(12) STANDARD PATENT (11) Application No. **AU 2016215676 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Anti-surrogate light chain antibodies

(51) International Patent Classification(s)
C07K 16/18 (2006.01) ***G01N 33/569*** (2006.01)
C07K 16/28 (2006.01) ***G01N 33/574*** (2006.01)
C07K 16/38 (2006.01)

(21) Application No: **2016215676** (22) Date of Filing: **2016.01.27**

(87) WIPO No: **WO16/126488**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/111,018	**2015.02.02**	**US**

(43) Publication Date: **2016.08.11**
(44) Accepted Journal Date: **2021.12.02**

(71) Applicant(s)
i2 Pharmaceuticals, Inc.

(72) Inventor(s)
Horowitz, Lawrence

(74) Agent / Attorney
Davies Collison Cave Pty Ltd, Level 15 1 Nicholson Street, MELBOURNE, VIC, 3000, AU

(56) Related Art
US 20030215453 A1
US 6335175 B1
SEON-HEE YIM ET AL, "The potential role ofgene copy number variation in susceptibility to rheumatoid arthritis", MOLECULAR IMMUNOLOGY, vol. 48, (2010-11-02), pages 1338 - 1343

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 August 2016 (11.08.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/126488 A1

(51) **International Patent Classification**:
C07K 16/18 (2006.01) ***G01N 33/574*** (2006.01)
C07K 16/28 (2006.01) ***G01N 33/569*** (2006.01)
C07K 16/38 (2006.01)

(21) **International Application Number**:
PCT/US2016/015166

(22) **International Filing Date**:
27 January 2016 (27.01.2016)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
62/111,018 2 February 2015 (02.02.2015) US

(71) **Applicant**: **SEA LANE BIOTECHNOLOGIES, LLC** [US/US]; 1400 Terra Bella Avenue, Suite K, Mountain View, CA 94043 (US).

(72) **Inventor**: **HOROWITZ, Lawrence**; 362 Selby Lane, Atherton, CA 94027 (US).

(74) **Agents**: **BRASNJO, Gabor** et al.; Arnold & Porter LLP, Three Embarcadero Center, 10th Floor, San Francisco, CA 94111 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2016/126488 A1

(54) **Title**: ANTI-SURROGATE LIGHT CHAIN ANTIBODIES

(57) **Abstract**: The present invention concerns anti-surrogate light chain antibodies and their uses. In particular, the present invention concerns anti-VpreBl antibodies and their uses.

ANTI-SURROGATE LIGHT CHAIN ANTIBODIES

Background of the Invention

Antibody (Ig) molecules produced by B-lymphocytes are built of heavy (H) and light (L) chains. The amino acid sequences of the amino terminal domains of the H and L chains are variable (V_H and V_L), especially at the three hypervariable regions (CDR1, CDR2, CDR3) that form the antigen combining site. The assembly of the H and L chains is stabilized by a disulfide bond between the constant region of the L chain (C_L) and the first constant region of the heavy chain (C_{H1}) and by non-covalent interactions between the V_H and V_L domains.

In humans and many animals, such as mice, the genes encoding the antibody H and L chains are assembled by stepwise somatic rearrangements of gene fragments encoding parts of the V regions. Various stages of B lymphocyte development are characterized by the rearrangement status of the Ig gene loci (see, e.g. Melchers, F. & Rolink, A., *B-Lymphocyte Development and Biology*, Paul, W.E., ed., 1999, Lippincott, Philadelphia).

Precursors of B cells (pre-B cells) have been identified in the bone marrow by their production of a set of genes called VpreB(1-3) and λ5, instead of the fully developed light chains, and coexpression of μ heavy chains.

The main isoform of human VpreB1 (CAG30495) is a 145 aa-long polypeptide (SEQ ID NO: 1). It has an Ig V domain-like structure, but lacks the last β-strand (β7) of a typical V domain, and has a carboxyl terminal end that shows no sequence homologies to any other proteins. VpreB2 has several isoforms, including a 142-amino acid mouse VpreB2 polypeptide (P13373; SEQ ID NO: 2), and a 171 amino acids long splice variant of the mouse VpreB2 sequence (CAA01964l SEQ ID NO: 3). VpreB1 and VpreB2 sequences have been disclosed in EP 0 269 127 and U.S. Patent No. 5,182,205; Collins et al., *Genome Biol.* 5(10):R84 (2004); and Hollins et al., *Proc. Natl. Acad. Sci. USA* 86(14):5552-5556 (1989). The main isoform of human VpreB3 (SEQ ID NO: 4) is a 123 aa-long protein (CAG30496), disclosed in Collins et al., *Genome Biol.* 5(10):R84 (2004).

VpreB(1-3) are non-covalently associated with another protein, λ5. The human λ5 is a 209-amino acid polypeptide (CAA01962; SEQ ID NO: 7), that carries an Ig C domain-like structure with strong homologies to antibody light chains and, towards its amino terminal end, two functionally distinct regions, one of which shows strong homology to the β7 strand of the Vλ domains. A human λ5-like protein has 213 amino acids (NP_064455; SEQ ID NO: 8) and shows about 84% sequence identity to the antibody λ light chain constant region.

For further details, see the following review papers: Karasuyama et al., *Adv. Immunol.* 63:1-41 (1996); Melchers et al., *Immunology Today* 14:60-68 (1993); and Melchers, *Proc. Natl. Acad. Sci. USA* 96:2571-2573 (1999).

The VpreB and λ5 polypeptides together form a non-covalently associated, Ig light chain-like structure, which is called the surrogate light chain or pseudo light chain. On the surface of early preB cells, the surrogate light chain is disulfide-linked to membrane-bound Ig μ heavy chain in association with a signal transducer CD79a/CD79b heterodimer to form a B cell receptor-like structure, the so-called preB cell receptor (pre-BCR).

Surrobodies are based on the pre-B cell receptor (pre-BCR), which is produced during normal development of antibody repertoire. Unlike antibodies, pre-BCR is a trimer, composed of an antibody heavy chain paired with two surrogate light chain components, VpreB and λ5. Both VpreB and λ5 are encoded by genes that do not undergo gene rearrangement and are expressed in early pre-B cells before V(D)J recombination begins. The pre-BCR is structurally different from a mature immunoglobulin in that it is composed of a heavy chain and two non-covalently associated proteins: VpreB and λ5, i.e., they have three components as opposed to two in antibodies. Furthermore, although VpreB is homologous to the Vλ Ig domain, and λ5 is homologous to the Cλ domain of antibodies, each has noncanonical peptide extensions: VpreB1 has additional 21 residues on its C terminus; λ5 has a 50 amino acid extension at its N terminus.

A κ-like B cell receptor (κ-like BCR) has been identified, utilizing a κ-like surrogate light chain (κ-like SLC) (Frances et al., *EMBO J* 13:5937-43 (1994); Thompson et al., *Immunogenetics* 48:305-11 (1998); Rangel et al., *J Biol Chem* 280:17807-14 (2005)).

Rangel et al., *J Biol Chem* 280(18):17807-17814 (2005) report the identification and molecular characterization of a Vκ-like protein that is the product of an unrearranged *V*κ gene, which turned out to the be identical to the cDNA sequence previously reported by Thompson et al., *Immunogenetics* 48:305-311 (1998). Whereas, Frances et al., *EMBO J* 13:5937-43 (1994) reported the identification and characterization of a rearranged germline JCk that has the capacity to associate with μ heavy chains at the surface of B cell precursors, thereby providing an alternative to the λ5 pathway for B cell development.

It has been proposed that κ-like and λ-like pre-BCRs work in concert to promote light chain rearrangement and ensure the maturation of B cell progenitors. For a review, see McKeller and Martinez-Valdez *Seminars in Immunology* 18:4043 (2006).

Further details of the design and production of Surrobodies are provided in Xu et al., *Proc. Natl. Acad. Sci. USA* 2008, 105(31):10756-61, in PCT Publication WO 2008/118970 published on October 2, 2008, in U.S. Provisional Application No.61/134,929 filed July 11, 2008, and in Xu et al., *J. Mol. Biol.* 2010, 397, 352-360, the entire disclosures of which are expressly incorporated by reference herein.

It has been described that the diversity of a filamentous phage-based combinatorial antibody library can be increased by shuffling of the heavy and light chain genes (Kang et al., *Proc. Natl. Acad. Sci. USA*, 88:11120-11123, (1991)) or by introducing random mutations into the library by error-prone polymerase chain reactions (PCR) (Gram et al., *Proc. Natl. Acad. Sci. USA*, 89:3576-3580, (1992)). The use of defined frameworks as the basis for generating antibody libraries has been described by Barbas et al., *Proc. Nat. Acad. Sci. USA* 89:4457-4461 (1992) (randomizing CD3-H3); Barbas et al., *Gene* 137:57-62 (2003) (extending randomization to V.sub..kappa. CDR3); and Hayanashi et al., *Biotechniques* 17:310 (1994) (simultaneous mutagenesis of antibody CDR regions by overlap extension and PCR). Others report combination of CDR-H3 libraries with a single V.sub.L gene (Nissim et al., *EMBO J.* 13:692-698 (1994)), a limited set of V_L genes (De Kruif et al., *J. Mol. Biol.* 248:97-105 (1995)); or a randomized repertoire of V_L genes (Griffiths et al., *EMBO J.* 13:3245-3260 (1994)).

See also U.S. Pat. Nos. 5,667,988; 6,096,551; 7,067,284 describing methods for producing antibody libraries using universal or randomized immunoglobulin light chains.

Knappik et al., *J. Mol. Biol.* 296:57-86 (2000) describe a different concept for designing and constructing human antibody libraries, designated HuCAL (Human Combinatorial Antibody Libraries). This approach is based on the finding that each of the human V.sub.H and V.sub.L subfamilies that is frequently used during an immune response is represented by one consensus framework, resulting in seven HuCAL consensus genes for heavy chains and seven HuCAL consensus genes for light chains, which yield 49 possible combinations. All genes are made by total synthesis, taking into consideration codon usage, unfavorable residues that promote protein aggregation, and unique and general restriction sites flanking all CDRs. The approach leads to the generation of modular antibody genes containing CDRs that can be converted into different antibody formats, as needed. The design and synthesis of HuCAL antibody libraries is described in U.S. Pat. Nos. 6,300,064; 6,696,248; 6,706,484; and 6,828,422.

The construction of diverse synthetics antibody libraries is described in U.S. Patent No. 8,131,480.

Summary of the Invention

The present invention concerns anti-Surrogate Light Chain (SLC) antibodies and their uses.

In one aspect, the present invention provides isolated antibodies capable of specifically binding to a surrogate light chain (SLC), or an antigen-binding fragment thereof. In one embodiment, the antibody, or antigen-binding fragment, specifically binds to the VpreB subunit of the SLC. In another embodiment, the VpreB subunit is human VpreB1 of SEQ ID NO: 1. In one other embodiment, the VpreB subunit is mouse VpreB2 of SEQ ID NO: 2 or SEQ ID NO: 3. In yet another embodiment, the VpreB subunit is human VpreB3 of SEQ ID NO: 4. In another embodiment, the antibody, or antigen-binding fragment, specifically binds to the λ5 subunit of the SLC. In one other embodiment, the λ5 subunit is human λ5 of SEQ ID NO: 7. In yet another embodiment, the λ5 subunit is human λ5 dTail of SEQ ID NO: 9.

In one aspect, the present invention provides an isolated antibody, or an antigen-binding fragment thereof, that specifically binds to the VpreB subunit of a surrogate light chain (SLC), wherein the antibody comprises a light chain variable region sequence from within SEQ ID NO:36 and a heavy chain variable region sequence of SEQ ID NO:52 and wherein the VpreB subunit is human VpreB1 of SEQ ID NO:1.

In one embodiment, the present invention provides an antibody, or antigen-binding fragment, comprising a light chain variable region sequence selected from the group consisting of SEQ ID NOs: 36 to 51. In another embodiment, the antibody or antigen-binding fragment comprises a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 52 to 67. In yet another embodiment, the antibody, or antigen-binding fragment, comprising a light chain variable region sequence selected from the group consisting of SEQ ID NOs: 36 to 51 further comprises a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 52 to 67. In one other embodiment, the antigen-binding fragment is selected from the group consisting of Fab, Fab′, $F(ab')_2$, scFv, and $(scFv)_2$ fragments.

In another aspect, the present invention provides compositions that contain an antibody, or antigen-binding fragment, described herein. In some embodiments, the composition is a diagnostic composition.

In one other aspect, the present invention provides methods for the diagnosis of autoimmune disease. In one embodiment, the method is used for the diagnosis of rheumatoid arthritis in a subject. In one other embodiment, the subject is a human patient. In another embodiment, the method comprises contacting a biological sample from the subject with an antibody specifically binding to human surrogate light chain (SLC) and determining the expression level of SLC.

In yet another aspect, the present invention provide methods for the diagnosis of a leukemia. In one embodiment, the leukemia is associated with aberrant SLC expression. In another embodiment, the method comprises contacting a biological sample from said subject with an antibody specifically binding to human surrogate light chain (SLC) and determining the expression level of SLC.

Brief Description of the Drawings

FIG. 1 shows the human VpreB1 amino acid sequence of SEQ ID NO: 1 (native leader sequence underlined); the mouse VpreB2 sequences of SEQ ID NOS: 2 and 3; the human VpreB3-like sequence of SEQ ID NO: 4, and the sequence of the truncated VpreB1 sequence in the "trimer" is shown as SEQ ID NO: 5; and the human VpreB1 amino acid sequence of SEQ ID

NO:6 (murine Ig kappa leader underlined). Underlining indicates the leader sequences within the VpreB amino acid sequences.

FIG. 2 shows the human λ5 sequence of SEQ ID NO: 7; the human λ5-like sequence of SEQ ID NO: 8; the sequence of the truncated λ5 sequence in the "trimer" designated as "λ5 dTail" (SEQ ID NO: 9); and the human λ5 dTail sequence of SEQ ID NO: 10 with a murine Ig κ leader sequence. Underlining indicates the leader sequences within the λ5 amino acid sequences.

FIG. 3 shows the human VpreB1-λ5 chimeric amino acid sequence as SEQ ID NO:35 (murine Ig κ leader sequence underlined).

FIG. 4A and 4B show (A) the human Vκ-like nucleotide sequence of SEQ ID NO:11 and the amino acid sequence of the encoded protein (AJ004956; SEQ ID NO:12) (native leader sequence underlined), and (B) the predicted mature amino acid sequences of Vκ-like proteins possible from all Vκ families, each bearing different lengths of extensions (SEQ ID NOS: 13-24) aligned with AJ004956 Vκ-like prototype sequence (SEQ ID NO:12).

FIG 5A-C shows (A) the human JCκ nucleotide sequence of SEQ ID NO:25 and the amino acid sequence of the encoded protein (SEQ ID NO:26) (unique sequence compared to predicted mature JCk proteins is doubly underlined and potential leader cleavage sequence singly underlined), (B) the predicted JCκ-like amino acid sequences from the remaining kappa J-constant region rearrangements (J1-J5Cκ) (SEQ ID NOS:27-31), and (C) the JCk engineered secretion optimized variants, including JCκ with an appended murine Ig κ leader sequence underlined (SEQ ID NO:32), a recombined JCκ only with an appended murine Ig κ leader sequence underlined (SEQ ID NO:33), and a predicted processed JCκ with an appended murine Ig κ leader sequence underlined (SEQ ID NO:34).

FIG. 6 shows the light chain sequences of anti-human VpreB1 Fab proteins (SEQ ID NOS: 36-51).

FIG. 7 shows the heavy chain sequences (VH) of anti-human VpreB1 Fab proteins (SEQ ID NOS: 52-67).

FIG. 8 illustrates an overview of the characterization methods used for anti-human VpreB1 IgG1 (2460B04 IgG1).

FIG. 9 demonstrates that anti-VpreB1 antibody (2460B04 IgG1) detects HGF-bound 2-piece Surrobody in 50% serum.

FIG. 10 demonstrates that anti-VpreB1 antibody (2460B04 IgG1) detects HGF-bound 3-piece Surrobody in 50% serum.

FIG. 11 demonstrates that anti-VpreB1 antibody (2460B04 IgG1) captures 2-piece Surrobody in 50% serum.

FIG. 12 demonstrates that anti-VpreB1 antibody (2460B04 IgG1) captures 3-piece Surrobody in 50% serum.

FIG. 13A-D demonstrates that anti-VpreB1 mAb (2460B04 IgG1) used as a detection reagent does not bind to IgG containing human VL ORF.

FIG. 14A-C demonstrates that anti-VpreB1 mAb Is unable to capture VL-contained IgG (448C12-HC).

FIG. 15A-C demonstrates that anti-VpreB1 mAb Is unable to capture VL-contained IgG (2547C02 HC)

FIG. 16A-C demonstrates that anti-VpreB1 mAb Is unable to capture VL-contained IgG (2211A01_N56H-HC).

FIG. 17 shows the PK serum ELISA results of the pre-immune serum samples.

FIG. 18 - shows the PK serum ELISA results for SL-541_αHGF SgG over the 5 min to 96hr timepoints.

FIG. 19 shows the PK serum ELISA results for SL-541_αHGF SgG over the 168hr to 672hr timepoints.

FIG. 20 shows the PK serum ELISA results for SL-656_αHGF SgG over the 5 min to 96hr timepoints.

FIG. 21 shows the PK serum ELISA results for SL-656_αHGF SgG over the 168hr to 672hr timepoints.

FIG.22 shows the PK properties of bispecific Surrobodies.

Detailed Description of the Invention

A. Definitions

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al.*, *Dictionary of Microbiology and Molecular Biology* 2nd ed., J. Wiley & Sons (New York, NY 1994), provides one skilled in the art with a general guide to many of the terms used in the present application.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

Throughout this application, the use of singular includes the plural unless expressly stated otherwise.

In this application, the use of "or" includes "and/or", unless expressly stated otherwise.

Furthermore, the terms, "include," "including," and "included," are not limiting.

In the context of the present invention, the term "antibody" (Ab) is used to refer to a native antibody from a classically recombined heavy chain derived from V(D)J gene

recombination and a classically recombined light chain also derived from VJ gene recombination, or a fragment thereof.

A "native antibody" is heterotetrameric glycoprotein of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by covalent disulfide bond(s), while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has, at one end, a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains, Chothia *et al.*, *J. Mol. Biol.* 186:651 (1985); Novotny and Haber, *Proc. Natl. Acad. Sci. U.S.A.* 82:4592 (1985).

The term "variable" with reference to antibody chains is used to refer to portions of the antibody chains which differ extensively in sequence among antibodies and participate in the binding and specificity of each particular antibody for its particular antigen. Such variability is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework region (FR). The variable domains of native heavy and light chains each comprise four FRs (FR1, FR2, FR3 and FR4, respectively), largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al., Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), pages 647-669). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.,* residues 30-36 (L1), 46-55 (L2) and 86-96 (L3) in the light chain variable domain and 30-35 (H1), 47-58 (H2) and 93-101 (H3) in the heavy chain variable domain; MacCallum *et al,. J Mol Biol.* 262(5):732-45 (1996).

The term "framework region" refers to the art recognized portions of an antibody variable region that exist between the more divergent CDR regions. Such framework regions are typically referred to as frameworks 1 through 4 (FR1, FR2, FR3, and FR4) and provide a scaffold for holding, in three-dimensional space, the three CDRs found in a heavy or light chain antibody variable region, such that the CDRs can form an antigen-binding surface.

Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes. There are five major classes of antibodies IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. In a preferred embodiment, the immunoglobulin sequences used in the construction of the immunoadhesins of the present invention are from an IgG immunoglobulin heavy chain domain. For human immunoadhesins, the use of human IgG1 and IgG3 immunoglobulin sequences is preferred. A major advantage of using the IgG1 is that IgG1 immunoadhesins can be purified efficiently on immobilized protein A. However, other structural and functional properties should be taken into account when choosing the Ig fusion partner for a particular immunoadhesin construction. For example, the IgG3 hinge is longer and more flexible, so that it can accommodate larger "adhesin" domains that may not fold or function properly when fused to IgG1. Another consideration may be valency; IgG immunoadhesins are bivalent homodimers, whereas Ig subtypes like IgA and IgM may give rise to dimeric or pentameric structures, respectively, of the basic Ig homodimer unit. For VEGF receptor Ig-like domain/immunoglobulin chimeras designed for in vivo applications, the pharmacokinetic properties and the effector functions specified by the Fc region are important as well. Although IgG1, IgG2 and IgG4 all have in vivo half-lives of 21 days, their relative potencies at activating the complement system are different. Moreover, various immunoglobulins possess varying numbers of allotypic isotypes.

The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

The "light chains" of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains. Any reference to an antibody light chain herein includes both κ and λ light chains.

"Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or a variable domain thereof. Examples of antibody fragments include, but are not limited to, Fab, Fab′, $F(ab')_2$, scFv, and $(scFv)_2$ fragments.

As used herein the term "antibody binding region" refers to one or more portions of an immunoglobulin or antibody variable region capable of binding an antigen(s). Typically, the antibody binding region is, for example, an antibody light chain (VL) (or variable region thereof), an antibody heavy chain (VH) (or variable region thereof), a heavy chain Fd region, a combined antibody light and heavy chain (or variable region thereof) such as a Fab, $F(ab')_2$, single domain, or single chain antibody (scFv), or a full length antibody, for example, an IgG (*e.g.*, an IgG1, IgG2, IgG3, or IgG4 subtype), IgA1, IgA2, IgD, IgE, or IgM antibody.

The term "epitope" as used herein, refers to a sequence of at least about 3 to 5, preferably at least about 5 to 10, or at least about 5 to 15 amino acids, and typically not more than about 500, or about 1,000 amino acids, which define a sequence that by itself, or as part of a larger sequence, binds to an antibody generated in response to such sequence. An epitope is not limited to a polypeptide having a sequence identical to the portion of the parent protein from which it is derived. Indeed, viral genomes are in a state of constant change and exhibit relatively high degrees of variability between isolates. Thus the term "epitope" encompasses sequences identical to the native sequence, as well as modifications, such as deletions, substitutions and/or insertions to the native sequence. Generally, such modifications are conservative in nature but non-conservative modifications are also contemplated. The term specifically includes "mimotopes," i.e. sequences that do not identify a continuous linear native sequence or do not

necessarily occur in a native protein, but functionally mimic an epitope on a native protein. The term "epitope" specifically includes linear and conformational epitopes.

The term "surrogate light chain polypeptide" or "SLC polypeptide" is used herein to refer to a VpreB polypeptide, a λ5 polypeptide, a Vκ-like polypeptide, a JCκ polypeptide, or variants thereof.

The term "non-surrogate light chain molecule" or "non-SLC molecule" is used herein to refer to a molecule that is not an SLC polypeptide. The non-SLC molecule may be a polypeptide, such as a cytokine or antibody fragment.

The term "VpreB" is used herein in the broadest sense and refers to any native sequence or variant VpreB polypeptide, specifically including, without limitation, human VpreB1 of SEQ ID NO: 1, mouse VpreB2 of SEQ ID NOS: 2 and 3, human VpreB3-like sequence of SEQ ID NO: 4, human VpreB dT of SEQ ID NO:5, human VpreB1 amino acid sequence of SEQ ID NO:6 and isoforms, including splice variants and variants formed by posttranslational modifications, other mammalian homologues thereof, as well as variants of such native sequence polypeptides. (Figure 1)

The term "λ5" is used herein in the broadest sense and refers to any native sequence or variant λ5 polypeptide, specifically including, without limitation, human λ5 of SEQ ID NO: 7, human λ5-like protein of SEQ ID NO: 8, the human λ5 dT shown as SEQ ID NOs: 9 and 10, and their isoforms, including splice variants and variants formed by posttranslational modifications, other mammalian homologous thereof, as well a variants of such native sequence polypeptides. (Figure 2)

Specific examples of λ-like Surrobodies include polypeptides in which a VpreB sequence, such as a VpreB1, VpreB2, or VpreB3 sequence, including fragments and variants of the native sequences, is conjugated to a λ5 sequence, including fragments and variants of the native sequence. Representative fusions of this type are provided in PCT Publication WO 2008/118970 published on October 2, 2008, the entire disclosures of which are expressly

incorporated by reference herein. An example of a fusion with a heterogeneous leader sequence is illustrated in Figure 3 (SEQ ID NO:35).

The terms "variant VpreB polypeptide" and "a variant of a VpreB polypeptide" are used interchangeably, and are defined herein as a polypeptide differing from a native sequence VpreB polypeptide at one or more amino acid positions as a result of an amino acid modification. The "variant VpreB polypeptide," as defined herein, will be different from a native antibody λ or κ light chain sequence, or a fragment thereof. The "variant VpreB polypeptide" will preferably retain at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 98% sequence identity with a native sequence VpreB polypeptide. In another preferred embodiment, the "variant VpreB polypeptide" will be less than 95%, or less than 90%, or less than 85%, or less than 80%, or less than 75%, or less than 70%, or less than 65%, or less than 60% identical in its amino acid sequence to a native antibody λ or κ light chain sequence. Variant VpreB polypeptides specifically include, without limitation, VpreB polypeptides in which the non-Ig-like unique tail at the C-terminus of the VpreB sequence is partially or completely removed.

The terms "variant λ5 polypeptide" and "a variant of a λ5 polypeptide" are used interchangeably, and are defined herein as a polypeptide differing from a native sequence λ5 polypeptide at one or more amino acid positions as a result of an amino acid modification. The "variant λ5 polypeptide," as defined herein, will be different from a native antibody λ or κ light chain sequence, or a fragment thereof. The "variant λ5 polypeptide" will preferably retain at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 98% sequence identity with a native sequence λ5 polypeptide. In another preferred embodiment, the "variant λ5 polypeptide" will be less than 95%, or less than 90%, or less than 85%, or less than 80%, or less than 75%, or less than 70%, or less than 65%, or less than 60% identical in its amino acid sequence to a native antibody λ or κ light chain sequence. Variant λ5 polypeptides specifically include, without limitation, λ5 polypeptides in which the unique tail at the N-terminus of the λ5 sequence is partially or completely removed.

The terms "variant Vκ-like polypeptide" and "a variant of a Vκ-like polypeptide" are used interchangeably, and are defined herein as a polypeptide differing from a native sequence Vκ-like polypeptide at one or more amino acid positions as a result of an amino acid modification. The "variant Vκ-like polypeptide," as defined herein, will be different from a native antibody λ or κ light chain sequence, or a fragment thereof. (Figure 4) The "variant Vκ-like polypeptide" will preferably retain at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 98% sequence identity with a native sequence Vκ-like polypeptide. In another preferred embodiment, the "variant Vκ-like polypeptide" will be less than 95%, or less than 90%, or less than 85%, or less than 80%, or less than 75%, or less than 70%, or less than 65%, or less than 60% identical in its amino acid sequence to a native antibody λ or κ light chain sequence. Variant Vκ-like polypeptides specifically include, without limitation, Vκ-like polypeptides in which the non-Ig-like unique tail at the C-terminus of the Vκ-like sequence is partially or completely removed.

The terms "variant JCκ polypeptide" and "a variant of a JCκ polypeptide" are used interchangeably, and are defined herein as a polypeptide differing from a native sequence JCκ polypeptide at one or more amino acid positions as a result of an amino acid modification. (Figure 5) The "variant JCκ polypeptide," as defined herein, will be different from a native antibody λ or κ light chain sequence, or a fragment thereof. The "variant JCκ polypeptide" will preferably retain at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 98% sequence identity with a native sequence JCκ polypeptide. In another preferred embodiment, the "variant JCκ polypeptide" will be less than 95%, or less than 90%, or less than 85%, or less than 80%, or less than 75%, or less than 70%, or less than 65%, or less than 60% identical in its amino acid sequence to a native antibody λ or κ light chain sequence. Variant JCκ polypeptides specifically include, without limitation, JCκ polypeptides in which the unique tail at the N-terminus of the JCκ sequence is partially or completely removed.

Percent amino acid sequence identity may be determined using the sequence comparison program NCBI-BLAST2 (Altschul et al., *Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-

BLAST2 sequence comparison program may be downloaded from http://www.ncbi.nlm.nih.gov or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

The term "VpreB sequence" is used herein to refer to the sequence of "VpreB," as hereinabove defined, or a fragment thereof.

The term "λ5 sequence" is used herein to refers to the sequence of "λ5," as hereinabove defined, or a fragment thereof.

The term "Vκ-like sequence" is used herein to refer to the sequence of "Vκ-like," as hereinabove defined, or a fragment thereof.

The term "JCκ sequence" is used herein to refer to the sequence of "JCκ," as hereinabove defined, or a fragment thereof.

The term "λ-like surrogate light chain," as used herein, refers to a dimer formed by the non-covalent association of a VpreB and a λ5 protein.

The term "κ-like surrogate light chain," as used herein, refers to a dimer formed by the non-covalent association of a Vκ-like and a JCκ protein.

The term "λ-like surrogate light chain sequence," as defined herein, means any polypeptide sequence that comprises a "VpreB sequence" and/or a "λ5 sequence," as hereinabove defined. The "λ-like surrogate light chain sequence," as defined herein, specifically includes, without limitation, the human VpreB1 sequence of SEQ ID NO 1, the mouse VpreB2 sequences of SEQ ID NOS: 2 and 3, and the human VpreB3 sequence of SEQ ID NO: 4, the human VpreB dT shown as SEQ ID NO: 5; and the human VpreB1 amino acid sequence of SEQ ID NO:6 and their various isoforms, including splice variants and variants formed by posttranslational modifications, homologues thereof in other mammalian species, as well as

fragments and variants thereof. The term "λ-like surrogate light chain sequence" additionally includes, without limitation, the human λ5 sequence of SEQ ID NO: 7, the human λ5-like sequence of SEQ ID NO: 8, the human λ5 dTail shown as SEQ ID NO: 9, the human λ5 dTail sequence of SEQ D NO: 10 and their isoforms, including splice variants and variants formed by posttranslational modifications, homologues thereof in other mammalian species, as well as fragments and variants thereof. The term "λ-like surrogate light chain sequence" additionally includes a sequence comprising both VpreB and λ5 sequences as hereinabove defined.

The term "κ-like surrogate light chain sequence," as defined herein, means any polypeptide sequence that comprises a "Vκ-like sequence" and/or a "JCκ," as hereinabove defined. The "κ-like surrogate light chain sequence," as defined herein, specifically includes, without limitation, the human Vκ-like sequence of any of SEQ ID NOS:12-24, and their various isoforms, including splice variants and variants formed by posttranslational modifications, homologues thereof in other mammalian species, as well as fragments and variants thereof. The term "κ-like surrogate light chain sequence" additionally includes, without limitation, the human Vκ-like sequence of any of SEQ ID NOS:12-24, the human JCκ sequence of any of SEQ ID NO:25-35, and their isoforms, including splice variants and variants formed by posttranslational modifications, homologues thereof in other mammalian species, as well as fragments and variants thereof. The term "κ-like surrogate light chain sequence" additionally includes a sequence comprising both Vκ-like and JCκ sequences as hereinabove defined.

The term, "surrogate light chain construct" is used in the broadest sense and includes any and all additional heterogeneous components, including a heterogeneous amino acid sequence, nucleic acid, and other molecules conjugated to a surrogate light chain sequence, wherein "conjugation" is defined below.

A "surrogate light chain construct" is also referred herein as a "Surrobody™," or "Surrobody" and the two terms are used interchangeably. Certain Surrobody™ λ-like surrogate light chain constructs are disclosed in Xu et al., *Proc. Natl. Acad. Sci. USA* 2008, 105(31):10756-61 and in PCT Publication WO 2008/118970 published on October 2, 2008. Also contemplated are κ-like surrogate light chain constructs as described in U.S. Patent Publication No. 2010-

0062950, and Xu et al., *J. Mol. Biol.* 2010, 397, 352-360, the entire disclosures of which are expressly incorporated by reference herein.

In the context of the polypeptides of the present invention, the term "heterogeneous amino acid sequence," relative to a first amino acid sequence, is used to refer to an amino acid sequence not naturally associated with the first amino acid sequence, at least not in the form it is present in the surrogate light chain constructs herein. Thus, a "heterogeneous amino acid sequence" relative to a VpreB, λ5, Vκ-like, or JCκ is any amino acid sequence not associated with native VpreB, λ5, Vκ-like, or JCκ in its native environment. These include, without limitation, i) λ5 sequences that are different from those λ5 sequences that, together with VpreB, form the surrogate light chain on developing B cells, such as amino acid sequence variants, e.g. truncated and/or derivatized λ5 sequences; ii) VpreB sequences that are different from those VpreB sequences that, together with λ5, form the surrogate light chain on developing B cells, such as amino acid sequence variants, e.g. truncated and/or derivatized VpreB sequences, iii) Vκ-like sequences that are different from those Vκ-like sequences that, together with JCκ, form the κ-like surrogate light chain on developing B cells, such as amino acid sequence variants, e.g. truncated and/or derivatized Vκ-like sequences; and iv) JCκ sequences that are different from those JCκ sequences that, together with Vκ-like, form the κ-like surrogate light chain on developing B cells, such as amino acid sequence variants, e.g. truncated and/or derivatized JCκ sequences.

A "heterogeneous amino acid sequence" relative to a VpreB or λ5 also includes VpreB or λ5 sequences covalently associated with, e.g. fused to, a corresponding VpreB or λ5, including native sequence VpreB or λ5, since in their native environment, the VpreB and λ5 sequences are not covalently associated, e.g. fused, to each other. Similarly, a "heterogeneous amino acid sequence" relative to a Vκ-like or JCκ also includes Vκ-like or JCκ sequences covalently associated with, e.g. fused to, a corresponding Vκ-like or JCκ, including native sequence Vκ-like or JCκ, since in their native environment, the Vκ-like or JCκ sequences are not covalently associated, e.g. fused, to each other. Heterogeneous amino acid sequences also include, without limitation, antibody sequences, including antibody and heavy chain sequences and fragments

thereof, such as, for example, antibody light and heavy chain variable region sequences, and antibody light and heavy chain constant region sequences.

The terms “conjugate,” “conjugated,” and “conjugation” refer to any and all forms of covalent or non-covalent linkage, and include, without limitation, direct genetic or chemical fusion, coupling through a linker or a cross-linking agent, and non-covalent association, for example through Van der Waals forces, or by using a leucine zipper.

The term “flexible linker” is used herein to refer to any linker that is not predicted, based on its chemical structure, to be fixed in three-dimensional space in its intended context and environment.

The term “fusion” is used herein to refer to the combination of amino acid sequences of different origin in one polypeptide chain by in-frame combination of their coding nucleotide sequences. The term "fusion" explicitly encompasses internal fusions, i.e., insertion of sequences of different origin within a polypeptide chain, in addition to fusion to one of its termini.

As used herein, the terms "peptide," "polypeptide" and "protein" all refer to a primary sequence of amino acids that are joined by covalent "peptide linkages." In general, a peptide consists of a few amino acids, typically from about 2 to about 50 amino acids, and is shorter than a protein. The term "polypeptide," as defined herein, encompasses peptides and proteins.

The term “amino acid” or “amino acid residue” typically refers to an amino acid having its art recognized definition such as an amino acid selected from the group consisting of: alanine (Ala); arginine (Arg); asparagine (Asn); aspartic acid (Asp); cysteine (Cys); glutamine (Gln); glutamic acid (Glu); glycine (Gly); histidine (His); isoleucine (Ile): leucine (Leu); lysine (Lys); methionine (Met); phenylalanine (Phe); proline (Pro); serine (Ser); threonine (Thr); tryptophan (Trp); tyrosine (Tyr); and valine (Val) although modified, synthetic, or rare amino acids may be used as desired. Thus, modified and unusual amino acids listed in 37 CFR 1.822(b)(4) are specifically included within this definition and expressly incorporated herein by reference. Amino acids can be subdivided into various sub-groups. Thus, amino acids can be grouped as having a nonpolar side chain (*e.g.*, Ala, Cys, Ile, Leu, Met, Phe, Pro, Val); a negatively charged side chain (*e.g.*, Asp, Glu); a positively charged side chain (*e.g.*, Arg, His, Lys); or an uncharged

polar side chain (*e.g.*, Asn, Cys, Gln, Gly, His, Met, Phe, Ser, Thr, Trp, and Tyr). Amino acids can also be grouped as small amino acids (Gly, Ala), nucleophilic amino acids (Ser, His, Thr, Cys), hydrophobic amino acids (Val, Leu, Ile, Met, Pro), aromatic amino acids (Phe, Tyr, Trp, Asp, Glu), amides (Asp, Glu), and basic amino acids (Lys, Arg).

The term "polynucleotide(s)" refers to nucleic acids such as DNA molecules and RNA molecules and analogs thereof (*e.g.*, DNA or RNA generated using nucleotide analogs or using nucleic acid chemistry). As desired, the polynucleotides may be made synthetically, *e.g.*, using art-recognized nucleic acid chemistry or enzymatically using, *e.g.*, a polymerase, and, if desired, be modified. Typical modifications include methylation, biotinylation, and other art-known modifications. In addition, the nucleic acid molecule can be single-stranded or double-stranded and, where desired, linked to a detectable moiety.

The term "variant" with respect to a reference polypeptide refers to a polypeptide that possesses at least one amino acid mutation or modification (i.e., alteration) as compared to a native polypeptide. Variants generated by "amino acid modifications" can be produced, for example, by substituting, deleting, inserting and/or chemically modifying at least one amino acid in the native amino acid sequence.

An "amino acid modification" refers to a change in the amino acid sequence of a predetermined amino acid sequence. Exemplary modifications include an amino acid substitution, insertion and/or deletion.

An "amino acid modification at" a specified position, refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. By insertion "adjacent" a specified residue is meant insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue.

An "amino acid substitution" refers to the replacement of at least one existing amino acid residue in a predetermined amino acid sequence with another different "replacement" amino acid residue. The replacement residue or residues may be "naturally occurring amino acid residues" (i.e. encoded by the genetic code) and selected from the group consisting of: alanine (Ala); arginine (Arg); asparagine (Asn); aspartic acid (Asp); cysteine (Cys); glutamine (Gln); glutamic

acid (Glu); glycine (Gly); histidine (His); isoleucine (Ile): leucine (Leu); lysine (Lys); methionine (Met); phenylalanine (Phe); proline (Pro); serine (Ser); threonine (Thr); tryptophan (Trp); tyrosine (Tyr); and valine (Val). Substitution with one or more non-naturally occurring amino acid residues is also encompassed by the definition of an amino acid substitution herein.

A "non-naturally occurring amino acid residue" refers to a residue, other than those naturally occurring amino acid residues listed above, which is able to covalently bind adjacent amino acid residues(s) in a polypeptide chain. Examples of non-naturally occurring amino acid residues include norleucine, ornithine, norvaline, homoserine and other amino acid residue analogues such as those described in Ellman et al. Meth. Enzym. 202:301 336 (1991). To generate such non-naturally occurring amino acid residues, the procedures of Noren et al. Science 244:182 (1989) and Ellman et al., supra, can be used. Briefly, these procedures involve chemically activating a suppressor tRNA with a non-naturally occurring amino acid residue followed by in vitro transcription and translation of the RNA.

An "amino acid insertion" refers to the incorporation of at least one amino acid into a predetermined amino acid sequence. While the insertion will usually consist of the insertion of one or two amino acid residues, the present application contemplates larger "peptide insertions", e.g. insertion of about three to about five or even up to about ten amino acid residues. The inserted residue(s) may be naturally occurring or non-naturally occurring as disclosed above.

An "amino acid deletion" refers to the removal of at least one amino acid residue from a predetermined amino acid sequence.

The term “mutagenesis” refers to, unless otherwise specified, any art recognized technique for altering a polynucleotide or polypeptide sequence. Preferred types of mutagenesis include error prone PCR mutagenesis, saturation mutagenesis, or other site directed mutagenesis.

"Site-directed mutagenesis" is a technique standard in the art, and is conducted using a synthetic oligonucleotide primer complementary to a single-stranded phage DNA to be mutagenized except for limited mismatching, representing the desired mutation. Briefly, the synthetic oligonucleotide is used as a primer to direct synthesis of a strand complementary to the single-stranded phage DNA, and the resulting double-stranded DNA is transformed into a phage-

supporting host bacterium. Cultures of the transformed bacteria are plated in top agar, permitting plaque formation from single cells that harbor the phage. Theoretically, 50% of the new plaques will contain the phage having, as a single strand, the mutated form; 50% will have the original sequence. Plaques of interest are selected by hybridizing with kinased synthetic primer at a temperature that permits hybridization of an exact match, but at which the mismatches with the original strand are sufficient to prevent hybridization. Plaques that hybridize with the probe are then selected, sequenced and cultured, and the DNA is recovered.

The term “vector” is used to refer to a rDNA molecule capable of autonomous replication in a cell and to which a DNA segment, e.g., gene or polynucleotide, can be operatively linked so as to bring about replication of the attached segment. Vectors capable of directing the expression of genes encoding for one or more polypeptides are referred to herein as "expression vectors. "The term “control sequences” refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers. A vector may be a “plasmid” referring to a circular double-stranded DNA loop into which additional DNA segments may be ligated. A vector may be a phage vector or a viral vector, in which additional DNA segements may be ligated into the viral genome. Suitable vectors are capable of autonomous replication in a host cell into which they are introduced, e.g., bacterial vector with a bacterial origin or replication and episomal mammalian vectors. A vector may be integrated into the host cell genome, e.g., a non-episomal mammalian vector, upon introduction into the host cell, and replicated along with the host genome.

Nucleic acid is “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, “operably linked” means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is

accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

A "phage display library" is a protein expression library that expresses a collection of cloned protein sequences as fusions with a phage coat protein. Thus, the phrase "phage display library" refers herein to a collection of phage (e.g., filamentous phage) wherein the phage express an external (typically heterologous) protein. The external protein is free to interact with (bind to) other moieties with which the phage are contacted. Each phage displaying an external protein is a "member" of the phage display library.

The term "filamentous phage" refers to a viral particle capable of displaying a heterogeneous polypeptide on its surface, and includes, without limitation, f1, fd, Pf1, and M13. The filamentous phage may contain a selectable marker such as tetracycline (e.g., "fd-tet"). Various filamentous phage display systems are well known to those of skill in the art (see, e.g., Zacher et al. Gene 9: 127-140 (1980), Smith et al. Science 228: 1315-1317 (1985); and Parmley and Smith Gene 73: 305-318 (1988)).

The term “panning” is used to refer to the multiple rounds of screening process in identification and isolation of phages carrying compounds, such as antibodies, with high affinity and specificity to a target.

A "host cell" includes an individual cell or cell culture which can be or has been a recipient for transformation of nucleic acid(s) and/or vector(s) containing nucleic acids encoding the molecules described herein. In methods of the present invention, a host cell can be a eukaryotic cell, such as a Chinese Hamster Ovary (CHO) cell, or a human embryonic kidney (HEK) 293 cell. Other suitable host cells are known to those skilled in the art.

B. Detailed Description

Techniques for performing the methods of the present invention are well known in the art and described in standard laboratory textbooks, including, for example, Ausubel *et al.*, *Current Protocols of Molecular Biology,* John Wiley and Sons (1997); *Molecular Cloning: A Laboratory Manual*, Third Edition, J. Sambrook and D. W. Russell, eds., Cold Spring Harbor, New York,

USA, Cold Spring Harbor Laboratory Press, 2001; O'Brian et al., *Analytical Chemistry of Bacillus Thuringiensis*, Hickle and Fitch, eds., Am. Chem. Soc., 1990; *Bacillus thuringiensis: biology, ecology and safety*, T.R. Glare and M. O'Callaghan, eds., John Wiley, 2000; *Antibody Phage Display, Methods and Protocols*, Humana Press, 2001; and *Antibodies*, G. Subramanian, ed., Kluwer Academic, 2004. Mutagenesis can, for example, be performed using site-directed mutagenesis (Kunkel et al., *Proc. Natl. Acad. Sci USA* 82:488-492 (1985)). PCR amplification methods are described in U.S. Pat. Nos. 4,683,192, 4,683,202, 4,800,159, and 4,965,188, and in several textbooks including *PCR Technology: Principles and Applications for DNA Amplification*, H. Erlich, ed., Stockton Press, New York (1989); and *PCR Protocols: A Guide to Methods and Applications*, Innis et al., eds., Academic Press, San Diego, Calif. (1990).

The present invention concerns antibodies against the Surrogate Light Chain (SLC). The antibodies can be directed against the VpreB subunit, the λ5 subunit, or the fusion junction as each of these antibodies will specifically bind the Surrogate Light Chain.

Anti-SLC antibodies may be used for a variety of applications. One application is detection of SLC-containing proteins in complex biological fluids in pharmacokinetic/pharmacodynamic studies, immunohistochemistry studies or for in vitro diagnostic uses.

Another application for anti-SLC antibodies is for diagnostic purposes. There are a number of conditions known in the art associated with the absence or deficiency of SLC. For example, λ5-deficient mice show a dramatic decrease in B-cell development, (Kitamura D, Kudo A, Schaal S, Muller W, Melchers F, Rajewsky K. A critical role of lambda 5 protein in B cell development. *Cell.* 1992;69(5):823-831) whereas mutations in the human λ5 gene result in agammaglobulinemia,(Minegishi Y, Coustan-Smith E, Wang YH, Cooper MD, Campana D, Conley ME. Mutations in the human lambda5/14.1 gene result in B cell deficiency and agammaglobulinemia. *J Exp Med.* 1998;187(1):71-77.) In humans, self-reactive B cells have been identified which express SLC and these cells have been shown to accumulate in the joints of patients with RA (Meffre et al., (2000) *Nature Immunology* 1, 207 - 213). Thus, SLC could be a marker for poly-reactive cells and aberrant pre-BCR function. Accordingly,

anti-SLC antibodies may be a useful reagent for diagnosing certain leukemias and autoimmune diseases that are associated with aberrant SLC expression and B cell function.

In addition, purification of SLC complexes as fusion proteins, non-covalent heteromers, or as cell populations can also be considered for the use of anti-SLC antibodies. As a research reagent, anti-SLC antibodies can be used to assess the biophysical and functional characteristics of SLC-containing complexes in vitro. Quantification, affinity determination, effective or inhibitory concentration, immunoprecipitation, and immunosorbent applications for SLC-complexes are examples of important uses for the anti-SLC antibodies.

The antibodies of the present invention can, for example, be obtained by screening antibody libraries, such as diverse antibody libraries described in U.S. Patent No. 8,131,480, which, in a preferred embodiment, use of phage vectors to express the diverse antibody libraries. The method generally involves the use of a filamentous phage (phagemid) surface expression vector system for cloning and expression. See, e.g., Kang et al., *Proc. Natl. Acad. Sci., USA*, 88:4363-4366 (1991); Barbas et al., *Proc. Natl. Acad. Sci., USA*, 88:7978-7982 (1991); Zebedee et al., *Proc. Natl. Acad. Sci., USA*, 89:3175-3179 (1992); Kang et al., *Proc. Natl. Acad. Sci., USA*, 88:11120-11123 (1991); Barbas et al., *Proc. Natl. Acad. Sci., USA*, 89:4457-4461 (1992); Gram et al., *Proc. Natl. Acad. Sci., USA*, 89:3576-3580 (1992); Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); and U.S. Pat. Nos. 5,698,426; 5,233,409; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,403,484; 5,571,698; 5,516,637; 5,780,225; 5,658,727; 5,733,743; 5,837,500; 5,969,108; 6,326,155; 5,885,793; 6,521,404; 6,492,160, 6,492,123; 6,489,123; 6,342,588; 6,291,650; 6,225,447; 6,180,336; 6,172,197; 6,140,471; 5,994,519; 6,969,108; 5,871,907; and 5,858,657.

The vector is used to transform a recombinant host cell, which is cultured to allow the introduced phage genes and display protein genes to be expressed, and for phage particles to be assembled and shed from the host cell. The shed phage particles are then harvested (collected) from the host cell culture media and screened for desirable antibody binding properties. Typically, the harvested particles are "panned" for binding with a preselected antigen. The

strongly binding particles are collected, and individual species of particles are clonally isolated and further screened for binding to the antigen. Phages which produce a binding site of desired antigen binding specificity are selected.

The invention is further described in the following non-limiting Examples.

EXAMPLES

Example 1 - Identification of anti-VpreB1 Fabs

Sea Lane's proprietary Fab fragment libraries were panned against human VpreB-1 for 4 rounds. Typically, after three to four rounds of panning, individual clones from enriched phage pools were analyzed by ELISA against human VpreB1, and the positive clones were sequenced to determine their heavy and light chain sequences. From these studies, Fab clonal analyses identified 16 unique human VpreB1 binders (Table 1). As further characterized in Table 2, both kappa and lambda light chains were identified with 4 different heavy chain frameworks.

Table 1 - Unique Positive Clones for Anti-human VpreB1

Unique Sequence	VL	VH
2462VpreBlam5E07	VK1_L8	VH3-321
2462VpreBlam5C04	VK1_L8	VH3-323
2462VpreBlam5B09	VK3_A27	VH1-102
2463VpreBlam5B05	VK1_L1	VH1-1e
2463VpreBlam5D07	VK1_L1	VH1-102
2460-VpreBlam5D10	VK1_L8	VH3-321
2460VpreBlam5B04	VK1_L8	VH3-321
2462VpreBlam5A07	VK1_L8	VH3-321
2460VpreBlam5E01	VK1_L8	VH1-102
2462VpreBlam5D05	VL2_2a2	VH1-1e
2462VpreBlam5A05	VL3_3m	VH1-102
2462VpreBlam5C09	VL2_2a2	VH3-323
2460-VpreBlam5F05	VL2_2a2	VH3-323
2462VpreBlam5E08	VL3_3L	VH3-323
2462VpreBlam5C05	VL2_2a2	VH3-321
2463VpreBlam5C04	VL2_2a2	VH1-102

Table 2 - Overview of light and heavy chain sequences among the anti-human VpreB1 Fab hits

	Lambda	Kappa
VH1_02	2	3
VH1-e	1	1
VH3_21	1	4
VH3_23	3	1

The amino acid sequence of the specific anti-human VpreB1 Fab clones was determined and the variable region sequences were identified and analyzed using the FASTA program. The light chain sequences (VL) of the anti-human VpreB1 Fab clones were aligned as depicted in Figure 6. Within this alignment, the variable light chain starts at residue number 3 and the point at which the variable light chain sequence transitions to the constant light chain (Cκ or Cλ) is

demarcated with arrows. The heavy chain sequences (VH) of anti-human VpreB1 Fab clones were aligned as depicted in Figure 7. Within this alignment, the variable heavy chain starts at residue number 1 and the point at which the variable heavy chain and J-chain sequence transitions to the constant heavy chain is demarcated with arrows.

Example 2 - Characterization of selected anti-VpreB1 IgG mAbs

ELISA assays were performed to characterize the SgG-binding affinity, sensitivity and serum background of individual panned phage Fab antibodies having affinity for human VpreB1. Figure 8 provides an overview of the characterization of anti-human VpreB1 IgG1 (2460B04 IgG1). SgG proteins refer to surrogate light chain (SLC) constructs, also referred herein as a "Surrobody," and the two terms are used interchangeably. In general, the 2-piece format includes an SLC fusion and an antibody heavy chain, while the 3-piece format includes two SLC polypeptides and an antibody heavy chain.

Detection

To assess the anti-human VpreB1 IgG1 (2460B04 IgG1) as a detection antibody, HGF-specific S2gG & S3gG were detected by unlabeled, biotin-labeled & DIG-labeled anti-VpreB1 antibodies in ELISA. In the assays to detect either the 2-piece or 3- piece surrobody, wells were coated with HGF (0.1 mL of 0.001 mg/mL), 1% BSA-PBST was used as a blocking and diluent buffer, and S2gG or S3gG were serial diluted in 50% serum. HGF-bound S2gG or S3gG was detected by biotinylated anti-VpreB1 IgG & HRP-conjugated streptavidin. As demonstrated in Figure 9, the anti-VpreB1 antibody detects the HGF-bound 2-Piece Surrobody in 50% Serum. Similarly, the anti-VpreB1 antibody also detects HGF-Bound 3-Piece Surrobody in 50% Serum (Figure 10). The EC50 values for the different SLC constructs used in the detection assays are provided in Table 3 below.

Table 3 - Unlabeled vs. Labeled Anti-Human VpreB1 IgG Antibody

	EC50 (nM) (ELISA)		
SgG	Unlabeled	Biotin-labeled	DIG-labeled
S2gG	0.605	0.549	0.535
S3gG	0.482	0.48	0.432

Capture

In the assays to assess 2-piece or 3- piece surrobody capture by anti-VpreB1 IgG (2460B04 IgG1), wells were coated with anti-VpreB1 IgG (0.1 mL of 0.001 mg/mL), 1% BSA-PBST was used as a blocking and diluent buffer, and S2gG or S3gG were serial diluted in 50% serum. HGF-bound S2gG was detected by HRP-conjugated goat anti-E tag Ab. As demonstrated in Figure 11, the anti-VpreB1 antibody captures 2-Piece surrobody in 50% Serum. The anti-VpreB1 Antibody also captures the 3-Piece Surrobody in 50% Serum (Figure 12).

Affinity

The binding affinity of anti-human VpreB1 IgG mAb (2460B04 IgG1) to SgG was tested by Biolayer Interferometry on a ForteBio Octet ("octet analysis"). Kinetic binding analysis was performed and the apparent affinities are reported in Table 4. The results indicate a sub-nanomolar affinity to bind to 2-piece or 3-piece surroglobulins.

Table 4 - Binding Affinity of Anti-Human VpreB1 IgG mAb to SgG Measured by Octet

Run	SA Biosensor Immobilization	Binding Titration Format	K_D [nM]	kobsRsq
1	0.75ug/ml aHGF S2gG-biotin	100ug/ml aVpreB_IgG	0.280 – 0.100	0.979 – 0.998
2	0.75ug/ml aHGF S2gG-biotin	50ug/ml aVpreB_IgG	0.120 – 0.066	0.981 – 0.998
3	0.75ug/ml aVpreB_IgG_biotin	50ug/ml aHGF S2gG	0.048 – 0.016	0.967 – 0.988
4	0.75ug/ml aVpreB_IgG_biotin	50ug/ml aHGF S3gG	0.058 – 0.032	0.983 – 0.993

The anti-human VpreB1 IgG mAb (2460B04 IgG1) demonstrated a clean background in the presence of human, mouse or *Cynomolgus* monkey serum. This suggests that the 2460B04 IgG1 antibody would be useful in pharmacokinetic (PK) studies. Further applications suggested by these results are as a detection reagent with minimal cross reactivity in human, mouse and monkey serum (15 ng/mL), as a capture reagent with minimal cross reactivity in human, mouse and monkey serum, and as an affinity antibody to enrich libraries in VpreB1-contained library construction.

Cross Reactivity

Cross-reactivities of the anti-VpreB1 mAb (2460B04 IgG1) to Human VL were examined by ELISA. Table 5 provides an overview of the methods used to examine the cross-reactivity of the Anti-VpreB1 mAb. Table 6 identifies the protein sequence similarity of IgG Containing Human VL ORFs.

Table 5 - Characterization of Cross-Reactivity of the Anti-VpreB1 mAb

Capture Reagent	Detection Reagent	Goal
Target (hHGF)	Donkey anti-human IgG Fcγ-HRP (Jackson 709-035-098)	Target binding
	Goat anti-human λ-HRP (Southern)	
	Anti-VpreB1 hIgG1-HRP	
Anti-VpreB1 mIgG2a	Mouse αhuman λ mAb-HRP (Clone JDC-10, Southern)	Complex formation
	Goat anti-human λ-HRP (Southern)	VpreB1-captured CL
Donkey anti-human IgG Fcγ (Jackson 709-005-098)	Goat anti-human λ-HRP (Southern)	Complex formation

Table 6 - Human VL Protein Sequence Similarities

	VpredT-λ5_J (F1)	VpreBdT-λ5-B11 JL (F2)	VL5e_JL	VL1b_JL	VL1c_JL	VL2a2_JL	VL3l_JL	VL3m_JL
VpredT-λ5_J (F1)	100	98	60	41	44	44	42	42
VpreBdT-λ5-B11 JL (F2)		100	62	43	46	46	44	44
VL5e_JL			100	52	58	55	50	55
VL1b_JL				100	62	68	68	62
VL1c_JL					100	69	62	59
VL2a2_JL						100	65	64
VL3l_JL							100	75
VL3m_JL								100

When used as a detection reagent, the anti-VpreB1 mAb does not bind to IgG containing human VL ORF (Figure 13 A-D). Likewise, when used as a capture reagent, the anti-VpreB1 mAb is unable to capture VL-contained IgG (448C12-HC) (Figure 14 A-C). The anti-VpreB1 mAb is also unable to capture the VL-contained IgGs represented by 2547C02 HC (Figure 15 A-C) or 2211A01_N56H-HC (Figure 16 A-C). Thus, there appears to be no cross reactivity to traditional light chain proteins.

Example 3 - Pharmacokinetic Study of Surrobodies in Cynomolgus Monkey

The utility of the anti-VpreB1 IgG mAbs was further demonstrated in pharmacokinetic (PK) studies of Surrobodies in the *Cynomolgus* monkey. The PK profile of Surrobodies in *Cynomolgus* monkeys was assessed by measurements of half life (T1/2), maximum plasma/serum concentration (Cmax), elimination rate constant, AUC0-t (area under the plasma concentration-time curve to the last observed data point), and clearance. We also examined the anti-SgG response that resulted from a single-injection. The Surrobody test articles used in the *Cynomolgus* monkey PK study are identified in Table 7.

Table 7 - Surrobody Test Articles Used in *Cynomolgus* Monkey PK Study

SL No	Target	SgG Format	SgG Structure
SL-349	HGF/PlGF	SgG1_SVD (Stacked Variable Domain)	
SL-521	HGF/PlGF	SgG1_Ball/Socket	
SL-541	HGF	SgG1	
SL-542	PlGF	SgG1	
SL-656	HGF	IgG1	

Detection of Target – Specific Surrobody in PK Cynomolgus Monkey Serum

Biologically naïve *Cynomolgus* monkeys were used to evaluate five SgG test articles, as identified in Figure 17. Three monkeys were studied in each of the test article groups. Among the five groups there was a total of 15 monkeys, weighing 3.37 – 5.67 kg each and health reports were maintained for each monkey. The mean weight of each group was 4.34 – 4.87 kg. A single 10 mg/mL injection was given by i.v. Each monkey serum was collected and prepared at up to 28 days. The time points for serum collection included short intervals (0 and 5 min), intermediate intervals (1, 4, 8 and 24 hrs) and long intervals (48hr (2d), 72hr (3d), 96hr (4d), 168hr (7d), 240hr (10d), 336hr (14d), 408hr (17d), 504hr (21d), 576hr (24d) and 672hr (28d)). ELISA assays were used for detecting SgG in the *Cynomolgus* monkey serum. One PK timepoint was assayed per plate and a serial dilution of standard/sample was performed in 2 mL deep-well blocks. The 1:3 serial dilution comprised 800 uL 1% BSA-PBST + 400 uL sample. One dilution was made for each of the HGF-binding, PlGF-binding and complex ELISAs.

The pre-immune serum samples from all monkeys in each of the 5 test groups were clean for SgG proteins (Figure 17). An example of the ELISA data gathered for SL-541_αHGF SgG over the 5min – 96hr timepoints is provided in Figure 18 and the data for this SgG structure over the 168hr - 672hr timepoints is provided in Figure 19. An example of the ELISA data gathered for SL-656_αHGF IgG over the 5min – 96hr timepoints is provided in Figure 20 and the data for this SgG structure over the 168hr - 672hr timepoints is provided in Figure 21. The results indicate that the half-life of the test subject ranged from 6-10 days. These results further demonstrate the utility of the VpreB1 IgG mAb (2460B04 IgG1) for determining the PK properties of therapeutic Surrobodies. PK studies help determine the distribution, metabolism, and elimination of potential therapeutic drugs. These data will provide a basis for historical comparison between the investigational therapeutic product and a licensed therapeutic, as well as help determine the optimum dosing schedule and withdrawal period for the product. Thus, the VpreB1 IgG mAbs are useful for determining the proper dosing of Surrobodies, by establishing both a clinically efficacious amount and the clearance time to achieve the clinically relevant dose.

We also looked at the PK properties of bispecific Surrobodies and found them to be comparable to parentals in Cynomolgus. (Figure22) Naive groups (n=3) of Cynomolgus monkeys were administered single IV doses of Surrobodies (10 mg/kg) and their serum was tested over a 28 day period. The overall PK properties of the bispecific Surrobodies were found to be very similar to the monspecific HGF and PIGF Surrobodies.

Although in the foregoing description the invention is illustrated with reference to certain embodiments, it is not so limited. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

All publications, patents and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent or patent application were specifically and individually indicated to be so incorporated by reference.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

CLAIMS:

1. An isolated antibody or an antigen-binding fragment thereof, that specifically binds to the VpreB subunit of a surrogate light chain (SLC), wherein the antibody comprises a light chain variable region sequence from within SEQ ID NO:36 and a heavy chain variable region sequence of SEQ ID NO:52 and wherein the VpreB subunit is human VpreB1 of SEQ ID NO:1.

2. The antibody of claim 1, wherein said antigen-binding fragment is selected from the group consisting of Fab, Fab', $F(ab')_2$, scFv, and $(scFv)_2$ fragments.

3. The antibody of claim 1 or claim 2, wherein the SLC further comprises a λ5 subunit.

4. The antibody of claim 3, wherein the λ5 subunit is the human λ5 subunit of SEQ ID NO:7.

5. The antibody of claim 3, wherein the λ5 subunit is the human λ5 dTail subunit of SEQ ID NO:9.

6. A composition comprising an antibody of any one of claims 1 to 5.

7. A method for the diagnosis of a rheumatoid arthritis, leukemia or autoimmune disease associated with aberrant SLC expression in a human subject, comprising contacting a biological sample from said subject with an antibody according to any one of claims 1 to 6, and determining the expression level of SLC.

8. A method for binding a human surrogate light chain (SLC) protein, comprising contacting the SLC protein with an antibody according to any one of claims 1 to 5.

9. A method for the detection of a human surrogate light chain (SLC) protein in a biological sample, comprising contacting the biological sample with an antibody according to any one of claims 1 to 5.

Figure 1

MSWAPVLLMLFVYCTGCGPQPVLHQPPAMSSALGTTIRLTCTLRNDHDIGVYSVYWYQ
QRPGHPPRFLLRYFSQSDKSQGPQVPPRFSGSKDVARNRGYLSISELQPEDEAMYYCAM
GARSSEKEERERERWEEEMEPTAARTRVP

SEQ ID NO:1)

MAWTSVLLMLLAHLTGCGPQPMVHQPPSASSSLGATIRLSCTLSNDHNIGIYSIYWYQQ
RPGHPPRFLLRYFSHSDKHQGPDIPPRFSGSKDTARNLGYLSISELQPEDEAVYYCAVGL
RSHEKKRMEREWEGEKSYTDLGS

(SEQ ID NO:2)

MAWTSVLLMLLAHLTGKGTLGVQGFLAPPVALLCPSDGHASIFSGCGPQPMVHQPPSA
SSSLGATIRLSCTLSNDHNIGIYSIYWYQQRPGHPPRFLLRYFSHSDKHQGPDIPPRFSGSK
DTARNLGYLSISELQPEDEAVYYCAVGLRSHEKKRMEREWEGEKSYTDLGS

(SEQ ID NO:3)

MACRCLSFLLMGTFLSVSQTVLAQLDALLVFPGQVAQLSCTLSPQHVTIRDYGVSWYQ
QRAGSAPRYLLYYRSEEDHHRPADIPDRFSAAKDEAHNACVLTISPVQPEDDADYYCSV
GYGFSP

(SEQ ID NO:4)

MSWAPVLLMLFVYCTGCGPQPVLHQPPAMSSALGTTIRLTCTLRNDHDIGVYSVYWYQ
QRPGHPPRFLLRYFSQSDKSQGPQVPPRFSGSKDVARNRGYLSISELQPEDEAMYYCAM
GA

(SEQ ID NO:5)

METDTLLLWVLLLWVPGSTGQPVLHQPPAMSSALGTTIRLTCTLRNDHDIGVYSVYWY
QQRPGHPPRFLLRYFSQSDKSQGPQVPPRFSGSKDVARNRGYLSISELQPEDEAMYYCA
MGARSSEKEERERERWEEEMEPTAARTRVP

(SEQ ID NO:6)

Figure 2

MKLRVGQTLGTIPRQCEVLLLLLLLGLVDGVHHILSPSSAERSRAVGPGASVGSNRPSL
WALPGRLLFQIIPRGAGPRCSPHRLPSKPQFWYVFGGGTQLTILGQPKSDPLVTLFLPSLK
NLQPTRPHVVCLVSEFYPGTLVVDWKVDGVPVTQGVETTQPSKQTNNKYMVSSYLTLI
SDQWMPHSRYSCRVTHEGNTVEKSVSPAECS

(SEQ ID NO:7)

MRPGTGQGGLEAPGEPGPNLRQRWPLLLLGLAVVTHGLLRPTAASQSRALGPGAPGGS
SRSSLRSRWGRFLLQRGSWTGPRCWPRGFQSKHNSVTHVFGSGTQLTVLSQPKATPSVT
LFPPSSEELQANKATLVCLMNDFYPGILTVTWKADGTPITQGVEMTTPSKQSNNKYAAS
SYLSLTPEQWRSRRSYSCQVMHEGSTVEKTVAPAECS

(SEQ ID NO:8)

MRPGTGQGGLEAPGEPGPNLRQRWPLLLLGLAVVTHGSVTHVFGSGTQLTVLSQPKAT
PSVTLFPPSSEELQANKATLVCLMNDFYPGILTVTWKADGTPITQGVEMTTPSKQSNNK
YAASSYLSLTPEQWRSRRSYSCQVMHEGSTVEKTVAPAECS

(SEQ ID NO:9)

METDTLLLWVLLLWVPGSTGSVTHVFGSGTQLTVLSQPKATPSVTLFPPSSEELQANKA
TLVCLMNDFYPGILTVTWKADGTPITQGVEMTTPSKQSNNKYAASSYLSLTPEQWRSRR
SYSCQVMHEGSTVEKTVAPAECS

(SEQ ID NO:10)

Figure 3

METDTLLLWVLLLWVPGSTGQPVLHQPPAMSSALGTTIRLTCTLRNDHDIGVYSVYWY
QQRPGHPPRFLLRYFSQSDKSQGPQVPPRFSGSKDVARNRGYLSISELQPEDEAMYYCA
MGARSSVTHVFGSGTQLTVLSQPKATPSVTLFPPSSEELQANKATLVCLMNDFYPGILTV
TWKADGTPITQGVEMTTPSKQSNNKYAASSYLSLTPEQWRSRRSYSCQVMHEGSTVEK
TVAPAECS

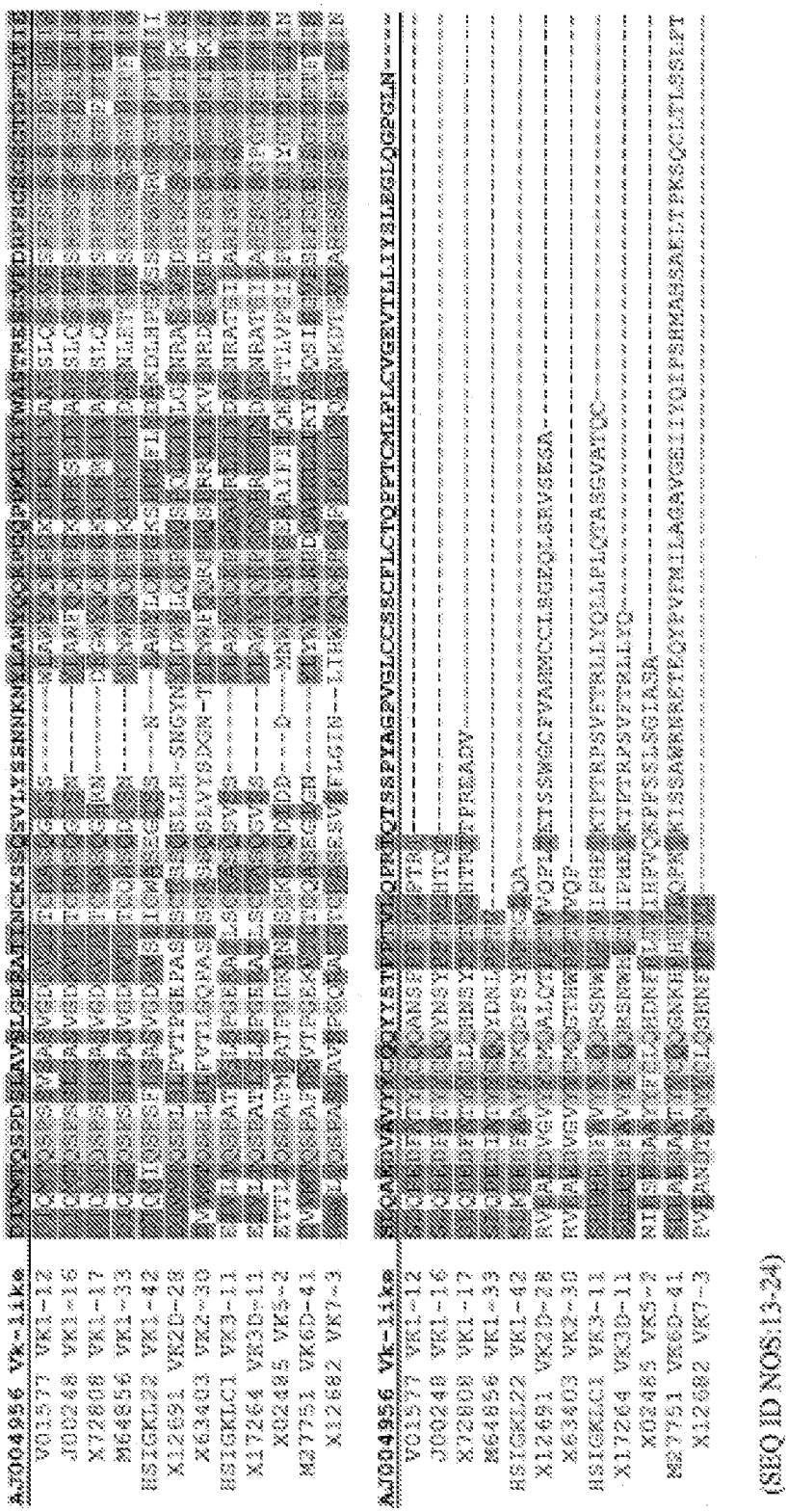
(SEQ ID NO:35)

Figure 4A

```
                M  V  L  Q  T  Q  V  F  I  S  L  L  L  W  I  S  G  A  Y  G  D  I  V  .
    1 CAGCAAGATGGTGTT GCAGACCCAGGTCTT CATTTCTCTTGTTGCT CTGGATCTCTGGTGC CTACGGGGACATCGT
      GTCGTTCTACCACAA CGTCTGGGTCCAGAA GTAAAGAGACAACGA GACCTAGAGACCACG GATGCCCCTGTAGCA

                .  M  T  Q  S  P  D  S  L  A  V  S  L  G  E  R  A  T  I  N  C  K  S  S  Q  S  .
   76 GATGACCCAGTCTCC AGACTGCCTGGCTGT GTCTCTGGGCGAGAG CGCCACCATCAACTG CAAGTCCAGCCAGAG
      CTACTGGGTCAGAGG TCTGACGGACCCGACA CAGAGACCCGCTCTC CCGGTGGTAGTTGAC GTTCAGGTCGGTCTC

                .  V  L  Y  S  S  N  N  K  N  Y  L  A  W  Y  Q  Q  K  P  G  Q  P  P  K  L  L  .
  151 TGTTTTATACAGCTC CAACAATAAGAACTA CTTAGCTTGGTACCA GCAGAACCAGGACA GCCTCCTAAGCTGCT
      ACAAAATATGTCGAG GTTGTTATTCTTGAT GAATCGAACCATGGT CGTCTTTGGTCCTGT CGGAGGATTCGACGA

                .  I  Y  W  A  S  T  R  E  S  G  V  P  D  R  F  S  G  S  G  S  G  T  D  F  T  .
  226 CATTTACTGGGCATC TACCCGGGAATCCGG GGTCCCTGACCGATT CAGTGGCAGCGGGTC TGGGACAGATTTCAC
      GTAAATGACCCGTAG ATGGGCCCTTAGGCC CCAGGGACTGGCTAA GTCACCGTCGCCCAG ACCCTGTCTAAAGTG

                .  L  T  I  S  S  L  Q  A  E  D  V  A  V  Y  Y  C  Q  Q  Y  Y  S  T  P  P  T  .
  301 TCTCACCATCAGCAG CCTGCAGGCTGAAGA TGTGGCAGTTTATTA CTGTCAGCAATATTA TAGTACTCCTCCCAC
      AGAGTGGTAGTCGTC GGACGTCCGACTTCT ACACCGTCAAATAAT GACAGTCGTTATAAT ATCATGAGGAGGGTG

                .  V  L  Q  P  R  T  Q  T  S  S  P  Y  A  G  P  V  G  L  C  C  S  S  C  F  L  .
  376 AGTGCTTCAGCCTCG AACACAAACCTCCTC CCCATACGCCTGGGCC AGTAGGTCTTTGCTG CAGCAGCTGCTTCCT
      TCACGAAGTCGGAGC TTGTGTTTGGAGGAG GGGTATGCGGACCCG TCATCCAGAAACGAC GTCGTCGACGAAGGA

                .  C  T  Q  P  P  T  C  M  L  P  L  C  V  G  E  V  T  L  L  I  Y  S  L  E  G  .
  451 CTGCACACAGCCCCC AACATGCATGCTTCC TCTGTGTGTTGGGGA GGTCACTCTCTTGAT TTATTGGTTGGAGGG
      GACGTGTGTCGGGGG TTGTACGTACGAAGG AGACACACAACCCCT CCAGTGAGAGAACTA AATAAGCAACCTCCC

                .  L  Q  G  P  G  L  N  *
  526 TTTGCAGGGACCCAGG ATTAAATTAAGAGAC TTGACTTTTGCTGGA TCTCTTTTTGTAGAA GATTATTAAGCAAA
      AAACGTCCCTGGGTCC TAATTTAATTCTCTG AACTGAAAACGACCT AGAGAAAAACATCTT CTAATAATTTCGTTT

  601 ATGTTGTAAAGATCC CTTAGAGACATTGTC AGGAGTTTTTGTGTT ACAGGAACCTGCATG TTTCACATGGACACA
      TACAACATTTCTAGG GAATCTCTGTAACAG TCCTCAAAAACACAA TGTCCTTGGACGTAC AAAGTGTACCTGTGT

  676 TCACATGACCGAGCC AAATAGATTTATCTT TACTCT
      AGTGTACTGGCTCGG TTTATCTAAATAGAA ATGAGA
```

(SEQ ID NOS:11-12)

Figure 4B



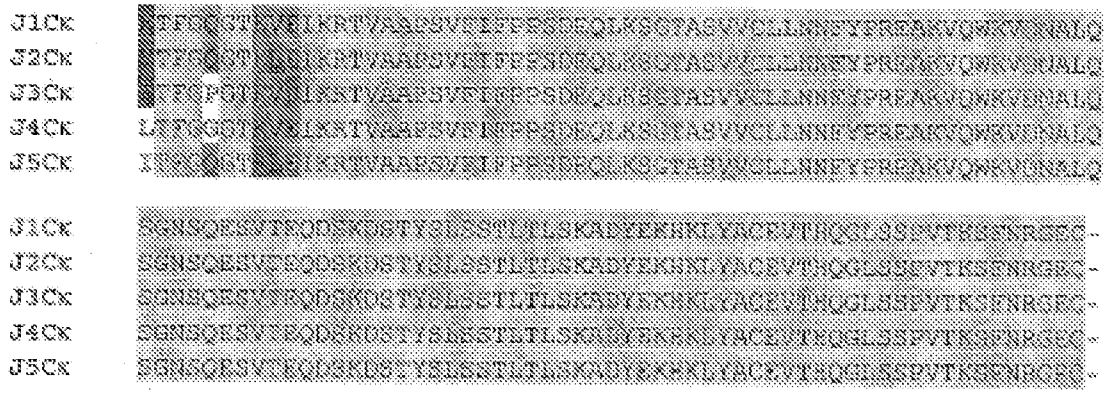
(SEQ ID NOS 13-24)

Figure 5A

```
                Y   R   R   V   F   V   Q   Q   D   N   G   E   L   T   L   W   W   T   F   G
1   GTGAGAAGGG TTTTGTTCA GCAAGACAAT GGAGAGCTCA CACTGTGGTG GACGTTCGGC
    CACTCTTCCC AAAAACAAGT CGTTCTGTTA CCTCTCGAGT GTGACACCAC CTGCAAGCCG

                Q   G   T   K   V   E   I   K   R   T   V   A   A   P   S   V   F   I   F   P
61  CAAGGGACCA AGGTGGAAAT CAAACGAACT GTGGCTGCAC CATCTGTCTT CATCTTCCCG
    GTTCCCTGGT TCCACCTTTA GTTTGCTTGA CACCGACGTG GTAGACAGAA GTAGAAGGGC

                P   S   D   E   Q   L   K   S   G   T   A   S   V   V   C   L   L   N   N   F
121 CCATCTGATG AGCAGTTGAA ATCTGGAACT GCCTCTGTTG TGTGCCTGCT GAATAACTTC
    GGTAGACTAC TCGTCAACTT TAGACCTTGA CGGAGACAAC ACACGGACGA CTTATTGAAG

                Y   P   R   E   A   K   V   Q   W   K   V   D   N   A   L   Q   S   G   N   S
181 TATCCCAGAG AGGCCAAAGT ACAGTGGAAG GTGGATAACG CCCTCCAATC GGGTAACTCC
    ATAGGGTCTC TCCGGTTTCA TGTCACCTTC CACCTATTGC CGGAGGTTAG CCCATTGAGG

                Q   E   S   V   T   E   Q   D   S   K   D   S   T   Y   S   L   S   S   T   L
241 CAGGAGAGTG TCACAGAGCA GGACAGCAAG GACAGCACCT ACAGCCTCAG CAGCACCCTG
    GTCCTCTCAC AGTGTCTCGT CCTGTCGTTC CTGTCGTGGA TGTCGGAGTC GTCGTGGGAC

                T   L   S   K   A   D   Y   E   K   H   K   L   Y   A   C   E   V   T   H   Q
301 ACGCTGAGCA AAGCAGACTA CGAGAAACAC AAACTCTACG CCTGCGAAGT CACCCATCAG
    TGCGACTCGT TTCGTCTGAT GCTCTTTGTG TTTGAGATGC GGACGCTTCA GTGGGTAGTC

                G   L   S   S   P   V   T   K   S   F   N   R   G   E   C   *
361 GGCCTCAGCT CGCCCGTCAC AAAGAGCTTC AACAGGGGAG AGTGTTAG
    CCGGAGTCGA GCGGGCAGTG TTTCTCGAAG TTGTCCCCTC TCACAATC
```

(SEQ ID NOS:25-26)

Figure 5B



(SEQ ID NOS: 27-31)

Figure 5C

METDTLLLWVLLLWVPGSTGVRRVEVQQDNGELTLWWTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQGNSQESVTEQDSKDSTYSLSS
TLTLSKADYEKHKLYACEVTHQGLSSPVTKSFNRGEC

METDTLLLWVLLLWVPGSTGWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC
LLNNFYPREAKVQWKVDNALQGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKLYA
CEVTHQGLSSPVTKSFNRGEC

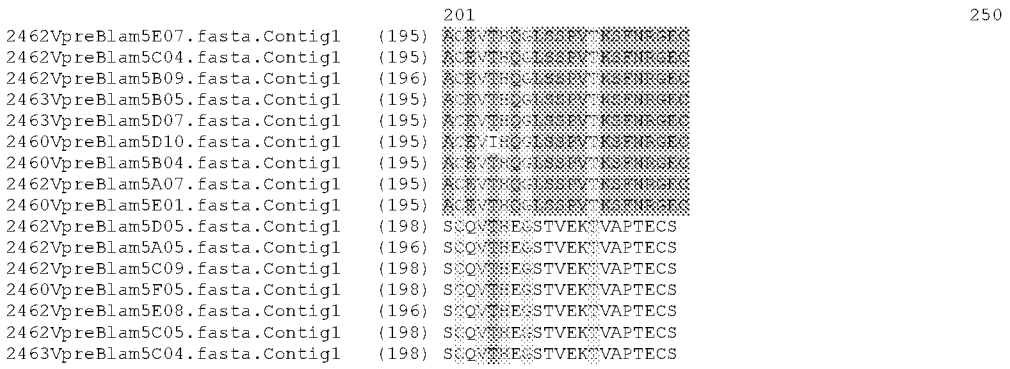
METDTLLLWVLLLWVPGSTGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKLYACEVTHQG
LSSPVTKSFNRGEC

(SEQ ID NOS:32-34)

Figure 6

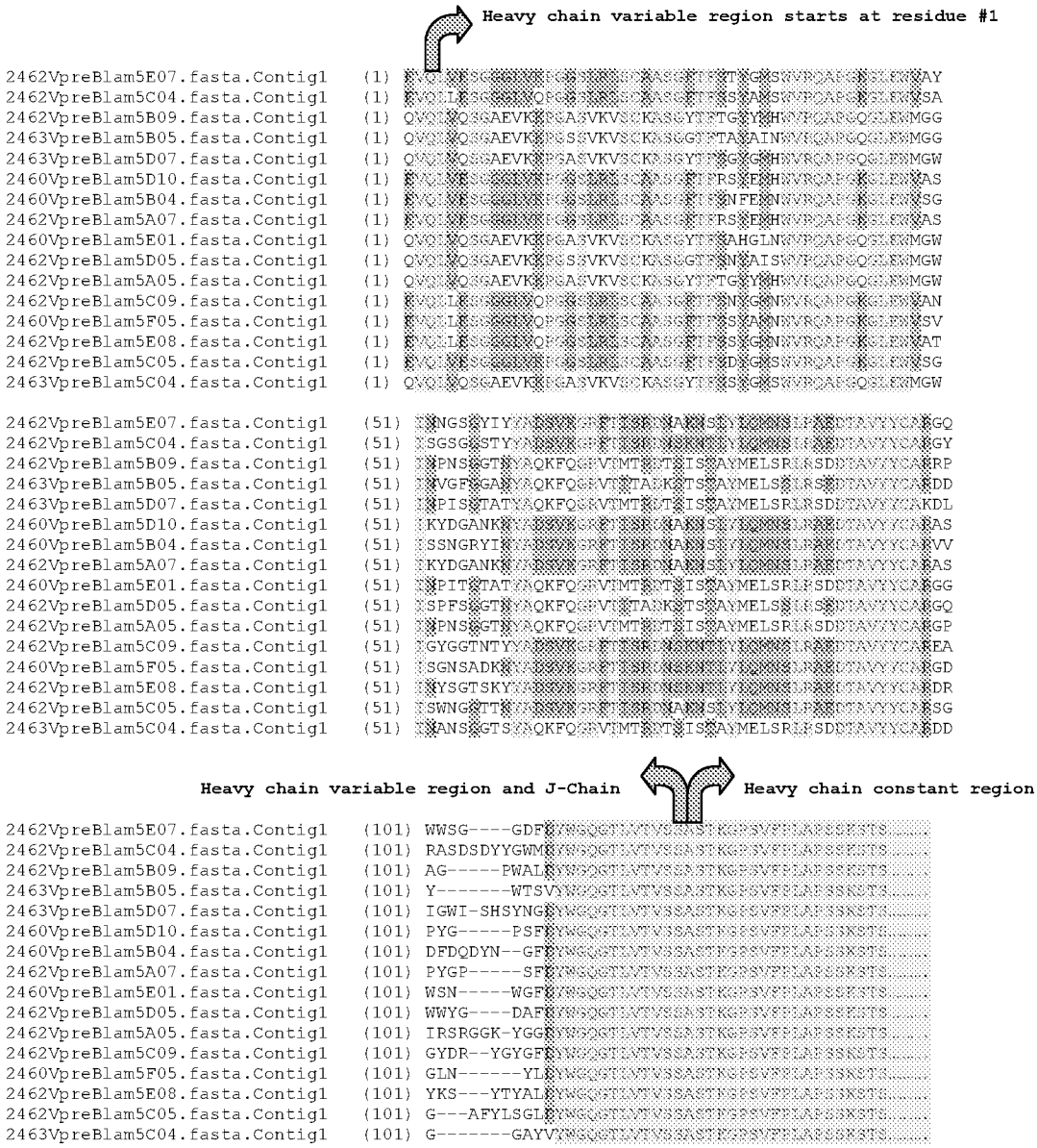

Light chain variable region starts at residue #3
Light chain variable region
Light chain constant region (Cκ or Cλ)

Figure 6 continued



(SEQ ID NOS: 36-51)

Figure 7



(SEQ ID NOS: 52-67)

Figure 8

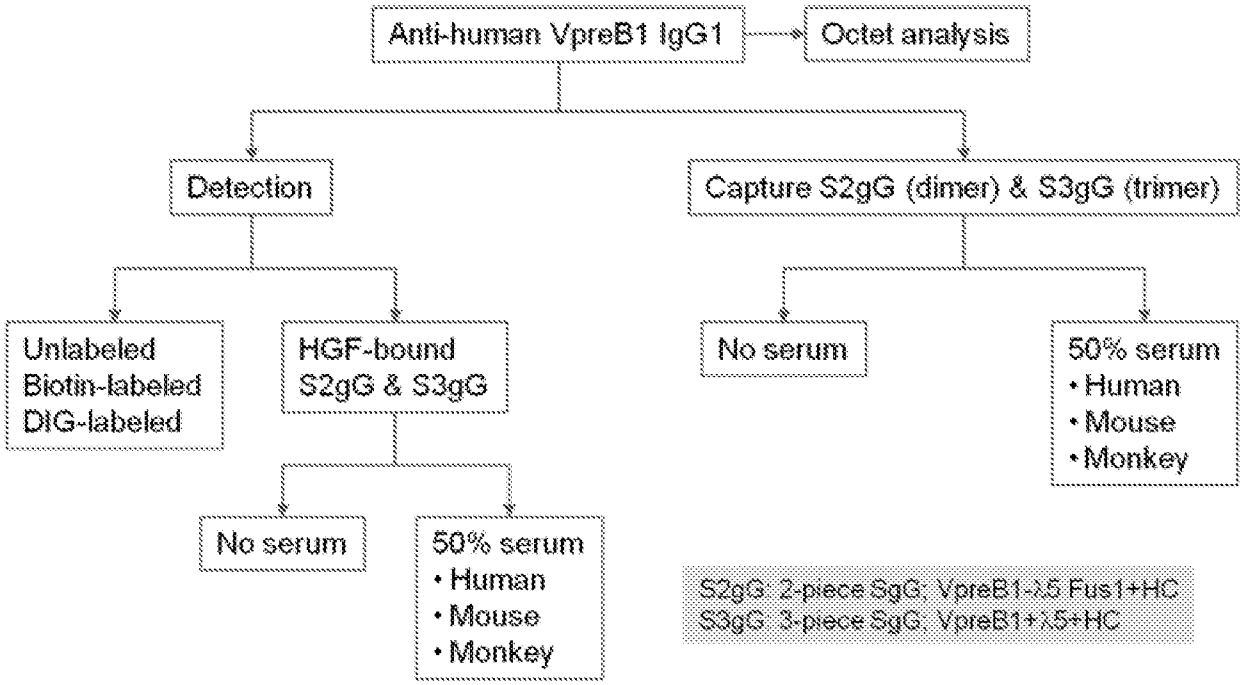

Anti-human VpreB1 IgG1
Octet analysis
Detection
Capture S2gG (dimer) & S3gG (trimer)
Unlabeled
Biotin-labeled
DIG-labeled
HGF-bound
S2gG & S3gG
No serum
50% serum
• Human
• Mouse
• Monkey
No serum
50% serum
• Human
• Mouse
• Monkey
S2gG: 2-piece SgG; VpreB1-λ5 Fus1+HC
S3gG: 3-piece SgG; VpreB1+λ5+HC

Figure 9

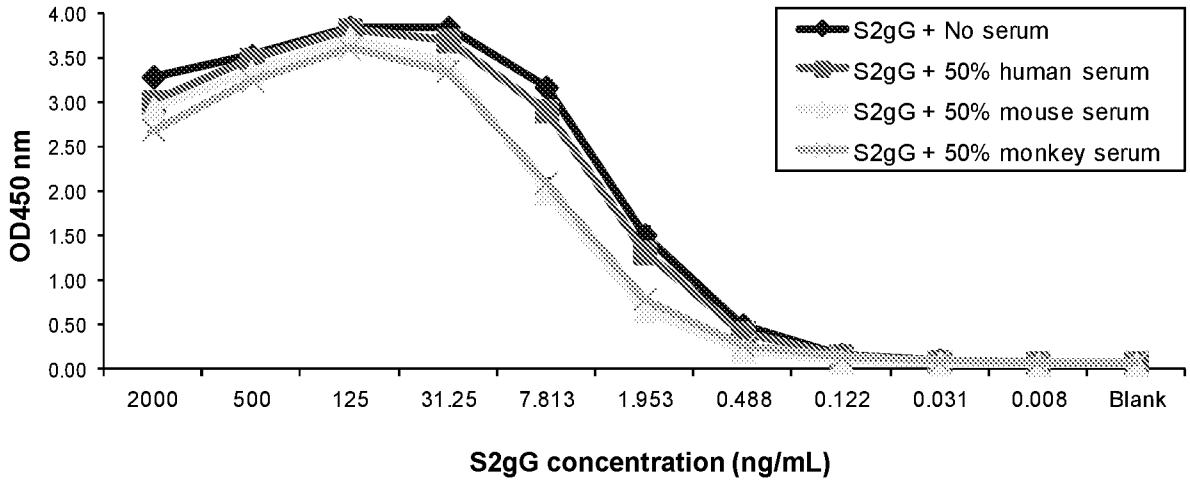

4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
OD450 nm
S2gG + No serum
S2gG + 50% human serum
S2gG + 50% mouse serum
S2gG + 50% monkey serum
2000
500
125
31.25
7.813
1.953
0.488
0.122
0.031
0.008
Blank
S2gG concentration (ng/mL)

Figure 10

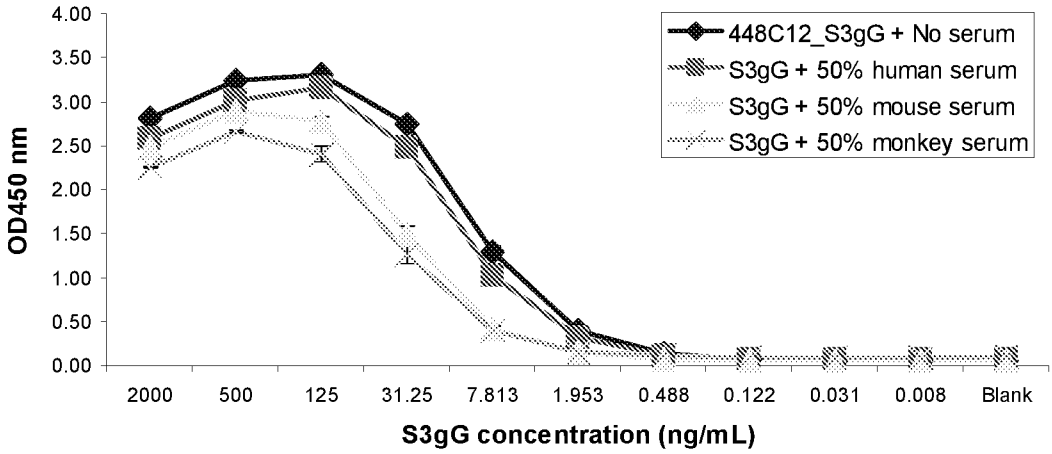

4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
OD450 nm
448C12_S3gG + No serum
S3gG + 50% human serum
S3gG + 50% mouse serum
S3gG + 50% monkey serum
2000
500
125
31.25
7.813
1.953
0.488
0.122
0.031
0.008
Blank
S3gG concentration (ng/mL)

Figure 11

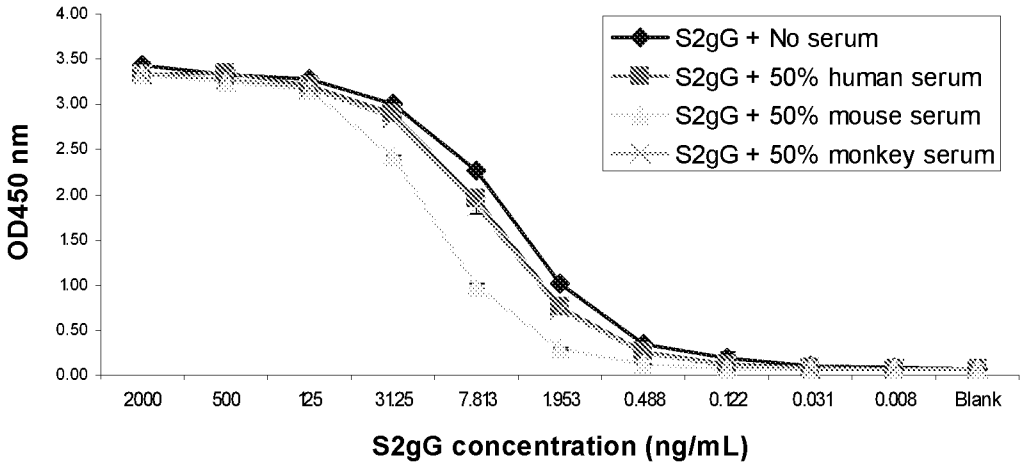

4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
OD450 nm
S2gG + No serum
S2gG + 50% human serum
S2gG + 50% mouse serum
S2gG + 50% monkey serum
2000
500
125
31.25
7.813
1.953
0.488
0.122
0.031
0.008
Blank
S2gG concentration (ng/mL)

Figure 12

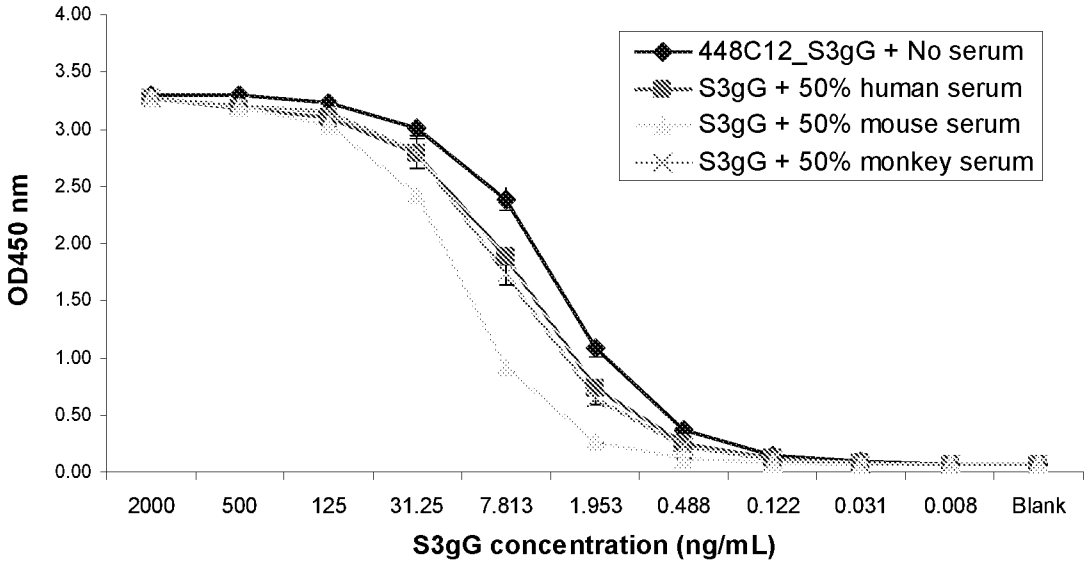

4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
OD450 nm
448C12_S3gG + No serum
S3gG + 50% human serum
S3gG + 50% mouse serum
S3gG + 50% monkey serum
2000
500
125
31.25
7.813
1.953
0.488
0.122
0.031
0.008
Blank
S3gG concentration (ng/mL)

Figure 13

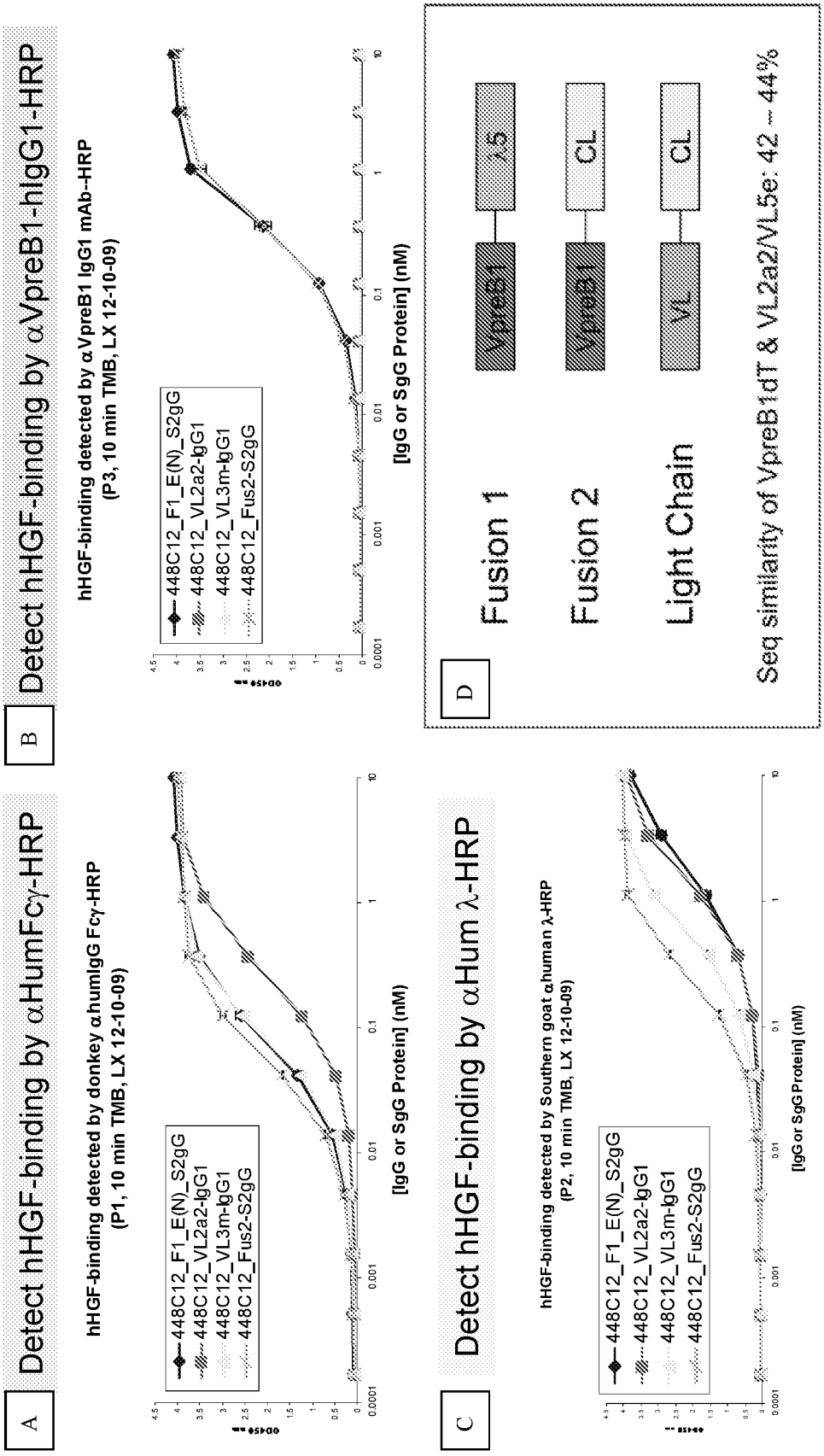
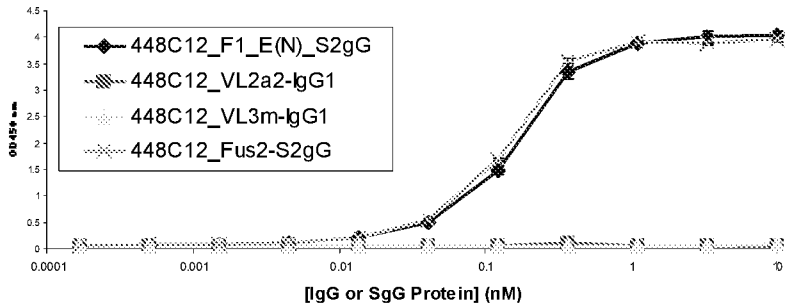

A
Detect hHGF-binding by αHumFcγ-HRP
hHGF-binding detected by donkey αhumIgG Fcγ-HRP
(P1, 10 min TMB, LX 12-10-09)
448C12_F1_E(N)_S2gG
448C12_VL2a2-lgG1
448C12_VL3m-lgG1
448C12_Fus2-S2gG
OD450 nm
[IgG or SgG Protein] (nM)
B
Detect hHGF-binding by αVpreB1-hIgG1-HRP
hHGF-binding detected by αVpreB1 IgG1 mAb-HRP
(P3, 10 min TMB, LX 12-10-09)
448C12_F1_E(N)_S2gG
448C12_VL2a2-lgG1
448C12_VL3m-lgG1
448C12_Fus2-S2gG
OD450 nm
[IgG or SgG Protein] (nM)
C
Detect hHGF-binding by αHum λ-HRP
hHGF-binding detected by Southern goat αhuman λ-HRP
(P2, 10 min TMB, LX 12-10-09)
448C12_F1_E(N)_S2gG
448C12_VL2a2-lgG1
448C12_VL3m-lgG1
448C12_Fus2-S2gG
OD450 nm
[IgG or SgG Protein] (nM)
D
Fusion 1
VpreB1
λ5
Fusion 2
VpreB1
CL
Light Chain
VL
CL
Seq similarity of VpreB1dT & VL2a2/VL5e: 42 ~ 44%

Figure 14

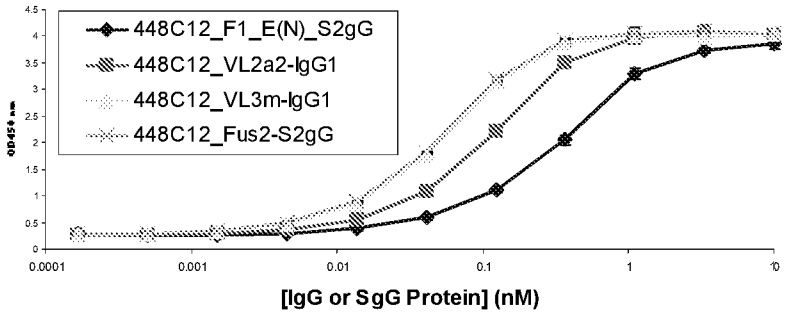
A αVpreB1-mGa2 capture & αHumFcγ mAb detection

Mouse αVpreB1-mIgG2a-captured complexes detected by mouse αhumFcγ mAb-HRP (P4, 10 min TMB, LX 12-10-09)



B Detect complexes in ELISA

Donkey αhum Fcγ-captured complexes detected by Southern goat αhum λ-HRP (P5, 10 min TMB, LX 12-10-09)



C

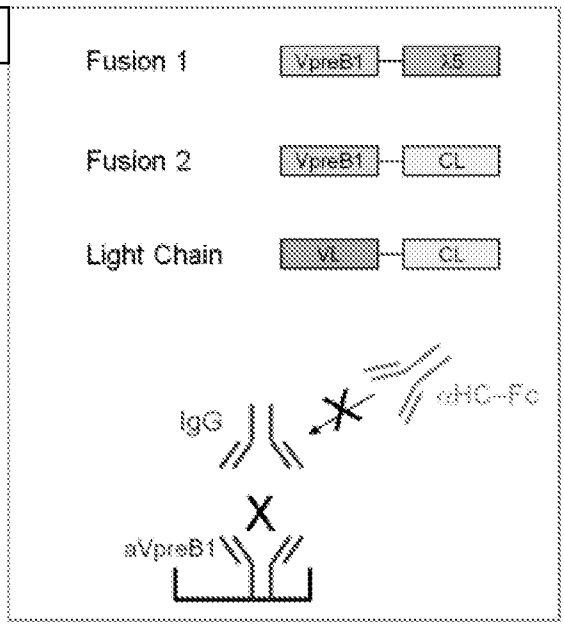
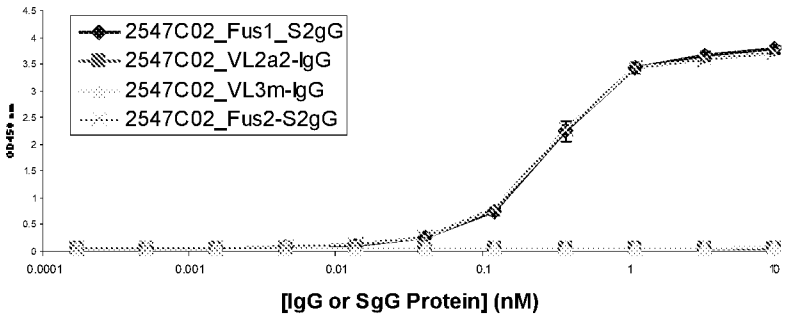


Figure 15

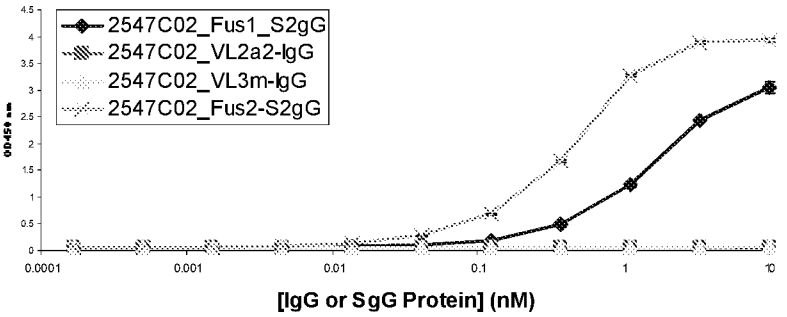
A

αVpreB1-mouse IgG2a-captured complexes detected by mouse αhumIgG Fcγ mAb-HRP (P3, 10 min TMB, LX 12-11-09)



B

αVpreB1-mouse IgG2a-captured complexes detected by Southern goat αhum λ-HRP (P4, 10 min TMB, LX 12-11-09)



C

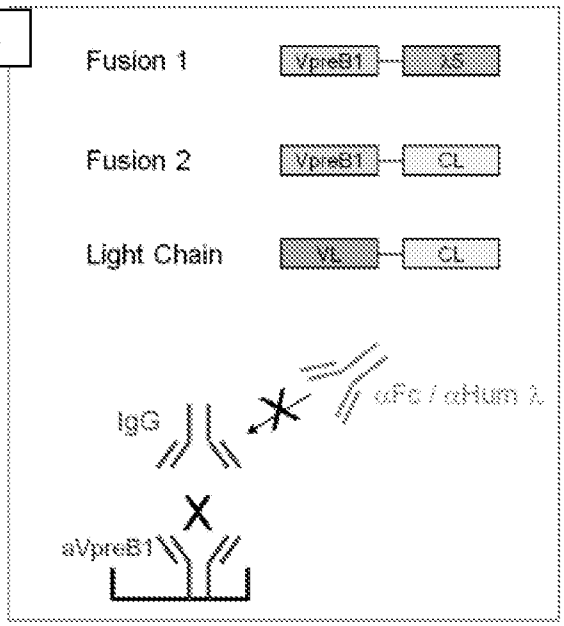
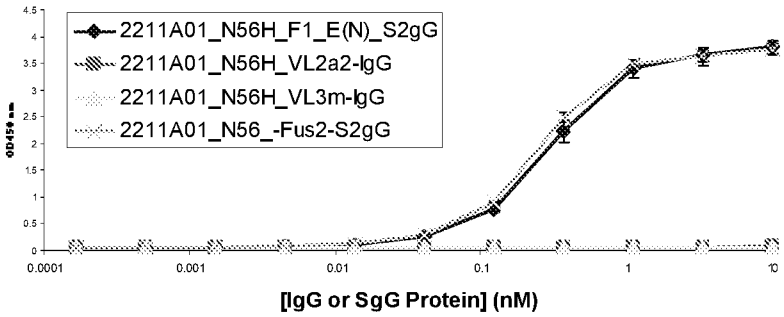


Figure 16

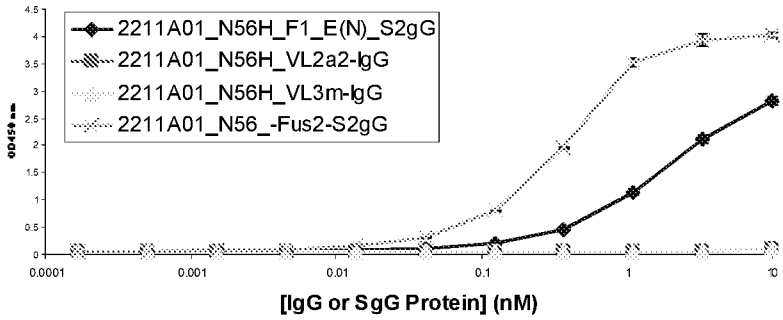
A

αVpreB1-mouse IgG2a-captured complexes detected by mouse αhumIgG Fcγ mAb-HRP (P1, 10 min TMB, LX 12-11-09)



B

αVpreB1-mouse IgG2a-captured complexes detected by Southern goat αhum λ-HRP (P2, 10 min TMB, LX 12-11-09)



C

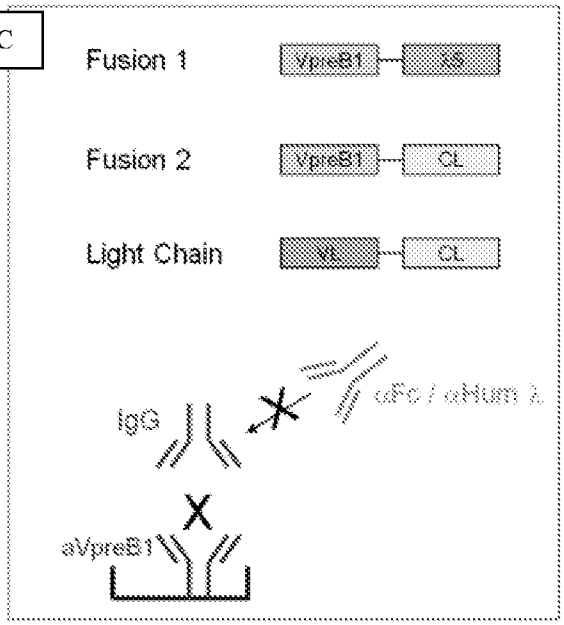


Figure 17

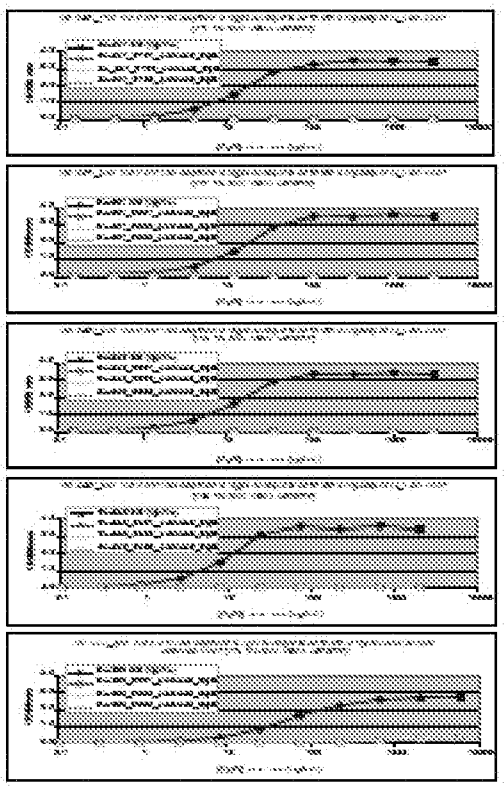

Group 1: SL-541_αHGF SgG
Group 2: SL-656_αHGF IgG
Group 3: SL-521_αHGF SgG_Ba/So
Group 4: SL-349_αHGF SgG_SVD
Group 5: SL-541_αPlGF SgG

Figure 18

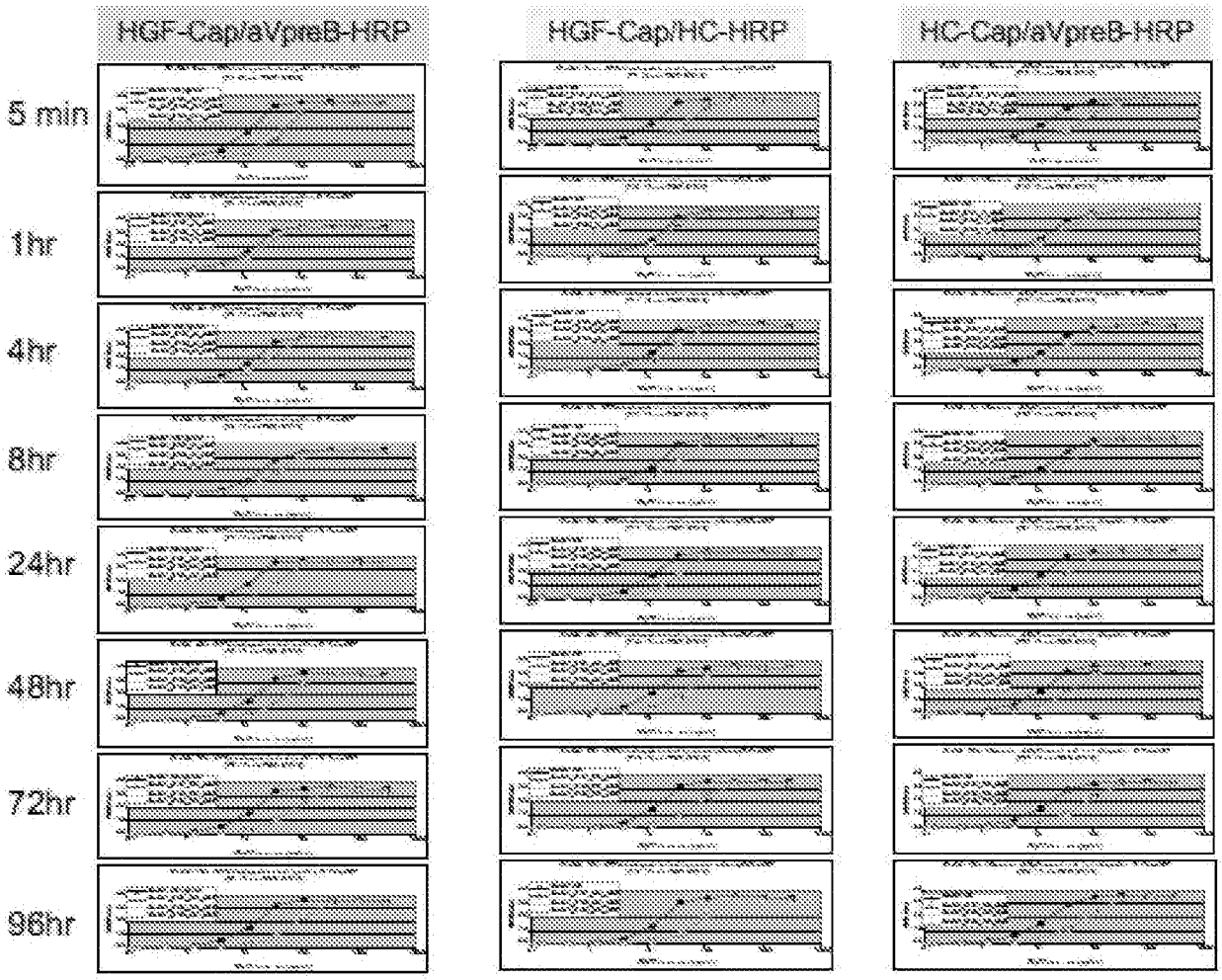

HGF-Cap/aVpreB-HRP
HGF-Cap/HC-HRP
HC-Cap/aVpreB-HRP
5 min
1hr
4hr
8hr
24hr
48hr
72hr
96hr

Figure 19

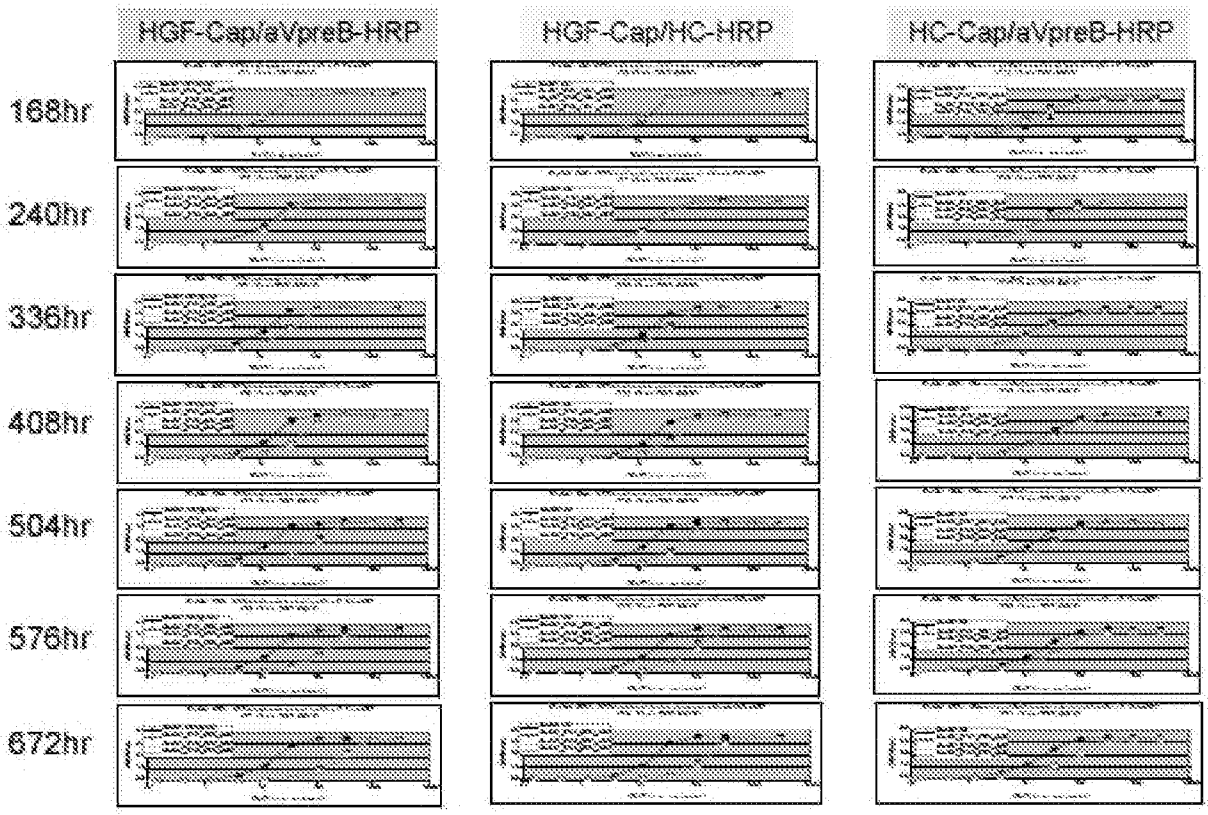

HGF-Cap/aVpreB-HRP
HGF-Cap/HC-HRP
HC-Cap/aVpreB-HRP
168hr
240hr
336hr
408hr
504hr
576hr
672hr

Figure 20

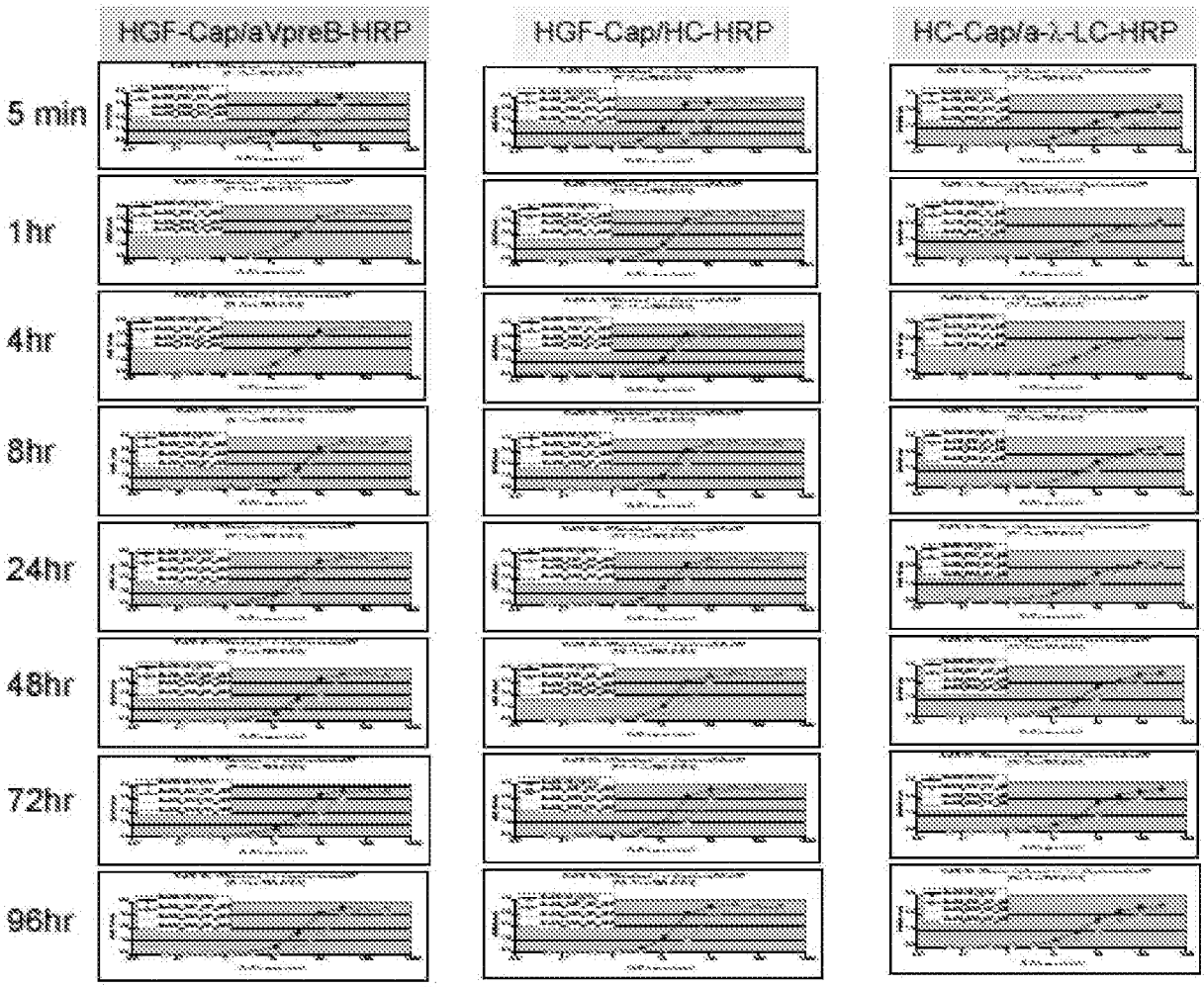

HGF-Cap/aVpreB-HRP
HGF-Cap/HC-HRP
HC-Cap/a-λ-LC-HRP
5 min
1hr
4hr
8hr
24hr
48hr
72hr
96hr

Figure 21

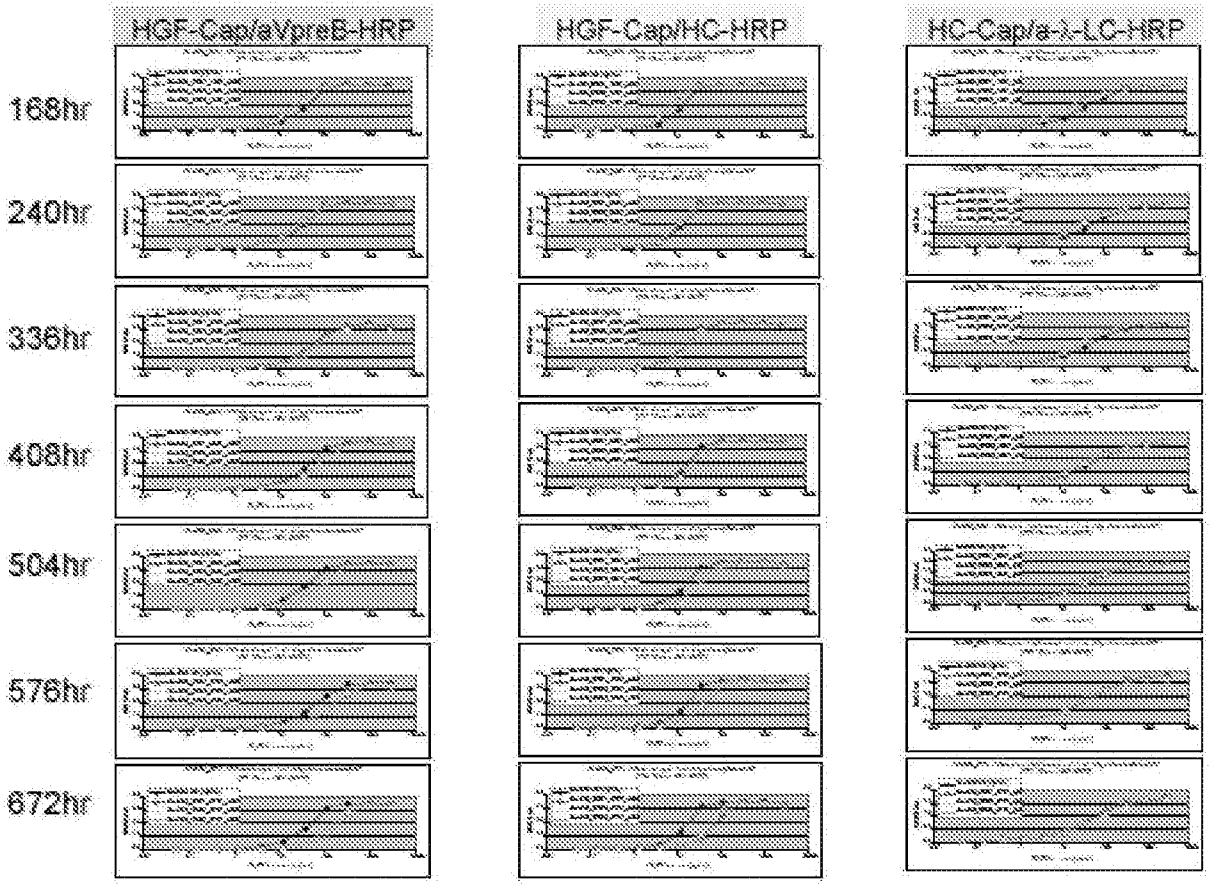
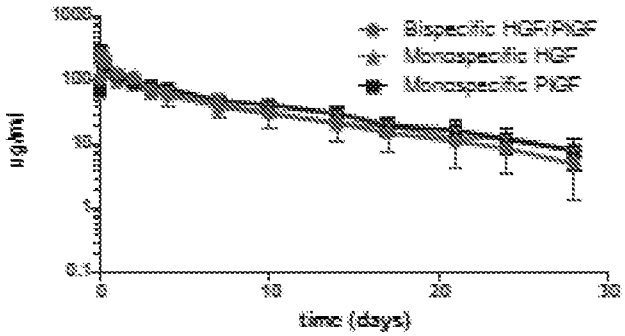

HGF-Cap/aVpreB-HRP
HGF-Cap/HC-HRP
HC-Cap/a-l-LC-HRP
168hr
240hr
336hr
408hr
504hr
576hr
672hr

Figure 22



Agent	Half-life (days)	Clearance (mL/hr/kg)	Vss (mL/kg)
SL-521_HGF/PlGF	6.04	0.42	77.2
SL-541_HGF	9.28	0.40	110
SL-542_PlGF	6.35	0.35	78.5