figure 3A (continued)**SEQ ID NO: 41**

```
1  ATGGGATGGA  GCTGTATCAT  CCTCTTCTTG  GTAGCAACAG  CTACAGGTGT
51  ACACTCCGCG  CGCGAGCTCG  TGATGACCCA  GACTCCACTC  TCCCTGCCCTG
101 TCAGTCTTGG  AGATCAAGCC  TCCATCTCTT  GCAGATCTAG  TCAGAGCCTT
151 GTACACAGTA  ATGGAACAC  CTATTTACAT  TGGTACCCTG  AGAAGCCAGG
201 CCAGTCTCCA  AAGCTCCTGA  TCTACAAAGT  TTCCAACCGA  TTTTCTGGGG
251 TCCAGACAG  GTTCAGTGGC  AGTGATCAG  GGACAGATTT  CACACTCAAG
301 ATCAGCAGAG  TGGAGGCTGA  GGATCTGGGA  GTTTATTTC  GCTCTCAAAG
351 TACACATGTT  CCGTACACGT  TCGGAGGGGG  GACCAAGCTT  GAGATCAAAG
401 GTGGTGGTGG  TTCTGGCGGC  GGCGGCTCCG  GTGGTGGTGG  TTCTGAGGTG
451 CAGCTGCTCG  AGCAGTCTGG  AGCTGAGCTG  GCGAGGCCCTG  GGGCTTCAGT
501 GAAGCTGTCC  TGCAAGGCTT  CTGGCTACAC  CTTCACAAAC  TATGGTTTAA
551 GCTGGGTGAA  GCAGAGGCCT  GGACAGGTCC  TTGAGTGGAT  TGGAGAGGTT
601 TATCCTAGAA  TTGGTAATGC  TTAATAAAT  GAGAAGTTCA  AGGCAAGGC
651 CACACTGACT  GCAGACAAAT  CCTCCAGCAC  AGCGTCCAAG  GAGCTCCGCA
701 GCCTGACCTC  TGAGGACTCT  GCGGTCTATT  TCTGTGCAAG  ACGGGATCC
751 TACGATACTA  ACTACGACTG  GTACTTCGAT  GTCTGGGGCC  AAGGGACCAC
801 GGTCAACGTC  TCCTCCGGAG  GTGGTGGATC  CGATATCAAA  CTGCAGCAGT
851 CAGGGGCTGA  ACTGGCAAGA  CCTGGGGCCT  CAGTGAAGAT  GTCCTGCAAG
```

Figure 3A (continued)

901	ACTTCTGGCT	ACACCTTTAC	TAGGTACACG	ATGCACTGGG	TAAACAGAG
951	GCCTGGACAG	GGTCTGGAAT	GGATTGGATA	CATTAAATCCT	AGCCGTGGTT
1001	ATACTAATTA	CAATCAGAAG	TTCAAGGACA	AGGCCACATT	GACTACAGAC
1051	AAATCCTCCA	GCACAGCCTA	CATGCAACTG	AGCAGCCTGA	CATCTGAGGA
1101	CTCTGCAGTC	TATTACTGTG	CAAGATATTA	TGATGATCAT	TACTGCCTTG
1151	ACTACTGGGG	CCAAGGCACC	ACTCTCACAG	TCTCCTCAGT	CGAAGGTGGA
1201	AGTGGAGGTT	CTGGTGGAAG	TGGAGGTTCA	GGTGGAGTCG	ACGACATTCA
1251	GCTGACCCAG	TCTCCAGCAA	TCAATGTCTGC	ATCTCCAGGG	GAGAAGGTCA
1301	CCATGACCCTG	CAGAGCCAGT	TCAAGTGTA	GTTACATGAA	CTGGTACCAG
1351	CAGAAGTCAG	GCACCTCCCC	CAAAAGATGG	ATTATGACA	CATCCAAAGT
1401	GGCTTCTGGA	GTCCCCTTATC	GCTTCAGTGG	CAGTGGGTCT	GGGACCTCAT
1451	ACTCTCTCAC	AATCAGCAGC	ATGGAGGCTG	AAGATGCTGC	CACCTATTAC
1501	TGCCAACAGT	GGAGTAGTAA	CCCGCTCACG	TTCGGTGCTG	GGACCAAGCT
1551	GGAGCTGAAA	CATCATCACC	ATCATCATTA	G	

Figure 3B

3-5 (vLvH) x anti-CD3 (SEQ ID NO: 30)

```
1  MGWSCIIILFL VATA'GVHSA RELVMTQTPL SLPVSLGDQA SISCRSSQSL
51  VHSNGNTYLH WYLQKPGQSP KLLIYKVSNR FSGVPDRFSG SGSGTDFTLK
101 ISRVEAEDLG VFCSQSTHV PYTFGGGTKL EIKGGGSGG GSGGGGSEV
151 QLEQSGAEL VRPGTSVKLS CKASGYTFTS YGLSWVKQRT GQGLEWIGEV
201 YPRIGNAYYN EKFKGKATLT ADKSSSTASM ELRLTSEDS AVYFCARRGS
251 YGSNYDWFED VWGQGT'TVTV SSGGGGSDIK LQQSGAELAR PGASVKMSCK
301 TSGYTFTRYT MHWVKQRPQG GLEWIGYINP SRGYTNYNQK FKDKATLT'D
351 KSSSTAYMQL SSLTSEDSAV YYCARYYDDH YCLDYWGQGT TLTVSSVEGG
401 SGGSGSGGS GGVD'IDIQLTQ SPAIMSASPG EKVTMTCRAS SSVSYMNWYQ
451 QKSGTSPKRW IYDTSKVASG VPYRFSGSGS GTSYSLTISS MEAEDAATYY
501 CQQWSSNPLT FGAGTKLELK HHHHHH*
```

Figure 3B (continued)**SEQ ID NO:29:**

```
1  ATGGGATGGA GCTGTATCAT CCTTCTTG GTAGCAACAG CTACAGGTGT
51  ACACTCCGG CGGAGCTCG TGATGACCCA GACTCCACTC TCCCTGCCCTG
101 TCAGTCTTG AGATCAAGCC TCCATCTCTT GCAGATCTAG TCAGAGCCTT
151 GTACACAGTA ATGGAACAC CTATTACAT TGGTACCCTG AGAAGCCAGG
201 CCAGTCTCCA AAGTCCCTGA TCTACAAAGT TTCCAACCGA TTTCTGGGG
251 TCCCAGACAG GTTCAGTGGC AGTGGATCAG GGACAGATTT CACACTCAAG
301 ATCAGCAGAG TGGAGGCTGA GGATCTGGA GTTTATTCT GCTCTCAAAG
351 TACACATGTT CCGTACACGT TCGGAGGGG GACCAAGCTT GAGATCAAAG
401 GTGGTGGTG TTCTGGCGG GCGGCTCCG GTGGTGGTG TTCTGAGGTG
451 CAGCTGCTCG AGCAGTCTGG AGCTGAGCTG GTAAGGCTG GGACTTCAGT
501 GAAGCTGTCC TGCAAGGCTT CTGGCTACAC CTTACAAGC TATGGTTTAA
551 GCTGGGTGAA GCAGAGAACT GGACAGGGCC TTGAGTGGAT TGGAGAGGTT
601 TATCCTAGAA TTGGTAATGC TTAATAAAT TTAATAAAT AGGCAAGGC
651 CACACTGACT GCAGACAAAT CCTCCAGCAC AGCTCCATG GAGCTCCGCA
701 GCCTGACATC TGAGGACTCT GCGGTCTATT TCTGTGCAAG ACGGGATCC
751 TACGGTAGTA ACTACGACTG GTACTTCGAT GTCGGGGCC AAGGGACCAC
801 GGTCACCGTC TCCTCCGGAG GTGGTGGATC CGATATCAAA CTGCAGCAGT
851 CAGGGGCTGA ACTGGCAAGA CCTGGGGCCT CAGTGAAGAT GTCCCTGCAAG
901 ACTTCTGGCT ACACCTTTAC TAGGTACACG ATGCACTGGG TAAAACAGAG
```

Figure 3B (continued)

```
951 GCCTGGACAG GGTC TGGAAT GGATTGGATA CATTAAATCCT AGCCGTGGTT
1001 ATACTAATTA CAATCAGAAG TTCAAGGACA AGCCACATT GACTACAGAC
1051 AAATCCTCCA GCACAGCCTA CATGCAACTG AGCAGCCTGA CATCTGAGGA
1101 CTCTGCAGTC TATTACTGTG CAAGATATTA TGATGATCAT TACTGCCTTG
1151 ACTACTGGGG CCAAGGCACC ACTCTCACAG TCTCCTCAGT CGAAGGTGGA
1201 AGTGGAGGTT CTGGTGGAAG TGGAGGTTCA GGTGGAGTCG ACGACATTCA
1251 GCTGACCCAG TCTCCAGCAA TCATGTCTGC ATCTCCAGGG GAGAAGGTCA
1301 CCATGACCCTG CAGAGCCAGT TCAAGTGTA GTTACATGAA CTGGTACCAG
1351 CAGAAGTCAG GCACCTCCCC CAAAAGATGG ATTTATGACA CATCCAAGT
1401 GGCTTCTGGA GTCCCTTATC GCTTCAGTGG CAGTGGGTCT GGGACCTCAT
1451 ACTCTCTCAC AATCAGCAGC ATGGAGGCTG AAGATGCTGC CACTTATTAC
1501 TGCCAAACAGT GGAGTAGTAA CCCGCTCAG TTCGGTGCTG GGACCAAGCT
1551 GGAGCTGAAA CATCATCACC ATCACATTA G
```

Figure 3C

3-1 (vLvH) x anti-CD3 (SEQ ID NO: 36)

```
1  MGWSCIILFL VATAATGVHSE LVMTQSPSYL AASPGETTTI NCRASKISK
51  YLAWYQEKPG KTNKLLIYSG STLQSGIPSR FSGSGGTDF TLTSSLEPE
101 DFAMYYCQQH NEYPYTFGGG TKLEIKGGG SGGGSGGGG SEVQLLEQSG
151 AELVKPGASV KISCKASGYA FTNYWLGVK QRPCHGLEWI GDLEFGSGNT
201 HYNFRFRGKA TLTADKSSST AFMQLSSLTS EDSAVYFCAR LRNWDEAMDY
251 WGQGTTVTVS SGGGSDIKL QQSGAELARP GASVKMSCKT SGYTFTRYTM
301 HWVKQRPQGQ LEWIGYINPS RGYTNYNQKF KDKATLTTDK SSSTAYMQLS
351 SLTSEDSAVY YCARYYDDHY CLDYWGQGT LTVSSVEGGS GSGGSGGSG
401 GVDDIQLTQS PAIMSASPGF KVTMTCRASS SVSYMNWYQQ KSGTSPKRWI
451 YDTSKVASGV PYRFSGSGSG TSYSLTISSM FAEDAATYYC QWSSNPETF
501 GAGTKLELKH HHHHH*
```

Figure 3C (continued)**SEQ ID NO: 35**

```
1  ATGGGATGGA  GCTGTATCAT  CCTCTTCTTG  GTAGCAACAG  CTACAGGTGT
51  ACACTCCGAG  CTCGTCATGA  CCCAGTCTCC  ATCTTATCTT  GCTGCATCTC
101 CTGGAGAAAC  CATTACTATT  AATTGCAGGG  CAAGTAAGAG  CATTAGCAAA
151 TATTTAGCCT  GGATCAAGA  GAAACCTGGG  AAAACTAATA  AGCTTCTTAT
201 CTACTCTGGA  TCCACTTTGC  AATCTGGAAT  TCCATCAAGG  TTCAGTGGCA
251 GTGGATCTGG  TACAGATTTC  ACTCTACCA  TCAGTAGCCT  GGAGCCTGAA
301 GATTTGCAA  TGTATTACTG  TCAACAGCAT  AATGAATATC  CGTACACGTT
351 CGGAGGGGG  ACCAAGCTTG  AGATCAAAGG  TGGTGGTGGT  TCTGGCGGCG
401 GCGGCTCCGG  TGGTGGTGGT  TCTGAGGTGC  AGCTGCTCGA  GCAGTCTGGA
451 GCTGAGCTGG  TGAAACCTGG  GCCCTCAGTG  AAGATATCCT  GCAAGGCTTC
501 TGGATACGCC  TTCACTAACT  ACTGGCTAGG  TTGGGTAAAG  CAGAGGCCCTG
551 GACATGGACT  TGAGTGGATT  GGAGATCTTT  TCCCTGGAAG  TGGTAATACT
601 CACTACAATG  AGAGGTCAG  GGGCAAAGCC  ACACTGACTG  CAGACAAATC
651 CTCGAGACA  GCCTTTATGC  AGCTCAGTAG  CCTGACATCT  GAGGACTCTG
701 CTGTCATTT  CTGTGCAAGA  TTGAGGAACT  GGGACGAGGC  TATGGACTAC
751 TGGGGCCAAG  GGACCACGGT  CACCGTCTCC  TCCGGAGGTG  GTGGATCCGA
801 TATCAAAC TG  CAGCAGTCAG  GGGCTGAACT  GGCAAGACCT  GGGGCCTCAG
851 TGAAGATGTC  CTGCAAGACT  TCTGGCTACA  CCTTACTAG  GTACACGATG
901 CACTGGGTAA  AACAGAGGCC  TGGACAGGGT  CTGGAATGGA  TTGGATACAT
```

Figure 3C (continued)

```
951 TAATCCTAGC CGTGGTTATA CTAATTACAA TCAGAAAGTTC AAGGACAAGG
1001 CCACATTGAC TACAGACAAA TCCTCCAGCA CAGCCTACAT GCAACTGAGC
1051 AGCCTGACAT CTGAGGACTC TGCAGTCTAT TACTGTGCAA GATATTATGA
1101 TGATCATTAC TGCCTTGACT ACTGGGGCCA AGGCACCACT CTCACAGTCT
1151 CCTCAGTCGA AGGTGGAAGT GGAGGTTCTG GTGGAAAGTG AGGTTCAGGT
1201 GGAGTCGACG ACATTCAGCT GACCCAGTCT CCAGCAATCA TGTCTGCATC
1251 TCCAGGGGAG AAGTCACCA TGACCCTGCAG AGCCAGTTCA AGTGTAAGTT
1301 ACATGAAC TG ACCAGCAG AAGTCAGGCA CCTCCCCAA AAGATGGATT
1351 TATGACACAT CCAAAGTGGC TTCTGGAGTC CCTTATCGCT TCAGTGGCAG
1401 TGGGTC TGGG ACCTCATACT CTCTCACAAT CAGCAGCATG GAGGCTGAAG
1451 ATGCTGCCAC TTATTACTGC CAACAGTGGA GTAGTAACCC GCTCACGTTT
1501 GGTGCTGGGA CCAAGCTGGA GCTGAAACAT CATCACCATC ATCATTAG
```

Figure 3D

4-1 (VLVH) x anti-CD3 (SEQ ID NO: 39)

1	MGWSCIIILFL	VATATGVHSE	LVMTQSPSSL	SVSAGEKVTM	SCKSSQSLLN
51	SGNQKNYLAW	YQQKPGQPPK	LLIYGASTRE	SGVPDRFTGS	GSGTDFTLTI
101	SSVQAEDLAV	YYCQNDYSYP	YTFGGGTKLE	IKGGGSGGG	GSGGGSEVQ
151	LLEQSGAELV	RPGTSVKISC	KASGYAFTNY	WLGWVKQRP	HGLEWVGDI
201	PGSGNAHYNE	KFKGKATLTA	DKSSYTAYMQ	LSSLTSEDSA	VYFCARLRNW
251	DEAMDYWGQG	TTVTVSSGGG	GSDIKLQQSG	AELARPGASV	KMSCKTSGYT
301	FTRYTMHWVK	QRPQGGLFWI	GYINPSRGYT	NYNQKFKDKA	TLTSDKSSST
351	AYMQLSSLTS	EDSAVYYCAR	YYDDHYCLDY	WGQGTTLTVS	SVEGGSGGSG
401	GSGGSGGVDD	IQLTQSPAIM	SASPGKVTM	TCRASSSVSY	MNWWYQQKSGT
451	SPKRWIYDTS	KVASGVPPYRF	SGSGSGTSYS	LTSSMEAED	AATYYCQQWS
501	SNPLTFGAGT	KLELKHHHHH	H*		

Figure 3D (continued)**SEQ ID NO: 38:**

1	ATGGGATGGA	GCTGTATCAT	CCTCTTCTTG	GTAGCAACAG	CTACAGGTGT
51	ACACTCCGAG	CTCGTGATGA	CACAGTCTCC	ATCCTCCCTG	AGTGTGTCAG
101	CAGGAGAGAA	GGTCACTATG	AGCTGCAAGT	CCAGTCAGAG	TCTGTAAAC
151	AGTGGAATC	AAAAGAACTA	CTTGCCCTGG	TACCAGCAGA	AACCAGGCA
201	GCCTCCTAAA	CTGTTGATCT	ACGGGGCATC	CAC TAGGAA	TCTGGGTCC
251	CTGATCGCTT	CACAGGCAGT	GGATCTGGAA	CAGATTTCAC	TCTCACCATC
301	AGCAGTGTGC	AGGCTGAAGA	CCTGGCAGTT	TATTACTGTC	AGAATGATTA
351	TAGTTATCCG	TACACGTTCC	GAGGGGGAC	CAAGCTTGAG	ATCAAAGGTG
401	GTGGTGGTTC	TGGCGGCGGC	GGCTCCGGTG	GTGGTGGTTC	TGAGGTGCAG
451	CTGCTCGAGC	AGTCTGGAGC	TGAGCTGGTA	AGGCTGGGA	CTTCAGTGAA
501	GATATCCTGC	AAGGCTTCTG	GATACGCCCTT	CACTAACTAC	TGGCTAGGTT
551	GGGTTAAGCA	GAGGCCCTGGA	CATGGACTTG	AATGGGTGG	AGATAATTTC
601	CCTGGAAGTG	GTAATGCTCA	CTACAATGAG	AAGTTC AAGG	GCAAAGCCAC
651	ACTGACTGCA	GACAAGTCCT	CGTACACAGC	CTATATGCAG	CTCAGTAGCC
701	TGACATCTGA	GGACTCTGCT	GTC TATTCT	GTGCAAGATT	GCGGA ACTGG
751	GACGAGGCTA	TGGACTACTG	GGGCCAAGGG	ACCACGGTCA	CCGTCTCCTC
801	CGGAGGTGGT	GGATCCGATA	TCAA AACTGCA	GCAGTCAGGG	GCTGAACTGG
851	CAAGACCCTGG	GGCCTCAGTG	AAGATGTCCT	GCAAGACTTC	TGGCTACACC
901	TTTACTAGGT	ACACGATGCA	CTGGGTAAAA	CAGAGGCCTG	GACAGGTCT

Figure 3D (continued)

```
951 GGAATGGATT GGATACATTA ATCTAGCCG TGGTTATACT AATTACAATC
1001 AGAAGTTCAA GGACAAGGCC ACATTGACTA CAGACAAATC CTCAGCACA
1051 GCCTACATGC AACTGAGCAG CCTGACATCT GAGGACTCTG CAGTCTATTA
1101 CTGTGCAAGA TATTATGATG ATCATTA CTGACTAC TGGGGCCAAG
1151 GCACCACTCT CACAGTCTCC TCAGTCGAAG GTGGAAGTGG AGGTTCTGGT
1201 GGAAGTGGAG GTTCAGGTGG AGTCGACGAC ATTCAGCTGA CCCAGTCTCC
1251 AGCAATCATG TCTGCATCTC CAGGGAGAGAA GGTCACCATG ACCTGCAGAG
1301 CCAGTTCAAG TGTAACTTAC ATGAACTGGT ACCAGCAGAA GTCAGGCACC
1351 TCCCCCAAAA GATGGATTTA TGACACATCC AAAGTGGCTT CTGGAGTCCC
1401 TTATCGCTTC AGTGGCAGTG GGTCTGGGAC CTCATACTCT CTCACAATCA
1451 GCAGCATGGA GGCTGAAGAT GCTGCCACTT ATTA CTGCCA ACAGTGGAGT
1501 AGTAACCCGC TCACGTTCCG TGCTGGGACC AAGCTGGAGC TGAAACATCA
1551 TCACCATCAT CATTAG
```

Figure 3E

5-10 (vLvH) x anti-CD3 (SEQ ID NO: 44)

```
1  MGWSCIILFL VATAGVHSE LVMTQSPSSL TVTAGEKVTM SCKSSQSLLN
51  SGNQKNYLTW YQKPGQPPK LLIYWASTRE SGVPRFTGS GSGTDFTLTI
101 SSVQAEDLAV YYCONDYYP LTFGAGTKLE IKGGGSGGG GSGGGSEVQ
151 LLEQSGAELV RPGTSVKISC KASGYAFTNY WLGWVKQRPG HGLEWIGDIF
201 PGSGNIHYNE KEKGKATLTA DKSSSTAYMQ LSSLTFEDSA VYFCARLRNW
251 DEPMDYWGQG TTVTVSSGGG GSDIKLQQSG AELARPGASV KMSCKTSGYT
301 FTRYTMHWVK QRPQGGLWI GYINPSRGYT NYNQKFKDKA TLTTDKSSST
351 AYMQLSSLTS EDSAVYYCAR YYDDHYCLDY WGQGTTLTVS SVEGGSGGSG
401 GSGGSGGVDD IQLTQSPAIM SASPGEKVTM TCRASSSVSY MNWYQQKSGT
451 SPKRWIYDTS KVASGVPYRF SGSGSGTSYS LTISSMEAED AATYYCQQWS
501 SNPLTFGAGT KLELKHHHHHH*
```

Figure 3E (continued)**SEQ ID NO: 43**

```
1  ATGGGATGGA  GCTGTATCAT  CCTCTTCTTG  GTAGCAACAG  CTACAGGTGT
51  ACACTCCGAG  CTCGTGATGA  CACAGTCTCC  ATCCTCCCTG  ACTGTGACAG
101  CAGGAGAGAA  GGTCACTATG  AGCTGCAAGT  CCAGTCAGAG  TCTGTAAAC
151  AGTGGAATC  AAAAGAACTA  CTTGACCCTGG  TACCAGCAGA  AACCAGGGCA
201  GCCTCCTAAA  CTGTTGATCT  ACTGGGCATC  CACTAGGGAA  TCTGGGGTCC
251  CTGATCGCTT  CACAGGCAGT  GGATCTGGAA  CAGATTTCAC  TCTCACCATC
301  AGCAGTGTGC  AGGCTGAAGA  CCTGGCAGTT  TATTACTGTC  AGAATGATTA
351  TAGTTATCCG  CTCACGTTCC  GTGCTGGGAC  CAAGCTTGAG  ATCAAAGGTG
401  GTGGTGGTTC  TGGCGCGGC  GGCTCCGGTG  GTGGTGGTTC  TGAGGTGCAG
451  CTGCTCGAGC  AGTCTGGAGC  TGAGCTGGTA  AGGCCTGGGA  CTTCAGTGAA
501  GATATCCTGC  AAGGCTTCTG  GATACGCCCTT  CACTAACTAC  TGGCTAGGTT
551  GGGTAAAGCA  GAGGCCCTGGA  CATGGACTTG  AGTGGATTGG  AGATAATTTC
601  CCTGGAAGTG  GTAATATCCA  CTACAAATGAG  AAGTTC AAGG  GCAAAGCCAC
651  ACTGACTGCA  GACAAATCTT  CGAGCACAGC  CTATATGCAG  CTCAGTAGCC
701  TGACATTTGA  GGACTCTGCT  GTCTAATTCT  GTGCAAGACT  GAGGAAGTGG
751  GACGAGCCTA  TGGACTACTG  GGGCCAAGGG  ACCACGGTCA  CCGTCTCCTC
801  CGGAGGTGGT  GGATCCGATA  TCAAACTGCA  GCAGTCAGGG  GCTGAAGTGG
851  CAAGACCCTGG  GGCCTCAGTG  AAGATGTCCT  GCAAGACTTC  TGGCTACACC
901  TTTACTAGGT  ACACGATGCA  CTGGGTAAAA  CAGAGGCCCTG  GACAGGCTCT
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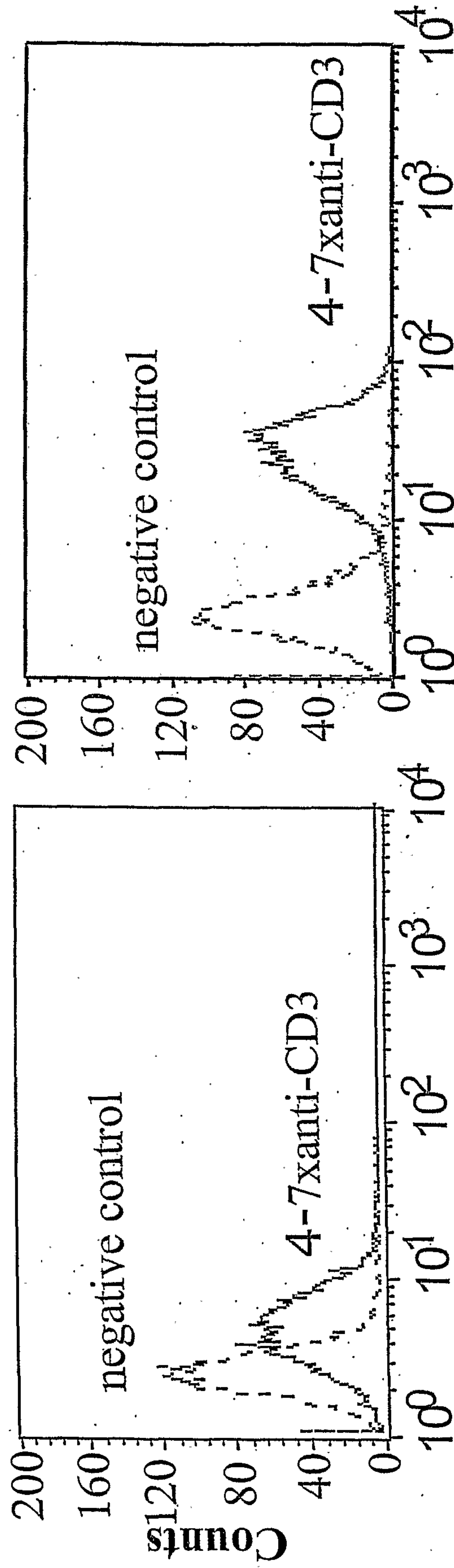
Figure 3E (continued)

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951 GGAATGGATT GGATACATTA ATCCTAGCCG TGGTTATACT AATTACAATC
1001 AGAAGTTCAA GGACAAGGCC ACATTGACTA CAGACAAATC CTCAGCACA
1051 GCCTACATGC AACTGAGCAG CCTGACATCT GAGGACTCTG CAGTCTATTA
1101 CTGTGCAAGA TATTATGATG ATCATTACTG CCTTGACTAC TGGGGCCAAG
1151 GCACCACTCT CACAGTCTCC TCAGTCGAAG GTGGAAGTGG AGGTCTGGT
1201 GGAAGTGGAG GTTCAGGTGG AGTCGACGAC ATTCAGCTGA CCCAGTCTCC
1251 AGCAATCATG TCTGCATCTC CAGGGAGAA GGTCACCATG ACCTGCAGAG
1301 CCAGTTCAAG TGTAACTTAC ATGAACTGGT ACCAGCAGAA GTCAGGCACC
1351 TCCCCCAA AA GATGGATT TA TGACACATCC AAAGTGGCTT CTGGAGTCCC
1401 TTATCGCTTC AGTGGCAGTG GGTCTGGGAC CTCATACTCT CTCACAATCA
1451 GCAGCATGGA GGCTGAAGAT GCTGCCACTT ATTA CTGCCA ACAGTGGAGT
1501 AGTAACCCGC TCACGTTTCGG TGCTGGGACC AAGCTGGAGC TGAAACATCA
1551 TCACCATCAT CATTAG
```

Figure 4A

CD3 Binding (Jurkat cells)

EpCAM Binding (Kato cells)

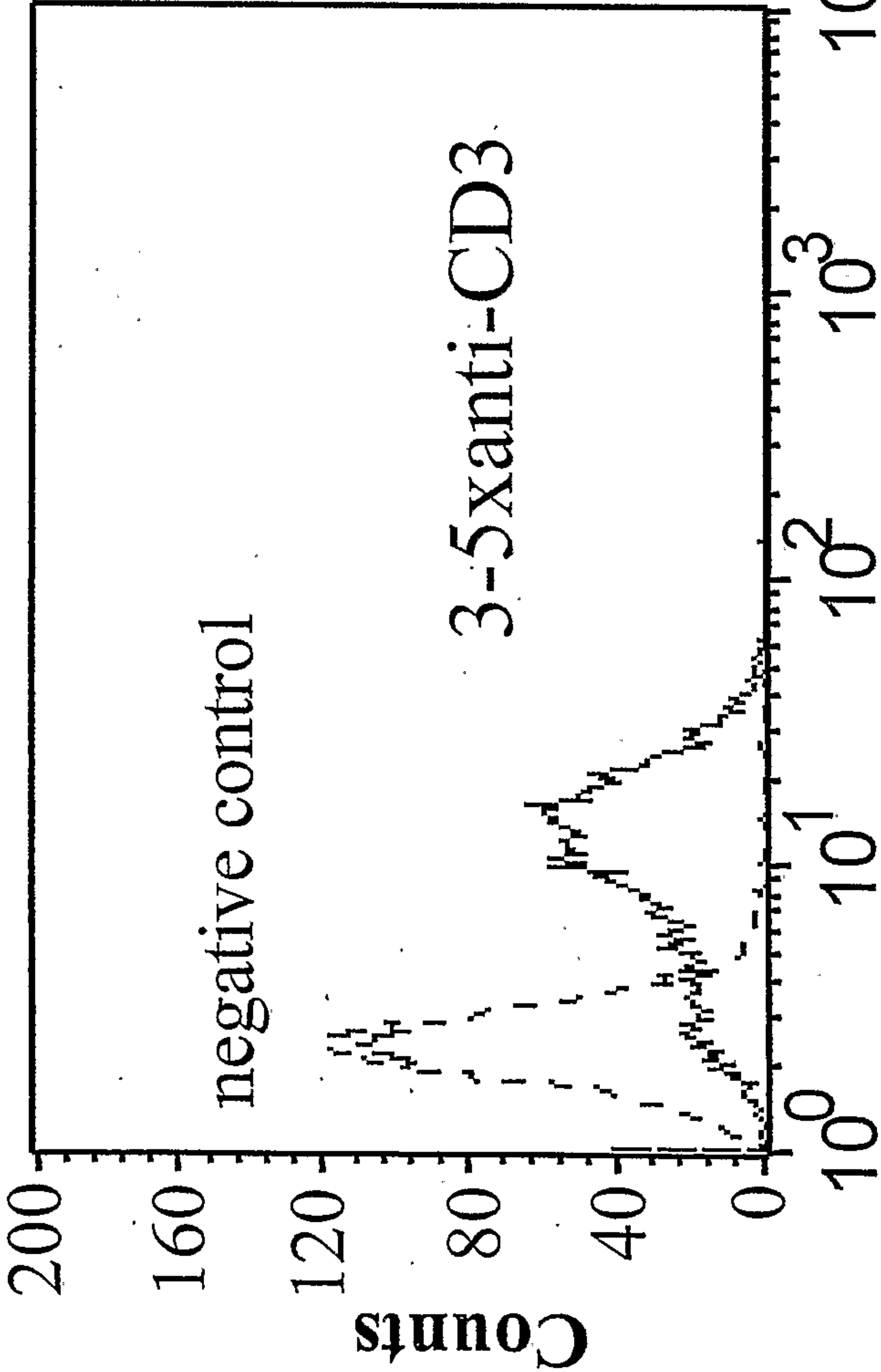


4-7xanti-CD3

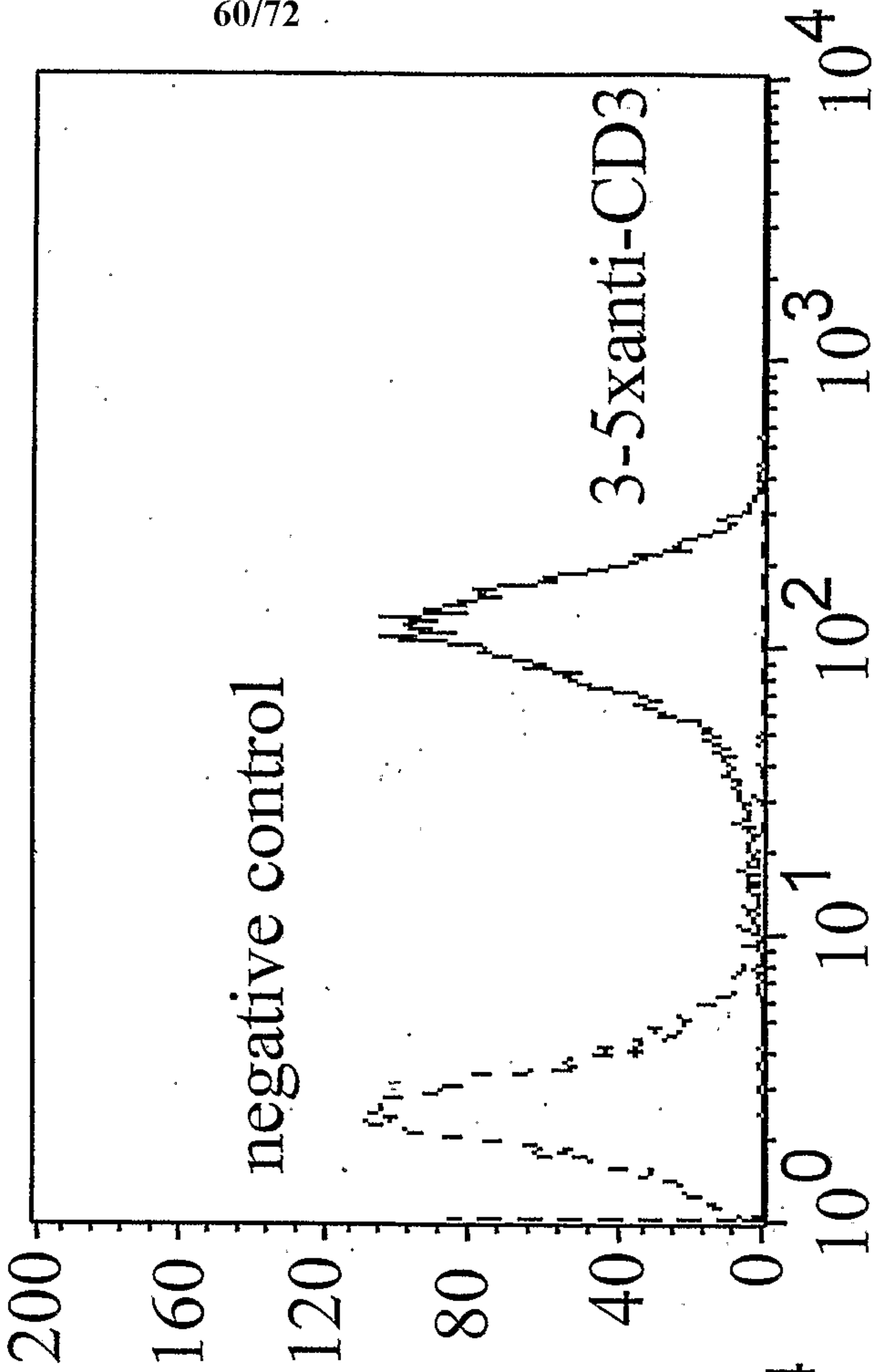
(SEQ ID NO:42)

Figure 4B

CD3 Binding (Jurkat cells)



EpCAM Binding (Kato cells)



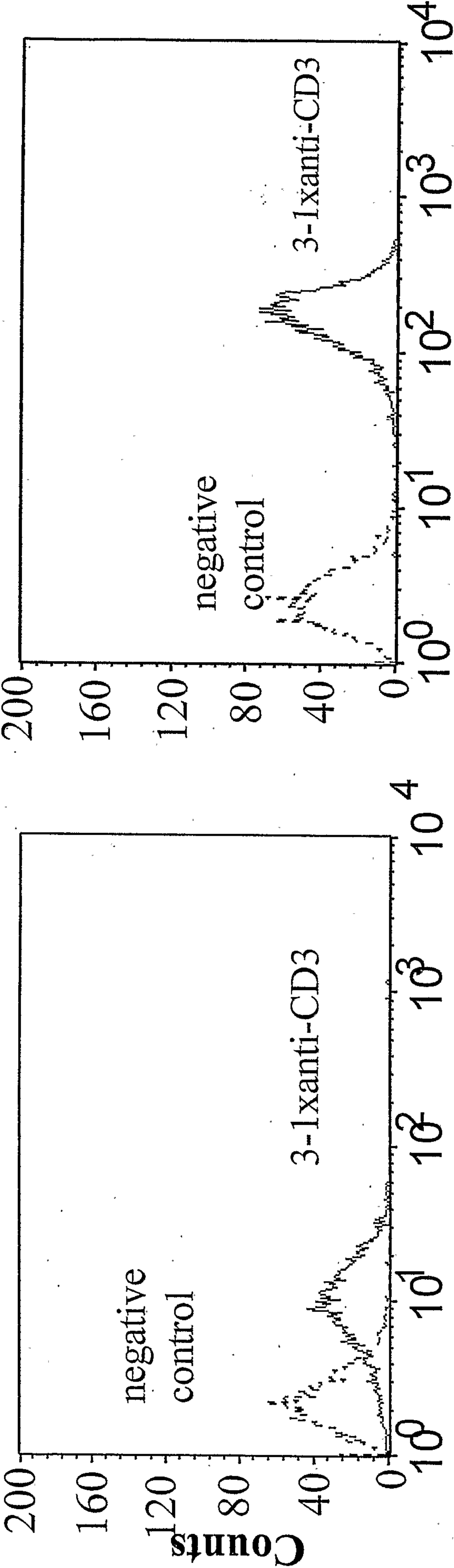
3-5xanti-CD3

(SEQ ID NO: 30)

Figure 4C

CD3 Binding (Jurkat cells)

EpCAM Binding (Kato cells)



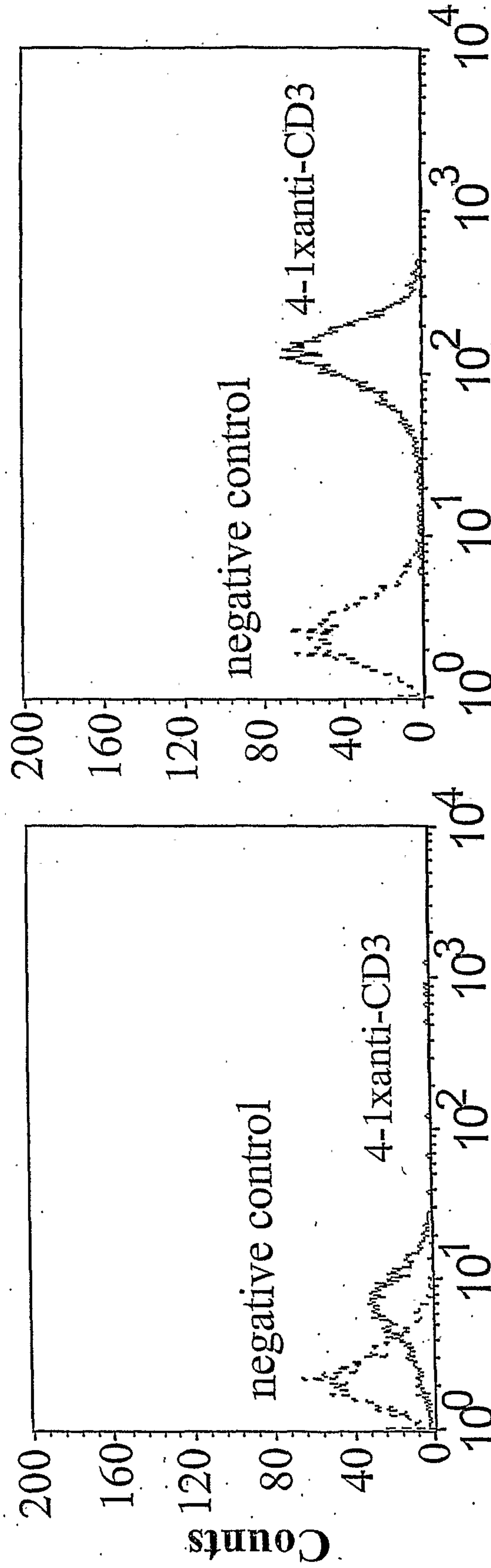
3-1xanti-CD3

(SEQ ID NO:36)

Figure 4D

CD3 Binding (Jurkat cells)

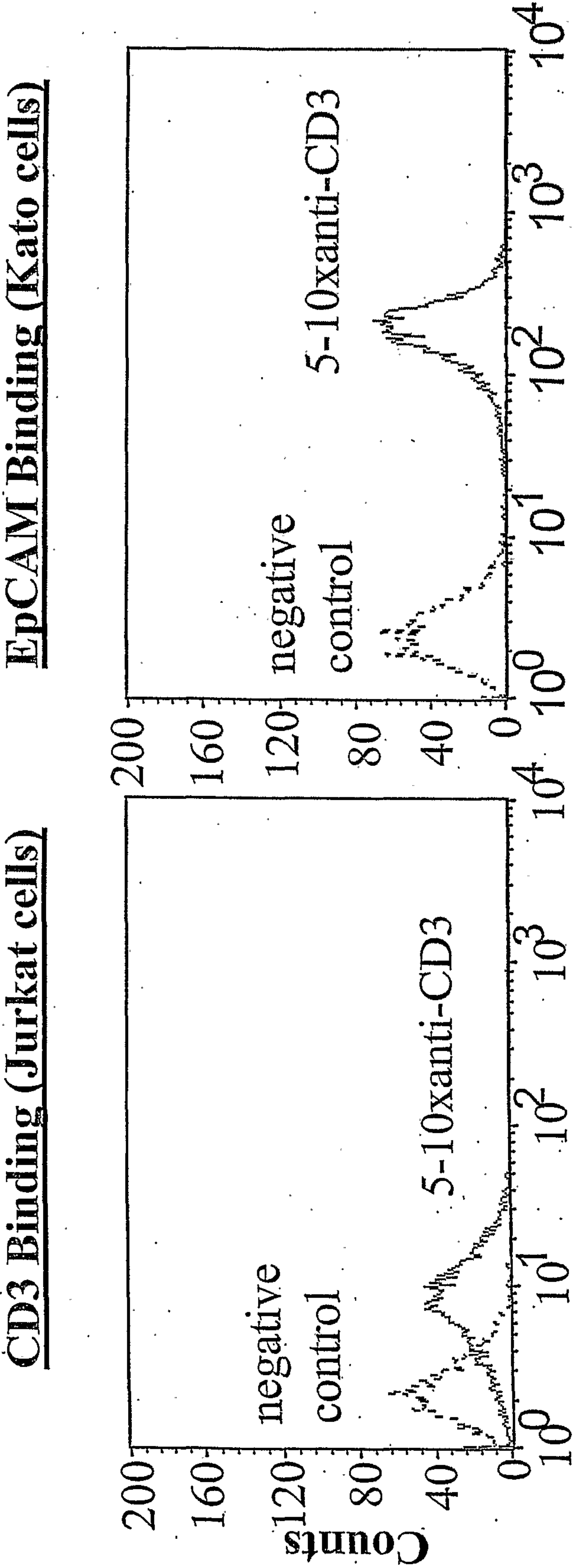
EpCAM Binding (Kato cells)



4-1xanti-CD3

(SEQ ID NO: 39)

Figure 4E



5-10xanti-CD3
(SEQ ID NO: 44)

Figure 5

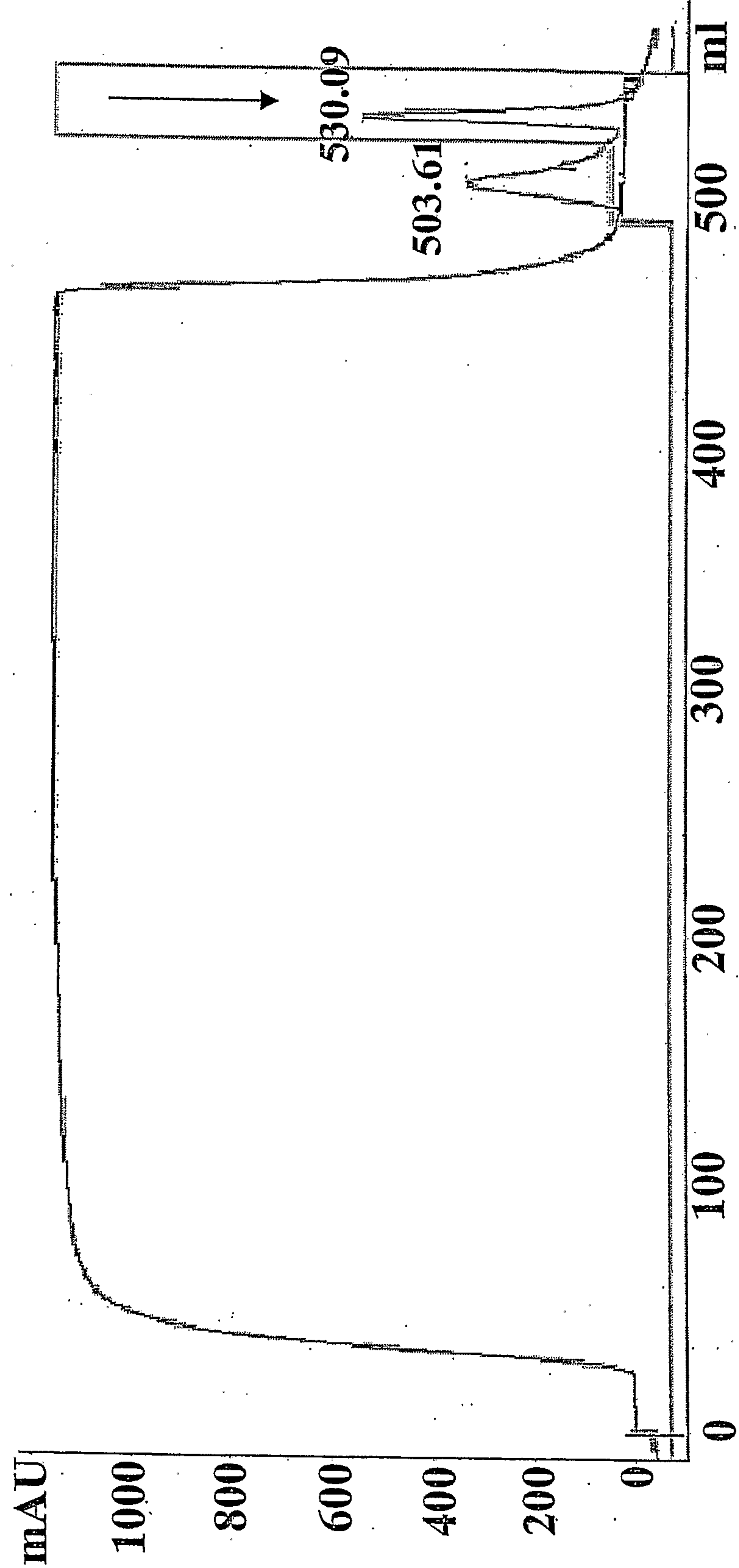


Figure 6

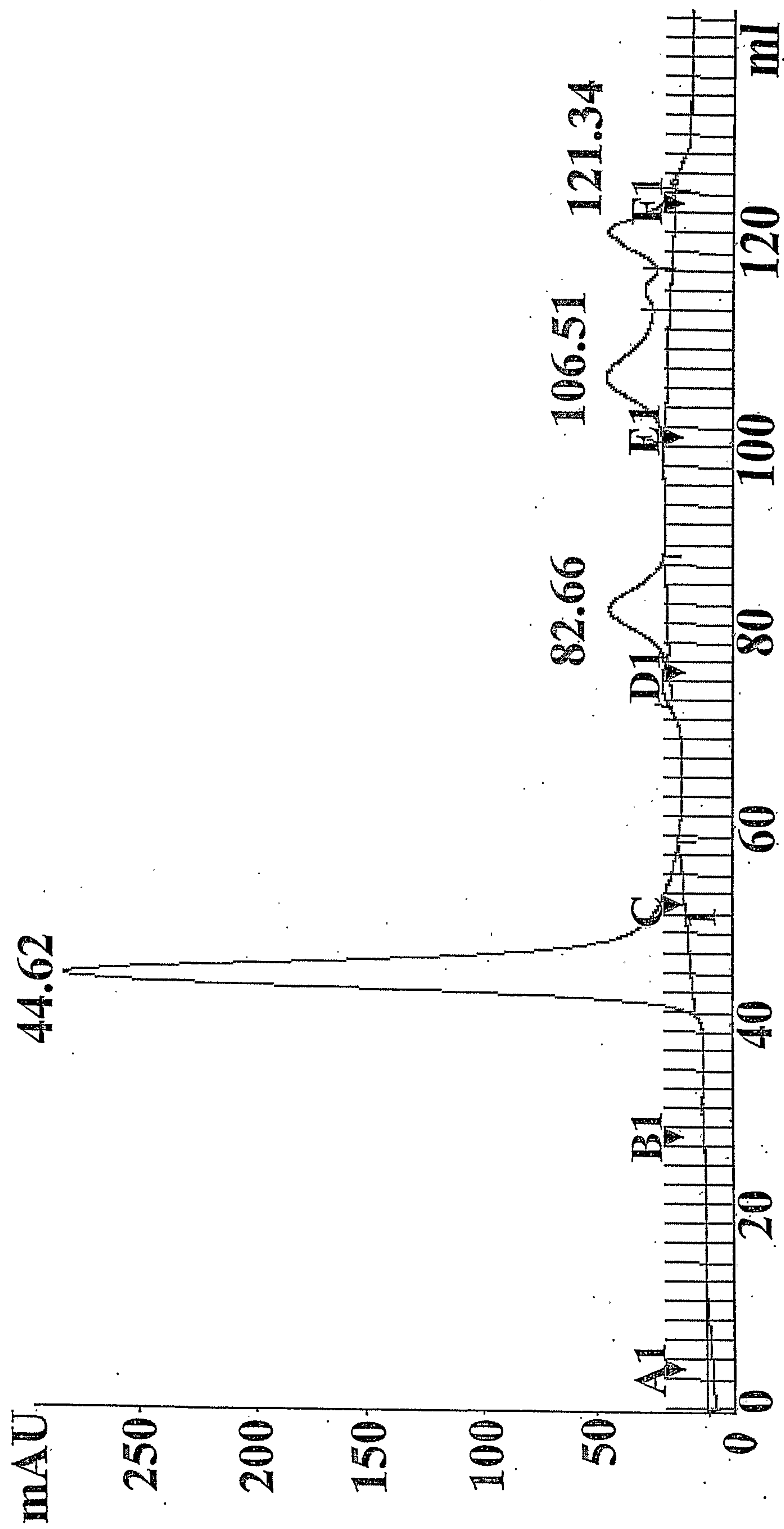


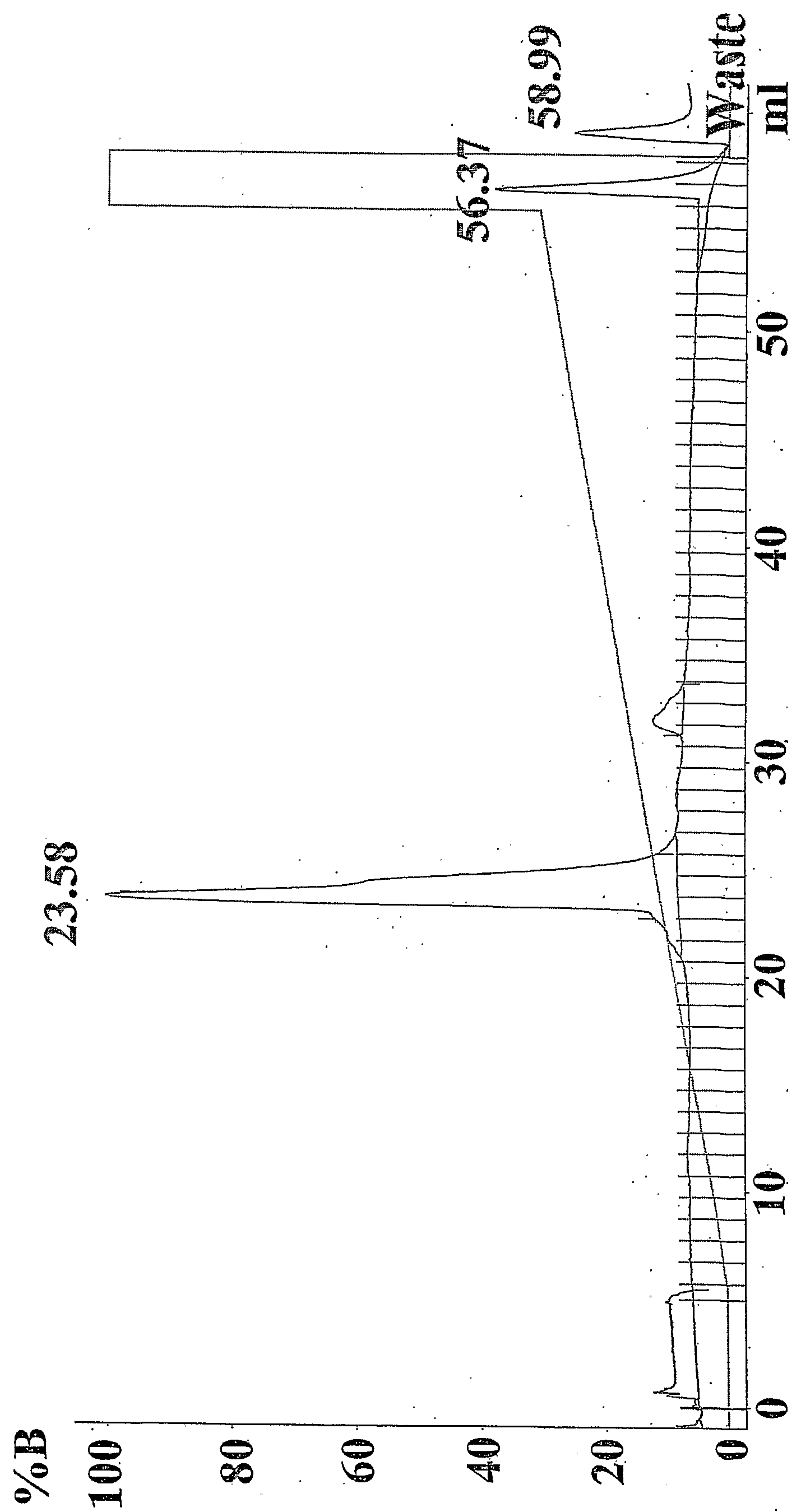
Figure 7A**3-1x anti-CD3**

Figure 7B

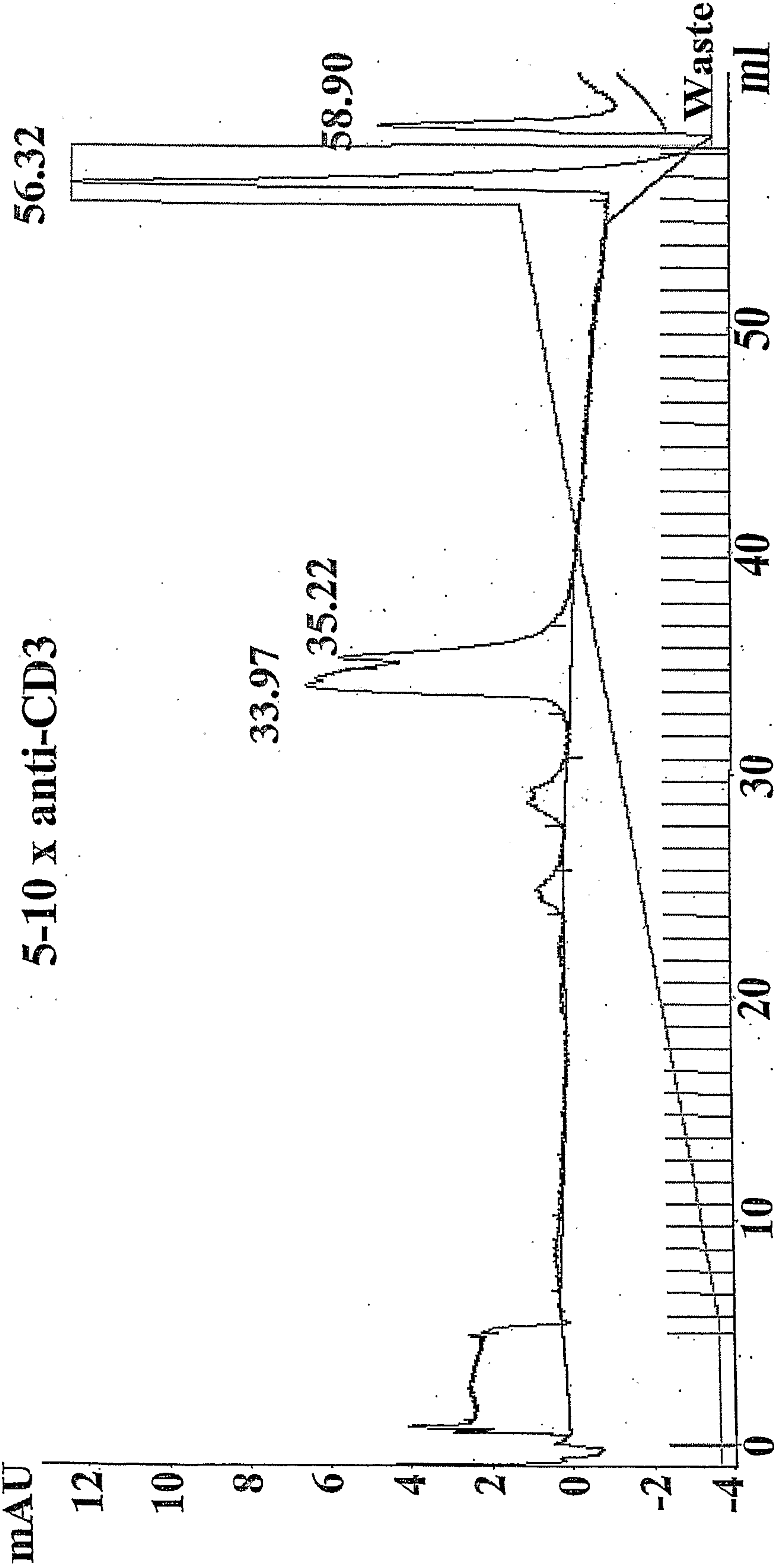


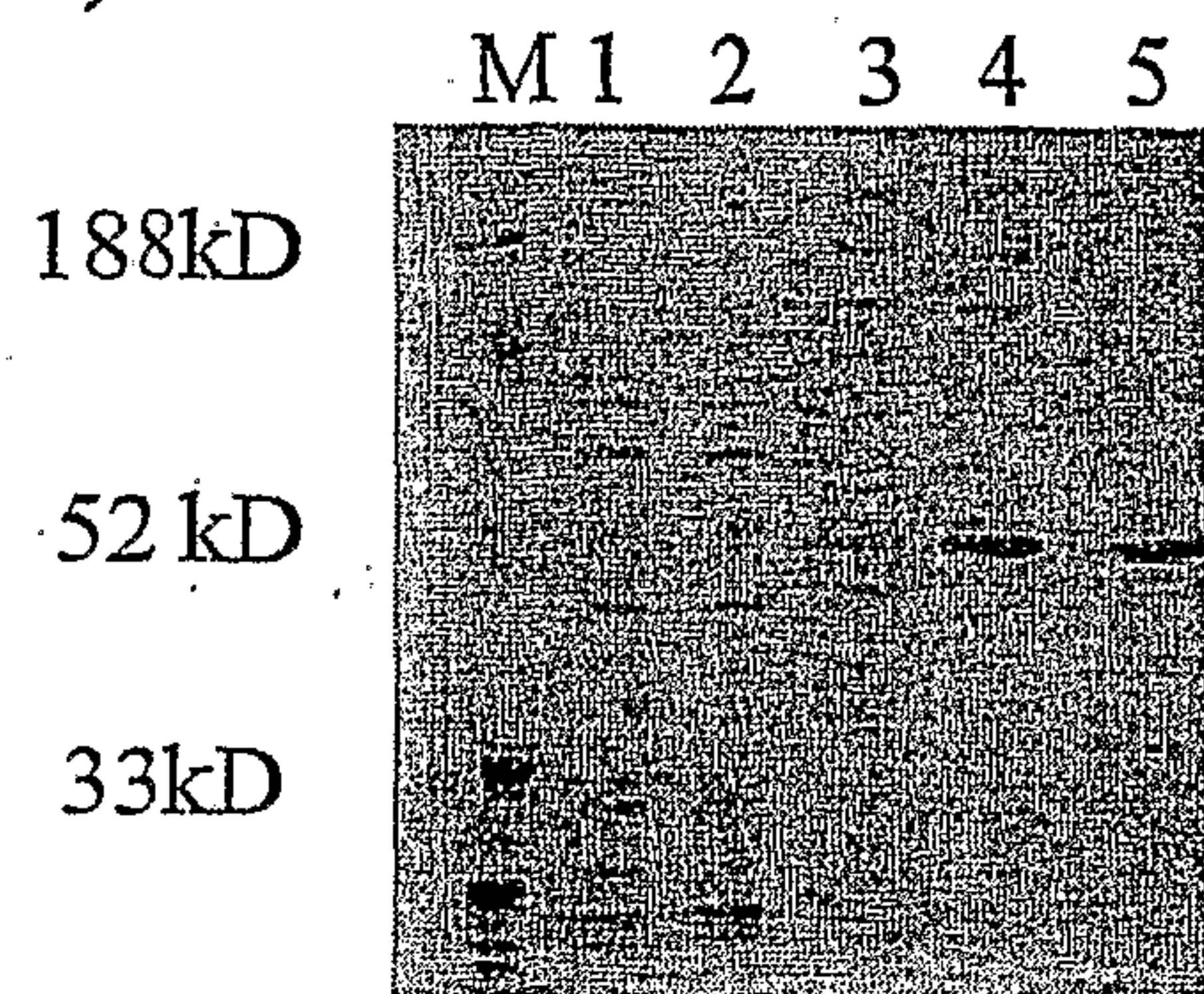
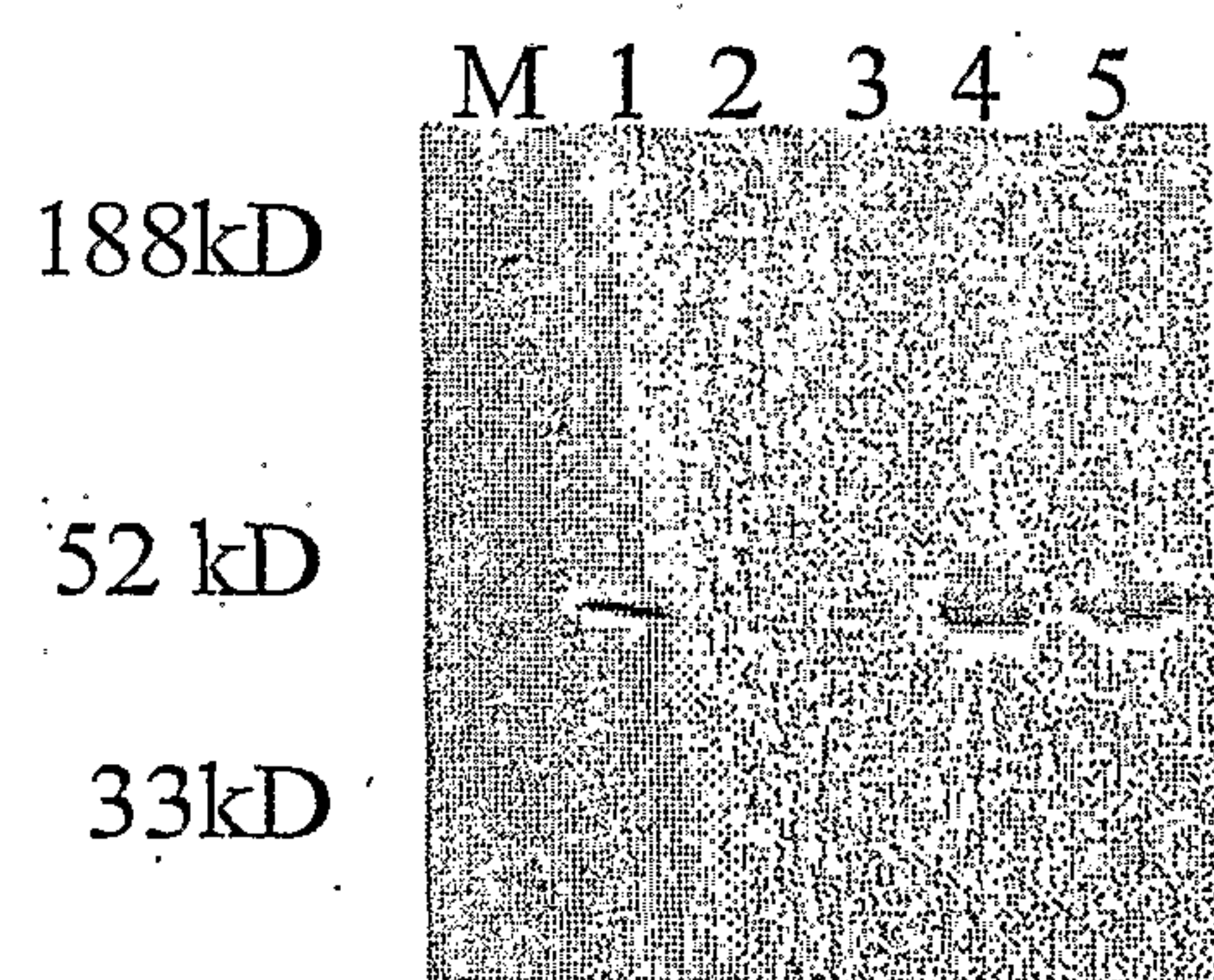
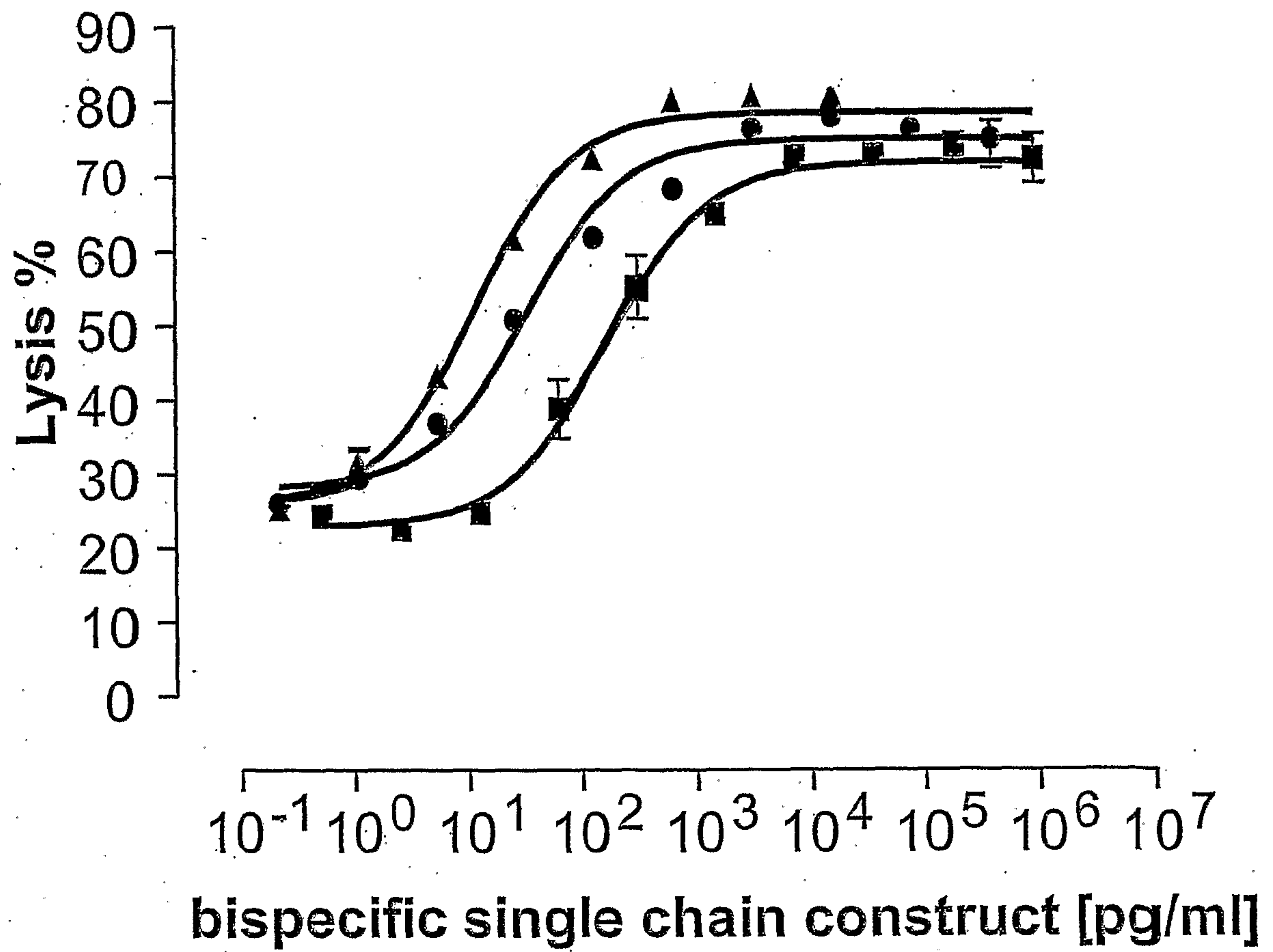
Figure 8**A)****B)**

Figure 9

- anti-CD3x3-1
- anti-CD3 x 5-10
- ▲ anti-CD3 x 4-7

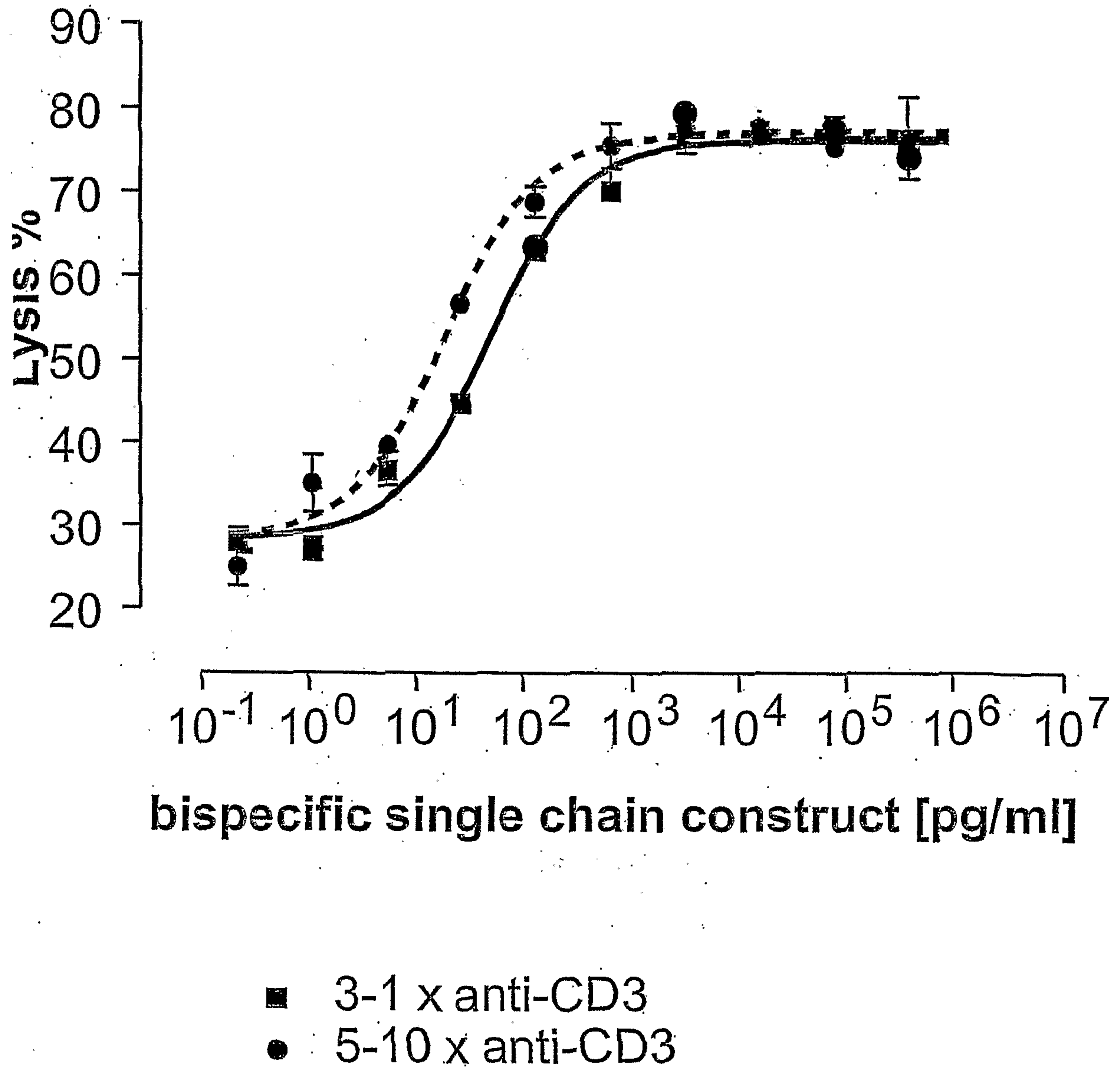
Figure 10

Figure 11A

3-1	LR NWD EAMDY
4-1	LR NWD EAMDY
5-10	LR NWD EPMDY
3-5	RGSYGS NYD WYFDV
4-7	RGSYDT NYD WYFDV
M79	MENWSFAY
HD70	DMGWGSGWRPYYYYGMDV
3B10	FTSPDY

Figure 11B

