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(54) Title: CYS80 CONJUGATED IMMUNOGLOBULINS

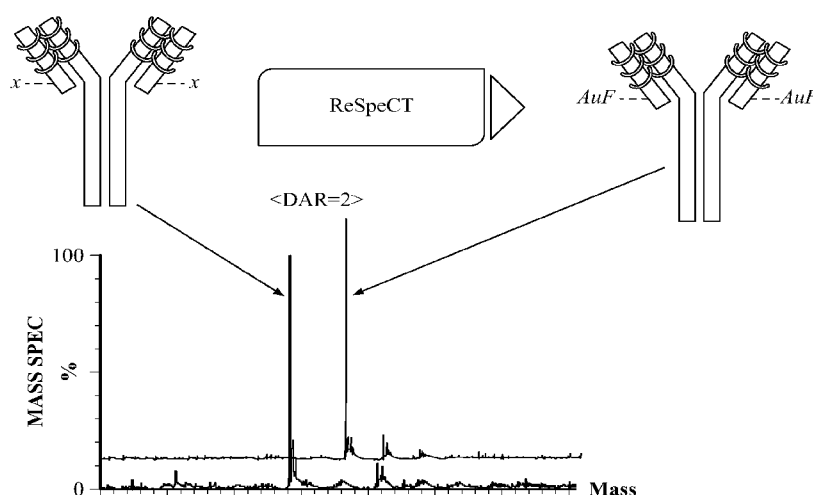


FIG. 13

(57) Abstract: Provided herein are methods for generating conjugated immunoglobulins, the method comprising: decapping a cysteine at amino acid position 80 ("Cys80") in a light chain variable region of an immunoglobulin, wherein the immunoglobulin comprises a heavy chain variable region and the light chain variable region; and conjugating a thiol-reactive compound to the Cys80, wherein the thiol-reactive compound comprises a thiol-reactive group. Antigen-binding molecules and methods for generating the same, immunoglobulins as well as nucleic acid molecules encoding the immunoglobulins and host cells comprising the nucleic acid molecules, conjugated immunoglobulins, and light chain variable regions for use in a conjugated immunoglobulin are also provided.

CYS80 CONJUGATED IMMUNOGLOBULINS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/182,020, filed June 19, 2015, the disclosure of which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The ASCII copy, created on June 16, 2016, is named 104018.000953_SL.txt and is 379,441 bytes in size.

TECHNICAL FIELD

[0003] Provided herein are Cys80 conjugated immunoglobulins and methods of creating the same.

BACKGROUND

[0004] The utility of monoclonal antibodies extends from basic research to therapeutic and diagnostic applications. The ability to conjugate antibodies to functional agents extends their functionality even further. The manufacture of conjugated antibodies usually involves conjugation of a linker, drug, or other functional agent to reactive lysine or cysteine residues on the heavy (HC) and light (LC) chains of a monoclonal antibody (mAb). Lysine conjugation is typically mediated by succinimide (NHS)-based or isothiocyanate-based chemistry. Given the number of exposed lysines on the surface of an antibody, amine-based conjugation approaches result in multiple lysines being modified, though not all lysine residues are modified to the same extent. Therefore, the final product is a heterogeneous mixture of mAbs with a distribution of drug-to-antibody (DAR) ratios.

[0005] Most cysteines within an antibody are involved in either inter- or intra-chain disulfide bonds. Conjugation to cysteines thus requires at least partial reduction of the antibody. Like lysine-based conjugation, cysteine-based conjugation results in a heterogeneous mixture of conjugated antibodies differing in drug load and conjugation site. Each species of conjugated antibody may have distinct properties, which in turn could lead to wide variation of *in vivo*

pharmacokinetic properties. Additionally, such heterogeneity can present challenges in manufacturing of the conjugated antibody.

SUMMARY

[0006] Disclosed herein are methods for generating a conjugated immunoglobulin, the methods comprising: decapping a cysteine at amino acid position 80 ("Cys80") in a light chain variable region of an immunoglobulin derived from rabbit, wherein the immunoglobulin comprises a heavy chain variable region and the light chain variable region; and conjugating a thiol-reactive compound to the Cys80, wherein the thiol-reactive compound comprises a thiol-reactive group.

[0007] Also provided are methods for generating an antigen-binding molecule, the methods comprising incubating a first conjugated immunoglobulin with a second conjugated immunoglobulin to generate the antigen-binding molecule, wherein: the first conjugated immunoglobulin comprises a first heavy chain variable region and a first light chain variable region, the first light chain variable region having a cysteine at position 80 ("Cys80¹") wherein Cys80¹ is conjugated to a first thiol-reactive compound comprising a first thiol-reactive group; and the second conjugated immunoglobulin comprises a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at position 80 ("Cys80²") wherein Cys80² is conjugated to a second thiol-reactive compound comprising a second thiol-reactive group.

[0008] Immunoglobulins comprising a heavy chain variable region and a light chain variable region, the light chain variable region having a cysteine at position 80 ("Cys80") and an amino acid other than Phe, Lys, or Cys at position 83 are also provided herein, as are nucleic acid molecules encoding the immunoglobulins and host cells comprising the nucleic acid molecules.

[0009] Further provided are conjugated immunoglobulins comprising the disclosed immunoglobulins, wherein the cysteine at position 80 is conjugated to a thiol-reactive compound, the thiol-reactive compound comprising a thiol-reactive group.

[0010] Also disclosed herein are methods of treating cancer in a subject comprising administering to the subject a pharmaceutically effective amount of a conjugated mesothelin immunoglobulin, wherein the conjugated mesothelin immunoglobulin comprises: any of the disclosed mesothelin immunoglobulins, and a thiol-reactive compound comprising a thiol-reactive group, a linker, and a functional agent.

[0011] Provided are antigen-binding molecules comprising: a first conjugated immunoglobulin comprising a first heavy chain variable region and a first light chain variable region, the first light chain variable region having a cysteine at position 80 ("Cys80¹"), wherein Cys80¹ is conjugated to a first thiol-reactive compound comprising a first thiol-reactive group, and a second conjugated immunoglobulin comprising a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at position 80 ("Cys80²") wherein Cys80² is conjugated to a second thiol-reactive compound comprising a second thiol-reactive group.

[0012] Light chain variable regions for use in a conjugated immunoglobulin, the light chain variable region having a cysteine at amino acid position 80 ("Cys80") and an amino acid residue other than Phe, Lys, or Cys at amino acid position 83, wherein the Cys80 is unpaired are also disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The summary, as well as the following detailed description, is further understood when read in conjunction with the appended drawings. For the purpose of illustrating the disclosed methods, conjugated immunoglobulins, antigen-binding molecules, immunoglobulins, and light chain variable regions, there are shown in the drawings exemplary embodiments; however, the methods, conjugated immunoglobulins, antigen-binding molecules, immunoglobulins, and light chain variable regions are not limited to the specific embodiments disclosed. In the drawings:

[0014] **FIG. 1** represents an alignment of rabbit and human light chain sequences. **(A)** Alignment of the germline V κ sequences from rabbit (IGKV1S2, X02336) and human (IGKV1-5, Z00001). The arrow indicates Cys80 (according to either Kabat or Chothia numbering) in the rabbit sequence. **(B)** Alignment of the germline C κ sequences from rabbit (IGKC1, K01360) and human (IGKC, J00241). The arrow indicates Cys171 (EU numbering) in the rabbit sequence.

[0015] **FIG. 2** represents structural models of **(A)** rabbit mAb, showing the Cys80-Cys171 disulfide bond, **(B)** human mAb, and **(C)** rabbit-human chimeric mAb showing the unpaired Cys80.

[0016] **FIG. 3** illustrates an alignment of rabbit germline V κ families. The residue at position 80 is indicated by the arrow.

[0017] **FIG. 4** illustrates an exemplary mass spectrometry analysis of xi155D5 light chain when **(A)** reduced using harsh conditions (20 mM DTT, 60°C, 5 minutes) and **(B)** reduced using mild conditions (100 µM DTT, 22°C, 30 minutes).

[0018] **FIG. 5** illustrates an exemplary SE-HPLC analysis of the stability of xi155D5. **(A)** Stability of xi155D5 stored at -80°C. **(B)** Stability of xi155D5 stored at 37°C for 1 week. Only a very slight increase in formation of aggregates or degradation products was observed. Y axis, mAU; x axis, retention time (minutes).

[0019] **FIG. 6** represents an exemplary decapping experiment showing that the cysteine capping Cys80 can be removed by mild reducing conditions (buffer containing 5 mM cysteine for 16 hours followed by washing with a cysteine-free Tris-containing buffer for 60 hours; all incubations carried out at 4°C). The mass of xi155D5 before **(A)** and after **(B)** decapping was 145,464 and 145,221 Da, respectively. The difference (243 Da) corresponding approximately to two free cysteines.

[0020] **FIG. 7** represents an exemplary conjugation experiment showing that an uncapped Cys80 can be conjugated to maleimide-PEG2-biotin. **(A)** The light chain of reduced xi155D5 (predicted mass of 23,399 Da) had a mass of 23,384 Da, and **(B)** after incubation with maleimide-PEG2-biotin, 94% of the product showed a mass increase by 526 Da (23,910 Da). Asterisks denote non-light chain peaks.

[0021] **FIG. 8** represents rabbit 155D5 V_K and V_H sequences aligned with the most homologous human germline variable domains. Framework region (FWR) and complementary determining region (CDR) based on Kabat numbering are identified above the sequences. CDRs based on Chothia numbering are underlined. The C-terminal half of Kabat CDR2 is not considered a CDR by Chothia numbering and is italicized.

[0022] **FIG. 9** illustrates an exemplary standard protein A purification of zu155D5-1. zu155D5-1 was found capped **(A)**, as evidenced by the change of mass after decapping **(B)** by 233 Da, approximately corresponding to two capping cysteines.

[0023] **FIG. 10** illustrates exemplary structural models of chimerized xi155D5. Modelling to determine Cys80 proximity was conducted as in FIG. 2. The residues differing between xi155D5 and zu155D5-1 were highlighted and the distance to Cys80 was measured for each. **(A)** Residues Val11, Ala14, Gly17, Thr18; **(B)** Lys63; **(C)** Thr76, Gly77, Val78, Ala83; and **(D)** Glu103 and Leu104, are within 11 Å of Cys80, except for Lys63 (18 Å). These residues were changed back to the rabbit amino acids in the presence of Cys80.

[0024] **FIG. 11** illustrates exemplary humanized mAbs of 155D5, 1E4, 166B3, and 33O11 light chain sequences.

[0025] **FIG. 12** represents (A-D) flow cytometry screening of sera from immunized animals and (E) ELISA screening of sera from immunized animals. (A-D) Cells were incubated with post-immunization bleed sera at the indicated dilutions (A: post bleed, rabbit 1 serum diluted in 1:1000; B: post bleed, rabbit 1 serum diluted in 1:5000; C: post bleed, rabbit 2 serum diluted in 1:1000; and D: post bleed, rabbit 2 serum diluted in 1:5000). Signal from cells transiently expressing human MSLN (+MSLN) and that from MSLN-negative cells (-MSLN) are shown. (E) ELISA plates were coated with 1 µg/mL of human MSLN at 4°C overnight and blocked using 1% BSA in PBS with 0.01% Tween (PBST) for 2 hours at room temperature. After blocking buffer was removed, serial diluted samples of pre- and post-immunization bleeds were added to wells. The plate was incubated for 2 hours at room temperature and then washed three times with PBST. HRP-conjugated goat anti-rabbit antibody was added in blocking buffer and incubated for 1 hour. The plate was washed three times and TMB substrate was added. The reaction was stopped and absorbance was measured at 450 nm.

[0026] **FIG. 13** represents an exemplary protein peak of deconvoluted mass spectrometry analysis before and after conjugation of maleimido-PEG2-aurostatin F (AuF) to Cys80.

[0027] **FIG. 14** illustrates (A) MSLN-AuF Cys80 conjugated mAbs cytotoxicity against MSLN-negative A431 cells and (B) MSLN-AuF Cys80 conjugated mAbs cytotoxicity against MSLN-positive A431-MSLN cells.

[0028] **FIG. 15** represents the average tumor volumes among different treatment groups.

[0029] **FIG. 16** represents (A) the average A431-MSLN (left flank) tumor volumes among different treatment groups and (B) the average tumor volumes among different treatment groups for A431 (right flank).

[0030] **FIG. 17** represents an SDS-PAGE analysis of an exemplary xi155D5-800CW conjugated antibody. (A) mwm, molecular weight marker; lane 1, xi155D5, unconjugated, non-reduced; lane 2, xi155D5, xi155D5-800CW, non-reduced; lane 3, blank; lane 4, unconjugated xi155D5, reduced; lane 5, xi155D5, xi155D5-800CW, reduced. All lanes contain 5 µg protein, Coomassie-stained. (B) Same gel as in A, imaged on IVIS system. Results indicate that IRDye 800CW is conjugated only on the light chain of xi155D5. ELISA analysis of xi155D5-800CW indicates that full binding to CA9 is retained (data not shown).

[0031] **FIG. 18** illustrates the tumor-specific localization of an exemplary DyeIR 800CW-conjugated antibody (xi155D5-800CW). Human colo205 (**A-D**) and HT-29 (**E-H**) cells were grafted into nude mice, which were later injected with xi155D5-800CW. Fluorescent signal (orange-red) was monitored at various times including 0 hr (**A and E**), 4 hr (**B and F**), 24 hr (**C and G**) and 72 hr (**D and H**) (shown only 0-72 hours and right flank). Approximate location of kidney (K) and tumor (T) is shown.

[0032] **FIG. 19** illustrates an exemplary purification of a xi155D5/xi1-55-2 bivalent/bispecific antigen-binding molecule. Gel-filtration chromatography graph showing the peak corresponding to the xi155D5/xi1-55-2 bivalent/bispecific antigen-binding molecule (referred to as “biFab”) (**A**). Fraction’s molecular size was analyzed by SDS-PAGE (**B**). The fractions containing the xi155D5/xi1-55-2 bivalent/bispecific antigen-binding molecule (biFab) were pooled and the mass was determined by mass spectrometry (**C**).

[0033] **FIG. 20** illustrates the bispecificity of an exemplary xi155D5/xi1-55-2 bivalent/bispecific antigen-binding molecule (biFab). Biotinylated human CA9 was captured on streptavidin Octet biosensor tips. Compounds were added as indicated by the first arrow (“compound”) and then allowed to bind. Subsequently, soluble human TEM-1 (second arrow; “TEM-1”) was added and its binding to the captured CA9/compound complexes was measured. The response shift (double arrow), indicating capturing of the soluble TEM-1, was observed only with xi155D5/xi1-55-2 bivalent/bispecific antigen-binding molecule (biFab).

[0034] **FIG. 21** illustrates an SDS-PAGE analysis of xi33O11-A β (1-16) Cys80 conjugated mAbs and xi1-55-2-A β (1-16) Cys80 conjugated mAbs. Shown are the products before (B) and after (A) conjugation of mAb-DBCO with peptide A β (1-16). MW, molecular weight marker. LC, light chain.

[0035] **FIG. 22** illustrates an exemplary mass spectrometry analysis of xi1-55-2 and xi33O11 parental light chain (LC) (**A and B**, respectively), xi1-55-2-DBCO Cys80 conjugated LC and xi33O11-DBCO Cys80 conjugated LC (**C and D**, respectively), and xi1-55-2-A β (1-16) Cys80 conjugated LC and xi33O11-A β (1-16) Cys80 conjugated LC (**E and F**, respectively).

[0036] **FIG. 23** illustrates an exemplary scheme for generating antigen binding molecules. Immunoglobulins derived from rabbit can be digested with papain to generate Fabs. A first Fab can be incubated with maleimido-DBCO and a second Fab can be incubated with maleimido-azide. The first and second Fabs can be combined to form a bi-specific-Fab-Fab binding molecule. SPAAC = strain promoted alkyne-azide conjugation.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0037] The disclosed methods, conjugated immunoglobulins, antigen-binding molecules, immunoglobulins, and light chain variable regions may be understood more readily by reference to the following detailed description taken in connection with the accompanying figures, which form a part of this disclosure. It is to be understood that the disclosed methods, conjugated immunoglobulins, antigen-binding molecules, immunoglobulins, and light chain variable regions are not limited to the specific embodiments described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed methods, conjugated immunoglobulins, antigen-binding molecules, immunoglobulins, and light chain variable regions.

[0038] Unless specifically stated otherwise, any description as to a possible mechanism or mode of action or reason for improvement is meant to be illustrative only, and the disclosed methods, conjugated immunoglobulins, antigen-binding molecules, immunoglobulins, and light chain variable regions are not to be constrained by the correctness or incorrectness of any such suggested mechanism or mode of action or reason for improvement.

[0039] Throughout this text, the descriptions refer to conjugated immunoglobulins, antigen-binding molecules, immunoglobulins, and light chain variable regions and methods of generating the same. Where the disclosure describes or claims a feature or embodiment associated with a conjugated immunoglobulin, antigen-binding molecule, immunoglobulin, or light chain variable region, such a feature or embodiment is equally applicable to the methods of generating the same. Likewise, where the disclosure describes or claims a feature or embodiment associated with a method of generating a conjugated immunoglobulin, antigen-binding molecule, immunoglobulin, or light chain variable region, such a feature or embodiment is equally applicable to the conjugated immunoglobulin, antigen-binding molecule, immunoglobulin, or light chain variable region.

[0040] Reference to a particular numerical value includes at least that particular value, unless the context clearly dictates otherwise. When a range of values is expressed, another embodiment includes from the one particular value and/or to the other particular value. Further, reference to values stated in ranges include each and every value within that range. All ranges are inclusive and combinable.

[0041] When values are expressed as approximations, by use of the antecedent “about,” it will be understood that the particular value forms another embodiment.

[0042] It is to be appreciated that certain features of the disclosed methods, conjugated immunoglobulins, antigen-binding molecules, immunoglobulins, and light chain variable regions which are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the disclosed methods, conjugated immunoglobulins, antigen-binding molecules, immunoglobulins, and light chain variable regions that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any subcombination.

[0043] As used herein, the singular forms “a,” “an,” and “the” include the plural.

[0044] The term “comprising” is intended to include examples encompassed by the terms “consisting essentially of” and “consisting of”; similarly, the term “consisting essentially of” is intended to include examples encompassed by the term “consisting of.”

[0045] Various terms relating to aspects of the description are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

[0046] The term “about” when used in reference to numerical ranges, cutoffs, or specific values is used to indicate that the recited values may vary by up to as much as 10% from the listed value. Thus, the term “about” is used to encompass variations of $\pm 10\%$ or less, variations of $\pm 5\%$ or less, variations of $\pm 1\%$ or less, variations of $\pm 0.5\%$ or less, or variations of $\pm 0.1\%$ or less from the specified value.

[0047] As used herein, the term “biological sample” refers to a sample obtained from a subject, including sample of biological tissue or fluid origin obtained *in vivo* or *in vitro*. Such samples can be, but are not limited to, body fluid (e.g., blood, blood plasma, serum, milk, spinal fluid, ascites, or urine), organs, tissues, fractions, and cells isolated from mammals including humans. Biological samples also may include sections of the sample obtained from a subject including tissues (e.g., sectional portions of an organ or tissue). Biological samples may also include extracts from a sample obtained from a subject, for example, an antigen from a biological fluid (e.g., blood or urine).

[0048] The term “capping cysteine” refers to a free cysteine from solution that forms a disulfide bond with Cys80 of the light chain variable region.

[0049] The term “chimerized,” “chimeric,” “chimeric antibody” and like terms refer to an immunoglobulin comprising a heavy chain variable region and light chain variable region, *i.e.*, antigen-binding region, from one source or species and at least a portion of a heavy chain

constant region and light chain constant region derived from a different source or species. These portions may be joined together chemically by conventional techniques (e.g., synthetic) or prepared as a contiguous polypeptide using genetic engineering techniques (e.g., DNA encoding the protein portions of the chimeric antibody may be expressed to produce a contiguous polypeptide chain). Exemplary chimeric immunoglobulins include those comprising a rabbit variable region and a human constant region. Such rabbit/human chimeric immunoglobulins are the product of expressed immunoglobulin genes comprising DNA segments encoding rabbit immunoglobulin variable regions and DNA segments encoding human immunoglobulin constant regions. Other forms of “chimeric immunoglobulins” encompassed by the present disclosure are those in which the class or subclass has been modified or changed from that of the original immunoglobulin (also referred to as “class-switched immunoglobulins”). Throughout the disclosure, chimeric immunoglobulins are designated “xi.” Herein, “chimeric immunoglobulin” and like terms refer to the sequence of the immunoglobulin rather than the process used to generate the antibody.

[0050] As used herein, “Cys80” refers to a cysteine residue at amino acid position 80 of the light chain variable region relative to a light chain variable region absent a leader sequence. For example, the light chain variable regions disclosed in Table 25 comprise a 19 amino acid (encoded by a 57 nucleotide) leader sequence. “Cys80” occurs at amino acid position 99 when the leader sequence is present and amino acid position 80 when the leader sequence is absent. The Cys80 numbering is based upon Kabat/Chothia numbering system.

[0051] The term “decapping” refers to removal of the capping cysteine using the methods provided herein under conditions that minimize disruption of the native intra- and inter-chain disulfides of the immunoglobulin.

[0052] The term “immunoglobulin derived from” refers to immunoglobulins, or portions thereof, having at least the CDR regions of a rabbit immunoglobulin. “Immunoglobulin derived from” includes rabbit/human chimeras or humanized rabbit immunoglobulins. The level of variability tolerated when deriving an immunoglobulin from a rabbit can be determined, for example, by the United States Adopted Names Counsel (USAN) of the American Medical Association (AMA).

[0053] As used herein, “functional agent” refers to an agent having therapeutic, diagnostic, or other functional property(ies). Various functional agents that fall within the scope of the disclosure are described elsewhere herein.

[0054] The term “humanized,” “humanized immunoglobulin” and like terms refer to immunoglobulins of rabbit origin in which the sequence of amino acids throughout the variable regions are changed to sequences having homology to a human variable region. Exemplary humanized immunoglobulins can comprise a rabbit variable domain whereby residues throughout the framework region (FWR) and/or the CDRs are replaced by sequences homologous to a human immunoglobulin. In some instances, FWR residues of the rabbit immunoglobulin are not replaced by corresponding human residues. Alternatively, “humanized,” “humanized immunoglobulin” and like terms can refer to immunoglobulins of human origin in which residues throughout the FWR and/or CDRs were replaced by sequences homologous to a rabbit immunoglobulin. For example, humanized immunoglobulins can be human immunoglobulins in which residues from a hypervariable region of the human immunoglobulin are replaced by residues from a hypervariable region of a rabbit immunoglobulin having the desired specificity, affinity, and capacity. Furthermore, humanized immunoglobulins may comprise residues that are not found in the recipient immunoglobulin or in the donor immunoglobulin. These modifications are made to further refine immunoglobulin performance. In general, the humanized immunoglobulin will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FWRs are those of a human immunoglobulin sequence. The humanized immunoglobulin can optionally also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. See, e.g., Riechmann, L., et al., *Nature* 332 (1988) 323-327; and Neuberger, M. S., et al., *Nature* 314 (1985) 268-270. Throughout the disclosure, “humanized immunoglobulins” are designated “zu.” Herein, “humanized immunoglobulin” and like terms refer to the sequence of the immunoglobulin rather than the process used to generate the immunoglobulin.

[0055] The term “donor immunoglobulin” refers to a non-human immunoglobulin that contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to the humanized immunoglobulin, and thereby provides the humanized immunoglobulin with the antigenic specificity and neutralizing activity characteristic of the donor immunoglobulin.

[0056] The term “recipient immunoglobulin” refers to an immunoglobulin heterologous to the donor immunoglobulin, which provides the amino acid sequences of its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the humanized

immunoglobulin. The recipient immunoglobulin may be derived from any mammal. In preferred embodiments, the recipient immunoglobulin is non-immunogenic in humans. Preferably the recipient immunoglobulin is a human immunoglobulin.

[0057] “Humanizing” refers to a process of generating a humanized immunoglobulin and includes any process for generating humanized immunoglobulins having the above characteristics, including, but not limited to, *in silico* humanization, engineering species/host CDRs into human immunoglobulins, substituting framework region residues of a chimeric immunoglobulin to match a corresponding human framework region, etc.

[0058] “Hydrophobic Amino Acid” refers to an amino acid exhibiting a hydrophobicity of greater than zero according to the normalized consensus hydrophobicity scale of Eisenberg, 1984, J. Mol. Biol. 179:125-142. Genetically encoded hydrophobic amino acids include Pro (P), Ile (I), Phe (F), Val (V), Leu (L), Trp (W), Met (M), Ala (A), Gly (G) and Tyr (Y).

[0059] “Immunoglobulin,” as used herein, refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes including the kappa and lambda light chains and the alpha, gamma, delta, epsilon and mu heavy chains. Full-length immunoglobulin “light chains” (about 25 Kd or 214 amino acids) are encoded by a variable region gene at the NH₂-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH - terminus. Full-length immunoglobulin “heavy chains” (about 50 Kd or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids). “Immunoglobulins” include: (a) immunoglobulin polypeptides, *i.e.*, polypeptides of the immunoglobulin family that contain an antigen binding site that specifically binds to a specific antigen (e.g., MSLN, CA9, TEM1, etc.), including all immunoglobulin isotypes (IgG, IgA, IgE, IgM, IgD, and IgY), classes (e.g. IgG1, IgG2, IgG3, IgG4, IgA1, IgA2), subclasses, and various monomeric and polymeric forms of each isotype, unless otherwise specified; and (b) conservatively substituted variants of such immunoglobulin polypeptides that immunospecifically bind to the antigen (e.g., MSLN, CA9, TEM1, etc.). Immunoglobulins are generally described in, for example, Harlow & Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1988).

[0060] One form of immunoglobulin disclosed herein constitutes the basic structural unit of an antibody. For example, an antibody can include a tetramer and consist of two identical pairs of immunoglobulin chains, each pair having one light chain and one heavy chain. Generally, in each pair, the light chain and heavy chain variable regions are together responsible

for binding to an antigen, and the constant regions are responsible for the antibody effector functions.

[0061] In addition to antibodies, immunoglobulins may exist in a variety of other forms including, for example: antigen-binding fragments or portions of an immunoglobulin, such as Fv, Fab, (Fab')₂ and Fv fragments; and alternative antibody formats such as single chain immunoglobulins (scFV and scFab), diabodies, triabodies, tetrabodies, linear antibodies, and multispecific antibodies, to name a few. See, for example, James D. Marks, *Antibody Engineering*, Chapter 2, Oxford University Press (1995) (Carl K. Borrebaeck, Ed.)

[0062] As used herein, the term “immunospecifically” refers to the ability of an immunoglobulin to specifically bind to an antigen against which the immunoglobulin was generated and not specifically bind to other peptides or proteins. An immunoglobulin that immunospecifically binds to an antigen against which the immunoglobulin was generated may not bind to other polypeptides or proteins, or may bind to other polypeptides or proteins with a lower binding affinity than the antigen against which the immunoglobulin was generated as determined by, for example, immunoassays, BIAcore, or other assays known in the art. An immunoglobulin binds immunospecifically to an antigen against which the immunoglobulin was generated when it binds to the antigen with a higher binding affinity than to any cross-reactive antigen as determined using experimental techniques, such as, but not limited to, radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (ELISAs) (See, for example, Paul, ed., *Fundamental Immunology*, 2nd ed., Raven Press, New York, pages 332-336 (1989) for a discussion regarding antibody specificity.).

[0063] “Linker,” as used herein, refers to a spacer, which may be a straight or branched chain, for connecting an immunoglobulin (through a thiol-reactive group on the unpaired Cys80) to a functional agent. Such linkers may be cleavable (e.g., acid labile or protease cleavable) or non-cleavable.

[0064] The term “monoclonal antibody” refers to an antibody that is derived from a single cell clone, including any eukaryotic or prokaryotic cell clone, or a phage clone, and not the method by which it is produced. A monoclonal antibody displays a single binding specificity and affinity for a particular epitope. The term “monoclonal antibody” is not limited to antibodies produced through hybridoma technology.

[0065] “Native” refers to the wild type immunoglobulin sequence from the species in which the immunoglobulin is derived. For example, in embodiments wherein a Cys80 is present

in the light chain variable region from the species from which it is derived, the Cys80 is said be present in the native light chain variable region.

[0066] As used herein, “percent identity” and like terms is used to describe the sequence relationships between two or more nucleic acids, polynucleotides, proteins, or polypeptides, and is understood in the context of and in conjunction with the terms including: (a) reference sequence, (b) comparison window, (c) sequence identity and (d) percentage of sequence identity.

- (a) A “reference sequence” is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, exemplary lengths of the reference polypeptide sequence include at least about 16 amino acids, at least about 20 amino acids, at least about 25 amino acids, at least about 35 amino acids, at least about 50 amino acids, or at least about 100 amino acids. For nucleic acids, exemplary length of the reference nucleic acid sequence include at least about 50 nucleotides, at least about 60 nucleotides, at least about 75 nucleotides, at least about 100 nucleotides, or at least about 300 nucleotides, or any integer thereabout or therebetween.
- (b) A “comparison window” includes reference to a contiguous and specified segment of a polynucleotide or polypeptide sequence, wherein the polynucleotide or polypeptide sequence may be compared to a reference sequence and wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions, substitutions, or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions, substitutions, or deletions) for optimal alignment of the two sequences. Exemplary comparison windows can be at least 20 contiguous nucleotides or amino acids in length, and optionally may be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a misleadingly high similarity to a reference sequence due to inclusion of gaps in the polynucleotide or polypeptide sequence a gap penalty is typically introduced and is subtracted from the number of matches.
- (c) Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, Adv. Appl. Math., 2: 482, 1981; by

the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol., 48: 443, 1970; by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. USA, 8: 2444, 1988; by computerized implementations of these algorithms, including, but not limited to: CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, Calif., GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 7 Science Dr., Madison, Wis., USA; the CLUSTAL program is well described by Higgins and Sharp, Gene, 73: 237-244, 1988; Corpet, et al., Nucleic Acids Research, 16:881-90, 1988; Huang, et al., Computer Applications in the Biosciences, 8:1-6, 1992; and Pearson, et al., Methods in Molecular Biology, 24:7-331, 1994. The BLAST family of programs which may be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences against nucleotide database sequences. See, Current Protocols in Molecular Biology, Chapter 19, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York, 1995. New versions of the above programs or new programs altogether will undoubtedly become available in the future, and may be used with the present disclosure.

- (d) “Percent identity” means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions, substitutions, or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions, substitutions, or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[0067] “Pharmaceutically effective amount” refers to an amount of an immunoglobulin that treats the subject.

[0068] “Polar Amino Acid” refers to a hydrophilic amino acid having a side chain that is uncharged at physiological pH, but which has at least one bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Genetically encoded polar amino acids include Asn (N), Gln (Q) Ser (S) and Thr (T).

[0069] The term “subject” as used herein refers to a human or non-human organism. Thus, the methods, immunoglobulins, and conjugated immunoglobulins described herein are applicable to both human and veterinary diseases and conditions. Subjects can be “patients,” *i.e.*, living humans or non-human organisms that are receiving medical care for a disease or condition, or humans or non-human organisms with no defined illness who are being investigated for signs of pathology or presence/absence of a particular condition.

[0070] “Substituting” refers to the replacement of one amino acid residue for another. “Substituting” includes, for example, missense mutations in one or more DNA base pairs encoding the amino acid residue or engineering the protein to exchange one amino acid with another.

[0071] As used herein, “treating” and like terms refer to reducing the severity and/or frequency of disease symptoms, eliminating disease symptoms and/or the underlying cause of said symptoms, reducing the frequency or likelihood of disease symptoms and/or their underlying cause, and improving or remediating damage caused, directly or indirectly, by disease. Exemplary diseases include, but are not limited to, cancer.

[0072] “Thiol-reactive group” refers to a reagent or group that can form a covalent bond with the thiol group in a cysteine.

[0073] “Unpaired Cys80” refers to a Cys80 present in an immunoglobulin that has a thiol functional group that is not involved in an intramolecular or intermolecular disulfide bond. For example, a thiol functional group of an “unpaired Cys80” is not involved in a disulfide bond with Cys171.

[0074] As used herein “90% identical to” encompasses at least 90% identical, 91% identical, 92% identical, 93% identical, 94% identical, 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 100% identical to the reference item (e.g., a biological sequence).

[0075] The following abbreviations are used throughout the disclosure: antibody drug conjugates (ADCs); drug-to-antibody (DAR); frame work region (FWR); complementary determining region (CDR); carbonic anhydrase IX (CA9); mesothelin (MSLN); auristatin F (AuF); variable heavy region (VH); variable light region (VL); variable kappa (Vκ); rabbit (Rb;

rabb); gamma constant region (C γ); kappa constant region (C κ); monoclonal antibody (mAb); cysteine at amino acid position 80 (Cys80).

Generation of conjugated immunoglobulins

[0076] Disclosed herein are methods for generating a conjugated immunoglobulin, the methods comprising:

decapping a cysteine at amino acid position 80 ("Cys80") in a light chain variable region of an immunoglobulin derived from rabbit, wherein the immunoglobulin comprises a heavy chain variable region and the light chain variable region; and
conjugating a thiol-reactive compound to the Cys80, wherein the thiol-reactive compound comprises a thiol-reactive group.

[0077] Suitable light chain variable regions include, for example, a kappa light chain variable region. The light chain variable region of the disclosed immunoglobulins are derived from rabbit. In some embodiments, the Cys80 can be present in the native light chain variable region of the rabbit immunoglobulin. Exemplary rabbits from which a light chain variable region having a Cys80 can be derived include, but are not limited to, *Oryctolagus cuniculus*. In some aspects, for example, the light chain variable region can be derived from a New Zealand White (NZW) rabbit. In other aspects, the light chain variable region can be derived from a b9 rabbit.

[0078] Exemplary methods of decapping a Cys80 in a light chain variable region of an immunoglobulin include incubating the immunoglobulin with a reducing buffer followed by incubating the immunoglobulin with an oxidizing buffer. Reducing buffers comprise one or more reducing agents. Suitable reducing agents include, for example, cysteine (including L-cysteine and D-cysteine), 2-mercaptoethylamine, Tris (2-carboxyethyl) phosphine, 2-mercaptoethanesulfonic acid, 2-mercaptopropionic acid, or combinations thereof. In preferred embodiments, the reducing buffer can comprise a mild reductant such as cysteine. The concentration of reducing agent can range from about 0.2 mM to about 100 mM, from about 1 mM to about 100 mM, from about 2 mM to about 100 mM, from about 5 mM to about 100 mM, from about 10 mM to about 100 mM, from about 20 mM to about 100 mM, from about 40 mM to about 100 mM, from about 50 mM to about 100 mM, from about 0.2 mM to about 90 mM, from about 0.2 mM to about 80 mM, from about 0.2 mM to about 70 mM, from about 0.2 mM to about 50 mM, from about 0.2 mM to about 30 mM, from about 0.2 mM to about 25 mM, from about 0.2 mM to about 10 mM, or from about 0.2 mM to about 5 mM. The concentration of

reducing agent can be about 0.2 mM. The concentration of reducing agent can be about 1 mM. The concentration of reducing agent can be about 2 mM. The concentration of reducing agent can be about 5 mM. The concentration of reducing agent can be about 10 mM. The concentration of reducing agent can be about 15 mM. The concentration of reducing agent can be about 20 mM. The concentration of reducing agent can be about 25 mM. The concentration of reducing agent can be about 30 mM. The concentration of reducing agent can be about 40 mM. The concentration of reducing agent can be about 50 mM. The concentration of reducing agent can be about 60 mM. The concentration of reducing agent can be about 70 mM. The concentration of reducing agent can be about 80 mM. The concentration of reducing agent can be about 90 mM. The concentration of reducing agent can be about 100 mM.

[0079] In some embodiments, for example, the reducing agent can comprise from about 2 mM to about 10 mM cysteine. In some embodiments, the reducing agent can comprise from about 2 mM to about 10 mM D-cysteine. In some embodiments, the reducing agent can comprise from about 2 mM to about 10 mM L-cysteine. In some embodiments, the reducing agent can comprise from about 10 mM to about 100 mM 2-mercaptoethylamine. In some embodiments, the reducing agent can comprise from about 0.2 mM to about 5 mM Tris (2-carboxyethyl) phosphine. In some embodiments, the reducing agent can comprise from about 2 mM to about 20 mM 2-mercaptoethanesulfonic acid. In some embodiments, the reducing agent can comprise from about 2 mM to about 20 mM 2-mercaptopropionic acid.

[0080] The reducing buffer can further comprise buffering agents such as sodium phosphate, potassium phosphate, MOPS, HEPES, sodium borate, potassium borate, or any combination thereof. Suitable buffering agent concentrations include, but are not limited to, from about 10 mM to about 100 mM, from about 15 mM to about 100 mM, from about 20 mM to about 100 mM, from about 30 mM to about 100 mM, from about 35 mM to about 100 mM, from about 40 mM to about 100 mM, from about 60 mM to about 100 mM, from about 80 mM to about 100 mM, from about 10 mM to about 90 mM, from about 10 mM to about 80 mM, from about 10 mM to about 60 mM, from about 10 mM to about 40 mM, from about 10 mM to about 30 mM, or from about 10 mM to about 20 mM.

[0081] In some embodiments, for example, the reducing buffer can contain from about 10 mM to about 100 mM sodium phosphate. In some embodiments, the reducing buffer can contain from about 10 mM to about 100 mM potassium phosphate. In some embodiments, the reducing buffer can contain from about 10 mM to about 100 mM MOPS. In some embodiments, the reducing buffer can contain from about 10 mM to about 100 mM HEPES. In some

embodiments, the reducing buffer can contain from about 10 mM to about 100 mM sodium borate. In some embodiments, the reducing buffer can contain from about 10 mM to about 100 mM potassium borate.

[0082] The reducing buffer can also contain a chelating agent including, but not limited to, EDTA (ethylenediaminetetraacetic acid), DTPA (diethylene triamine pentaacetic acid), or a combination thereof. Suitable concentrations of chelating agents include from about 10 mM to about 100 mM, from about 10 mM to about 80 mM, from about 10 mM to about 60 mM, from about 10 mM to about 40 mM, from about 10 mM to about 30 mM, from about 10 mM to about 20 mM, from about 20 mM to about 100 mM, from about 30 mM to about 100 mM, from about 40 mM to about 100 mM, from about 50 mM to about 100 mM, from about 60 mM to about 100 mM, or from about 80 mM to about 100 mM.

[0083] Suitable pH ranges of the reducing buffer include from about 6.8 to about 8.0. In some embodiments, the pH of the reducing buffer can be about 6.8. In some embodiments, the pH of the reducing buffer can be about 6.9. In some embodiments, the pH of the reducing buffer can be about 7.0. In some embodiments, the pH of the reducing buffer can be about 7.1. In some embodiments, the pH of the reducing buffer can be about 7.2. In some embodiments, the pH of the reducing buffer can be about 7.3. In some embodiments, the pH of the reducing buffer can be about 7.4. In some embodiments, the pH of the reducing buffer can be about 7.5. In some embodiments, the pH of the reducing buffer can be about 7.6. In some embodiments, the pH of the reducing buffer can be about 7.7. In some embodiments, the pH of the reducing buffer can be about 7.8. In some embodiments, the pH of the reducing buffer can be about 7.9. In some embodiments, the pH of the reducing buffer can be about 8.0.

[0084] The immunoglobulin can be incubated with the reducing buffer for about 12 hours to about 96 hours, from about 18 hours to about 96 hours, from about 24 hours to about 96 hours, from about 30 hours to about 96 hours, from about 36 hours to about 96 hours, from about 42 hours to about 96 hours, from about 48 hours to about 96 hours, from about 54 hours to about 96 hours, from about 60 hours to about 96 hours, from about 12 hours to about 90 hours, from about 12 hours to about 84 hours, from about 12 hours to about 78 hours, from about 12 hours to about 72 hours, from about 12 hours to about 66 hours, from about 12 hours to about 60 hours, from about 12 hours to about 54 hours, from about 12 hours to about 48 hours, from about 12 hours to about 42 hours, from about 12 hours to about 36 hours, or from about 12 hours to about 30 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 12 hours. In some embodiments, the immunoglobulin can be incubated with the

reducing buffer for about 18 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 24 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 30 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 36 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 42 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 48 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 54 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 60 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 66 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 72 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 78 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 84 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 90 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for about 96 hours. In some embodiments, the immunoglobulin can be incubated with the reducing buffer for greater than 96 hours.

[0085] Suitable oxidizing buffers include, but are not limited to, Tris-based, glutamine-based, arginine-based or other amino acid-based, or primary amine-based buffers. The concentration of oxidizing buffer can range from about 20 mM to about 100 mM, from about 40 mM to about 100 mM, from about 60 mM to about 100 mM, from about 80 mM to about 100 mM, from about 20 mM to about 80 mM, from about 20 mM to about 60 mM, or from about 20 mM to about 40 mM. The concentration of oxidizing buffer can be about 20 mM. The concentration of oxidizing buffer can be about 25 mM. The concentration of oxidizing buffer can be about 30 mM. The concentration of oxidizing buffer can be about 40 mM. The concentration of oxidizing buffer can be about 50 mM. The concentration of oxidizing buffer can be about 60 mM. The concentration of oxidizing buffer can be about 70 mM. The concentration of oxidizing buffer can be about 80 mM. The concentration of oxidizing buffer can be about 90 mM. The concentration of reducing agent can be about 100 mM.

[0086] The immunoglobulin can be incubated with the oxidizing buffer for about 24 hours to about 96 hours, from about 30 hours to about 96 hours, from about 36 hours to about 96 hours, from about 42 hours to about 96 hours, from about 48 hours to about 96 hours, from about 54 hours to about 96 hours, from about 60 hours to about 96 hours, from about 24 hours to about

90 hours, from about 24 hours to about 84 hours, from about 24 hours to about 78 hours, from about 24 hours to about 72 hours, from about 24 hours to about 66 hours, from about 24 hours to about 60 hours, from about 24 hours to about 54 hours, from about 24 hours to about 48 hours, from about 24 hours to about 42 hours, or from about 24 hours to about 36 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 24 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 30 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 36 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 42 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 48 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 54 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 60 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 66 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 72 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 78 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 84 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 90 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for about 96 hours. In some embodiments, the immunoglobulin can be incubated with the oxidizing buffer for greater than 96 hours.

[0087] Suitable pH ranges of the oxidizing buffer include from about 7.5 to about 9.0. In some embodiments, the pH of the oxidizing buffer can be about 7.5. In some embodiments, the pH of the oxidizing buffer can be about 7.6. In some embodiments, the pH of the oxidizing buffer can be about 7.7. In some embodiments, the pH of the oxidizing buffer can be about 7.8. In some embodiments, the pH of the oxidizing buffer can be about 7.9. In some embodiments, the pH of the oxidizing buffer can be about 8.0. In some embodiments, the pH of the oxidizing buffer can be about 8.1. In some embodiments, the pH of the oxidizing buffer can be about 8.2. In some embodiments, the pH of the oxidizing buffer can be about 8.3. In some embodiments, the pH of the oxidizing buffer can be about 8.4. In some embodiments, the pH of the oxidizing buffer can be about 8.5. In some embodiments, the pH of the oxidizing buffer can be about 8.6. In some embodiments, the pH of the oxidizing buffer can be about 8.7. In some embodiments,

the pH of the oxidizing buffer can be about 8.8. In some embodiments, the pH of the oxidizing buffer can be about 8.9. In some embodiments, the pH of the oxidizing buffer can be about 9.0.

[0088] The method can further comprise immobilizing the immunoglobulin on a matrix prior to the incubating with the reducing agent and eluting the immunoglobulin from the matrix following the incubating with the oxidizing buffer. Suitable matrices include any surface to which an immunoglobulin can be bound and eluted from including, but not limited to, protein A, protein G, protein L, anti-Fab antibody, anti-Fc antibody, anti-Mab-based affinity supports, and strong cation exchange resins. In some embodiments, the matrix can be protein A. In some embodiments, the matrix can be protein G. In some embodiments, the matrix can be protein L. In some embodiments, the matrix can comprise an anti-Fab antibody. In some embodiments, the matrix can comprise an anti-Fc antibody. In some embodiments, the matrix can comprise an anti-MAb. In some embodiments, the matrix can comprise a strong cation exchange resin.

[0089] The disclosed methods for decapping an immunoglobulin can comprise: equilibrating a matrix; immobilizing the immunoglobulin onto the matrix; incubating the immobilized immunoglobulin on the matrix with a reducing buffer to remove capping group; incubating the immobilized immunoglobulin on the matrix with an oxidizing buffer; eluting the immunoglobulin from the matrix, and neutralizing the immunoglobulin.

[0090] Those skilled in the art would recognize that the buffer, concentration, pH, and time for eluting the immunoglobulin from the matrix will depend, at least in part, on the matrix. For example, in embodiments wherein the matrix is protein A, the immunoglobulin can be eluted from the protein A using glycine (for example, 0.1 M at pH 2.9). In some embodiments, the eluting can be performed in a low pH buffer.

[0091] Neutralizing the immunoglobulin can comprise incubating the immunoglobulin in a Tris-based, sodium phosphate-based, or potassium phosphate-based buffer (herein referred to as “neutralization buffer”). The neutralization buffer can have a concentration from about 0.5 M to about 2 M, and a pH from about 8.0 to about 9.5.

[0092] Conjugation can be performed by dissolving a thiol-reactive compound in a dissolution solution and incubating the dissolved thiol-reactive compound with the immunoglobulin in a conjugation buffer.

[0093] For aqueous-insoluble thiol-reactive compounds, which may include, but are not be limited to, maleimide-based compounds, suitable dissolution solutions include organic, water-miscible solvents such as dimethylsulfoxide (DMSO). For aqueous-soluble thiol-reactive

compounds, suitable dissolution solutions include, but are not limited to, water or buffered aqueous solutions, such as phosphate-buffered saline, pH 7.2 (1 x PBS).

[0094] Suitable concentrations of thiol-reactive compounds include from about 5 mM to about 100 mM, from about 10 mM to about 100 mM, from about 25 mM to about 100 mM, from about 40 mM to about 100 mM, from about 55 mM to about 100 mM, from about 70 mM to about 100 mM, from about 10 mM to about 90 mM, from about 10 mM to about 75 mM, from about 10 mM to about 60 mM, from about 10 mM to about 50 mM, from about 10 mM to about 40 mM, or from about 10 mM to about 30 mM. In some embodiments, the concentration of the thiol-reactive compound can be about 10 mM. In some embodiments, the concentration of the thiol-reactive compound can be about 20 mM. In some embodiments, the concentration of the thiol-reactive compound can be about 30 mM. In some embodiments, the concentration of the thiol-reactive compound can be about 40 mM. In some embodiments, the concentration of the thiol-reactive compound can be about 50 mM. In some embodiments, the concentration of the thiol-reactive compound can be about 60 mM. In some embodiments, the concentration of the thiol-reactive compound can be about 70 mM. In some embodiments, the concentration of the thiol-reactive compound can be about 80 mM. In some embodiments, the concentration of the thiol-reactive compound can be about 90 mM. In some embodiments, the concentration of the thiol-reactive compound can be about 100 mM.

[0095] Suitable concentrations of immunoglobulin include from about 0.1 mg/ml to about 20 mg/ml, from about 0.5 mg/ml to about 20 mg/ml, from about 1 mg/ml to about 20 mg/ml, from about 5 mg/ml to about 20 mg/ml, from about 10 mg/ml to about 20 mg/ml, from about 0.1 mg/ml to about 15 mg/ml, from about 0.1 mg/ml to about 12 mg/ml, from about 0.1 mg/ml to about 10 mg/ml, from about 0.1 mg/ml to about 5 mg/ml, or from about 0.1 mg/ml to about 2 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 0.1 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 0.5 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 1 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 2 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 5 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 10 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 15 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 20 mg/ml.

[0096] Suitable ratios of thiol-reactive compound:immunoglobulin include from about 3:1 to 20:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be

3:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 4:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 5:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 6:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 7:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 8:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 9:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 10:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 11:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 12:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 13:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 14:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 15:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 16:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 17:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 18:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 19:1. In some embodiments, the ratio of thiol-reactive compound:immunoglobulin can be 20:1.

[0097] The incubating can be performed in a number of suitable conjugation buffers including, for example, 1xPBS, pH 7.2, sodium phosphate, potassium phosphate, sodium borate, and HEPES, to name a few. The concentration of conjugation buffer include from about 5 mM to about 100 mM, from about 10 mM to about 100 mM, from about 20 mM to about 100 mM, from about 30 mM to about 100 mM, from about 45 mM to about 100 mM, from about 60 mM to about 100 mM, from about 75 mM to about 100 mM, from about 10 mM to about 90 mM, from about 10 mM to about 75 mM, from about 10 mM to about 60 mM, from about 10 mM to about 45 mM, or from about 10 mM to about 30 mM. In some embodiments, the concentration of the conjugation buffer can be about 10 mM. In some embodiments, the concentration of the conjugation buffer can be about 20 mM. In some embodiments, the concentration of the conjugation buffer can be about 30 mM. In some embodiments, the concentration of the conjugation buffer can be about 40 mM. In some embodiments, the concentration of the conjugation buffer can be about 50 mM. In some embodiments, the concentration of the conjugation buffer can be about 60 mM. In some embodiments, the concentration of the conjugation buffer can be about 70 mM. In some embodiments, the concentration of the conjugation buffer can be about 80 mM. In some embodiments, the concentration of the

conjugation buffer can be about 90 mM. In some embodiments, the concentration of the conjugation buffer can be about 100 mM.

[0098] The conjugation buffer can further include sodium chloride. Suitable concentrations of sodium chloride include from about 0 mM to about 500 mM, from about 25 mM to about 500 mM, from about 50 mM to about 500 mM, from about 75 mM to about 500 mM, from about 100 mM to about 500 mM, from about 150 mM to about 500 mM, from about 200 mM to about 500 mM, from about 250 mM to about 500 mM, from about 300 mM to about 500 mM, from about 350 mM to about 500 mM, from about 400 mM to about 500 mM, from about 0 mM to about 400 mM, from about 0 mM to about 350 mM, from about 0 mM to about 300 mM, from about 0 mM to about 250 mM, from about 0 mM to about 200 mM, from about 0 mM to about 150 mM, from about 0 mM to about 100 mM, from about 0 mM to about 50 mM, or from about 0 mM to about 25 mM. In some embodiments, the concentration of sodium chloride can be about 25 mM. In some embodiments, the concentration of sodium chloride can be about 50 mM. In some embodiments, the concentration of sodium chloride can be about 75 mM. In some embodiments, the concentration of sodium chloride can be about 100 mM. In some embodiments, the concentration of sodium chloride can be about 150 mM. In some embodiments, the concentration of sodium chloride can be about 200 mM. In some embodiments, the concentration of sodium chloride can be about 250 mM. In some embodiments, the concentration of sodium chloride can be about 300 mM. In some embodiments, the concentration of sodium chloride can be about 350 mM. In some embodiments, the concentration of sodium chloride can be about 400 mM. In some embodiments, the concentration of sodium chloride can be about 500 mM.

[0099] The pH of the conjugation buffer can be from about 6.5 to about 8.5. In some embodiments, the pH of the conjugation buffer can be about 6.5. In some embodiments, the pH of the conjugation buffer can be about 6.6. In some embodiments, the pH of the conjugation buffer can be about 6.7. In some embodiments, the pH of the conjugation buffer can be about 6.8. In some embodiments, the pH of the conjugation buffer can be about 6.9. In some embodiments, the pH of the conjugation buffer can be about 7.0. In some embodiments, the pH of the conjugation buffer can be about 7.1. In some embodiments, the pH of the conjugation buffer can be about 7.2. In some embodiments, the pH of the conjugation buffer can be about 7.3. In some embodiments, the pH of the conjugation buffer can be about 7.4. In some embodiments, the pH of the conjugation buffer can be about 7.5. In some embodiments, the pH of the conjugation buffer can be about 7.6. In some embodiments, the pH of the conjugation

buffer can be about 7.7. In some embodiments, the pH of the conjugation buffer can be about 7.8. In some embodiments, the pH of the conjugation buffer can be about 7.9. In some embodiments, the pH of the conjugation buffer can be about 8.0. In some embodiments, the pH of the conjugation buffer can be about 8.1. In some embodiments, the pH of the conjugation buffer can be about 8.2. In some embodiments, the pH of the conjugation buffer can be about 8.3. In some embodiments, the pH of the conjugation buffer can be about 8.4. In some embodiments, the pH of the conjugation buffer can be about 8.5.

[00100] To facilitate solubility of the thiol-reactive compound in the conjugation buffer, a final concentration of organic, water-miscible solvent in the conjugation buffer may be from about 0% to about 20%, from about 2% to about 20%, from about 5% to about 20%, from about 8% to about 20%, from about 11% to about 20%, from about 16% to about 20%, from about 0% to about 18%, from about 0% to about 15%, from about 0% to about 12%, from about 0% to about 10%, from about 0% to about 8%, from about 0% to about 6%, or from about 0% to about 2%.

[0101] The conjugation buffer can further comprise propylene glycol to facilitate solubility of the thiol-reactive compound in the conjugation buffer. Suitable concentrations of propylene glycol include from about 10% to about 50%, from about 20% to about 50%, from about 30% to about 50%, from about 40% to about 50%, from about 10% to about 40%, from about 10% to about 30%, or from about 10% to about 20%. In some embodiments, the concentration of propylene glycol can be about 10%. In some embodiments, the concentration of propylene glycol can be about 20%. In some embodiments, the concentration of propylene glycol can be about 30%. In some embodiments, the concentration of propylene glycol can be about 40%. In some embodiments, the concentration of propylene glycol can be about 50%.

[0102] The conjugation buffer can further comprise a non-ionic detergent to facilitate solubility of the conjugated immunoglobulin in the conjugation buffer. Exemplary non-ionic detergents include, but are not limited to, polysorbate-20 or polysorbate-80. Suitable concentrations of non-ionic detergent include from about 0% to about 1%, from about 0.1% to about 1%, from about 0.3% to about 1%, from about 0.5% to about 1%, from about 0.7% to about 1%, from about 0% to about 0.8%, from about 0% to about 0.6%, from about 0% to about 0.4%, or from about 0% to about 0.2%. In some embodiments, the concentration of non-ionic detergent can be about 0.1%. In some embodiments, the concentration of non-ionic detergent can be about 0.2%. In some embodiments, the concentration of non-ionic detergent can be about 0.3%. In some embodiments, the concentration of non-ionic detergent can be about 0.4%. In

some embodiments, the concentration of non-ionic detergent can be about 0.5%. In some embodiments, the concentration of non-ionic detergent can be about 0.6%. In some embodiments, the concentration of non-ionic detergent can be about 0.7%. In some embodiments, the concentration of non-ionic detergent can be about 0.8%. In some embodiments, the concentration of non-ionic detergent can be about 0.9%. In some embodiments, the concentration of non-ionic detergent can be about 1.0%.

[0103] The incubating can be performed for about 2 hours to about 48 hours, for about 6 hours to about 48 hours, for about 12 hours to about 48 hours, for about 24 hours to about 48 hours, for about 30 hours to about 48 hours, for about 36 hours to about 48 hours, for about 42 hours to about 48 hours, for about 2 hours to about 42 hours, for about 2 hours to about 36 hours, for about 2 hours to about 30 hours, for about 2 hours to about 24 hours, for about 2 hours to about 18 hours, for about 2 hours to about 12 hours, or for about 2 hours to about 6 hours. In some embodiments, the incubating can be performed for 2 hours. In some embodiments, the incubating can be performed for 6 hours. In some embodiments, the incubating can be performed for 12 hours. In some embodiments, the incubating can be performed for 18 hours. In some embodiments, the incubating can be performed for 24 hours. In some embodiments, the incubating can be performed for 30 hours. In some embodiments, the incubating can be performed for 36 hours. In some embodiments, the incubating can be performed for 42 hours. In some embodiments, the incubating can be performed for 48 hours.

[0104] The temperature of the incubating can be from about 18°C to about 37°C, from about 20°C to about 37°C, from about 22°C to about 37°C, from about 24°C to about 37°C, from about 26°C to about 37°C, from about 28°C to about 37°C, from about 30°C to about 37°C, from about 32°C to about 37°C, from about 34°C to about 37°C, from about 18°C to about 34°C, from about 18°C to about 32°C, from about 18°C to about 30°C, from about 18°C to about 28°C, from about 18°C to about 26°C, or from about 18°C to about 24°C. In some embodiments, the incubating can be performed at 18°C. In some embodiments, the incubating can be performed at 20°C. In some embodiments, the incubating can be performed at 22°C. In some embodiments, the incubating can be performed at 24°C. In some embodiments, the incubating can be performed at 26°C. In some embodiments, the incubating can be performed at 28°C. In some embodiments, the incubating can be performed at 30°C. In some embodiments, the incubating can be performed at 32°C. In some embodiments, the incubating can be performed at 34°C. In some embodiments, the incubating can be performed at 37°C.

[0105] Unincorporated thiol-reactive compounds can be separated from the conjugated immunoglobulin by desalting chromatography using a number of suitable resins including, but not limited to, G-25 resin, G-50 resin, Biogel P10, or other resins with exclusion limits of ranges 5,000-10,000 Da. Chromatography can be performed in column format or spin-column format, depending on scale. Suitable buffers for desalting include, for example, 1xPBS, sodium phosphate, potassium phosphate, sodium borate, or HEPES-based buffers may substitute for 1x PBS.

[0106] In an exemplary embodiment, the conjugating can be performed by dissolving a maleimido-based thiol reactive compound in 100% dimethylsulfoxide (DMSO) at a final concentration of thiol-reactive compound of 10 mM. The dissolved thiol-reactive compound can then be incubated with an immunoglobulin at an immunoglobulin concentration of 5 mg/ml in 1xPBS, pH 7.2 at a molar ratio of 5:1 thiol-reactive compound:immunoglobulin and mixed thoroughly. The incubating can be performed for 24 hours at 22°C. Unincorporated thiol-reactive compound can be removed from the conjugated immunoglobulin by desalting chromatography using G-25 resin with 1x PBS as running buffer.

[0107] Preferably, the thiol-reactive compound is conjugated to the Cys80 via the thiol-reactive group. Thiol-reactive groups include haloacetyls, maleimides, aziridines, acryloyls, arylating agents, vinylsulfones, pyridyl disulfides, TNB-thiols and disulfide reducing agents. In some embodiments, the thiol-reactive group can comprise a maleimide. In some embodiments, the thiol-reactive group can comprise a haloacetyl. In some embodiments, the thiol-reactive group can comprise an aziridine. In some embodiments, the thiol-reactive group can comprise an acryloyl. In some embodiments, the thiol-reactive group can comprise an arylating agent. In some embodiments, the thiol-reactive group can comprise a vinylsulfone. In some embodiments, the thiol-reactive group can comprise a pyridyl disulfide. In some embodiments, the thiol-reactive group can comprise a TNB-thiol. In some embodiments, the thiol-reactive group can comprise a disulfide reducing agent.

[0108] Thiol reactive groups can be derived from a number of suitable reagents including iodoacetamides, maleimides, benzylic halides and bromomethylketones, which can react by S-alkylation of thiols to generate stable thioether products.

[0109] The thiol-reactive group can be appended to a linker. Linkers can be non-cleavable linkers or cleavable linkers. Exemplary linkers include, for example, disulfide containing linkers, acetal-based linkers, and ketal-based linkers. In some aspects, the linker can be a non-cleavable linker. Suitable non-cleavable linkers include, but are not limited to,

polyethylene glycol (PEG) or an alkyl. In some embodiments, the linker can comprise PEG. In some aspects, the linker can be a cleavable linker. Suitable cleavable linkers include, for example, valine-citrulline-para aminobenzyl. In some aspects, the linker can be a disulfide containing linker. In some aspects, the linker can be an acetal-based linker. In some aspects, the linker can be a ketal-based linker. Examples of linkers covalently appended to a thiol-reactive group are provided, for example, in U.S. Publ. No. 20140050746.

[0110] The thiol-reactive compound can further comprise a functional agent. Suitable functional agents include, for example, fluorophores, fluorescent dyes, polypeptides, immunoglobulins, antibiotics, nucleic acids, radionuclides, chemical linkers, small molecules, chelators, lipids, and drugs. In some aspects, the functional agent can comprise a fluorophore. In some aspects, the functional agent can comprise a fluorescent dye. In some aspects, the functional agent can comprise a polypeptide. In some aspects, the functional agent can comprise an immunoglobulin. In some aspects, the functional agent can comprise an antibiotic. In some aspects, the functional agent can comprise a nucleic acid (such as DNA or RNA). In some aspects, the functional agent can comprise a radionuclide. In some aspects, the functional agent can comprise a chemical linker (for example dibenzylcyclooctyne (DBCO) or azide). In some aspects, the functional agent can comprise a small molecule. In some aspects, the functional agent can comprise a chelator (for example, DOTA, CHX-A"-DTPA, NOTA, among others). In some aspects, the functional agent can comprise a lipid. In some aspects, the functional agent can comprise a drug. In some aspects, the functional agent can comprise a combination of any of the above listed functional agents.

[0111] The thiol-reactive compound (i.e. a first thiol-reactive compound) can be bound to a second thiol-reactive compound, the second thiol-reactive compound being bound to a second immunoglobulin having a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at amino acid position 80 ("Cys80²"), wherein the second thiol-reactive compound comprises a second thiol-reactive group bound to the Cys80². For example, the first thiol-reactive compound and the second thiol-reactive compounds can have a first and second chemical linker as the first and second functional agents, respectively. The first and second chemical linkers can be bound to each other by a number of suitable means including, for example, by click chemistry.

[0112] In preferred embodiments, the Cys80 can be unpaired. Suitable means for unpairing Cys80 include, for example, chimerizing a light chain variable region having Cys80 with a constant domain having an amino acid residue other than cysteine at position 171.

[0113] The disclosed methods can be performed on a chimerized immunoglobulin. Thus, in some embodiments, the immunoglobulin can be a chimerized immunoglobulin. In embodiments wherein the immunoglobulin is chimerized, the methods for generating a conjugated immunoglobulin can comprise: decapping a Cys80 in a light chain variable region of a chimerized immunoglobulin, wherein the chimerized immunoglobulin comprises a heavy chain variable region and the light chain variable region; and conjugating a thiol-reactive compound to the Cys80, wherein the thiol-reactive compound comprises a thiol-reactive group.

[0114] Alternatively, the disclosed methods can further comprise chimerizing an immunoglobulin prior to the decapping. For example, the methods for generating a conjugated immunoglobulin can comprise: chimerizing an immunoglobulin comprising a heavy chain variable region and a light chain variable region, the light chain variable region having a Cys80; decapping the Cys80; and conjugating a thiol-reactive compound to the Cys80, wherein the thiol-reactive compound comprises a thiol-reactive group.

[0115] The disclosed methods can be performed on a humanized immunoglobulin. Thus, in some embodiments, the immunoglobulin can be a humanized immunoglobulin. In embodiments wherein the immunoglobulin is humanized, the methods for generating a conjugated immunoglobulin can comprise: decapping a Cys80 in a light chain variable region of a humanized immunoglobulin, wherein the humanized immunoglobulin comprises a heavy chain variable region and the light chain variable region; and conjugating a thiol-reactive compound to the Cys80, wherein the thiol-reactive compound comprises a thiol-reactive group.

[0116] Alternatively, the disclosed methods can further comprise humanizing an immunoglobulin prior to the decapping. For example, the methods for generating a conjugated immunoglobulin can comprise: humanizing an immunoglobulin comprising a heavy chain variable region and a light chain variable region, the light chain variable region having a Cys80; decapping the Cys80; and conjugating a thiol-reactive compound to the Cys80, wherein the thiol-reactive compound comprises a thiol-reactive group.

[0117] The methods can further comprise substituting an amino acid at position 83 with an amino acid residue other than Phe, Lys, or Cys. In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with alanine ("Ala83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with valine ("Val83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with isoleucine ("Ile83"). In some aspects, the methods can comprise substituting the phenylalanine

at position 83 of the light chain variable region with threonine ("Thr83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with arginine ("Arg83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with asparagine ("Asn83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with aspartic acid ("Asp83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with glutamic acid ("Glu83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with glutamine ("Gln83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with glycine ("Gly83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with histidine ("His83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with leucine ("Leu83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with methionine ("Met83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with proline ("Pro83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with serine ("Ser83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with tryptophan ("Trp83"). In some aspects, the methods can comprise substituting the phenylalanine at position 83 of the light chain variable region with tyrosine ("Tyr83"). In some embodiments, the methods can comprise substituting an amino acid at position 83 with a polar or hydrophobic amino acid including, but not limited to, alanine, valine, isoleucine, or threonine.

[0118] The amino acid residue other than Phe, Lys, or Cys at amino acid position 83 in combination with the disclosed decapping methods can decrease the aggregation, and increase the Cys80 conjugation efficiency, of the immunoglobulin. Suitable immunoglobulin aggregation achieved by the disclosed methods include, for example, less than about 5%, less than about 7%, less than about 10%, less than about 12%, less than about 15%, less than about 17%, less than about 20%, less than about 22%, or less than about 25%. Suitable conjugation efficiencies achieved by the disclosed methods include, for example, greater than about 70%, greater than about 73%, greater than about 76%, greater than about 79%, greater than about 82%, greater than

about 85%, greater than about 88%, greater than about 91%, greater than about 94%, greater than about 97%, or greater than about 99%.

Methods of generating antigen-binding molecules

[0119] Also provided herein are methods for generating an antigen-binding molecule, the method comprising incubating a first conjugated immunoglobulin with a second conjugated immunoglobulin to generate the antigen-binding molecule, wherein:

the first conjugated immunoglobulin comprises a first heavy chain variable region and a first light chain variable region, the first light chain variable region having a cysteine at position 80 ("Cys80¹") wherein Cys80¹ is conjugated to a first thiol-reactive compound comprising a first thiol-reactive group; and

the second conjugated immunoglobulin comprises a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at position 80 ("Cys80²") wherein Cys80² is conjugated to a second thiol-reactive compound comprising a second thiol-reactive group.

[0120] Antigen-binding molecules include multivalent and/or multispecific antigen-binding molecules. For example, antigen-binding molecules include bivalent, trivalent, and tetravalent antigen-binding molecules that are monospecific or bispecific. In some aspects, the antigen-binding molecule can be bivalent and monospecific. In some aspects, the antigen-binding molecule can be bivalent and bispecific. In some aspects, the antigen-binding molecule can be trivalent and monospecific. In some aspects, the antigen-binding molecule can be trivalent and bispecific. In some aspects, the antigen-binding molecule can be tetravalent and monospecific. In some aspects, the antigen-binding molecule can be tetravalent and bispecific. In some aspects, the valency can be greater than tetravalent. In some aspects, the specificity can be greater than bispecific.

[0121] The Cys80¹, the Cys80², or both, can be unpaired. Suitable means for unpairing Cys80 include, for example, chimerizing a light chain variable region having Cys80 with a constant domain having an amino acid residue other than cysteine at position 171.

[0122] In some aspects, the methods can further comprise decapping the Cys80¹. In some aspects, the methods can further comprise decapping the Cys80². In other aspects, the methods can further comprise decapping the Cys80¹ and Cys80².

[0123] The decapping can comprise incubating the first immunoglobulin, the second immunoglobulin, or both, with a reducing buffer followed by incubating the first

immunoglobulin, the second immunoglobulin, or both, with an oxidizing buffer. In some aspects of the methods for generating antigen-binding molecules, the decapping can further comprise immobilizing the first immunoglobulin, the second immunoglobulin, or both on a matrix prior to the incubating with the reducing buffer and eluting the first immunoglobulin, the second immunoglobulin, or both from the matrix following the incubating with the oxidizing buffer.

[0124] Suitable decapping and conjugating conditions, including reducing buffers, oxidizing buffers, concentrations, pHs, times and matrices, are disclosed in the section entitled “*generation of conjugated immunoglobulins*” and are equally applicable herein.

[0125] In some aspects, the methods can further comprise conjugating a first thiol-reactive compound to the Cys80¹, wherein the first thiol-reactive compound comprises a first thiol-reactive group. In some aspects, the methods can further comprise conjugating a second thiol-reactive compound to the Cys80², wherein the second thiol-reactive compound comprises a second thiol-reactive group. In yet other aspects, the methods can further comprise conjugating a first thiol-reactive compound to the Cys80¹ and a second thiol-reactive compound to the Cys80², wherein the first thiol-reactive compound comprises a first thiol-reactive group and the second thiol-reactive compound comprises a second thiol-reactive group.

[0126] The methods can further comprise both decapping and conjugating. For example, the methods can further comprise, prior to the incubating step,

decapping the Cys80¹, Cys80², or both; and

conjugating a first thiol-reactive compound to the Cys80¹, a second thiol-reactive compound to the Cys80², or both, wherein the first thiol-reactive compound comprises a first thiol-reactive group and the second thiol-reactive compound comprises a second thiol-reactive group.

[0127] The first immunoglobulin, the second immunoglobulin, or both, can be chimerized. Conversely, the methods can further comprise chimerizing the first immunoglobulin, chimerizing the second immunoglobulin, or chimerizing both the first immunoglobulin and the second immunoglobulin. For example, and without intending to be limiting, the methods can further comprise, prior to the incubating step:

chimerizing a first immunoglobulin comprising a Cys80¹ to generate a first chimeric immunoglobulin;

chimerizing the second immunoglobulin comprising a Cys80² to generate a second chimeric immunoglobulin;

decapping the Cys80¹, Cys80², or both; and

conjugating a first thiol-reactive compound to the Cys80¹, a second thiol-reactive compound to the Cys80², or both, wherein the first thiol-reactive compound comprises a first thiol-reactive group and the second thiol-reactive compound comprises a second thiol-reactive group.

[0128] The first immunoglobulin, the second immunoglobulin, or both, can be humanized. Conversely, the methods can further comprise humanizing the first immunoglobulin, humanizing the second immunoglobulin, or humanizing both the first immunoglobulin and the second immunoglobulin. For example, and without intending to be limiting, the methods can further comprise, prior to the incubating step:

humanizing a first immunoglobulin comprising a Cys80¹ to generate a first humanized immunoglobulin;

humanizing the second immunoglobulin comprising a Cys80² to generate a second humanized immunoglobulin;

decapping the Cys80¹, Cys80², or both; and

conjugating a first thiol-reactive compound to the Cys80¹, a second thiol-reactive compound to the Cys80², or both, wherein the first thiol-reactive compound comprises a first thiol-reactive group and the second thiol-reactive compound comprises a second thiol-reactive group.

[0129] Preferably, the first and second thiol-reactive compounds are conjugated to the Cys80¹ and Cys80², respectively, via the first thiol-reactive group and the second thiol-reactive group. Suitable, thiol-reactive groups include haloacetyls, maleimides, aziridines, acryloyls, arylating agents, vinylsulfones, pyridyl disulfides, TNB-thiols and disulfide reducing agents. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise a maleimide. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise a haloacetyl. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise an aziridine. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise an acryloyl. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise an arylating agent. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise a vinylsulfone. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise a pyridyl disulfide. In some embodiments, the first thiol-reactive group, the second-

thiol reactive group, or both, can comprise a TNB-thiol. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise a disulfide reducing agent.

[0130] The first thiol-reactive group, the second-thiol reactive group, or both can be appended to a linker. In some aspects, the first thiol-reactive group can be appended to a linker (“first linker”). In some aspects, the second thiol-reactive group can be appended to a linker (“second linker”). In yet other aspects the first thiol-reactive group can be appended to a first linker and the second thiol-reactive group can be appended to a second linker. Suitable first and second linkers can be non-cleavable linkers or cleavable linkers. Exemplary first and second linkers include, for example, disulfide containing linkers, acetal-based linkers, and ketal-based linkers. In some aspects, the first linker, second linker, or both, can be a non-cleavable linker. Suitable non-cleavable linkers include, but are not limited to, polyethylene glycol (PEG) or an alkyl. In some embodiments, the first linker, second linker, or both, can comprise PEG. In some aspects, the first linker, second linker, or both, can be a cleavable linker. Suitable cleavable linkers include, for example, valine-citrulline-para aminobenzyl. In some aspects, the first linker, second linker, or both, can be a disulfide containing linker. In some aspects, the first linker, second linker, or both can be an acetal-based linker. In some aspects, the first linker, second linker, or both, can be a ketal-based linker. Examples of linkers covalently appended to a thiol-reactive group are provided, for example, in U.S. Publ. No. 20140050746.

[0131] The first thiol-reactive compound, the second thiol-reactive compound, or both, can further comprise a functional agent. In some aspects, the first thiol-reactive compound can further comprise a functional agent (“first functional agent”). In some aspects, the second thiol-reactive compound can further comprise a functional agent (“second functional agent”). In yet other aspects, the first thiol-reactive compound can further comprise a first functional agent and the second thiol-reactive compound can further comprise a second functional agent.

[0132] Suitable functional agents include, for example, chemical linkers. Preferably, the chemical linker of the first thiol-reactive compound (“first chemical linker”) and the chemical linker of the second thiol-reactive compound (“second chemical linker”) can be coupled. For example, and without intent to be limiting, one of the first or second chemical linkers can be dibenzylcyclooctyne (DBCO) and the other of the first or second chemical linkers can be azide. In some embodiments, for example, the first chemical linker can be DBCO and the second chemical linker can be azide. Conversely, the first chemical linker can be azide and the second chemical linker can be DBCO. The DBCO and azide can be coupled, this resulting in the conjugation of the first immunoglobulin and the second immunoglobulin. For example, the first

immunoglobulin and the second immunoglobulin can be conjugated to each other by click chemistry.

[0133] In an exemplary embodiment, thiol-reactive compounds can include maleimido-PEG4-azide and maleimido-PEG4-dibenzocyclooctyne. In some aspects, for example, the first thiol-reactive compound can be maleimido-PEG4-azide and the second thiol-reactive compound can be maleimido-PEG4-dibenzocyclooctyne. In some aspects, the first thiol-reactive compound can be maleimido-PEG4-dibenzocyclooctyne and the second thiol-reactive compound can be maleimido-PEG4-azide.

[0134] The first immunoglobulin, second immunoglobulin, or both, can be Fabs. In some embodiments, the first immunoglobulin can be a Fab (“first Fab”). In some embodiments, the second immunoglobulin can be a Fab (“second Fab”). In yet other embodiments, the first immunoglobulin can be a first Fab and the second immunoglobulin can be a second Fab.

[0135] In some embodiments, the methods comprise generating a first Fab, a second Fab, or both, prior to the incubating. Suitable techniques for generating Fabs are known in the art and include, for example, digesting a full or partial immunoglobulin to produce a Fab or recombinantly expressing the immunoglobulin as a Fab. For example, the methods of generating antigen-binding molecules can further comprise, prior to the incubating,

digesting a first immunoglobulin, a second immunoglobulin, or both, with papain to generate a first Fab, second Fab, or first and second Fab, wherein the first immunoglobulin comprises a first heavy chain and a first light chain, the first light chain having a cysteine at position 80 (“Cys80¹”), and wherein the second immunoglobulin comprises a second heavy chain and a second light chain, the second light chain having a cysteine at position 80 (“Cys80²”); or

recombinantly expressing a first Fab comprising a first heavy chain and a first light chain having a cysteine at position 80 (Cys80¹), recombinantly expressing a second Fab comprising a second heavy chain and a second light chain having a cysteine at position 80 (Cys80²), or both;

and conjugating the first Fab at Cys80¹ to a first thiol-reactive compound to generate a first conjugated Fab, conjugating the second Fab at Cys80² to a second thiol-reactive compound to generate a second conjugated Fab, or both.

[0136] The methods of generating antigen-binding molecules can further comprise substituting an amino acid at position 83 of the first light chain variable region with an amino acid residue other than Phe, Lys, or Cys. The methods of generating antigen-binding molecules

can further comprise substituting an amino acid at position 83 of the second light chain variable region with an amino acid residue other than Phe, Lys, or Cys. The methods of generating antigen-binding molecules can further comprise substituting an amino acid at position 83 of the first light chain variable region with an amino acid residue other than Phe, Lys, or Cys and substituting an amino acid at position 83 of the second light chain variable region with an amino acid residue other than Phe, Lys, or Cys.

[0137] In some aspects, the methods can comprise substituting an amino acid at position 83 of the first light chain variable region, substituting an amino acid at position 83 of the second light chain variable region, or substituting an amino acid at position 83 of the first light chain variable region and the second light chain variable region with alanine ("Ala83"), valine ("Val83"), isoleucine ("Ile83"), threonine ("Thr83"), arginine ("Arg83"), asparagine ("Asn83"), aspartic acid ("Asp83"), glutamic acid ("Glu83"), glutamine ("Gln83"), glycine ("Gly83"), histidine ("His83"), leucine ("Leu83"), methionine ("Met83"), proline ("Pro83"), serine ("Ser83"), tryptophan ("Trp83"), or tyrosine ("Tyr83").

[0138] The amino acid at position 83 in the first light chain variable region can be the same as the amino acid at position 83 in the second light chain variable region. Conversely, the amino acid at position 83 in the first light chain variable region can be different from the polar or hydrophobic amino acid at position 83 in the second light chain variable region. The amino acid at position 83 other than Phe, Lys, or Cys in the first light chain variable region and/or the amino acid at position 83 other than Phe, Lys, or Cys in the second light chain variable region can be a polar or hydrophobic amino acid including, but not limited to, alanine, valine, isoleucine, or threonine. In some aspects, the methods can comprise substituting an amino acid at position 83 of the first light chain variable region, substituting an amino acid at position 83 of the second light chain variable region, or substituting an amino acid at position 83 of the first light chain variable region and the second light chain variable region with valine ("Val83"). In some aspects, the methods can comprise substituting an amino acid at position 83 of the first light chain variable region, substituting an amino acid at position 83 of the second light chain variable region, or substituting an amino acid at position 83 of the first light chain variable region and the second light chain variable region with isoleucine ("Ile83"). In some aspects, the methods can comprise substituting an amino acid at position 83 of the first light chain variable region, substituting an amino acid at position 83 of the second light chain variable region, or substituting an amino acid at position 83 of the first light chain variable region and the second light chain variable region with threonine ("Thr83"). The polar or hydrophobic amino

acid at position 83 in the first light chain variable region can be the same as or different from the polar or hydrophobic amino acid at position 83 in the second light chain variable region.

[0139] Suitable light chain variable regions include, for example, a kappa light chain variable region. In some embodiments, the Cys80¹, Cys80², or both, can be present in the native light chain variable region. The first light chain variable region and second light chain variable region are derived from rabbit. Exemplary rabbits from which a first light chain variable region, second light chain variable region, or both, can be derived from include, but are not limited to, *Oryctolagus cuniculus*. In some aspects, for example, the light chain variable region(s) can be derived from a New Zealand White (NZW) rabbit. In other aspects, the light chain variable region(s) can be derived from a b9 rabbit.

Immunoglobulin components of conjugated immunoglobulins

[0140] Disclosed herein are immunoglobulins comprising a heavy chain variable region and a light chain variable region, the light chain variable region having a cysteine at position 80 ("Cys80") and an amino acid other than Phe, Lys, or Cys at position 83.

[0141] Suitable light chain variable regions include, for example, a kappa light chain variable region. The light chain variable region is derived from rabbit. In some embodiments, the Cys80 can be present in the native light chain variable region of the rabbit immunoglobulin. Exemplary rabbits from which a light chain variable region having a Cys80 can be derived include, but are not limited to, *Oryctolagus cuniculus*. In some aspects, for example, the light chain variable region can be derived from a New Zealand White (NZW) rabbit. In other aspects, the light chain variable region can be derived from a b9 rabbit.

[0142] The amino acid other than Phe, Lys, or Cys at position 83 includes alanine ("Ala83"), valine ("Val83"), isoleucine ("Ile83"), threonine ("Thr83"), arginine ("Arg83"), asparagine ("Asn83"), aspartic acid ("Asp83"), glutamic acid ("Glu83"), glutamine ("Gln83"), glycine ("Gly83"), histidine ("His83"), leucine ("Leu83"), methionine ("Met83"), proline ("Pro83"), serine ("Ser83"), tryptophan ("Trp83"), or tyrosine ("Tyr83").

[0143] In some embodiments, the amino acid other than Phe, Lys, or Cys at position 83 can be a polar or hydrophobic amino acid including, but not limited to, alanine, valine, isoleucine, or threonine. In some aspects, the polar or hydrophobic amino acid other than Phe at position 83 is alanine ("Ala83"). In some aspects, the polar or hydrophobic amino acid other than Phe at position 83 is valine ("Val83"). In some aspects, the polar or hydrophobic amino

acid other than Phe at position 83 is isoleucine (“Ile83”). In some aspects, the polar or hydrophobic amino acid other than Phe at position 83 is threonine (“Thr83”).

[0144] The Cys80 can be unpaired. For example, a light chain variable region having Cys80 can be chimerized with a constant domain having an amino acid residue other than cysteine at position 171.

[0145] Preferably, the Cys80 is decapped.

[0146] In some embodiments, the immunoglobulins can be chimerized. In other embodiments, the immunoglobulins can be humanized.

[0147] In some embodiment, the disclosed immunoglobulin immunospecifically binds to human CA9. In some embodiments, the immunoglobulin that immunospecifically binds to human CA9 comprises:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-141 of xi155D5HC (SEQ ID NO:52) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi155D5LC (SEQ ID NO:78);
- b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-144 of zu155D5HC (SEQ ID NO:54) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu155D5LC-3 (SEQ ID NO:84), zu155D5LC-4 (SEQ ID NO:86), zu155D5LC-5 (SEQ ID NO:88), zu155D5LC-6 (SEQ ID NO:90), zu155D5LC-7 (SEQ ID NO:92), zu155D5LC-huVK2-40 (SEQ ID NO:96), zu155D5LC-huVK4-1 (SEQ ID NO:100), zu155D5LC-huVK6-21 (SEQ ID NO:102), zu155D5LC-huVK6D-41 (SEQ ID NO:104); or zu155D5LC-huVK7-3-Glu81 (SEQ ID NO:106);
- c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-138 of xi1E4HC (SEQ ID NO:58) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi1E4LC (SEQ ID NO:110);
- d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-140 of zu1E4HC (SEQ ID NO:60) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu1E4LC-CXXA (SEQ ID NO:114);
- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi166B3HC (SEQ ID NO:74) and a light chain variable region

having an amino acid sequence at least 90% identical to amino acids 20-130 of xi166B3LC (SEQ ID NO:132); or

- f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu166B3HC (SEQ ID NO:76) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu166B3LC-CXXA (SEQ ID NO:136).

[0148] In some embodiments, the immunoglobulin that immunospecifically binds to human CA9 comprises:

- a. a heavy chain variable region as set forth as amino acids 20-141 of xi155D5HC (SEQ ID NO:52) and a light chain variable region as set forth as amino acids 20-130 of xi155D5LC (SEQ ID NO:78);
- b. a heavy chain variable region as set forth as amino acids 20-144 of zu155D5HC (SEQ ID NO:54) and a light chain variable region as set forth as amino acids 20-130 of zu155D5LC-3 (SEQ ID NO:84), zu155D5LC-4 (SEQ ID NO:86), zu155D5LC-5 (SEQ ID NO:88), zu155D5LC-6 (SEQ ID NO:90), zu155D5LC-7 (SEQ ID NO:92), zu155D5LC-huVK2-40 (SEQ ID NO:96), zu155D5LC-huVK4-1 (SEQ ID NO:100), zu155D5LC-huVK6-21 (SEQ ID NO:102), zu155D5LC-huVK6D-41 (SEQ ID NO:104); or zu155D5LC-huVK7-3-Glu81 (SEQ ID NO:106);
- c. a heavy chain variable region as set forth as amino acids 20-138 of xi1E4HC (SEQ ID NO:58) and a light chain variable as set forth as amino acids 20-130 of xi1E4LC (SEQ ID NO:110);
- d. a heavy chain variable region as set forth as amino acids 20-140 of zu1E4HC (SEQ ID NO:60) and a light chain variable region as set forth as amino acids 20-130 of zu1E4LC-CXXA (SEQ ID NO:114);
- e. a heavy chain variable region as set forth as amino acids 20-142 of xi166B3HC (SEQ ID NO:74) and a light chain variable region as set forth as amino acids 20-130 of xi166B3LC (SEQ ID NO:132); or
- f. a heavy chain variable region as set forth as amino acids 20-145 of zu166B3HC (SEQ ID NO:76) and a light chain variable region as set forth as amino acids 20-130 of zu166B3LC-CXXA (SEQ ID NO:136).

[0149] In some embodiments, the immunoglobulin that immunospecifically binds to human CA9 comprises:

- a. a heavy chain CDR1, CDR2, and CDR3 of xi155D5HC as set forth as SEQ ID NO:146, 148, and 150, respectively, and a light chain CDR1, CDR2, and CDR3 of xi155D5LC as set forth as SEQ ID NO:224, 226, and 228, respectively;
- b. a heavy chain CDR1, CDR2, and CDR3 of zu155D5HC as set forth as SEQ ID NO:152, 154, and 156, respectively, and a light chain CDR1, CDR2, and CDR3 of zu155D5LC-3 as set forth as SEQ ID NO:242, 244, and 246, respectively, zu155D5LC-4 as set forth as SEQ ID NO:248, 250, and 252, respectively, zu155D5LC-5 as set forth as SEQ ID NO:254, 256, and 258, respectively, zu155D5LC-6 as set forth as SEQ ID NO:260, 262, and 264, respectively, zu155D5LC-7 as set forth as SEQ ID NO:266, 268, and 270, respectively, zu155D5LC-huVK2-40 as set forth as SEQ ID NO 278, 280, and 282, respectively, zu155D5LC-huVK4-1 as set forth as SEQ ID NO 290, 292, and 294, respectively, zu155D5LC-huVK6-21 as set forth as SEQ ID NO 296, 298, and 300, respectively, zu155D5LC-huVK6D-41 as set forth as SEQ ID NO 302, 304, and 306, respectively; or zu155D5LC-huVK7-3-Glu81 as set forth as SEQ ID NO 308, 310, and 312, respectively;
- c. a heavy chain CDR1, CDR2, and CDR3 of xi1E4HC as set forth as SEQ ID NO 164, 166, and 168, respectively and a light chain CDR1, CDR2, and CDR3 of xi1E4LC as set forth as SEQ ID NO 320, 322, and 324, respectively;
- d. a heavy chain CDR1, CDR2, and CDR3 of zu1E4HC as set forth as SEQ ID NO:170, 172, and 174, respectively, and a light chain CDR1, CDR2, and CDR3 of zu1E4LC-CXXA as set forth as SEQ ID NO:332, 334, and 336, respectively;
- e. a heavy chain CDR1, CDR2, and CDR3 of xi166B3HC as set forth as SEQ ID NO:212, 214, and 216, respectively and a light chain CDR1, CDR2, and CDR3 of xi166B3LC as set forth as SEQ ID NO:386, 388, and 390, respectively; or
- f. a heavy chain CDR1, CDR2, and CDR3 of zu166B3HC as set forth as SEQ ID NO:218, 220, and 222, respectively, and a light chain CDR1, CDR2, and CDR3 of zu166B3LC-CXXA as set forth as SEQ ID NO:398, 400, and 402, respectively.

[0150] In some embodiments, the disclosed immunoglobulins immunospecifically bind to human TEM1. In some embodiments, the immunoglobulin that immunospecifically binds to human TEM1 comprises a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-139 of xi1-55-2HC (SEQ ID NO:56) and a light chain variable

region having an amino acid sequence at least 90% identical to amino acids 20-129 of xi1-55-2LC (SEQ ID NO:108).

[0151] In some embodiments, the immunoglobulin that immunospecifically binds to human TEM1 comprises a heavy chain variable region as set forth as amino acids 20-139 of xi1-55-2HC (SEQ ID NO:56) and a light chain variable region as set forth as amino acids 20-129 of xi1-55-2LC (SEQ ID NO:108).

[0152] In some embodiments, the immunoglobulin that immunospecifically binds to human TEM1 comprises a heavy chain CDR1, CDR2, and CDR3 of xi1-55-2HC as set forth as SEQ ID NO:158, 160, and 162, respectively, and a light chain CDR1, CDR2, and CDR3 of xi1-55-2LC as set forth as SEQ ID NO:314, 316, and 318, respectively.

[0153] In some embodiments, the disclosed immunoglobulins immunospecifically bind to human mesothelin. In some embodiments, the immunoglobulin that immunospecifically binds to human mesothelin comprises:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi33O11HC (SEQ ID NO:62) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of xi33O11LC (SEQ ID NO:116);
- b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu33O11HC (SEQ ID NO:64) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of zu33O11LC-CXXA (SEQ ID NO:120) or zu33O11LC-CXXI (SEQ ID NO:122);
- c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi324O5HC (SEQ ID NO:66) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi324O5LC (SEQ ID NO:124);
- d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi178F16HC (SEQ ID NO:68) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi178F16LC (SEQ ID NO:126);
- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-132 of xi237N18HC (SEQ ID NO:70) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi237N18LC (SEQ ID NO:128); or

- f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi383I18HC (SEQ ID NO:72) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi383I18LC (SEQ ID NO:130).

[0154] In some embodiments, the immunoglobulin that immunospecifically binds to human mesothelin comprises:

- a. a heavy chain variable region as set forth as amino acids 20-142 of xi33O11HC (SEQ ID NO:62) and a light chain variable region as set forth as amino acids 20-131 of xi33O11LC (SEQ ID NO:116);
- b. a heavy chain variable region as set forth as amino acids 20-145 of zu33O11HC (SEQ ID NO:64) and a light chain variable region as set forth as amino acids 20-131 of zu33O11LC-CXXA (SEQ ID NO:120) or zu33O11LC-CXXI (SEQ ID NO:122);
- c. a heavy chain variable region as set forth as amino acids 20-137 of xi324O5HC (SEQ ID NO:66) and a light chain variable region as set forth as amino acids 20-127 of xi324O5LC (SEQ ID NO:124);
- d. a heavy chain variable region as set forth as amino acids 20-137 of xi178F16HC (SEQ ID NO:68) and a light chain variable region as set forth as amino acids 20-127 of xi178F16LC (SEQ ID NO:126);
- e. a heavy chain variable region as set forth as amino acids 20-132 of xi237N18HC (SEQ ID NO:70) and a light chain variable region as set forth as amino acids 20-127 of xi237N18LC (SEQ ID NO:128); or
- f. a heavy chain variable region as set forth as amino acids 20-137 of xi383I18HC (SEQ ID NO:72) and a light chain variable region as set forth as amino acids 20-127 of xi383I18LC (SEQ ID NO:130).

[0155] In some embodiments, the immunoglobulin that immunospecifically binds to human mesothelin comprises:

- a. a heavy chain CDR1, CDR2, and CDR3 of xi33O11HC as set forth as SEQ ID NO: 176, 178, and 180, respectively, and a light chain CDR1, CDR2, and CDR3 of xi33O11LC as set forth as SEQ ID NO:338, 340, and 342, respectively;
- b. a heavy chain CDR1, CDR2, and CDR3 of zu33O11HC as set forth as SEQ ID NO:182, 184, and 186, respectively, and a light chain CDR1, CDR2, and CDR3 of zu33O11LC-CXXA as set forth as SEQ ID NO:350, 352, and 354, respectively or zu33O11LC-CXXI as set forth as SEQ ID NO:356, 358, and 360, respectively;

- c. a heavy chain CDR1, CDR2, and CDR3 of xi324O5HC as set forth as SEQ ID NO:188, 190, and 192, respectively, and a light chain CDR1, CDR2, and CDR3 of xi324O5LC as set forth as SEQ ID NO:362, 364, and 366, respectively;
- d. a heavy chain CDR1, CDR2, and CDR3 of xi178F16HC as set forth as SEQ ID NO:194, 196, and 198, respectively, and a light chain CDR1, CDR2, and CDR3 of xi178F16LC as set forth as SEQ ID NO:368, 370, and 372, respectively;
- e. a heavy chain CDR1, CDR2, and CDR3 of xi237N18HC as set forth as SEQ ID NO:200, 202, and 204, respectively, and a light chain CDR1, CDR2, and CDR3 of xi237N18LC as set forth as SEQ ID NO:374, 376, and 378, respectively; or
- f. a heavy chain CDR1, CDR2, and CDR3 of xi383I18HC as set forth as SEQ ID NO:206, 208, and 210, respectively, and a light chain CDR1, CDR2, and CDR3 of xi383I18LC as set forth as SEQ ID NO:380, 382, and 384, respectively.

Conjugated immunoglobulins

[0156] Also disclosed herein are conjugated immunoglobulins comprising any of the immunoglobulins disclosed herein, wherein the cysteine at position 80 ("Cys80") is conjugated to a thiol-reactive compound, the thiol-reactive compound comprising a thiol-reactive group.

[0157] In some embodiments, the conjugated immunoglobulins comprise an immunoglobulin comprising a heavy chain variable region and a light chain variable region, the light chain variable region having a Cys80 and an amino acid other than Phe, Lys, or Cys at position 83, wherein Cys80 is conjugated to a thiol-reactive compound, the thiol-reactive compound comprising a thiol-reactive group. In some embodiments, the light chain variable region can have a Cys80 and a polar or hydrophobic amino acid other than Phe, Lys, or Cys at position 83.

[0158] The immunoglobulin comprises a heavy chain variable region and a light chain variable region. Suitable light chain variable regions include, for example, a kappa light chain variable region. The light chain variable region is derived from rabbit. In some embodiments, the Cys80 can be present in the native light chain variable region of the rabbit immunoglobulin. Exemplary rabbits from which a light chain variable region having a Cys80 can be derived include, but is not limited to, *Oryctolagus cuniculus*. In some aspects, for example, the light chain variable region can be derived from a New Zealand White (NZW) rabbit. In other aspects, the light chain variable region can be derived from a b9 rabbit.

[0159] The light chain variable region can have a Cys80 and an amino acid other than Phe, Lys, or Cys at position 83. The amino acid other than Phe, Lys, or Cys at position 83 includes alanine ("Ala83"), valine ("Val83"), isoleucine ("Ile83"), threonine ("Thr83"), arginine ("Arg83"), asparagine ("Asn83"), aspartic acid ("Asp83"), glutamic acid ("Glu83"), glutamine ("Gln83"), glycine ("Gly83"), histidine ("His83"), leucine ("Leu83"), methionine ("Met83"), proline ("Pro83"), serine ("Ser83"), tryptophan ("Trp83"), or tyrosine ("Tyr83"). In some embodiments, the light chain variable region can have a Cys80 and a polar or hydrophobic amino acid other than Phe, Lys, or Cys at position 83. Suitable polar or hydrophobic amino acids include, but are not limited to, alanine, valine, isoleucine, or threonine. In some aspects, the polar or hydrophobic amino acid other than Phe at position 83 is alanine ("Ala83"). In some aspects, the polar or hydrophobic amino acid other than Phe at position 83 is valine ("Val83"). In some aspects, the polar or hydrophobic amino acid other than Phe at position 83 is isoleucine ("Ile83"). In some aspects, the polar or hydrophobic amino acid other than Phe at position 83 is threonine ("Thr83").

[0160] The Cys80 can be unpaired. For example, the light chain variable region having Cys80 can be chimerized with a constant domain having an amino acid residue other than cysteine at position 171.

[0161] Preferably, the Cys80 is decapped.

[0162] In some embodiments, the immunoglobulin can be chimerized. In other embodiments, the immunoglobulin can be humanized.

[0163] Preferably, the thiol-reactive compound is conjugated to the Cys80 via the thiol-reactive group. Thiol-reactive groups include haloacetyls, maleimides, aziridines, acryloyls, arylating agents, vinylsulfones, pyridyl disulfides, TNB-thiols and disulfide reducing agents. In some embodiments, the thiol-reactive group can comprise a maleimide. In some embodiments, the thiol-reactive group can comprise a haloacetyl. In some embodiments, the thiol-reactive group can comprise an aziridine. In some embodiments, the thiol-reactive group can comprise an acryloyl. In some embodiments, the thiol-reactive group can comprise an arylating agent. In some embodiments, the thiol-reactive group can comprise a vinylsulfone. In some embodiments, the thiol-reactive group can comprise a pyridyl disulfide. In some embodiments, the thiol-reactive group can comprise a TNB-thiol. In some embodiments, the thiol-reactive group can comprise a disulfide reducing agent.

[0164] The thiol-reactive group can be appended to a linker. Linkers can be non-cleavable linkers or cleavable linkers. Exemplary linkers include, for example, disulfide

containing linkers, acetal-based linkers, and ketal-based linkers. In some aspects, the linker can be a non-cleavable linker. Suitable non-cleavable linkers include, but are not limited to, polyethylene glycol (PEG) or an alkyl. In some embodiments, the linker can comprise PEG. In some aspects, the linker can be a cleavable linker. Suitable cleavable linkers include, for example, valine-citrulline-para aminobenzyl. In some aspects, the linker can be a disulfide containing linker. In some aspects, the linker can be an acetal-based linker. In some aspects, the linker can be a ketal-based linker. Examples of linkers covalently appended to a thiol-reactive group are provided, for example, in U.S. Publ. No. 20140050746.

[0165] The thiol-reactive compound can further comprise a functional agent. Suitable functional agents include, for example, fluorophores, fluorescent dyes, polypeptides, immunoglobulins, antibiotics, nucleic acids, radionuclides, chemical linkers, small molecules, chelators, lipids, and drugs. In some aspects, the functional agent can comprise a fluorophore. In some aspects, the functional agent can comprise a fluorescent dye. In some aspects, the functional agent can comprise a polypeptide. In some aspects, the functional agent can comprise an immunoglobulin. In some aspects, the functional agent can comprise an antibiotic. In some aspects, the functional agent can comprise a nucleic acid (such as DNA or RNA). In some aspects, the functional agent can comprise a radionuclide. In some aspects, the functional agent can comprise a chemical linker (for example dibenzylcyclooctyne (DBCO) or azide). In some aspects, the functional agent can comprise a small molecule. In some aspects, the functional agent can comprise a chelator (for example, DOTA, CHX-A"-DTPA, NOTA, among others). In some aspects, the functional agent can comprise a lipid. In some aspects, the functional agent can comprise a drug. In some aspects, the functional agent can comprise a combination of any of the above listed functional agents.

[0166] Accordingly, the disclosed conjugated immunoglobulins include: immunoglobulin-fluorophore Cys80 conjugates, immunoglobulin-fluorescent dye Cys80 conjugates, immunoglobulin-polypeptide Cys80 conjugates, immunoglobulin-immunoglobulin Cys80 conjugates, immunoglobulin-antibiotic Cys80 conjugates, immunoglobulin-nucleic acid Cys80 conjugates, immunoglobulin-radionuclide Cys80 conjugates, immunoglobulin-chemical linker Cys80 conjugates, immunoglobulin-small molecule Cys80 conjugates, immunoglobulin-chelator Cys80 conjugates, immunoglobulin-lipid Cys80 conjugates, and immunoglobulin-drug Cys80 conjugates.

[0167] Any of the immunoglobulins disclosed herein can be conjugated to any of the functional agents disclosed herein. For example, the conjugated immunoglobulin can comprise

an immunoglobulin that immunospecifically binds to human CA9 and a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug. In some embodiments, the conjugated immunoglobulin is a CA9-fluorophore Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a CA9-fluorescent dye Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a CA9-polypeptide Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a CA9-immunoglobulin Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a CA9-antibiotic Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a CA9-nucleic acid Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a CA9-radionuclide Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a CA9-chemical linker Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a CA9-small molecule Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a CA9-chelator Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a CA9-lipid Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a CA9-drug Cys80 conjugate.

[0168] Suitable immunoglobulins that immunospecifically bind to human CA9 that can be conjugated at Cys80 to any of the above functional agents include:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-141 of xi155D5HC (SEQ ID NO:52) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi155D5LC (SEQ ID NO:78);
- b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-144 of zu155D5HC (SEQ ID NO:54) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu155D5LC-3 (SEQ ID NO:84), zu155D5LC-4 (SEQ ID NO:86), zu155D5LC-5 (SEQ ID NO:88), zu155D5LC-6 (SEQ ID NO:90), zu155D5LC-7 (SEQ ID NO:92), zu155D5LC-huVK2-40 (SEQ ID NO:96), zu155D5LC-huVK4-1 (SEQ ID NO:100), zu155D5LC-huVK6-21 (SEQ ID NO:102), zu155D5LC-huVK6D-41 (SEQ ID NO:104); or zu155D5LC-huVK7-3-Glu81 (SEQ ID NO:106);
- c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-138 of xi1E4HC (SEQ ID NO:58) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi1E4LC (SEQ ID NO:110);

- d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-140 of zu1E4HC (SEQ ID NO:60) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu1E4LC-CXXA (SEQ ID NO:114);
- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi166B3HC (SEQ ID NO:74) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi166B3LC (SEQ ID NO:132);
- f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu166B3HC (SEQ ID NO:76) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu166B3LC-CXXA (SEQ ID NO:136);
- g. a heavy chain variable region as set forth as amino acids 20-141 of xi155D5HC (SEQ ID NO:52) and a light chain variable region as set forth as amino acids 20-130 of xi155D5LC (SEQ ID NO:78);
- h. a heavy chain variable region as set forth as amino acids 20-144 of zu155D5HC (SEQ ID NO:54) and a light chain variable region as set forth as amino acids 20-130 of zu155D5LC-3 (SEQ ID NO:84), zu155D5LC-4 (SEQ ID NO:86), zu155D5LC-5 (SEQ ID NO:88), zu155D5LC-6 (SEQ ID NO:90), zu155D5LC-7 (SEQ ID NO:92), zu155D5LC-huVK2-40 (SEQ ID NO:96), zu155D5LC-huVK4-1 (SEQ ID NO:100), zu155D5LC-huVK6-21 (SEQ ID NO:102), zu155D5LC-huVK6D-41 (SEQ ID NO:104); or zu155D5LC-huVK7-3-Glu81 (SEQ ID NO:106);
- i. a heavy chain variable region as set forth as amino acids 20-138 of xi1E4HC (SEQ ID NO:58) and a light chain variable as set forth as amino acids 20-130 of xi1E4LC (SEQ ID NO:110);
- j. a heavy chain variable region as set forth as amino acids 20-140 of zu1E4HC (SEQ ID NO:60) and a light chain variable region as set forth as amino acids 20-130 of zu1E4LC-CXXA (SEQ ID NO:114);
- k. a heavy chain variable region as set forth as amino acids 20-142 of xi166B3HC (SEQ ID NO:74) and a light chain variable region as set forth as amino acids 20-130 of xi166B3LC (SEQ ID NO:132);

- l. a heavy chain variable region as set forth as amino acids 20-145 of zu166B3HC (SEQ ID NO:76) and a light chain variable region as set forth as amino acids 20-130 of zu166B3LC-CXXA (SEQ ID NO:136);
- m. a heavy chain CDR1, CDR2, and CDR3 of xi155D5HC as set forth as SEQ ID NO:146, 148, and 150, respectively, and a light chain CDR1, CDR2, and CDR3 of xi155D5LC as set forth as SEQ ID NO:224, 226, and 228, respectively;
- n. a heavy chain CDR1, CDR2, and CDR3 of zu155D5HC as set forth as SEQ ID NO:152, 154, and 156, respectively, and a light chain CDR1, CDR2, and CDR3 of zu155D5LC-3 as set forth as SEQ ID NO:242, 244, and 246, respectively, zu155D5LC-4 as set forth as SEQ ID NO:248, 250, and 252, respectively, zu155D5LC-5 as set forth as SEQ ID NO:254, 256, and 258, respectively, zu155D5LC-6 as set forth as SEQ ID NO:260, 262, and 264, respectively, zu155D5LC-7 as set forth as SEQ ID NO:266, 268, and 270, respectively, zu155D5LC-huVK2-40 as set forth as SEQ ID NO 278, 280, and 282, respectively, zu155D5LC-huVK4-1 as set forth as SEQ ID NO 290, 292, and 294, respectively, zu155D5LC-huVK6-21 as set forth as SEQ ID NO 296, 298, and 300, respectively, zu155D5LC-huVK6D-41 as set forth as SEQ ID NO 302, 304, and 306, respectively; or zu155D5LC-huVK7-3-Glu81 as set forth as SEQ ID NO 308, 310, and 312, respectively;
- o. a heavy chain CDR1, CDR2, and CDR3 of xi1E4HC as set forth as SEQ ID NO:164, 166, and 168, respectively, and a light chain CDR1, CDR2, and CDR3 of xi1E4LC as set forth as SEQ ID NO:320, 322, and 324, respectively;
- p. a heavy chain CDR1, CDR2, and CDR3 of zu1E4HC as set forth as SEQ ID NO:170, 172, and 174, respectively, and a light chain CDR1, CDR2, and CDR3 of zu1E4LC-CXXA as set forth as SEQ ID NO:332, 334, and 336, respectively;
- q. a heavy chain CDR1, CDR2, and CDR3 of xi166B3HC as set forth as SEQ ID NO:212, 214, and 216, respectively, and a light chain CDR1, CDR2, and CDR3 of xi166B3LC as set forth as SEQ ID NO:386, 388, and 390, respectively; or
- r. a heavy chain CDR1, CDR2, and CDR3 of zu166B3HC as set forth as SEQ ID NO:218, 220, and 222, respectively, and a light chain CDR1, CDR2, and CDR3 of zu166B3LC-CXXA as set forth as SEQ ID NO:398, 400, and 402, respectively.

[0169] The conjugated immunoglobulin can comprise an immunoglobulin that immunospecifically binds to human TEM1 and a fluorophore, fluorescent dye, polypeptide,

immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug. In some embodiments, the conjugated immunoglobulin is a TEM1-fluorophore Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a TEM1-fluorescent dye Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a TEM1-polypeptide Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a TEM1-immunoglobulin Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a TEM1-antibiotic Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a TEM1-nucleic acid Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a TEM1-radionuclide Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a TEM1-chemical linker Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a TEM1-small molecule Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a TEM1-chelator Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a TEM1-lipid Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a TEM1-drug Cys80 conjugate.

[0170] Suitable immunoglobulins that immunospecifically bind to human TEM1 that can be conjugated at Cys80 to any of the above functional agents include:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-139 of xi1-55-2HC (SEQ ID NO:56) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-129 of xi1-55-2LC (SEQ ID NO:108);
- b. a heavy chain variable region as set forth as amino acids 20-139 of xi1-55-2HC (SEQ ID NO:56) and a light chain variable region as set forth as amino acids 20-129 of xi1-55-2LC (SEQ ID NO:108); or
- c. a heavy chain CDR1, CDR2, and CDR3 of xi1-55-2HC as set forth as SEQ ID NO:158, 160, and 162, respectively, and a light chain CDR1, CDR2, and CDR3 of xi1-55-2LC as set forth as SEQ ID NO:314, 316, and 318, respectively.

[0171] The conjugated immunoglobulin can comprise an immunoglobulin that immunospecifically binds to human MSLN and a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug. In some embodiments, the conjugated immunoglobulin is a MSLN-fluorophore Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a MSLN-fluorescent dye Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a MSLN-polypeptide Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a

MSLN-immunoglobulin Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a MSLN-antibiotic Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a MSLN-nucleic acid Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a MSLN-radionuclide Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a MSLN-chemical linker Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a MSLN-small molecule Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a MSLN-chelator Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a MSLN-lipid Cys80 conjugate. In some embodiments, the conjugated immunoglobulin is a MSLN-drug Cys80 conjugate.

[0172] Suitable immunoglobulins that immunospecifically bind to human MSLN that can be conjugated at Cys80 to any of the above functional agents include:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi33O11HC (SEQ ID NO:62) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of xi33O11LC (SEQ ID NO:116);
- b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu33O11HC (SEQ ID NO:64) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of zu33O11LC-CXXA (SEQ ID NO:120) or zu33O11LC-CXXI (SEQ ID NO:122);
- c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi324O5HC (SEQ ID NO:66) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi324O5LC (SEQ ID NO:124);
- d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi178F16HC (SEQ ID NO:68) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi178F16LC (SEQ ID NO:126);
- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-132 of xi237N18HC (SEQ ID NO:70) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi237N18LC (SEQ ID NO:128);
- f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi383I18HC (SEQ ID NO:72) and a light chain variable region

- having an amino acid sequence at least 90% identical to amino acids 20-127 of xi383I18LC (SEQ ID NO:130);
- g. a heavy chain variable region as set forth as amino acids 20-142 of xi33O11HC (SEQ ID NO:62) and a light chain variable region as set forth as amino acids 20-131 of xi33O11LC (SEQ ID NO:116);
 - h. a heavy chain variable region as set forth as amino acids 20-145 of zu33O11HC (SEQ ID NO:64) and a light chain variable region as set forth as amino acids 20-131 of zu33O11LC-CXXA (SEQ ID NO:120) or zu33O11LC-CXXI (SEQ ID NO:122);
 - i. a heavy chain variable region as set forth as amino acids 20-137 of xi324O5HC (SEQ ID NO:66) and a light chain variable region as set forth as amino acids 20-127 of xi324O5LC (SEQ ID NO:124);
 - j. a heavy chain variable region as set forth as amino acids 20-137 of xi178F16HC (SEQ ID NO:68) and a light chain variable region as set forth as amino acids 20-127 of xi178F16LC (SEQ ID NO:126);
 - k. a heavy chain variable region as set forth as amino acids 20-132 of xi237N18HC (SEQ ID NO:70) and a light chain variable region as set forth as amino acids 20-127 of xi237N18LC (SEQ ID NO:128);
 - l. a heavy chain variable region as set forth as amino acids 20-137 of xi383I18HC (SEQ ID NO:72) and a light chain variable region as set forth as amino acids 20-127 of xi383I18LC (SEQ ID NO:130);
 - m. a heavy chain CDR1, CDR2, and CDR3 of xi33O11HC as set forth as SEQ ID NO: 176, 178, and 180, respectively, and a light chain CDR1, CDR2, and CDR3 of xi33O11LC as set forth in SEQ ID NO:338, 340, and 342, respectively;
 - n. a heavy chain CDR1, CDR2, and CDR3 of zu33O11HC as set forth as SEQ ID NO:182, 184, and 186, respectively, and a light chain CDR1, CDR2, and CDR3 of zu33O11LC-CXXA as set forth as SEQ ID NO:350, 352, and 354, respectively or zu33O11LC-CXXI as set forth as SEQ ID NO:356, 358, and 360, respectively;
 - o. a heavy chain CDR1, CDR2, and CDR3 of xi324O5HC as set forth as SEQ ID NO:188, 190, and 192, respectively, and a light chain CDR1, CDR2, and CDR3 of xi324O5LC as set forth as SEQ ID NO:362, 364, and 366, respectively;
 - p. a heavy chain CDR1, CDR2, and CDR3 of xi178F16HC as set forth as SEQ ID NO:194, 196, and 198, respectively, and a light chain CDR1, CDR2, and CDR3 of xi178F16LC as set forth as SEQ ID NO:368, 370, and 372, respectively;

- q. a heavy chain CDR1, CDR2, and CDR3 of xi237N18HC as set forth as SEQ ID NO:200, 202, and 204, respectively, and a light chain CDR1, CDR2, and CDR3 of xi237N18LC as set forth as SEQ ID NO:374, 376, and 378, respectively; or
- r. a heavy chain CDR1, CDR2, and CDR3 of xi383I18HC as set forth as SEQ ID NO:206, 208, and 210, respectively, and a light chain CDR1, CDR2, and CDR3 of xi383I18LC as set forth as SEQ ID NO:380, 382, and 384, respectively.

[0173] In some embodiments, the immunoglobulin that immunospecifically binds to human MSLN can be conjugated to a small molecule antineoplastic agent such as an auristatin. In some aspects, the functional agent can be auristatin F (AuF). Thus, the disclosed conjugated immunoglobulins include any of the above disclosed immunoglobulins that immunospecifically bind to human MSLN, wherein the immunoglobulin is conjugated to auristatin F (MSLN-AuF Cys80 conjugate).

[0174] In embodiments wherein the immunoglobulin comprises two light chain variable regions, the conjugated immunoglobulin can have an immunoglobulin:functional agent ratio of 2:1, with each light chain having a functional agent conjugated at Cys80.

Antigen-binding molecules

[0175] Further provided herein are antigen-binding molecules comprising:

a first conjugated immunoglobulin comprising a first heavy chain variable region and a first light chain variable region, the first light chain variable region having a cysteine at position 80 (“Cys80¹”), wherein Cys80¹ is conjugated to a first thiol-reactive compound comprising a first thiol-reactive group, and

a second conjugated immunoglobulin comprising a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at position 80 (“Cys80²”) wherein Cys80² is conjugated to a second thiol-reactive compound comprising a second thiol-reactive group.

[0176] The first conjugated immunoglobulin and second conjugated immunoglobulin can be any one of the conjugated immunoglobulins disclosed herein.

[0177] Suitable light chain variable regions include, for example, a kappa light chain variable region. The first light chain variable region and the second light chain variable region are derived from rabbit. In some embodiments, the Cys80¹, Cys80², or both, can be present in the native light chain variable region of the rabbit immunoglobulin. Exemplary rabbits from which a first light chain variable region, second light chain variable region, or both, can be

derived from include, but are not limited to, *Oryctolagus cuniculus*. In some aspects, for example, the light chain variable region(s) can be derived from a New Zealand White (NZW) rabbit. In other aspects, the light chain variable region(s) can be derived from a b9 rabbit.

[0178] The Cys80¹, the Cys80², or both, can be unpaired. Suitable means for unpairing Cys80¹ and/or Cys80² include, for example, chimerizing a light chain variable region (a first light chain variable region, a second light chain variable region, or both) having a Cys80 with a constant domain having an amino acid residue other than cysteine at position 171.

[0179] The first immunoglobulin, the second immunoglobulin, or both, can be chimerized. In some embodiments, the first immunoglobulin can be chimerized. In some embodiments, the second immunoglobulin can be chimerized. In some embodiments, the first immunoglobulin and the second immunoglobulin can be chimerized.

[0180] The first immunoglobulin, the second immunoglobulin, or both, can be humanized. In some embodiments, the first immunoglobulin can be humanized. In some embodiments, the second immunoglobulin can be humanized. In some embodiments, the first immunoglobulin and the second immunoglobulin can be humanized.

[0181] In some embodiments, the first immunoglobulin can be chimerized and the second immunoglobulin can be humanized. In some embodiments, the first immunoglobulin can be humanized and the second immunoglobulin can be chimerized.

[0182] The amino acid at position 83 of the first light chain variable region can be an amino acid other than Phe, Lys, or Cys if the amino acid at position 83 is Phe. The amino acid at position 83 of the second light chain variable region can be an amino acid other than Phe, Lys, or Cys if the amino acid at position 83 is Phe. The amino acid at position 83 of the first light chain variable region can be an amino acid other than Phe, Lys, or Cys if the amino acid at position 83 is Phe and the amino acid at position 83 of the second light chain variable region can be an amino acid other than Phe, Lys, or Cys if the amino acid at position 83 is Phe. The amino acid at position 83 of the first light chain variable region and/or second light chain variable region can be alanine ("Ala83"), valine ("Val83"), isoleucine ("Ile83"), threonine ("Thr83"), arginine ("Arg83"), asparagine ("Asn83"), aspartic acid ("Asp83"), glutamic acid ("Glu83"), glutamine ("Gln83"), glycine ("Gly83"), histidine ("His83"), leucine ("Leu83"), methionine ("Met83"), proline ("Pro83"), serine ("Ser83"), tryptophan ("Trp83"), or tyrosine ("Tyr83"). The amino acid at position 83 of the first light chain variable region can be the same as the amino acid at position 83 of the second light chain variable region. Conversely, the amino acid at position 83

of the first light chain variable region can be different from the amino acid at position 83 of the second light chain variable region.

[0183] In some embodiments, the amino acid at position 83 of the first light chain variable region can be a polar or hydrophobic residue other than Phe if the amino acid at position 83 is Phe. In some embodiments, the amino acid at position 83 of the second light chain variable region can be a polar or hydrophobic residue other than Phe if the amino acid at position 83 is Phe. In some embodiments, the amino acid at position 83 of the first light chain variable region can be a polar or hydrophobic residue other than Phe if the amino acid at position 83 is Phe and the amino acid at position 83 of the second light chain variable region can be a polar or hydrophobic residue other than Phe if the amino acid at position 83 is Phe. Suitable polar or hydrophobic amino acids include, but are not limited to alanine, valine, isoleucine, or threonine. In some aspects, the amino acid at position 83 of the first light chain variable region, the amino acid at position 83 of the second light chain variable region, or the amino acid at position 83 of the first light chain variable region and the amino acid at position 83 of the second light chain variable region can be alanine (“Ala83”). In some aspects, the amino acid at position 83 of the first light chain variable region, the amino acid at position 83 of the second light chain variable region, or the amino acid at position 83 of the first light chain variable region and the amino acid at position 83 of the second light chain variable region can be valine (“Val83”). In some aspects, the amino acid at position 83 of the first light chain variable region, the amino acid at position 83 of the second light chain variable region, or the amino acid at position 83 of the first light chain variable region and the amino acid at position 83 of the second light chain variable region can be isoleucine (“Ile83”). In some aspects, the amino acid at position 83 of the first light chain variable region, the amino acid at position 83 of the second light chain variable region, or the amino acid at position 83 of the first light chain variable region and the amino acid at position 83 of the second light chain variable region can be Threonine (“Thr83”). The polar or hydrophobic amino acid at position 83 in the first light chain variable region can be the same as, or different from, the polar or hydrophobic amino acid at position 83 in the second light chain variable region.

[0184] The first immunoglobulin and the second immunoglobulin can bind to the same antigens. In some aspects, the first immunoglobulin and the second immunoglobulin can bind to the same epitope of the same antigen. In other aspects, the first immunoglobulin and the second immunoglobulin can bind to different epitopes of the same antigen. In some embodiments, for example, the first immunoglobulin and the second immunoglobulin can be an immunoglobulin

that immunospecifically binds to human CA9, wherein the first immunoglobulin, second immunoglobulin, or both are conjugated to any one of a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug. In some embodiments, the first immunoglobulin and the second immunoglobulin can be an immunoglobulin that immunospecifically binds to human TEM1, wherein the first immunoglobulin, second immunoglobulin, or both are conjugated to any one of a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug. In some embodiments, the first immunoglobulin and the second immunoglobulin can be an immunoglobulin that immunospecifically binds to human MSLN, wherein the first immunoglobulin, second immunoglobulin, or both are conjugated to any one of a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug.

[0185] The first immunoglobulin and the second immunoglobulin can bind to different antigens. In some embodiments, for example, the first conjugated immunoglobulin can be an immunoglobulin that immunospecifically binds to human CA9, wherein the first immunoglobulin that binds to human CA9 is conjugated to any one of a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug, whereas the second immunoglobulin can be an immunoglobulin that immunospecifically binds to human TEM1 or human MSLN. In such embodiments, the second immunoglobulin can be conjugated to any one of a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug. In some embodiments, the first conjugated immunoglobulin can be an immunoglobulin that immunospecifically binds to human TEM1, wherein the immunoglobulin is conjugated to any one of a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug, whereas the second immunoglobulin can be an immunoglobulin that immunospecifically binds to human CA9 or human MSLN. In such embodiments, the second immunoglobulin can be conjugated to any one of a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug. In some embodiments, the first conjugated immunoglobulin can be an immunoglobulin that immunospecifically binds to human MSLN, wherein the immunoglobulin is conjugated to any one of a fluorophore, fluorescent dye,

polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug, whereas the second immunoglobulin can be an immunoglobulin that immunospecifically binds to human CA9 or human TEM1. In such embodiments, the second immunoglobulin can be conjugated to any one of a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug.

[0186] Suitable, thiol-reactive groups include haloacetyls, maleimides, aziridines, acryloyls, arylating agents, vinylsulfones, pyridyl disulfides, TNB-thiols and disulfide reducing agents. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise a maleimide. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise a haloacetyl. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise an aziridine. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise an acryloyl. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise an arylating agent. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise a vinylsulfone. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise a pyridyl disulfide. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise a TNB-thiol. In some embodiments, the first thiol-reactive group, the second-thiol reactive group, or both, can comprise a disulfide reducing agent.

[0187] The first thiol-reactive group, the second-thiol reactive group, or both can be appended to a linker. In some aspects, the first thiol-reactive group can be appended to a linker ("first linker"). In some aspects, the second thiol-reactive group can be appended to a linker ("second linker"). In yet other aspects the first thiol-reactive group can be appended to a first linker and the second thiol-reactive group can be appended to a second linker. Suitable first and second linkers can be non-cleavable linkers or cleavable linkers. Exemplary first and second linkers include, for example, disulfide containing linkers, acetal-based linkers, and ketal-based linkers. In some aspects, the first linker, second linker, or both, can be a non-cleavable linker. Suitable non-cleavable linkers include, but are not limited to, polyethylene glycol (PEG) or an alkyl. In some embodiments, the first linker, second linker, or both, can comprise PEG. In some aspects, the first linker, second linker, or both, can be a cleavable linker. Suitable cleavable linkers include, for example, valine-citrulline-para aminobenzyl. In some aspects, the first linker, second linker, or both, can be a disulfide containing linker. In some aspects, the first

linker, second linker, or both can be an acetal-based linker. In some aspects, the first linker, second linker, or both, can be a ketal-based linker. Examples of linkers covalently appended to a thiol-reactive group are provided, for example, in U.S. Publ. No. 20140050746.

[0188] The first thiol-reactive compound, the second thiol-reactive compound, or both, can further comprise a functional agent. In some aspects, the first thiol-reactive compound can further comprise a functional agent (“first functional agent”). In some aspects, the second thiol-reactive compound can further comprise a functional agent (“second functional agent”). In yet other aspects, the first thiol-reactive compound can further comprise a first functional agent and the second thiol-reactive compound can further comprise a second functional agent.

[0189] Suitable functional agents include, for example, chemical linkers. Preferably, the chemical linker of the first thiol-reactive compound (“first chemical linker”) and the chemical linker of the second thiol-reactive compound (“second chemical linker”) can be coupled. For example, and without intent to be limiting, one of the first or second chemical linkers can be dibenzylcyclooctyne (DBCO) and the other of the first or second chemical linkers can be azide. In some embodiments, for example, the first chemical linker can be DBCO and the second chemical linker can be azide. Conversely, the first chemical linker can be azide and the second chemical linker can be DBCO. The DBCO and azide can be coupled, thus resulting in the conjugation of the first immunoglobulin and the second immunoglobulin. For example, the first immunoglobulin and the second immunoglobulin can be conjugated to each other by click chemistry.

[0190] In an exemplary embodiment, thiol-reactive compounds can include maleimido-PEG4-azide and maleimido-PEG4-dibenzocyclooctyne. In some aspects, for example, the first thiol-reactive compound can be maleimido-PEG4-azide and the second thiol-reactive compound can be maleimido-PEG4-dibenzocyclooctyne. In some aspects, the first thiol-reactive compound can be maleimido-PEG4-dibenzocyclooctyne and the second thiol-reactive compound can be maleimido-PEG4-azide. Thus, the first thiol-reactive compound can differ from the second thiol-reactive compound.

[0191] The first immunoglobulin, second immunoglobulin, or both, can be Fabs. In some embodiments, the first immunoglobulin can be a Fab (“first Fab”). In some embodiments, the second immunoglobulin can be a Fab (“second Fab”). In yet other embodiments, the first immunoglobulin can be a first Fab and the second immunoglobulin can be a second Fab.

Methods of treating cancer in a subject

[0192] Also disclosed herein are methods of treating cancer in a subject comprising administering to the subject a pharmaceutically effective amount of a conjugated mesothelin immunoglobulin, wherein the conjugated mesothelin immunoglobulin comprises:

any of the conjugated mesothelin immunoglobulins disclosed herein, and
a thiol-reactive compound comprising a thiol-reactive group, a linker, and a functional agent.

[0193] It is to be understood that any of the characteristics, features, and embodiments relating to the disclosed conjugated immunoglobulins are equally applicable to those conjugated immunoglobulins used in the disclosed methods of treating cancer. Accordingly, the disclosed methods can comprise administering to the subject a pharmaceutically effective amount of a conjugated mesothelin immunoglobulin, wherein the conjugated mesothelin immunoglobulin comprises a heavy chain variable region and a light chain variable region, the light chain variable region having a cysteine at position 80 (“Cys80”) and an amino acid other than Phe, Lys, or Cys at position 83, wherein the Cys80 is conjugated to a thiol-reactive compound, the thiol-reactive compound comprising a thiol-reactive group, a linker, and a functional agent. In some embodiments, the amino acid other than Phe, Lys, or Cys at position 83 is a polar or hydrophobic amino acid.

[0194] Preferably, the cancer is a mesothelin-expressing cancer. In some embodiments, the conjugated antibodies for use in the disclosed methods can comprise:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi33O11HC (SEQ ID NO:62) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of xi33O11LC (SEQ ID NO:116);
- b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu33O11HC (SEQ ID NO:64) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of zu33O11LC-CXXA (SEQ ID NO:120) or zu33O11LC-CXXI (SEQ ID NO:122);
- c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi324O5HC (SEQ ID NO:66) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi324O5LC (SEQ ID NO:124);
- d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi178F16HC (SEQ ID NO:68) and a light chain variable

region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi178F16LC (SEQ ID NO:126);

- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-132 of xi237N18HC (SEQ ID NO:70) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi237N18LC (SEQ ID NO:128); or
- f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi383I18HC (SEQ ID NO:72) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi383I18LC (SEQ ID NO:130).

[0195] Antibodies (a)-(f) can be conjugated to a number of suitable thiol-reactive compounds including, but not limited to, those having an antineoplastic agent, such as an auristatin, as the functional agent. Thus, in some embodiments, the methods can comprise administering to the subject a pharmaceutically effective amount of a conjugated immunoglobulin, wherein the conjugated immunoglobulin comprises one or more of immunoglobulins (a)-(f), each being conjugated to a thiol-reactive compound comprising auristatin F, wherein the thiol-reactive compound is conjugated to the light chain variable region of the immunoglobulin at the Cys80.

[0196] In some embodiments, the conjugated antibodies for use in the disclosed methods can comprise:

- a. a heavy chain variable region as set forth as amino acids 20-142 of xi33O11HC (SEQ ID NO:62) and a light chain variable region as set forth as amino acids 20-131 of xi33O11LC (SEQ ID NO:116);
- b. a heavy chain variable region as set forth as amino acids 20-145 of zu33O11HC (SEQ ID NO:64) and a light chain variable region as set forth as amino acids 20-131 of zu33O11LC-CXXA (SEQ ID NO:120) or zu33O11LC-CXXI (SEQ ID NO:122);
- c. a heavy chain variable region as set forth as amino acids 20-137 of xi324O5HC (SEQ ID NO:66) and a light chain variable region as set forth as amino acids 20-127 of xi324O5LC (SEQ ID NO:124);
- d. a heavy chain variable region as set forth as amino acids 20-137 of xi178F16HC (SEQ ID NO:68) and a light chain variable region as set forth as amino acids 20-127 of xi178F16LC (SEQ ID NO:126);

- e. a heavy chain variable region as set forth as amino acids 20-132 of xi237N18HC (SEQ ID NO:70) and a light chain variable region as set forth as amino acids 20-127 of xi237N18LC (SEQ ID NO:128); or
- f. a heavy chain variable region as set forth as amino acids 20-137 of xi383I18HC (SEQ ID NO:72) and a light chain variable region as set forth as amino acids 20-127 of xi383I18LC (SEQ ID NO:130).

[0197] Antibodies (a)-(f) can be conjugated to a number of suitable thiol-reactive compounds including, but not limited to, those having an antineoplastic agent, such as an auristatin, as the functional agent. Thus, in some embodiments, the methods can comprise administering to the subject a pharmaceutically effective amount of a conjugated immunoglobulin, wherein the conjugated immunoglobulin comprises one or more of immunoglobulins (a)-(f), each being conjugated to a thiol-reactive compound comprising auristatin F, wherein the thiol-reactive compound is conjugated to the light chain variable region of the immunoglobulin at the Cys80.

[0198] In some embodiments, the conjugated antibodies for use in the disclosed methods can comprise:

- a. a heavy chain CDR1, CDR2, and CDR3 of xi33O11HC as set forth as SEQ ID NO: 176, 178, and 180, respectively, and a light chain CDR1, CDR2, and CDR3 of xi33O11LC as set forth in SEQ ID NO:338, 340, and 342, respectively;
- b. a heavy chain CDR1, CDR2, and CDR3 of zu33O11HC as set forth as SEQ ID NO:182, 184, and 186, respectively, and a light chain CDR1, CDR2, and CDR3 of zu33O11LC-CXXA as set forth as SEQ ID NO:350, 352, and 354, respectively or zu33O11LC-CXXI as set forth as SEQ ID NO:356, 358, and 360, respectively;
- c. a heavy chain CDR1, CDR2, and CDR3 of xi324O5HC as set forth as SEQ ID NO:188, 190, and 192, respectively, and a light chain CDR1, CDR2, and CDR3 of xi324O5LC as set forth as SEQ ID NO:362, 364, and 366, respectively;
- d. a heavy chain CDR1, CDR2, and CDR3 of xi178F16HC as set forth as SEQ ID NO:194, 196, and 198, respectively, and a light chain CDR1, CDR2, and CDR3 of xi178F16LC as set forth as SEQ ID NO:368, 370, and 372, respectively;
- e. a heavy chain CDR1, CDR2, and CDR3 of xi237N18HC as set forth as SEQ ID NO:200, 202, and 204, respectively, and a light chain CDR1, CDR2, and CDR3 of xi237N18LC as set forth as SEQ ID NO:374, 376, and 378, respectively; or

- f. a heavy chain CDR1, CDR2, and CDR3 of xi383I18HC as set forth as SEQ ID NO:206, 208, and 210, respectively, and a light chain CDR1, CDR2, and CDR3 of xi383I18LC as set forth as SEQ ID NO:380, 382, and 384, respectively.

[0199] Antibodies (a)-(f) can be conjugated to a number of suitable thiol-reactive compounds including, but not limited to, those having an antineoplastic agent, such as an auristatin, as the functional agent. Thus, in some embodiments, the methods can comprise administering to the subject a pharmaceutically effective amount of a conjugated immunoglobulin, wherein the conjugated immunoglobulin comprises one or more of immunoglobulins (a)-(f), each being conjugated to a thiol-reactive compound comprising auristatin F, wherein the thiol-reactive compound is conjugated to the light chain variable region of the immunoglobulin at the Cys80.

Methods for detecting cancer

[0200] Also disclosed herein are methods of detecting cancer in a subject. In some embodiments, the methods can be performed on the subject. For example, the methods can comprise administering to the subject a pharmaceutically effective amount of a conjugated immunoglobulin, wherein the conjugated immunoglobulin comprises a heavy chain variable region and a light chain variable region, the light chain variable region having a cysteine at position 80 ("Cys80") and an amino acid other than Phe, Lys, or Cys at position 83, wherein the Cys80 is conjugated to a thiol-reactive compound, the thiol-reactive compound comprising a thiol-reactive group, a linker, and a functional agent. In some embodiments, the amino acid other than Phe, Lys, or Cys at position 83 is a polar or hydrophobic.

[0201] Alternatively, the methods can be performed on a biological sample obtained from the subject. For example, the methods can comprise contacting a biological sample with a conjugated immunoglobulin, wherein the conjugated immunoglobulin comprises a heavy chain variable region and a light chain variable region, the light chain variable region having a cysteine at position 80 ("Cys80") and an amino acid other than Phe, Lys, or Cys at position 83, wherein the Cys80 is conjugated to a thiol-reactive compound, the thiol-reactive compound comprising a thiol-reactive group, a linker, and a functional agent. The amino acid other than Phe, Lys, or Cys at position 83 is a polar or hydrophobic. In some embodiments, the methods can be performed *ex vivo*. In some embodiments, the methods can be performed *in vivo*.

[0202] The functional agent is a fluorophore or fluorescent dye.

[0203] Any of the immunoglobulins disclosed herein can be conjugated to a fluorophore or fluorescent dye and used in the disclosed methods of detecting cancer. In some embodiments, the cancer is a CA9-expressing cancer and the conjugated immunoglobulin is a CA9-fluorophore Cys80 conjugate or a CA9-fluorescent dye Cys80 conjugate comprising:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-141 of xi155D5HC (SEQ ID NO:52) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi155D5LC (SEQ ID NO:78);
- b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-144 of zu155D5HC (SEQ ID NO:54) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu155D5LC-3 (SEQ ID NO:84), zu155D5LC-4 (SEQ ID NO:86), zu155D5LC-5 (SEQ ID NO:88), zu155D5LC-6 (SEQ ID NO:90), zu155D5LC-7 (SEQ ID NO:92), zu155D5LC-huVK2-40 (SEQ ID NO:96), zu155D5LC-huVK4-1 (SEQ ID NO:100), zu155D5LC-huVK6-21 (SEQ ID NO:102), zu155D5LC-huVK6D-41 (SEQ ID NO:104); or zu155D5LC-huVK7-3-Glu81 (SEQ ID NO:106);
- c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-138 of xi1E4HC (SEQ ID NO:58) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi1E4LC (SEQ ID NO:110);
- d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-140 of zu1E4HC (SEQ ID NO:60) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu1E4LC-CXXA (SEQ ID NO:114);
- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi166B3HC (SEQ ID NO:74) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi166B3LC (SEQ ID NO:132);
- f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu166B3HC (SEQ ID NO:76) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu166B3LC-CXXA (SEQ ID NO:136);

- g. a heavy chain variable region as set forth as amino acids 20-141 of xi155D5HC (SEQ ID NO:52) and a light chain variable region as set forth as amino acids 20-130 of xi155D5LC (SEQ ID NO:78);
- h. a heavy chain variable region as set forth as amino acids 20-144 of zu155D5HC (SEQ ID NO:54) and a light chain variable region as set forth as amino acids 20-130 of zu155D5LC-3 (SEQ ID NO:84), zu155D5LC-4 (SEQ ID NO:86), zu155D5LC-5 (SEQ ID NO:88), zu155D5LC-6 (SEQ ID NO:90), zu155D5LC-7 (SEQ ID NO:92), zu155D5LC-huVK2-40 (SEQ ID NO:96), zu155D5LC-huVK4-1 (SEQ ID NO:100), zu155D5LC-huVK6-21 (SEQ ID NO:102), zu155D5LC-huVK6D-41 (SEQ ID NO:104); or zu155D5LC-huVK7-3-Glu81 (SEQ ID NO:106);
- i. a heavy chain variable region as set forth as amino acids 20-138 of xi1E4HC (SEQ ID NO:58) and a light chain variable as set forth as amino acids 20-130 of xi1E4LC (SEQ ID NO:110);
- j. a heavy chain variable region as set forth as amino acids 20-140 of zu1E4HC (SEQ ID NO:60) and a light chain variable region as set forth as amino acids 20-130 of zu1E4LC-CXXA (SEQ ID NO:114);
- k. a heavy chain variable region as set forth as amino acids 20-142 of xi166B3HC (SEQ ID NO:74) and a light chain variable region as set forth as amino acids 20-130 of xi166B3LC (SEQ ID NO:132);
- l. a heavy chain variable region as set forth as amino acids 20-145 of zu166B3HC (SEQ ID NO:76) and a light chain variable region as set forth as amino acids 20-130 of zu166B3LC-CXXA (SEQ ID NO:136);
- m. a heavy chain CDR1, CDR2, and CDR3 of xi155D5HC as set forth as SEQ ID NO:146, 148, and 150, respectively, and a light chain CDR1, CDR2, and CDR3 of xi155D5LC as set forth as SEQ ID NO:224, 226, and 228, respectively;
- n. a heavy chain CDR1, CDR2, and CDR3 of zu155D5HC as set forth as SEQ ID NO:152, 154, and 156, respectively, and a light chain CDR1, CDR2, and CDR3 of zu155D5LC-3 as set forth as SEQ ID NO:242, 244, and 246, respectively, zu155D5LC-4 as set forth as SEQ ID NO:248, 250, and 252, respectively, zu155D5LC-5 as set forth as SEQ ID NO:254, 256, and 258, respectively, zu155D5LC-6 as set forth as SEQ ID NO:260, 262, and 264, respectively, zu155D5LC-7 as set forth as SEQ ID NO:266, 268, and 270, respectively, zu155D5LC-huVK2-40 as set forth as SEQ ID NO 278, 280, and 282, respectively,

zu155D5LC-huVK4-1 as set forth as SEQ ID NO 290, 292, and 294, respectively,
 zu155D5LC-huVK6-21 as set forth as SEQ ID NO 296, 298, and 300, respectively,
 zu155D5LC-huVK6D-41 as set forth as SEQ ID NO 302, 304, and 306, respectively;
 or zu155D5LC-huVK7-3-Glu81 as set forth as SEQ ID NO 308, 310, and 312,
 respectively;

- o. a heavy chain CDR1, CDR2, and CDR3 of xi1E4HC as set forth as SEQ ID NO:164, 166, and 168, respectively, and a light chain CDR1, CDR2, and CDR3 of xi1E4LC as set forth as SEQ ID NO:320, 322, and 324, respectively;
- p. a heavy chain CDR1, CDR2, and CDR3 of zu1E4HC as set forth as SEQ ID NO:170, 172, and 174, respectively, and a light chain CDR1, CDR2, and CDR3 of zu1E4LC-CXXA as set forth as SEQ ID NO:332, 334, and 336, respectively;
- q. a heavy chain CDR1, CDR2, and CDR3 of xi166B3HC as set forth as SEQ ID NO:212, 214, and 216, respectively, and a light chain CDR1, CDR2, and CDR3 of xi166B3LC as set forth as SEQ ID NO:386, 388, and 390, respectively; or
- r. a heavy chain CDR1, CDR2, and CDR3 of zu166B3HC as set forth as SEQ ID NO:218, 220, and 222, respectively, and a light chain CDR1, CDR2, and CDR3 of zu166B3LC-CXXA as set forth as SEQ ID NO:398, 400, and 402, respectively.

[0204] In some embodiments, the cancer is a TEM1-expressing cancer and the conjugated immunoglobulin is a TEM1-fluorophore Cys80 conjugate or a TEM1-fluorescent dye Cys80 conjugate comprising:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-139 of xi1-55-2HC (SEQ ID NO:56) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-129 of xi1-55-2LC (SEQ ID NO:108);
- b. a heavy chain variable region as set forth as amino acids 20-139 of xi1-55-2HC (SEQ ID NO:56) and a light chain variable region as set forth as amino acids 20-129 of xi1-55-2LC (SEQ ID NO:108); or
- c. a heavy chain CDR1, CDR2, and CDR3 of xi1-55-2HC as set forth as SEQ ID NO:158, 160, and 162, respectively, and a light chain CDR1, CDR2, and CDR3 of xi1-55-2LC as set forth as SEQ ID NO:314, 316, and 318, respectively.

[0205] In some embodiments, the cancer is a MSLN-expressing cancer and the conjugated immunoglobulin is a MSLN-fluorophore Cys80 conjugate or a MSLN-fluorescent dye Cys80 conjugate comprising:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi33O11HC (SEQ ID NO:62) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of xi33O11LC (SEQ ID NO:116);
- b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu33O11HC (SEQ ID NO:64) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of zu33O11LC-CXXA (SEQ ID NO:120) or zu33O11LC-CXXI (SEQ ID NO:122);
- c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi324O5HC (SEQ ID NO:66) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi324O5LC (SEQ ID NO:124);
- d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi178F16HC (SEQ ID NO:68) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi178F16LC (SEQ ID NO:126);
- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-132 of xi237N18HC (SEQ ID NO:70) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi237N18LC (SEQ ID NO:128);
- f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi383I18HC (SEQ ID NO:72) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi383I18LC (SEQ ID NO:130);
- g. a heavy chain variable region as set forth as amino acids 20-142 of xi33O11HC (SEQ ID NO:62) and a light chain variable region as set forth as amino acids 20-131 of xi33O11LC (SEQ ID NO:116);
- h. a heavy chain variable region as set forth as amino acids 20-145 of zu33O11HC (SEQ ID NO:64) and a light chain variable region as set forth as amino acids 20-131 of zu33O11LC-CXXA (SEQ ID NO:120) or zu33O11LC-CXXI (SEQ ID NO:122);
- i. a heavy chain variable region as set forth as amino acids 20-137 of xi324O5HC (SEQ ID NO:66) and a light chain variable region as set forth as amino acids 20-127 of xi324O5LC (SEQ ID NO:124);

- j. a heavy chain variable region as set forth as amino acids 20-137 of xi178F16HC (SEQ ID NO:68) and a light chain variable region as set forth as amino acids 20-127 of xi178F16LC (SEQ ID NO:126);
- k. a heavy chain variable region as set forth as amino acids 20-132 of xi237N18HC (SEQ ID NO:70) and a light chain variable region as set forth as amino acids 20-127 of xi237N18LC (SEQ ID NO:128);
- l. a heavy chain variable region as set forth as amino acids 20-137 of xi383I18HC (SEQ ID NO:72) and a light chain variable region as set forth as amino acids 20-127 of xi383I18LC (SEQ ID NO:130);
- m. a heavy chain CDR1, CDR2, and CDR3 of xi330I1HC as set forth as SEQ ID NO: 176, 178, and 180, respectively, and a light chain CDR1, CDR2, and CDR3 of xi330I1LC as set forth in SEQ ID NO:338, 340, and 342, respectively;
- n. a heavy chain CDR1, CDR2, and CDR3 of zu330I1HC as set forth as SEQ ID NO:182, 184, and 186, respectively, and a light chain CDR1, CDR2, and CDR3 of zu330I1LC-CXXA as set forth as SEQ ID NO:350, 352, and 354, respectively or zu330I1LC-CXXI as set forth as SEQ ID NO:356, 358, and 360, respectively;
- o. a heavy chain CDR1, CDR2, and CDR3 of xi324O5HC as set forth as SEQ ID NO:188, 190, and 192, respectively, and a light chain CDR1, CDR2, and CDR3 of xi324O5LC as set forth as SEQ ID NO:362, 364, and 366, respectively;
- p. a heavy chain CDR1, CDR2, and CDR3 of xi178F16HC as set forth as SEQ ID NO:194, 196, and 198, respectively, and a light chain CDR1, CDR2, and CDR3 of xi178F16LC as set forth as SEQ ID NO:368, 370, and 372, respectively;
- q. a heavy chain CDR1, CDR2, and CDR3 of xi237N18HC as set forth as SEQ ID NO:200, 202, and 204, respectively, and a light chain CDR1, CDR2, and CDR3 of xi237N18LC as set forth as SEQ ID NO:374, 376, and 378, respectively; or
- r. a heavy chain CDR1, CDR2, and CDR3 of xi383I18HC as set forth as SEQ ID NO:206, 208, and 210, respectively, and a light chain CDR1, CDR2, and CDR3 of xi383I18LC as set forth as SEQ ID NO:380, 382, and 384, respectively.

[0206] Exemplary fluorophores for conjugation to the immunoglobulin include, for example, IRDye-800CW.

[0207] The methods can comprise administering the conjugated immunoglobulin to the subject or contacting the biological sample with the conjugated immunoglobulin and detecting binding of the conjugated immunoglobulin to an antigen (CA9, TEM1, or MSLN) present in the

subject or in the biological sample, respectively. Suitable methods of detection include, for example, fluorescent imaging. Detection of binding of the conjugated immunoglobulin to the antigen (through the emission of a fluorescent signal, for example) is indicative of cancer.

Pharmaceutical compositions

[0208] Also provided herein are pharmaceutical compositions. In some embodiments, the pharmaceutical compositions can comprise any of the immunoglobulins disclosed herein. In some embodiments, the pharmaceutical compositions can comprise any of the conjugated immunoglobulins disclosed herein.

[0209] Administration of a conjugated immunoglobulin in accordance with the methods of treatment or diagnosis described herein may be by any means known in the art.

Light chain variable regions for use in conjugated immunoglobulins

[0210] Provided herein are light chain variable regions for use in a conjugated immunoglobulin, the light chain variable region having a cysteine at amino acid position 80 ("Cys80") and an amino acid residue other than Phe, Lys, or Cys at amino acid position 83, wherein the Cys80 is unpaired. In some embodiments, the amino acid other than Phe, Lys, or Cys at position 83 is a polar or hydrophobic.

[0211] In preferred embodiments, the light chain has a Cys80-Xaa₁-Xaa₂-Xaa₃ motif, wherein Xaa₃ is an amino acid other than Phe, Lys, or Cys.

[0212] Suitable light chain variable regions include, for example, a kappa light chain variable region. The light chain variable region is derived from rabbit. In some embodiments, the Cys80 can be present in the native light chain variable region of the rabbit immunoglobulin. Exemplary rabbits from which a light chain variable region having a Cys80 can be derived include, but is not limited to, *Oryctolagus cuniculus*. In some aspects, for example, the light chain variable region can be derived from a New Zealand White (NZW) rabbit. In other aspects, the light chain variable region can be derived from a b9 rabbit.

[0213] The Cys80 can be uncapped, can be involved in an intramolecular or intermolecular disulfide bond, or can have a capping cysteine.

[0214] In some embodiments, the light chain variable region can be chimerized. In other embodiments, the light chain variable region can be humanized.

[0215] The light chain variable region can comprise, consist of, or consist essentially of:

- a. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi155D5LC (SEQ ID NO:78);
- b. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu155D5LC-3 (SEQ ID NO:84), zu155D5LC-4 (SEQ ID NO:86), zu155D5LC-5 (SEQ ID NO:88), zu155D5LC-6 (SEQ ID NO:90), zu155D5LC-7 (SEQ ID NO:92), zu155D5LC-huVK2-40 (SEQ ID NO:96), zu155D5LC-huVK4-1 (SEQ ID NO:100), zu155D5LC-huVK6-21 (SEQ ID NO:102), zu155D5LC-huVK6D-41 (SEQ ID NO:104); or zu155D5LC-huVK7-3-Glu81 (SEQ ID NO:106);
- c. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi1E4LC (SEQ ID NO:110);
- d. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu1E4LC-CXXA (SEQ ID NO:114);
- e. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi166B3LC (SEQ ID NO:132);
- f. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu166B3LC-CXXA (SEQ ID NO:136);
- g. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-129 of xi1-55-2LC (SEQ ID NO:108);
- h. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of xi33O11LC (SEQ ID NO:116);
- i. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of zu33O11LC-CXXA (SEQ ID NO:120) or zu33O11LC-CXXI (SEQ ID NO:122);
- j. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi324O5LC (SEQ ID NO:124);
- k. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi178F16LC (SEQ ID NO:126);
- l. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi237N18LC (SEQ ID NO:128); or
- m. a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi383I18LC (SEQ ID NO:130).

Nucleic acid molecules encoding immunoglobulins and host cells comprising the same

[0216] Also provided herein are nucleic acid molecules encoding any of the above disclosed immunoglobulins. In some embodiments, the nucleic acid molecules encode an immunoglobulin comprising a heavy chain variable region and a light chain variable region, the light chain variable region having a cysteine at position 80 (“Cys80”) and an amino acid other than Phe, Lys, or Cys at position 83. In some embodiments, the amino acid other than Phe, Lys, or Cys at position 83 is polar or hydrophobic.

[0217] The disclosed nucleic acid molecules can encode an immunoglobulin that can immunospecifically bind to human CA9. In some embodiments, the nucleic acid molecule encodes:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-141 of xi155D5HC (SEQ ID NO:52) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi155D5LC (SEQ ID NO:78);
- b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-144 of zu155D5HC (SEQ ID NO:54) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu155D5LC-3 (SEQ ID NO:84), zu155D5LC-4 (SEQ ID NO:86), zu155D5LC-5 (SEQ ID NO:88), zu155D5LC-6 (SEQ ID NO:90), zu155D5LC-7 (SEQ ID NO:92), zu155D5LC-huVK2-40 (SEQ ID NO:96), zu155D5LC-huVK4-1 (SEQ ID NO:100), zu155D5LC-huVK6-21 (SEQ ID NO:102), zu155D5LC-huVK6D-41 (SEQ ID NO:104); or zu155D5LC-huVK7-3-Glu81 (SEQ ID NO:106);
- c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-138 of xi1E4HC (SEQ ID NO:58) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi1E4LC (SEQ ID NO:110);
- d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-140 of zu1E4HC (SEQ ID NO:60) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu1E4LC-CXXA (SEQ ID NO:114);
- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi166B3HC (SEQ ID NO:74) and a light chain variable region

having an amino acid sequence at least 90% identical to amino acids 20-130 of xi166B3LC (SEQ ID NO:132); or

- f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu166B3HC (SEQ ID NO:76) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu166B3LC-CXXA (SEQ ID NO:136).

[0218] In some embodiments, the nucleic acid molecule encodes:

- a. a heavy chain variable region as set forth as amino acids 20-141 of xi155D5HC (SEQ ID NO:52) and a light chain variable region as set forth as amino acids 20-130 of xi155D5LC (SEQ ID NO:78);
- b. a heavy chain variable region as set forth as amino acids 20-144 of zu155D5HC (SEQ ID NO:54) and a light chain variable region as set forth as amino acids 20-130 of zu155D5LC-3 (SEQ ID NO:84), zu155D5LC-4 (SEQ ID NO:86), zu155D5LC-5 (SEQ ID NO:88), zu155D5LC-6 (SEQ ID NO:90), zu155D5LC-7 (SEQ ID NO:92), zu155D5LC-huVK2-40 (SEQ ID NO:96), zu155D5LC-huVK4-1 (SEQ ID NO:100), zu155D5LC-huVK6-21 (SEQ ID NO:102), zu155D5LC-huVK6D-41 (SEQ ID NO:104); or zu155D5LC-huVK7-3-Glu81 (SEQ ID NO:106);
- c. a heavy chain variable region as set forth as amino acids 20-138 of xi1E4HC (SEQ ID NO:58) and a light chain variable as set forth as amino acids 20-130 of xi1E4LC (SEQ ID NO:110);
- d. a heavy chain variable region as set forth as amino acids 20-140 of zu1E4HC (SEQ ID NO:60) and a light chain variable region as set forth as amino acids 20-130 of zu1E4LC-CXXA (SEQ ID NO:114);
- e. a heavy chain variable region as set forth as amino acids 20-142 of xi166B3HC (SEQ ID NO:74) and a light chain variable region as set forth as amino acids 20-130 of xi166B3LC (SEQ ID NO:132); or
- f. a heavy chain variable region as set forth as amino acids 20-145 of zu166B3HC (SEQ ID NO:76) and a light chain variable region as set forth as amino acids 20-130 of zu166B3LC-CXXA (SEQ ID NO:136).

[0219] In some embodiments, the nucleic acid molecule encodes:

- a. a heavy chain CDR1, CDR2, and CDR3 of xi155D5HC as set forth as SEQ ID NO:146, 148, and 150, respectively, and a light chain CDR1, CDR2, and CDR3 of xi155D5LC as set forth as SEQ ID NO:224, 226, and 228, respectively;

- b. a heavy chain CDR1, CDR2, and CDR3 of zu155D5HC as set forth as SEQ ID NO:152, 154, and 156, respectively, and a light chain CDR1, CDR2, and CDR3 of zu155D5LC-3 as set forth as SEQ ID NO:242, 244, and 246, respectively, zu155D5LC-4 as set forth as SEQ ID NO:248, 250, and 252, respectively, zu155D5LC-5 as set forth as SEQ ID NO:254, 256, and 258, respectively, zu155D5LC-6 as set forth as SEQ ID NO:260, 262, and 264, respectively, zu155D5LC-7 as set forth as SEQ ID NO:266, 268, and 270, respectively, zu155D5LC-huVK2-40 as set forth as SEQ ID NO 278, 280, and 282, respectively, zu155D5LC-huVK4-1 as set forth as SEQ ID NO 290, 292, and 294, respectively, zu155D5LC-huVK6-21 as set forth as SEQ ID NO 296, 298, and 300, respectively, zu155D5LC-huVK6D-41 as set forth as SEQ ID NO 302, 304, and 306, respectively; or zu155D5LC-huVK7-3-Glu81 as set forth as SEQ ID NO 308, 310, and 312, respectively;
- c. a heavy chain CDR1, CDR2, and CDR3 of xi1E4HC as set forth as SEQ ID NO:164, 166, and 168, respectively, and a light chain CDR1, CDR2, and CDR3 of xi1E4LC as set forth as SEQ ID NO:320, 322, and 324, respectively;
- d. a heavy chain CDR1, CDR2, and CDR3 of zu1E4HC as set forth as SEQ ID NO:170, 172, and 174, respectively, and a light chain CDR1, CDR2, and CDR3 of zu1E4LC-CXXA as set forth as SEQ ID NO:332, 334, and 336, respectively;
- e. a heavy chain CDR1, CDR2, and CDR3 of xi166B3HC as set forth as SEQ ID NO:212, 214, and 216, respectively, and a light chain CDR1, CDR2, and CDR3 of xi166B3LC as set forth as SEQ ID NO:386, 388, and 390, respectively; or
- f. a heavy chain CDR1, CDR2, and CDR3 of zu166B3HC as set forth as SEQ ID NO:218, 220, and 222, respectively, and a light chain CDR1, CDR2, and CDR3 of zu166B3LC-CXXA as set forth as SEQ ID NO:398, 400, and 402, respectively.

[0220] The disclosed nucleic acid molecules can encode an immunoglobulin that can immunospecifically bind to human TEM1. In some embodiments, the nucleic acid molecule encodes a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-139 of xi1-55-2HC (SEQ ID NO:56) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-129 of xi1-55-2LC (SEQ ID NO:108). In some embodiments, the nucleic acid molecule encodes a heavy chain variable region as set forth as amino acids 20-139 of xi1-55-2HC (SEQ ID NO:56) and a light chain variable region as set forth as amino acids 20-129 of xi1-55-2LC (SEQ ID NO:108). In some

embodiments, the nucleic acid molecule encodes a heavy chain CDR1, CDR2, and CDR3 of xi1-55-2HC as set forth as SEQ ID NO:158, 160, and 162, respectively, and a light chain CDR1, CDR2, and CDR3 of xi1-55-2LC as set forth as SEQ ID NO:314, 316, and 318, respectively.

[0221] The disclosed nucleic acid molecules can encode an immunoglobulin that can immunospecifically bind to human MSLN. In some embodiments, the nucleic acid molecule encodes:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi33O11HC (SEQ ID NO:62) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of xi33O11LC (SEQ ID NO:116);
- b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu33O11HC (SEQ ID NO:64) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of zu33O11LC-CXXA (SEQ ID NO:120) or zu33O11LC-CXXI (SEQ ID NO:122);
- c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi324O5HC (SEQ ID NO:66) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi324O5LC (SEQ ID NO:124);
- d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi178F16HC (SEQ ID NO:68) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi178F16LC (SEQ ID NO:126);
- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-132 of xi237N18HC (SEQ ID NO:70) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi237N18LC (SEQ ID NO:128); or
- f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi383I18HC (SEQ ID NO:72) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi383I18LC (SEQ ID NO:130).

[0222] In some embodiments, the nucleic acid molecule encodes:

- a. a heavy chain variable region as set forth as amino acids 20-142 of xi33011HC (SEQ ID NO:62) and a light chain variable region as set forth as amino acids 20-131 of xi33011LC (SEQ ID NO:116);
- b. a heavy chain variable region as set forth as amino acids 20-145 of zu33011HC (SEQ ID NO:64) and a light chain variable region as set forth as amino acids 20-131 of zu33011LC-CXXA (SEQ ID NO:120) or zu33011LC-CXXI (SEQ ID NO:122);
- c. a heavy chain variable region as set forth as amino acids 20-137 of xi32405HC (SEQ ID NO:66) and a light chain variable region as set forth as amino acids 20-127 of xi32405LC (SEQ ID NO:124);
- d. a heavy chain variable region as set forth as amino acids 20-137 of xi178F16HC (SEQ ID NO:68) and a light chain variable region as set forth as amino acids 20-127 of xi178F16LC (SEQ ID NO:126);
- e. a heavy chain variable region as set forth as amino acids 20-132 of xi237N18HC (SEQ ID NO:70) and a light chain variable region as set forth as amino acids 20-127 of xi237N18LC (SEQ ID NO:128); or
- f. a heavy chain variable region as set forth as amino acids 20-137 of xi383I18HC (SEQ ID NO:72) and a light chain variable region as set forth as amino acids 20-127 of xi383I18LC (SEQ ID NO:130).

[0223] In some embodiments, the nucleic acid molecule encodes:

- a. a heavy chain CDR1, CDR2, and CDR3 of xi33011HC as set forth as SEQ ID NO: 176, 178, and 180, respectively, and a light chain CDR1, CDR2, and CDR3 of xi33011LC as set forth in SEQ ID NO:338, 340, and 342, respectively;
- b. a heavy chain CDR1, CDR2, and CDR3 of zu33011HC as set forth as SEQ ID NO:182, 184, and 186, respectively, and a light chain CDR1, CDR2, and CDR3 of zu33011LC-CXXA as set forth as SEQ ID NO:350, 352, and 354, respectively or zu33011LC-CXXI as set forth as SEQ ID NO:356, 358, and 360, respectively;
- c. a heavy chain CDR1, CDR2, and CDR3 of xi32405HC as set forth as SEQ ID NO:188, 190, and 192, respectively, and a light chain CDR1, CDR2, and CDR3 of xi32405LC as set forth as SEQ ID NO:362, 364, and 366, respectively;
- d. a heavy chain CDR1, CDR2, and CDR3 of xi178F16HC as set forth as SEQ ID NO:194, 196, and 198, respectively, and a light chain CDR1, CDR2, and CDR3 of xi178F16LC as set forth as SEQ ID NO:368, 370, and 372, respectively;

- e. a heavy chain CDR1, CDR2, and CDR3 of xi237N18HC as set forth as SEQ ID NO:200, 202, and 204, respectively, and a light chain CDR1, CDR2, and CDR3 of xi237N18LC as set forth as SEQ ID NO:374, 376, and 378, respectively; or
- f. a heavy chain CDR1, CDR2, and CDR3 of xi383I18HC as set forth as SEQ ID NO:206, 208, and 210, respectively, and a light chain CDR1, CDR2, and CDR3 of xi383I18LC as set forth as SEQ ID NO:380, 382, and 384, respectively.

[0224] Also disclosed are host cells comprising any of the disclosed nucleic acid molecules. Suitable host cells include, but are not limited to, mammalian cells, bacterial cells, yeast cells, insect cells, to name a few.

[0225] The following examples are provided to further describe some of the embodiments disclosed herein. The examples are intended to illustrate, not to limit, the disclosed embodiments.

EXAMPLES

Example 1 – Exemplary Methods

Generation of rabbit mAbs specific to human TEM1 (endosialin/CD248)

[0226] Rabbit immunization: To generate rabbit mAbs specific to human TEM1 (hTEM1), a soluble human endosialin extracellular domain-mouse Fc fusion protein was prepared (“human endosialin/TE M1 extracellular domain fused to mouse IgG2b Fc”). The extracellular domain of hTEM1 was cloned in-frame EcoRI/HpaI to pEF6-EK-IgG2b, which contained an enterokinase cleavage site followed by the murine IgG2b Fc gamma fragment. CHO-K1 cells were transfected with this construct and selected with 5 µg/mL blasticidin. Secreted TEM1-Fc was electrophoresed on a 4-12% PAGE gel and Coomassie stained, followed by excision of the bands. The gel slices were emulsified in complete/incomplete adjuvant, and injected into New Zealand White rabbits every 3 to 4 weeks, four injections. The spleen from a rabbit showing the best titers against hTEM1 as assessed by ELISA was harvested for the generation of hybridomas.

[0227] Generation of hybridomas: Fusions were performed as follows: spleen cells ($1.5\text{--}3 \times 10^8$) of immunized rabbits and the fusion partner 240E 1-1-2 were fused at a ratio of 2:1 with 50% PEG 4000 (EM Science, Cherry Hill, NJ) at 37°C in serum-free medium. The cells were plated in 48-well microtiter plates, at approximately 2×10^5 spleen cells per well, in medium with 15% FCS. After 72 hr, hypoxanthine-aminopterin-thymidine (HAT) was added. Medium

was changed every 5-6 days. Supernatants were screened by ELISA for the presence of antibody specific for TEM-1 using TEM1-Fc coated plates and counter-screened against mouse Fc. Supernatants from hybridomas were screened for hTEM1 reactivity by ELISA and clone 1-55-2 was chosen for recombinant cloning.

[0228] Amplification of anti-hTEM1 1-55-2 light and heavy chain variable regions:

RNA was isolated from rabbit hybridoma 1-55-2 using the RNeasy mini kit (Qiagen, Valencia, CA). Two µg RNA was used for RT-PCR using SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity (Invitrogen). The rabbit variable heavy chain and full length light chain gene fragments were amplified using primer pairs N02937/N02898 and N02937/N02347 respectively (Table 1). The cycling parameters for the RT-PCR amplification were as follows: 55°C 30 min; 94°C 2 min; 30 cycles of (94°C 15 sec, 55°C 30 sec, 68°C 1 min); 68°C 2 min.

[0229] These PCR products were subsequently used in a second round PCR to amplify fragments amenable to generating chimeric rabbit/human IgGs using primer pairs N02416/N02761 and N02417/N02764 (Table 1). The cycling parameters for the second round PCR were as follows: 94°C for 2 min; 30 cycles of (94°C 30 sec, 55°C 30 sec, 68°C 1 min); 68°C 2 min,

Table 1. Primers used for RT-PCR and cloning of anti-hTEM1 1-55-2

Primer ID	Primer sequence	Fragment
N02937	GATCAAGCTTGCCGCCACCATGGGCTGGTCCTGCATCATCCTGTT TCTGGTGGCGGCCGCCACCGGCGTGCACTCC (SEQ ID NO:1)	Rabbit VH
N02898	GTGCCTTTGGCTGGCCTGARGAGAYGGTGACCAGGGTGCC (SEQ ID NO:2)	Rabbit VH
N02937	GATCAAGCTTGCCGCCACCATGGGCTGGTCCTGCATCATCCTGTT TCTGGTGGCGGCCGCCACCGGCGTGCACTCC (SEQ ID NO:3)	Rabbit LC
N02347	GATCGGCGCGCCTCACTTGCCGGGGCTCCGG (SEQ ID NO:4)	Rabbit LC
N02416	GCCACCGGCGTGCACTCCCAGTCGGTGRAGGAGTCCRGGGG (SEQ ID NO:5)	xi rb-hu HC
N02761	GGGCCCTTGGTGGATGCTGARGAGAYGGTGACCAGGGTGCC (SEQ ID NO:6)	xi rb-hu HC
N02417	GCCACCGGCGTGCACTCCGAGCTCGTGATGACCCAGACTCCA (SEQ ID NO:7)	xi rb-hu LC
N02764	AGCCACAGTTCGTTTGACSAACCTCGGTCCC (SEQ ID NO:8)	xi rb-hu LC

[0230] PCR products were then separated by electrophoresis in an agarose gel. PCR products having the correct molecular sizes for the VL and VH products were purified by QIAquick® Gel Extraction Kit (Qiagen, Valencia, CA) and cloned as described below.

Generation of rabbit mAbs specific to human CA9

[0231] Rabbit Immunization: To generate rabbit antibodies specific to human CA9, human CA9 extracellular domain ("human CA9 extracellular domain" or "CA9-ECD") was recombinantly generated. Two b9 rabbits were immunized using CA9-ECD. Briefly, the rabbits were subcutaneously injected with the antigens every 21 days. Each rabbit received 400 µg of CA9-ECD and Freund's complete adjuvant (FCA) in the first injection and 200 µg of CA9-ECD and Freund's Incomplete Adjuvant (FIA) in the subsequent boosts. The pre- and test-bleed were collected for the antibody titer testing.

[0232] The pre- and post-immunization blood was tested for CA9 binding using an Enzyme-Linked Immunosorbent Assay (ELISA) as described herein. The bleeds were serially diluted and added to CA9-ECD protein-coated microplates. When the titer reached 1:15,000 after four injections, the rabbits were finally boosted by intravenous injection of 400 µg of CA9-ECD without adjuvant. Rabbit spleens were collected one week after the final boosting. Up to 100 mL exsanguination bleeds were collected in the presence of anti-coagulant and the lymphocytes from spleens and lymph nodes were isolated from each rabbit.

[0233] Generation of hybridomas: Rabbit splenocytes were quickly thawed, spun down at 1200 rpm at room temperature for 5 min, and re-suspended in cIMDM plus 10% FBS containing 100 µg/mL DNase. Cells were stimulated with 2.5 µg/mL pokeweed mitogen at 37°C for at least 1 hour. After stimulation, cells were spun down at 1200 rpm at room temperature for 5 min and re-suspended in fresh media. Cell counts and viability were determined.

[0234] Fusion partner cells CBF7 were thawed out and cultured at 37°C with 5% CO₂ for one week before fusion. An appropriate amount of rabbit splenocytes and fusion partner cells CBF7 were mixed at the desired ratio (1:1.55 ~ 1:4) in 50 mL tubes. The mixture of cells was spun down at 1000 rpm at room temperature for 5 min and washed twice with ice-cold 20 mL CytoPulse Fusion Medium (CPFM Formula C: CytoPulse Sciences #LCM-C) at 4°C. The cells were re-suspended in CPFM to 10⁶ cells/mL.

[0235] CytoPulse cell fusion apparatus CEEF-50 (CytoPulse Sciences) was used for the fusion. An appropriate volume of cells was moved to the fusion chamber and fusion was

performed by activating high voltage connection. After fusion, the cells were incubated in the chamber at RT for 5 min, gently re-suspended in Post-Fusion Medium (RPMI1640 with 10% FBS, containing glutamate, pyruvate, non-essential amino acids, β -mercaptoethanol, penicillin, streptomycin, and no Phenol Red) and then transferred to a flask. The chamber was washed with the same volume of post-fusion media to obtain additional cells. The cells were incubated at room temperature for 25 min and then overnight at 37°C, 5% CO₂.

[0236] One day after fusion, the cells were diluted in pre-warmed seeding media (cIMDM plus 10% FBS containing 1X hypoxanthine-aminopterin-thymidine) to the desired density (35,000 cells/mL) and plated at 200 μ L/well in 96-well microplates. The plates were incubated at 37°C, 5% CO₂ and fed with fresh medium weekly for 3-4 weeks.

[0237] Screening of anti-CA9 mAbs: B-cells from rabbit splenocytes were fused to fusion partner cells CBF7 to generate hybridomas as described herein. Four weeks after plating the cells, the supernatants from individual hybridoma cultures were collected and screened using a CA9-specific ELISA. The assay plates (Greiner Bio-One High Binding 384-well clear plate, cat #655081) were coated with 1 μ g/ml CA9 ECD overnight at 4°C and blocked with 1X Assay Buffer (PBS plus 1% BSA, containing 0.05% Tween-20). Then, 25 μ L/well of supernatants and controls were added to the blocked plates and incubated overnight at 4°C. The assay plates were washed three times and 25 μ L/well of secondary antibodies (HRP-conjugated goat anti-mouse IgG, Jackson # 115-035-146) diluted 1:10,000 in Assay Buffer was added to the plates. After incubation at room temperature for one hour, the assay plates were washed three times and 25 μ L/well of TMB Substrate (KPL #52-000-04) was added to the plates. After incubation at room temperature for 5 minutes, 25 μ L/well of 1X Stop Solution (1:10 H₂SO₄, VWR #EM-SX1244-75) was added. Sample absorbance at 450 nm was measured by using Paradigm (Beckman) plate reader. The positive hits from the primary screen were confirmed by a second CA9-specific ELISA.

Cloning and mutagenesis

[0238] Amplification of VH and V κ regions of CA9 and hTEM1 mAbs: Hybridoma cells secreting rabbit mAbs of interest were lysed to extract RNA. RNA was then used for DNA amplification of variable kappa (V κ) and heavy chain variable (VH) regions by using the reverse transcriptase-polymerase chain reaction (RT-PCR) method. One hundred to 10,000 cultured hybridoma cells were washed with ice cold PBS and lysed by adding 100 μ L of Lysis/Binding Solution (Ambion, 8540G5) and pipetting. The lysed cells were quickly frozen on dry ice. RNA

was isolated with Ambion RNAqueous Kit according to manufacture procedure. About 5 ng RNAs were subject to first round of RT-PCR using the primers listed on Table 2 in each reaction.

Table 2. Primers used for first round of RT-PCR

Rabb.VHA1.F	5'-CAGTCGCTGCTCGAGTCCGGGGGT-3' (SEQ ID NO:9)
Rabb.VHB1.F	5'-CTCTGGCACAGGAGCTC-3' (SEQ ID NO:10)
Rabb.IgM_CH1.R	5'-GGAGACGAGCGGGTACAGAGT-3' (SEQ ID NO:11)
Rabb.IgG_Hinge.R	5'-CGTGGGCTTGCTGCATGTCG-3' (SEQ ID NO:12)
Rabb.Vκ.F	5'-GTGATGACCCAGACTCCA-3' (SEQ ID NO:13)
Rabb.Vκ1B4.R	5'-ACAGTCACCCCTATTGAAGCTCTGG-3' (SEQ ID NO:14)
Rabb.Vκ2B4.R	5'-GCAGTCACCCCTGTTGAAGCTCTG-3' (SEQ ID NO:15)

[0239] The cycling parameters for the RT-PCR amplification were as follows: 55°C 30 min; 95°C 2 min; 30 cycles of (94°C 1 min, 54°C 50 sec, 68°C 1.5 min); 68°C 10 min.

[0240] The products from the first round RT-PCR were subjected to a second round of PCR amplification in separate reaction for heavy chain and light chain, using the primers listed in Table 3.

Table 3. Primers used for second round of PCR amplification

Heavy Chain	
ldr-Rabb.VHA1.F	gccaccggcgctgcactccCAGTCGGTGRAGGAGTCCRGGGG (SEQ ID NO:16)
R-Rb-VH1-hu-gamma	gggcccttggtggatgcTGARGAGAYGGTGACCAGGGTGCC (SEQ ID NO:17)
Light Chain	
ldr-Rabb.Vκ.F	gccaccggcgctgcactccGAGCTCGTGATGACCCAGACTCCA (SEQ ID NO:18)
R-Rb-Vκ1m-hu-kappa	agccacagttcgTTTGATCTCCAGCTCGGTCCC (SEQ ID NO:19)
R-Rb-Vκ2-hu-kappa	agccacagttcgTTTGATTTCACATTGGTGCC (SEQ ID NO:20)

R-Rb-Vκ3-hu-kappa	agccacagttcgTTTGACsACCACCTCGGTCCC (SEQ ID NO:21)
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[0241] The cycling parameters for the second round of PCR amplification were as follows: 95°C 5 min; 40 cycles of (94°C 1 min, 54°C 50 sec, 68°C 1.5 min); 68°C 10 min; 4°C Soak.

[0242] PCR products were then separated by electrophoresis on agarose gel. PCR products having the correct molecular sizes for the VL and VH products were purified by QIAquick® Gel Extraction Kit (Qiagen, Valencia, CA), and the fragments were subcloned into an expression plasmid containing a human gamma (Cγ) or kappa (Cκ) constant region using an InFusion HD cloning kit (Clontech). All clones were sequenced to confirm the presence and fidelity of the inserts.

[0243] Gene synthesis: Humanized VH domains and zu155D5LC-1, -huVK1-39, -huVK2-40, -huVK3-11, -huVK4-1, -huVK5-2, -huVK6-21, -huVK6D-41, -huVK7-3, zu1E4LC-1, and zu166B3LC-1 Vκ domains were codon-optimized for expression in human cells and were synthesized by DNA 2.0. The variable domains were synthesized with a Kozak sequence and an Ig leader sequence, and included 15 base-pairs at the 5' and 3' ends homologous to the cloning site within the subcloning vector. Following excision from the DNA 2.0 vector, the fragments were subcloned into an expression plasmid containing a human Cγ or Cκ region using an InFusion HD cloning kit. All clones were sequenced to confirm the presence and fidelity of the inserts.

[0244] QuikChange: Mutagenesis of the codon-optimized Vκ domains was performed using Stratagene's QuikChange XL according to the manufacturer's protocol. All clones were sequenced to confirm the presence of the mutation.

Cell Culture

[0245] Transfection and stable cell line generation: One day prior to transfection, 293F cells were seeded at 6.0×10^5 cells/mL in 293FreeStyle medium (Thermo Fisher Scientific) in a shake flask and incubated at 37°C, 8% CO₂, with shaking at 125 rpm. On the day of transfection, cells were seeded at 1×10^6 cells/mL as above. Cells were transfected using PEI (25 kDa, linear; Polysciences) or ExpiFectamine (Thermo Fisher Scientific). For the PEI transfections, 166.7 ng HC plasmid, 166.7 ng LC plasmid, 2.2 μg PEI, and 50 μL OptiPro (Thermo Fisher Scientific) per mL of transfected cells were incubated for 15 min at 22°C. The DNA:PEI mixture was added to the cells while swirling and incubated at 37°C, 8% CO₂, shaking

at 125 rpm. After 48-72 h, cells were fed at a final concentration of 10 g/L Yeastolate (BD Biosciences), 5 mM valeric acid (Sigma Aldrich), and 1:100 CD Lipid Concentrate (Thermo Fisher Scientific).

[0246] For each mL of cells to be transfected with ExpiFectamine, 333.3 ng HC plasmid and 333.3 ng LC plasmid were incubated for 10 min in 50 μ L Opti-MEM (Thermo Fisher Scientific). Likewise, 2.67 μ L ExpiFectamine was incubated in 50 μ L Opti-MEM. The ExpiFectamine solution was added to the DNA mixture, and incubated for 30 min at 22°C. The DNA:ExpiFectamine mixture was added to the cells while swirling and incubated at 37°C, 8% CO₂, shaking at 125 rpm. The following day, 3 μ L of enhancer 1 and 30 μ L of enhancer 2 per mL of cells were added to the transfection with continued to incubate for another 7 or 10 days, depending on cell density.

[0247] Antibody-expressing stable pools were selected by adding 3 mL of transfectants to 12 mL DMEM in a T75 flask with 5 μ g/mL blasticidin and 400 μ g/mL zeocin (Thermo Fisher Scientific) one to three days after transfection. After drug-resistant cells grew to confluency, the medium was replaced with FreeStyle 293 expression medium. After 24 or 48 h, cells were physically dislodged by tapping the flask (trypsinization resulted in low viability; data not shown) and were then seeded at 6×10^5 cells/mL in 30 mL FreeStyle 293 expression medium in a 125-mL shake flask. Cultures were incubated at 37°C in 8% CO₂ with shaking at 125 rpm.

[0248] mAb production: Antibody production from stable pools was performed by one of two methods:

1. Stable-transfected cell line pools were seeded at 0.6 to 1×10^6 cells/mL in 293FreeStyle medium. Two days after the culture reached a density of 1×10^6 cells/mL, cultures were fed as described herein; or
2. Stable-transfected cell line pools were centrifuged at 1000 rpm in a Beckman Allegra 6 centrifuge for 5 min. The supernatant was removed, and the cells were resuspended in 1 L expi293 medium (Gibco) at 0.5 - 0.8×10^6 cells/mL in a 2.8-L shake flask. Cells were incubated at 37 °C, 8% CO₂, shaking at 125 rpm.

[0249] For both methods, the cultures were incubated at 37°C in 8% CO₂ with shaking at 125 rpm for 7-10 days, depending on when cell viability dropped to about 50%, at which time the cultures were centrifuged for 1 h at 8000 rpm in a Beckman JLA8.1000 rotor. The supernatant was then filtered through a 0.2 μ m PES filter and stored at 4°C or -20°C until purification.

mAb purification

[0250] Antibody purification by protein A affinity chromatography: Using an ÄKTA Explorer (GE Healthcare), a protein A column (GE Healthcare) was equilibrated with 10 column volumes (CV) of 20 mM sodium phosphate, 10 mM EDTA, pH 7.2. The sample was then loaded, followed by washing unbound material with 10 CV of equilibration buffer. The sample was eluted using 5 CV of 0.1 M Glycine pH 2.9. The fractions containing the mAb were pooled and dialyzed in Dulbecco's phosphate buffer (DPBS) using a MWCO 20K Slide-A-Lyzer (Thermo Fisher Scientific).

[0251] Cysteine decapping: Using an ÄKTA Explorer (GE Healthcare), a protein A column (GE Healthcare) was equilibrated with 10 CV of 20 mM sodium phosphate, 10 mM EDTA, pH 7.2 (equilibration buffer). The sample was then loaded, followed by washing unbound material with 10 CV of equilibration buffer. The column was washed with 16 CV of 20 mM sodium phosphate, 10 mM EDTA, 5 mM cysteine, pH 7.2 at 0.5 mL/min for 16 h at 4°C to remove capping group. The column was then washed with 60 CV of 20 mM Tris, pH 7.5 at 0.5 mL/min for 60 h at 4°C. The sample was eluted using 5 CV of 0.1 M glycine pH 2.9 and immediately neutralized using 5% volume of 2M Tris, pH 9.0. The fractions containing mAb were pooled and dialyzed in DPBS using a MWCO 20K Slide-A-Lyzer (Thermo Fisher Scientific).

LC-MS/MS cysteinylolation and disulfide bond mapping analyses

[0252] The mAb was buffer-exchanged to 50 mM ammonium bicarbonate buffer, pH 7.8 using a Zeba spin desalting column (Thermo-Fisher). The concentration was adjusted to 1 mg/mL and RapiGest (Waters) was added to 0.1%. The mAb was then digested with Glu-C (New England BioLabs) (25:1 w/w) at 37 °C for 4 h, followed by digestion with Asp-N (New England BioLabs) (25:1 w/w) at 37°C for 18 h. Following digestion, 5% trifluoroacetic acid (TFA) was added to 0.5% and incubated at 37°C for 90 min. The sample was centrifuged at 13,000 rpm for 30 min to remove pellets and analyzed by LC-MS/MS using MS^E methodology in the second ionization phase. MS^E methodology uses a ramped voltage rather than a fixed voltage in the second ionization phase to generate a more complete ion profile. Samples were analyzed using a Waters Acquity UPLC and Q-ToF Premier mass spectrometer. Samples were injected onto a Waters BEH 300 C18, 1.7 µm pore size, 2.1 x 100 mm, eluted from the column with a 3 min equilibration in 97% of mobile phase A (0.1% formic acid in H₂O), a 55 min linear gradient (3-45% mobile phase B (0.1% formic acid in acetonitrile)), a 5 min linear gradient

(45%-90% mobile phase B), a 5 min isocratic phase (90% mobile phase B), a 5 min linear gradient (90%-3% mobile phase B), and a 5 min re-equilibration in 97% of mobile phase A, at 0.05 mL/min. The Q-ToF mass spectrometer was run in positive ion, V-mode with detection in the range of 200-2000 m/z. The source parameters were as follows: capillary voltage, 3.0 kV, sampling cone voltage, 40 V; source temperature, 120°C; desolvation temperature, 250°C; desolvation gas flow, 600 L/hr. Lockspray mass reference standard was glu-fib. MS^E method was as follows: acquisition time, 3-70 mins; data range, 200-2000m/z; scan time, 1.5 sec; expression, low energy 6V, ramp high energy from 10-30V.

[0253] The antibody aggregation was analyzed by size-exclusion, high-performance liquid chromatography method (SEC-HPLC) using an Agilent 1100. The mAb was diluted to 1 mg/mL in DPBS. The antibody (20 µL) was injected onto a TSKgel SuperSW guard column (4.6 mm x 3.5 cm, 4 µm pore size, Tosoh Bioscience), followed by a TSKgel SuperSW3000 column (4.6 mm x 30 cm, 4 µm pore size), eluted from the column with 0.1 M PBS containing 0.15 M NaCl and 0.05% NaN₃, at pH 7.4, at a flow rate of 0.3 mL/min for 20 min. All data were analyzed using Agilent ChemStation software. Percent aggregation was calculated as $[PA_{\text{aggregate}}/PA_{\text{total}}]*100$, where PA = integrated peak area.

UPLC/ESI-MS analysis of maleimide-biotin:mAb conjugation

[0254] Purified antibodies were diluted to 1 mg/mL in DPBS (samples were left at original concentration if below 1.0 mg/mL). Maleimide-PEG2-Biotin ((mal)-PEG2-Biotin) (Thermo Fisher Scientific) was dissolved in DPBS to yield a 20 mM stock solution, followed by dilution to 1 mM in DPBS. Mal-PEG2-Biotin was added to 1 mL of decapped mAb at a 5:1 conjugation ratio and incubated at 22°C with gentle rotating for 2 hr. The reaction was desalted using a Zeba spin desalting column. The mAbs were then deglycosylated using PNGase F (New England BioLabs). G7 buffer (10 µL) and PNGase F (2 µL) were added to the mAb (90 µL). The reaction was incubated in a Discover microwave (CEM) for 2 cycles: 1) microwave power 10 W, 37°C, 10 min, followed by a 5-min pause; and 2) microwave power 2 W, 37°C, 10 min. A portion of the sample was reduced by adding dithiothreitol (DTT) to a final concentration of 20 mM, followed by incubation at 60°C for 3 min.

[0255] Samples were then analyzed using a Waters Acquity UPLC and Q-ToF Premier mass spectrometer. Samples (0.5-2 µg each) were injected onto a MassPrep micro desalting column at 65°C, eluted from the column with a 5 min equilibration in 95% of mobile phase A, a 10 min gradient (5-90% B), and a 10 min re-equilibration in 95% of mobile phase A, at 0.05

mL/min. Mobile phase A was 0.1% formic acid in water. Mobile phase B was 0.1% formic acid in acetonitrile. The Q-ToF mass spectrometer was run in positive ion, V-mode with detection in the range of 500-4000 m/z. The source parameters were as follows: capillary voltage, 2.25 kV (intact antibody)-2.50 kV (reduced antibody); sampling cone voltage, 65.0 V (intact antibody) or 50.0 V (reduced antibody); source temperature, 100°C; desolvation temperature, 250°C; desolvation gas flow, 550 L/hr. The protein peak was deconvoluted using the MassLynx MaxEnt 1 function. Conjugation efficiency was calculated as $[I_{\text{biotinylated}}/(I_{\text{biotinylated}}+I_{\text{unmodified}})]*100$ of the deconvoluted mass spectrum, where I = mass peak intensity.

BIAcore analysis of mAb:antigen affinity

[0256] Antibody concentrations were adjusted to generate 30-40 RU signal when bound to the antigen. Humanized mAbs purified by standard protein A affinity chromatography or by the decapping method were injected over an anti-human IgG sensor on a BIAcore T100 (GE Healthcare) for 1 min at a flow rate of 10 $\mu\text{L}/\text{min}$. The sensor surface was washed by injecting HBS-P buffer for 1 min at a flow rate of 50 $\mu\text{L}/\text{min}$. To record the antigen association to the captured mAb, a series of increasing concentrations of antigen was injected for 60 sec at a flow rate of 50 $\mu\text{L}/\text{min}$. The dissociation of antigen was monitored for 30 min at the same flow rate. The sensor surface was regenerated by injecting 3 M MgCl_2 for 1 min and then 30 sec at a flow rate of 30 $\mu\text{L}/\text{min}$. Sensograms were analyzed with Biacore T100 Evaluation Software using a 1:1 Langmuir binding model.

Bivalent/Bispecific Fab preparation

[0257] mAb-derived Fab fragments were prepared separately using immobilized papain, followed by isolation of the pure Fab fragments from Fc/undigested mAb using Protein A chromatography. Maleimido-PEG4-azide was synthesized by combining NHS-maleimide and azido-PEG4-amine in DMSO for 1 hr in a 1:1 molar ratio. Unreacted NHS was quenched by the addition of Tris-HCl buffer to prevent homodimerization. Fabs were conjugated to either maleimido-PEG4-azide or maleimido-PEG4-dibenzocyclooctyne (DBCO) at a 5:1 molar ratio of maleimide:Fab and reacted for 4 hr at 22°C. The modified Fab fragments were desalted twice each in DPBS to remove all unreacted products, and the Fab fragments were combined at a molar ratio of 1:1 at 2 mg/mL final concentration and allowed to form dimers overnight at 22°C. The reaction was analyzed by SDS-PAGE and dimerization efficiency was estimated at 20%.

The dimer preparation was purified from unreacted monomer by S-200 gel filtration chromatography.

Bivalent/Bispecific Octet assay

[0258] Biotinylated human CA9 was captured on streptavidin Biosensor tips (Pall) for 4 min. Following incubation in PBS for 2 min, the tips were incubated with the bivalent/bispecific Fabs, mAb alone, or Fab alone for 5 min. Following incubation in PBS for 2 min, the tips were incubated with human endosialin/TEM-1 for 5 min. Finally, the tips were incubated in PBS for another 2 min. Association and disassociation protein to the tips was measured throughout.

Example 2 – Cys80 conjugation

Objective

[0259] Site-specific conjugation technologies are desirable to produce a homogeneous product with a defined drug-to-antibody ratio (DAR). The V κ domain of a rabbit mAb, such as that derived from *Oryctolagus cuniculus*, may contain a cysteine in position 80 (referred to as “Cys80”) (FIG. 1A) and the C κ region may contain a cysteine in position 171 (“Cys171”) (FIG. 1B). In-silico modelling predicted that Cys80 and Cys171 might be forming a disulfide bond, as the two S atoms are predicted to be approximately 1.6 Å apart (FIG. 2A). Human mAbs have proline, serine, or alanine residues in position 80 (FIG. 1A), and serine in position 171 (FIG. 1B), thus there is no disulfide bridge between the variable and constant region (FIG. 2B).

[0260] The crystal structure closest to rabbit or human V κ and C κ sequences was identified using BLAST pdb database and used as a template for modeling 155D5 mAb structure. Models were generated using Discovery Studio's “Build Homology Models” tool (Accelrys). The model with the lowest total energy was selected, typed with the CHARMM forcefield, and the energy was further minimized through two rounds of energy minimization using the “Minimize” tool. The CDR loops were then refined using the “Model Antibody Loops” tool. The model with the lowest total energy was selected, typed with the CHARMM forcefield, and the energy was further minimized as above. The proximity of Cys80 and Cys171 (FIG. 2A) predicts that these cysteines may be forming a disulfide bond. The “Build Mutants” tool was used to represent this disulfide bond.

[0261] Since disulfide bonds are critical for maintaining secondary and tertiary structural integrity, which in turn is necessary for an antibody's biological activity, it was

important to prove whether the predicted Cys80-Cys171 bond actually existed. Therefore, *ad hoc* experiments were conducted that unequivocally demonstrated that the rabbit mAbs contained such a bond (Table 4).

Table 4. Demonstration of the existence of Cys80-Cys171 disulfide bond

Peptide Sequence (position)	Predicted mass (Da)	Observed mass (Da)
DCTYNLSSTLSLTK (170-183) (SEQ ID NO:22)	1545.7465	Not observed
FTLTITGVQCD (71-81) (SEQ ID NO:23)	1197.582	Not observed
DCTYNLSSTLSLTK=FTLTITGVQCD (SEQ ID NO:22) (SEQ ID NO:23) (disulfide-linked peptides as above)	2741.3285	2740.2659

LC-MS/MS analysis was performed on a Glu-C/Asp-N digest of rabbit 155D5 MAb (from NZW rabbit). Only masses corresponding to disulfide-linked cys80-cys171 were found, indicating that cys80 forms a disulfide bond with cys171 in rabbit IgG. A similar analysis was performed using 1-55-2 mAb (from b9 rabbit).

[0262] A species-human chimerized mAb is made through the fusion between: i) the variable region from the species where the mAb was generated; and ii) the human constant region. This process is called chimerization. A humanized mAb is mostly made of human variable and constant regions, except for those residues necessary for antigen binding, which are from the same species of the host from which the mAb was generated. This process is called humanization. To engineer human chimerized or humanized mAbs, whereby the mAbs were generated in hosts belonging to the species *Oryctolagus cuniculus* the entire constant domains as well as most of the variable regions (if humanized) were genetically replaced with the human variable and human constant sequences. After either chimerization or humanization, the Cys80 in the V κ no longer formed a disulfide bond with position 171 in the C κ (FIG. 2C), and is therefore unpaired.

[0263] Germline NZW rabbit V κ families have a cysteine at position 80 as shown in FIG. 3 (the CDR regions were deleted, and frameworks (FWR) 1, 2, and 3 were aligned).

Discovery and characterization of an unpaired cysteine at position 80

[0264] Rabbit constant regions of 155D5 and 1E4 (anti-CA9), 1-55-2 (anti-hTEM1), as well as 33O11 (anti-MSLN), all of which contain Cys80 and generated as described in Example 1, were replaced with the human constant regions of an IgG1 κ to generate rabbit/human

chimerized mAb, as described elsewhere herein. Specifically, the rabbit VH region of 155D5 was fused with the human C γ region to generate xi155D5HC, and the rabbit V κ region of 155D5 was fused with the human C κ region to generate xi155D5LC. The rabbit/human chimerized 155D5 mAb with the unpaired Cys80 is referred to herein as xi155D5.

[0265] VH region of 1-55-2 was fused with the human C γ region to generate xi1-55-2H, and the rabbit V κ region of 1-55-2 was fused with the human C κ region to generate xi1-55-2LC. The rabbit/human chimerized 1-55-2 mAb with the unpaired Cys80 is referred to herein as xi1-55-2.

[0266] The rabbit VH region of 1E4 was fused with the human C γ region to generate xi1E4HC, and the rabbit V κ region of 1E4 was fused with the human C κ region to generate xi1E4LC. The rabbit/human chimerized 1E4 mAb with the unpaired Cys80 is referred to herein as xi1E4.

[0267] The rabbit VH region of 33O11 was fused with the human C γ region to generate xi33O11HC, and the rabbit V κ region of 33O11 was fused with the human C κ region to generate xi33O11LC. The rabbit/human chimerized 33O11 mAb (xi33O11) with the unpaired Cys80 is referred to herein as xi33O11.

[0268] Because the Cys171 was substituted with Ser171 during chimerization, the chimerized antibodies (xi155D5, xi1-55-2, xi1E4, and xi33O11) contained an unpaired cysteine at position 80 in the V κ (referred to as “Cys80”). When reduced using harsh conditions (20 mM DTT at 60 °C for 5 min), the molecular weight (mass) of the protein A-purified mAb xi155D5 light chain was 23,382 Da (FIG. 4A). However, when subjected to mild reducing conditions (100 μ M DTT, RT, 30 min) the mass increased by 120 Da (FIG. 4B). This mass increase suggested that Cys80 might be forming a disulfide bond with a free cysteine, referred to as “capping” cysteine. This molecular structure, which results from a reaction called “cysteinylation”, is referred to as “capped” Cys80. To confirm this hypothesis, xi155D5 mAb was digested with Asp-N and Glu-C, and the masses of the peptides were analyzed. Mass spectrometry analysis of peptide fragments corresponding to residues 71 through Cys80 (FTLTITGVQC) (SEQ ID NO:24) indicated an increased molecular weight by 119 Da (Table 5), thereby confirming that Cys80 was capped.

Table 5. Mass spectrometry of xi155D5 peptide 71-Cys80 fragments

Peptide Sequence	Fragment mass (Da)	Observed mass (Da)	Δ mass (Da)
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FTLTITGVQC (SEQ ID NO:24)	1082.555	1201.5544	118.9994
TLTITGVQC (SEQ ID NO:25)	935.4866	1054.4946	119.008
LTITGVQC (SEQ ID NO:26)	834.4389	953.4397	119.0008
TITGVQC (SEQ ID NO:27)	721.3549	840.3552	119.0003
ITGVQC (SEQ ID NO:28)	620.3072	739.3067	118.9995
TGVQC (SEQ ID NO:29)	507.2231	626.2235	119.0004
GVQC (SEQ ID NO:30)	405.47	525.1809	119.7109
QC (SEQ ID NO:31)	249.28	369.0883	119.8083

[0269] Because the lack of the Cys80-Cys171 disulfide bond could have led to antibody instability, disruption of antigen binding, or both, antibody stability and antigen binding tests were conducted. The stability of xi155D5 was tested using a SE-HPLC assay. This assay tests whether the lack of Cys80-Cys171 disulfide bond could lead to aggregation (due to possible intermolecular Cys80-Cys80 bonds), or degradation (due to increased sensitivity to proteases). Purified antibody at 1 mg/mL in 1X PBS was stored at -80°C or 37°C for 1 week. Ten µL of xi155D5 was injected onto a SuperSW3000 column (TOSOH Biosciences, 4.6 mm X 30 cm, 4 µm particle size) equipped with an in-line TSKgel 4.6 mm X 3.5 cm guard column at a flow rate at 0.3 mL/min with 0.1 M sodium phosphate, 0.15 M NaCl, 0.05% NaN₃ as mobile phase. No significant change in aggregation was observed between the two storage conditions (FIG. 5A and 5B). The level of aggregation was in the 3-4% range and hence within the normal range of a typical human IgG1 (data not shown). Little or no degradation products were observed in any storage conditions (FIG. 5). These results suggest that xi155D5 lacking the Cys80-Cys171 disulfide bond is a stable protein under the storage conditions tested.

[0270] To determine if chimerization, and therefore the loss of Cys80-Cys171 disulfide bond, results in structural perturbations leading to loss of antigen binding, the binding affinity of mAbs 155D5, xi155D5, 1-55-2, and xi1-55-2 by surface plasmon resonance was evaluated. Biotinylated ligand (biotin-hTEM1 for 1-55-2, biotin-CA9 for 155D5) was captured on a coated biotin CAP BIAcore chip (GE Healthcare, Piscataway, NJ) using HBS-EP as running buffer. Final antigen capture levels were 130 RU and 280 RU, respectively, for biotin-TEM1 and biotin-CA9. Serial dilutions of antibody (120 µL of 0-50 nM) were passed over the ligand-coated chip.

Dissociation was observed for 25 min. The chip surface was regenerated with 6 M GuHCl, 250 mM NaOH. Sensograms were double referenced and kinetic parameters were determined using BIAEvaluation software (ver. 4.1). Little or no loss of binding affinity was observed due to chimerization of two different mAb (Table 6), suggesting that the lack of the Cys80-Cys171 disulfide bond does not lead to disruption of the binding region.

Table 6. Kinetic constants of chimerized and rabbit mAbs

mAb	k_a ($M^{-1}sec^{-1}$)	k_d (sec^{-1})	K_D (M)
1-55-2	3.7×10^6	5.8×10^{-5}	1.5×10^{-11}
xi1-55-2	1.5×10^6	7.1×10^{-5}	4.6×10^{-11}
155D5	5.1×10^5	2.1×10^{-5}	4.1×10^{-11}
xi155D5	4.6×10^5	2.4×10^{-5}	5.1×10^{-11}

Assessment of the utility of Cys80 for conjugations of functional agents

[0271] After having established that the lack of Cys80-Cys171 disulfide bond does not lead to structural perturbations, the possibility of replacing the capping cysteine with a thiol-reactive compound was explored. A thiol-reactive group can be attached to a linker, which in turn can be attached to a molecule of diagnostic or therapeutic utility, referred to herein as “functional agent.” Functional agents may include fluorophores, fluorescent dyes, polypeptides, immunoglobulins, antibiotics, nucleic acids, radionuclides, chemical linkers, small molecules (such as chemotherapeutic agents), chelators, lipids, and drugs.

[0272] To substitute the capping cysteine with a functional agent, the capping cysteine was first removed. Exposing purified mAbs to reducing conditions could break the disulfide bond between Cys80 and the capping cysteine, referred to herein as “decapping.” However, suboptimal reducing conditions, for example harsh reducing conditions, could also break the inter- and intra-chain disulfide bonds, thereby compromising the mAb structure and activity. Therefore, a decapping method involving removal of the capping cysteine using mild reduction, followed by reoxidation with Tris-containing buffer that does not alter the mAb structure and activity, while still allowing removal of the capping cysteine, was developed. A number of reducing agents were initially evaluated, including reduced glutathione, cysteine, TCEP, and DTT. Glutathione did not efficiently remove the capping cysteine (data not shown). Both DTT and TCEP efficiently removed the capping cysteine, but higher concentrations also resulted in the near-complete breakage of inter-chain disulfides and likely some intra-chain disulfides as well (data not shown). The mild reductant cysteine efficiently removed the capping cysteine and

only limited inter-chain breakage was observed. Reoxidation was examined using phosphate buffer, Tris buffer, and the strong oxidant CuSO_4 . No reoxidation of the disrupted inter-chain disulfides was observed with phosphate buffer, while CuSO_4 efficiently and rapidly reformed the disulfides, but was not evaluated further, due to its inherent toxicity compared with Tris. Optimized conditions were adapted to a column format to allow for sequential purification and decapping from feedstock. With this method, the antibody was bound to protein A resin and incubated with limited flow (0.5 mL/min) with a buffer containing 5 mM cysteine for 16 h to reduce (break) the Cys80-cysteine disulfide bond, followed by washing with a cysteine-free Tris-containing buffer for 60 h to remove the cysteines released by this treatment and re-oxidize any reduced interchain disulfide bonds. The mAb was then eluted in a low pH glycine buffer. In an exemplary experiment whereby the decapping method was applied to xi155D5, the mass of the non-reduced, purified mAb was determined and ~99% of the mAb was found decapped, as demonstrated by the drop in mass equivalent to two free cysteines (FIG. 6A and 6B). Free thiol assay confirmed the presence of two thiol groups per mAb, also demonstrating efficient re-oxidation (data not shown).

Decapped Cys80 can be conjugated to maleimide

[0273] Cysteine is an α -amino acid with a nonpolar side chain (thiol; -SH). The reduced thiol side chain in an unpaired cysteine could serve as a nucleophile that can react with an electrophile molecule such as maleimide, a chemical compound with the formula $\text{H}_2\text{C}_2(\text{CO})_2\text{NH}$. The electrophile double bond in maleimide readily reacts with the nucleophile thiol group found on cysteine to form a stable carbon-sulfur thioether bond. The nonpolarity of the thiol side chain, depending on the surrounding residues, might confer a hydrophobic property to a cysteine that may prevent solvent exposure necessary for chemical modifications. In addition, the location of the cysteine in the context of the secondary structure of the peptide in which it is located may further prevent access of thiol-reactive molecule. Experimental testing to determine whether Cys80 could react with a thiol-reactive molecule after decapping was performed. The decapped xi155D5 was incubated with maleimide-PEG2-biotin as described elsewhere herein. Mass spectrometry analysis showed that 94% of the mAb was conjugated with maleimide-PEG2-biotin as indicated by an increase in molecular mass by 526 Da (FIG. 7), corresponding to the functional agent mass. As each light chain was found conjugated (single mass peak, FIG. 7), the maleimide-PEG2-biotin to xi155D5 ratio was homogeneously equal to

2:1. One maleimide-PEG2-biotin was conjugated to a Cys80 in each of the two light chains in the chimerized mAb (Cys80¹ and Cys80²).

[0274] These results demonstrate that Cys80 and Cys171 form a disulfide bond that links the V κ and C κ regions of a rabbit mAb. When rabbit mAbs were chimerized, Cys171 was substituted by Ser171 present in the human C κ region. This substitution abolished the Cys80-Cys171 disulfide bond. When the effects of losing this disulfide bridge on the structural stability and activity of the resulting chimerized mAb compared with the parental rabbit mAb were evaluated, it was observed that the chimerized mAb was stable and active. It was discovered that both Cys80¹ and Cys80², which remained unpaired in the chimerized mAb, were capped by a free cysteine (capping cysteine). Subsequently, a method to remove the capping cysteine (decapping), while maintaining structural stability and activity of the resulting chimera mAb, was developed. Additionally, it was demonstrated that high yields of mAb conjugated to maleimide-PEG2-biotin could be achieved with a functional agent to mAb ratio equal to 2:1.

Humanization of rabbit mAbs

[0275] Chimerized mAbs could be immunogenic when administered to humans and therefore it is desirable to humanize rabbit mAbs by substituting rabbit sequences with human sequences in the V κ and V H regions. The amino acid sequence of mAb 155D5 was analyzed using a BLAST search against a human variable domain database at <http://www.ncbi.nlm.nih.gov/igblast/> to identify the human sequence with highest homology to the rabbit sequence. IGHV3-64*04 and IGKV1-5*03 were identified as the best sequences for humanization, as their use would result in the least number of rabbit residue substitutions (FIG. 8).

[0276] The 155D5 sequences corresponding to the antigen binding domains as identified by Kabat and Chothia CDRH1, Chothia CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 were inserted into the framework (FWR) regions of human IGHV3-64*04 or IGKV1-5*03 to generate the humanized 155D5 mAb, named zu155D5-1 (Table 7 and Table 8).

[0277] During the humanization of 155D5LC (zu155D5LC), Cys80 was maintained, which was unpaired since the human kappa sequence has Ser171 as opposed to Cys171. zu155D5-1 was produced and purified using standard protein A purification, and found to be capped, as evidenced by the change of mass after decapping by 233 Da, approximately corresponding to two capping cysteines (FIG. 9). As observed with xi155D5, zu155D5-1 could also be decapped with efficiency close to 100% (Table 9). However, the decapping led to

massive levels of aggregation (70%) versus only 14% in xi155D5. When zu155D5-1 was reacted with maleimide-PEG2-biotin, 0% conjugation was observed while xi155D5 was 93% conjugated (Table 9). These results were surprising, as they suggest that: 1) having a cysteine in position 80, although necessary, is not sufficient to allow for site-specific conjugation of a functional agent; and 2) in some conditions, attempting conjugation on Cys80 could lead to high aggregation not compatible with drug manufacturability. However, since the disclosed studies, which characterized xi155D5, demonstrated that at least in some conditions conjugation of a functional agent on Cys80 could be very efficient, it was next investigated how residues surrounding Cys80 may influence conjugation efficiency. Consequently, structural models of chimerized xi155D5 were generated (FIG. 10), which indicated that residues Val11, Ala14, Gly17, Thr18, Lys63, Thr76, Gly77, Val78, Ala83, Glu103, and Leu104 were in close proximity (within 18 Å) to Cys80 and therefore could potentially be affecting the efficiency of its conjugation.

[0278] Two versions of FWR1 (FWR1a-b), one version of FWR2, three versions of FWR3 (FWR3a-c), and two version of FWR4 (FWR4a-b) were designed based on the aforementioned residues (Table 7).

Table 7. Versions of frameworks derived from human V κ family IGKV1-5

human V κ family	FWR version code	FWR1	FWR2	FWR3	FWR4
IGKV1-5	A	DIQMTQSPSTLSA SVG DR VTITC (SEQ ID NO:32)	WYQQKPGKAP KLLIY (SEQ ID NO:33)	GVPSRFSGSGSGTEFTL TISSLQCDDFATYYC (SEQ ID NO:34)	FGGGT K VEIK (SEQ ID NO:35)
IGKV1-5	B	DIQMTQSPSTVSA AVG GT VTITC (SEQ ID NO:36)	n/a	GVPSRFSGSGSGTEFTL TIT GV QCDDFATYYC (SEQ ID NO:37)	FGGGT E LEIK (SEQ ID NO:38)
IGKV1-5	C	n/a	n/a	GVPSRF K GSGSGTEFT LTIT GV QCDDAATYYC (SEQ ID NO:39)	n/a

Residues in bold font indicate differences between the framework variants. n/a, not applicable.

[0279] A series of humanized 155D5 variants were generated that contained combinations of these frameworks and either Cys80-Xaa₁-Xaa₂-Phe83 (also referred to as C-X-X-F or CXXF) or Cys80-Xaa₁-Xaa₂-Ala83 (also referred to as C-X-X-A or CXXA), whereby “Xaa” or “X” indicates amino acids in position 81 and 82 (Table 8).

Table 8. Humanized 155D5 variants derived from human V κ family IGKV1-5

mAb name	FWR combination	aa 80-83
zu155D5-1	FWR1a,FWR2a,FWR3a,FWR4a	CXXF
zu155D5-2	FWR1a,FWR2a,FWR3b,FWR4a	CXXF
zu155D5-3	FWR1a,FWR2a,FWR3a,FWR4a	CXXA
zu155D5-4	FWR1a,FWR2a,FWR3b,FWR4a	CXXA
zu155D5-5	FWR1a,FWR2a,FWR3c,FWR4a	CXXA
zu155D5-6	FWR1b,FWR2a,FWR3b,FWR4a	CXXA
zu155D5-7	FWR1a,FWR2a,FWR3b,FWR4b	CXXA

[0280] It was observed that, irrespective of the FWR version used, humanized mAbs having the C-X-X-F motif showed high aggregation (after decapping) and poor conjugation (Table 9). Conversely, 4 of the 5 mAb variants containing C-X-X-A motif, and irrespective of the FWR version used, showed high percent of conjugation efficiency ($\geq 80\%$) and low aggregation (after decapping) of $<18\%$ (Table 9). zu155D5-4 is an outlier that exhibited a low percentage of conjugation efficiency and a high aggregation after decapping. It is noted that the zu155D5-4 antibody has a propensity to aggregate independently of decapping, which may account for the observed results. These data suggested that Phe83 is involved in causing high aggregation after decapping and is not conducive to conjugation on Cys80.

Table 9. Aggregation levels and conjugation efficiency of xi155D5 versus different variants of zu155D5

mAb name	% Aggregates		% Decapped	% Conjugated	aa 80-83
	ProA	Decapped			
xi155D5	ND	14.20%	100.00%	93.90%	CXXA
zu155D5-1	1.10%	70.10%	100.00%	0.00%	CXXF
zu155D5-2	10.10%	44.50%	100.00%	0.00%	CXXF
zu155D5-3	0.00%	17.30%	100.00%	80.10%	CXXA
zu155D5-4	10.80%	28.30%	100.00%	25.90%	CXXA
zu155D5-5	7.40%	13.30%	100.00%	86.90%	CXXA
zu155D5-6	1.60%	6.70%	100.00%	88.20%	CXXA
zu155D5-7	4.90%	12.30%	100.00%	89.30%	CXXA

In bold are indicated the C-X-X-F motif and values not meeting the conjugation specifications.

[0281] It is desirable to achieve aggregation of 25% or less as a starting point of downstream process optimization, whereby further optimization of fermentation parameters, purification conditions and drug formulations can achieve a more desirable aggregation level of 5% or less. It is also desirable to achieve 70% or higher conjugation efficiency to minimize product waste, cost of goods, and maximize product homogeneity. Henceforth, the investigation focused on meeting and exceeding these specifications by extrapolating rules to apply to the humanization methods of rabbit mAbs.

[0282] 155D5-1 was generated by following a standard practice, which involves utilizing the human germline sequences most homologous to the parent sequence. Because of this practice, the human V κ subfamily IGKV1-5 was used for humanizing 155D5, having an percent identity of 70.5% (data not shown) and containing Phe83. The alternative V κ subfamilies, which have similar percent of identity (data not shown), also contained Phe83 (FIG. 8). As this residue appeared to negatively influence Cys80 conjugation efficiency and cause high aggregation, the presence or absence of Phe83 in other human V κ families was evaluated, despite the fact that the highest identity found in these V κ families was lower (68.4%) and therefore they would not typically be used for humanizing 155D5. Since all of the human V κ families except IGKV4, IGKV5, and IGKV7 have multiple subfamilies, all subfamilies were aligned within their family (data not shown). After removal of redundant sequences, only one sequence remained for each of the IGKV-4, -5, -6, -6D, and -7 families, which could be used for humanization. For families IGKV-1, -2, and -3, the subfamily with closest homology to the consensus was chosen as the framework to humanize 155D5. A preliminary analysis indicated that while some of these V κ families contained Phe83 others did not (and Table 10).

[0283] To study the effect of the presence or absence of Phe83 in the context of these V κ families, the CDR regions of 155D5 were genetically grafted onto the human frameworks IGKV1-39*01, IGKV2-40*01, IGKV3-11*01, IGKV4-1*01, IGKV5-2*01, IGKV6-21*01, IGKV6D-41*01, and IGKV7-3*01. IGKV5-2*01 Asn20, which contains an N-linked glycosylation site at residues 20, and its Thr20 variant were not included in this analysis because the former could not be analyzed by mass spectrometry due to heterogeneity, and the latter did not express well. IGKV7-3*01 Asn81 was not included in the analysis because it could not be analyzed by mass spectrometry. However, the variant IGKV7-3*01-Glu81 was included in the analysis. The following results were obtained from the analysis (Table 10):

1. The humanized mAb with the huIGKV1-39 sequence and containing Phe83 showed an aggregation increase from 0 to 26% after decapping; the conjugation efficiency was

- borderline acceptable (68%) but aggregation being >25% was not;
- The humanized mAb with the huIGKV2-40 sequence contained a human germline Val83; this mAb showed aggregation <25% after decapping (11%) and conjugation efficiency >70% (92%). These parameters were acceptable, suggesting that the human germline Val83 is conducive to pairing with Cys80 to allow Cys80 conjugation;
 - The humanized mAb with the huIGKV3-11 sequence contained a human germline Phe83; while aggregation was below 25%, conjugation efficiency was 55%, therefore not meeting the criterion of >70%;
 - The humanized mAb with the huIGKV4-1 sequence contained a human germline Val83; this mAb showed aggregation <25% after decapping (6%) and conjugation efficiency >70% (82%). These parameters were acceptable, suggesting that the human Val83 is conducive to pairing with Cys80 to allow Cys80 conjugation, as seen with the huIGKV2-40 sequence;
 - The humanized mAbs with the huIGKV6-21 or huIGKV6D-41 sequences both contained a human germline Ala83; these mAbs showed aggregation <25% after decapping and conjugation efficiency >70%. These parameters were acceptable, suggesting that the human germline Ala83 is conducive to pairing with Cys80 to allow Cys80 conjugation, as seen with the xi155D5 sequence; and
 - The humanized mAb with the huIGKV7-3-Glu81 sequence contain a human germline Thr83; this mAb showed aggregation <25% after decapping (6%) and conjugation efficiency close to 100%. These parameters were acceptable, suggesting that the human germline Thr83 is conducive to pairing with Cys80 to allow Cys80 conjugation.

Table 10. Aggregation levels and conjugation efficiency of different variants of zu155D5 generated by using various human V κ subfamilies

humanized mAb	aa 80-83	% Aggregates		% Decapped	% Conjugated
		ProA	Decapped		
zu155D5-1 (huVK1-5)	CXXF	1.10%	70.10%	100.00%	0.00%
zu155D5-huVK1-39	CXXF	0.00%	26.00%	100.00%	68.70%
zu155D5-huVK2-40	CXXV	9.60%	11.70%	100.00%	92.70%
zu155D5-huVK3-11	CXXF	6.20%	16.50%	100.00%	55.00%
zu155D5-huVK4-1	CXXV	0.00%	6.30%	100.00%	82.70%
zu155D5-huVK6-21	CXXA	13.80%	15.00%	61.70%	81.50%
zu155D5-huVK6D-41	CXXA	9.30%	11.90%	62.00%	79.80%

zu155D5-huVK7-3-Glu81	CXXT	0.00%	6.30%	100.00%	100.00%
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In bold are indicated the C-X-X-F motif and values not meeting the conjugation specifications.

[0284] These results support the discovery that position 83 influences Cys80 conjugation efficiency negatively when occupied by phenylalanine, and indicate that, in addition to alanine, valine and threonine can substitute Phe83 to allow Cys80 conjugation.

[0285] To confirm that, in the context of other mAbs, Phe83 is involved with causing high aggregation after decapping and is not conducive to conjugation on Cys80, humanized mAb variants of 1E4 (anti-CA9), 166B3 (anti-CA9), and 33O11 (anti-MSLN) were generated containing either C-X-X-F or C-X-X-A.

[0286] Monoclonal antibody variants having C-X-X-F motif met the conjugation specifications but not the aggregation specifications, whereas all humanized mAb variants having C-X-X-A showed aggregation less than 25% and conjugation efficiency greater than 70% (Tables 11 and 12). These studies demonstrate that the C-X-X-(non) F or K is a motif that allows meeting conjugation specifications.

Table 11. Aggregation levels and conjugation efficiency of different variants of humanized mAbs comparing C-X-X-F versus C-X-X-A and C-X-X-I motifs

humanized mAb	% Aggregates		% Decapped	% Conjugated
	ProA	Decapped		
zu155D5-CXXF (zu155D5-1)	1.10%	70.10%	100.00%	0.00%
zu155D5-CXXA (zu155D5-3)	0.00%	17.30%	100.00%	80.10%
zu166B3-CXXF	22.10%	51.50%	77.70%	34.70%
zu166B3-CXXA	2.30%	6.90%	100.00%	89.90%
zu33O11-CXXF	2.10%	27.50%	100.00%	76.10%
zu33O11-CXXA	1.50%	4.30%	100.00%	93.20%
zu33O11-CXXI	1.40%	4.30%	100.00%	100.00%

In bold are indicated the C-X-X-F motif and values not meeting the conjugation specifications.

Table 12. Aggregation levels and conjugation efficiency of different variants of z1E4 mAbs comparing C-X-X-F versus C-X-X-(non)F or K motifs

Antibody	Protein A		Decapped		% Decapping	% Conjugated	fold increase
	% Aggregates	% Monomer	% Aggregates	% Monomer			
1E4-CXXG	7.10%	93.00%	ND*	ND*	ND*	ND*	
1E4-CXXA	13.20%	86.80%	21.90%	78.10%	100.00%	71.00%	1.66
1E4-CXXL	8.30%	91.70%	13.10%	86.90%	100.00%	88.30%	1.59
1E4-CXXV	12.10%	87.90%	18.00%	82.00%	100.00%	95.60%	1.49
1E4-CXXP	11.60%	88.40%	23.30%	76.70%	100.00%	87.50%	2.01
1E4-CXXM	10.70%	89.30%	17.60%	82.40%	100.00%	91.10%	1.65
1E4-CXXF	22.10%	77.90%	47.20%	52.80%	77.70%	43.60%	2.14
1E4-CXXY	7.20%	92.80%	16.40%	83.70%	100.00%	90.90%	2.28
1E4-CXXW	11.50%	88.50%	19.50%	80.50%	100.00%	89.80%	1.7
1E4-CXXS	9.90%	90.10%	14.60%	85.40%	100.00%	97.20%	1.48
1E4-CXXT	7.10%	92.90%	11.70%	88.40%	100.00%	97.20%	1.64
1E4-CXXN	7.30%	92.70%	13.40%	86.70%	100.00%	96.60%	1.82
1E4-CXXQ	11.10%	88.90%	12.80%	87.20%	100.00%	93.40%	1.15
1E4-CXXD	10.60%	89.40%	14.90%	85.10%	100.00%	73.00%	1.4
1E4-CXXE	7.80%	92.20%	13.20%	86.80%	100.00%	70.60%	1.69
1E4-CXXK	8.40%	91.70%	15.10%	84.90%	50.00%	43.70%	1.81
1E4-CXXR	12.60%	87.40%	20.50%	79.50%	100.00%	87.10%	1.63
1E4-CXXH	11.20%	88.80%	16.30%	83.70%	100.00%	83.70%	1.45

In bold are indicated the C-X-X-F and C-X-X-K motifs and values not meeting the aggregation or conjugation specifications.

[0287] In addition to Ala83, Val83, and Thr83, which can be found in V κ sequences belonging to huIGKV1-7 germline subfamilies, Ile83 can also be found, albeit rarely, in the huIGKV1 germline family. Because Ala83, Val83 and Thr83 were already found conducive for Cys80 conjugation (Table 9, and Table 11), it remained to be determined whether Ile83 would be a favorable or unfavorable residue with respect to Cys80 conjugation. Hence, the humanized mAb variant of 33O11 was generated containing the Cys80-Xaa₁-Xaa₂-Ile83 (also referred to as C-X-X-I or CXXI) motif, which showed aggregation less than 25% and conjugation efficiency greater than 70% (Table 11), consistent with previous C-X-X-(non)F motifs tested. This result supports the discovery that, in addition to Ala83, Val83 and Thr83, Ile83 can also substitute Phe83 to allow Cys80 conjugation while meeting conjugation specifications.

[0288] The disclosed studies indicate that, while chimerized rabbit mAbs are suitable for site-specific conjugation on Cys80 only after applying the disclosed decapping method as discussed above, humanized rabbit mAbs having the C-X-X-F motif or C-X-X-K motif are not well suited for such modifications due to high aggregation after decapping and/or low conjugation efficiency. It was hypothesized that the residues surrounding Cys80 may play a role in this phenomenon. Because the V κ region encompasses more than 100 residues, understanding the interplay between key surrounding residues and Cys80 required the use of structural modelling paired with experimental testing. It was discovered that among the residues in close proximity to Cys80, Phe83 exerted a negative effect on Cys80 conjugation efficiency. It was also observed that all of the rabbit mAbs contained Phe83 after humanization using a classical humanization approach (FIG. 11), despite the fact that human V κ sequences can also contain other amino acids in position 83, including alanine, threonine, valine, and isoleucine. When these V κ families were used for humanization, it was confirmed that Phe83 and Lys83 are sufficient to endow the humanized mAb with undesirable properties, such as high aggregation and/or low conjugation efficiency, while the remaining amino acid residues (with the exception of Cys, which was not tested due to the desire to obtain a single Cys for conjugation) were conducive to Cys80 conjugation.

[0289] These results suggest that the C-X-X-F motif and C-X-X-K motif are to be avoided when conjugating at Cys80. Using a C-X-X-(not)F or K motif, for example the motif C-X-X-A, C-X-X-T, C-X-X-V, and C-X-X-I through the substitution of Phe83, chimerized as well as humanized mAbs were generated that met the desired conjugation specifications.

Affinity of xi155D5 and the humanized variants

[0290] xi155D5 and the humanized variants were purified by standard protein A chromatography or the decapping method, and their affinity was analyzed using BIAcore (Table 13). There was less than a 2-fold difference in the KD between chimeric and humanized 155D5, and little difference between the samples purified by the two different methods.

Table 13. BIAcore analysis of antigen binding of humanized and chimeric 155D5 mAbs

Antibodies	ProA			Decapped		
	ka (1/Ms)	kd (1/s)	KD (M)	ka (1/Ms)	kd (1/s)	KD (M)
xi155D5	4.58E+05	1.49E-04	3.24E-10			
zu155D5-1	1.01E+06	4.95E-04	4.98E-10	7.70E+05	3.55E-04	5.00E-10
zu155D5-2	6.63E+05	3.76E-04	5.69E-10	4.58E+05	2.70E-04	5.89E-10
zu155D5-3	7.27E+05	4.01E-04	5.62E-10	7.26E+05	3.51E-04	5.07E-10
zu155D5-4	5.77E+05	3.82E-04	6.64E-10	6.41E+05	3.78E-04	5.90E-10
zu155D5-5	5.87E+05	3.73E-04	6.39E-10	5.72E+05	2.73E-04	4.78E-10
zu155D5-6	8.98E+05	4.77E-04	5.33E-10	5.58E+05	3.19E-04	5.68E-10
zu155D5-7	8.69E+05	4.58E-04	5.34E-10	4.85E+05	3.19E-04	6.80E-10

Example 3 - Generation of mesothelin-auristatin conjugated monoclonal antibodies

[0291] Mesothelin (MSLN) is a cell-surface protein expressed in cancer, including certain ovarian, lung, pancreatic, and mesothelioma tumors. To improve the potency of agents targeting MSLN, *de novo* anti-MSLN rabbit monoclonal antibodies (mAbs) were developed and subsequently engineered and conjugated with auristatin F (AuF) at Cys80 to generate a panel of MSLN-AuF conjugated mAbs.

Generation and characterization of rabbit anti-MSLN mAbs

[0292] New Zealand rabbits (*Oryctolagus cuniculus*) were immunized at Aldevron (Germany) using plasmid DNA encoding the human MSLN protein ("MSLN"). On day 52, animal sera were collected and later tested for MSLN binding by flow cytometry using mammalian cells transiently expressing human MSLN. FIG. 12-D shows that sera from both animals contained MSLN-binding antibodies. An ELISA assay later confirmed this result (FIG.

12E). Then, animals were sacrificed and PBMCs from blood as well as the lymphocytes from spleens and lymph nodes were collected and cryopreserved.

[0293] The lymphocytes from lymph nodes previously frozen were recovered and treated with DNase I and Pokeweed mitogen for one hour at 37°C/5% CO₂. Cells were counted and seeded at 5 cells/40 µL/well in complete IMDM medium containing 10% fetal bovine serum (FBS) and cytokines (IL-2 and IL-21 at 10.5 ng/mL) in 384-well plates pre-seeded with CHO-K1 cells expressing CD154 as feeder cells. Cells were fed again by adding 20 µL/well of the same medium as above after one week. Two weeks after seeding, B cell culture supernatants were collected for screening to identify clones with specific reactivity to human MSLN. The plates with cells were frozen and stored at -80°C for future RNA isolation and gene amplification. B cell culture supernatants were first screened for IgG production by rabbit IgG FRET assay. IgG-producing clones (5,775) were selected and screened by using ELISA to determine binding to human MSLN (1st screening). Some of the anti-MSLN-reactive clones were re-tested (2nd screening) for reactivity to MSLN but not to a control antigen (human CD73). Five mAbs were selected and are shown in Table 14.

Table 14. Clones with specific reactivity to human MSLN

rabbit mAb name	1 st screening Reading (Optical Density)		2 nd screening Reading (Optical Density)	
	IgG production	Anti-MSLN reactivity	Anti-MSLN reactivity	Anti-CD73 reactivity
33O11	0.524	0.2821	0.5039	0.0818
178F16	1.534	0.9661	1.879	0.1411
237N18	0.586	0.362	0.6683	0.0918
324O5	2.207	1.3982	2.218	0.0801
383I18	0.586	0.249	0.2965	0.0744

[0294] The plates containing the selected mAbs were thawed, and the B cells were lysed to isolate RNAs using RNAqueous Kit (Ambion). The RNAs were used to set up RT-PCR reactions to amplify the antibody variable regions. The resulting DNAs were sequenced, and primers were designed for compatibility with the InFusion HD[®] cloning system as described by the manufacturer (Clontech, Mountain View, CA). The variable region PCR fragments were cloned into an expression plasmid containing either a human gamma or kappa constant region.

These plasmids were transfected using the FreeStyle 293 expression system (Thermo Fisher Scientific) to produce mAbs as described elsewhere herein.

Generation and characterization of MSLN-AuF Cys80 conjugated mAbs

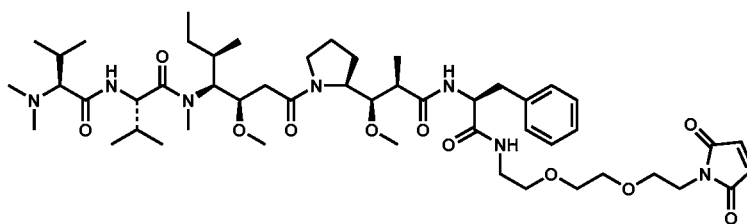
[0295] Chimerized mAbs were generated as disclosed in Example 2, wherein xi33O11 is one of the five anti-MSLN mAbs and the other four mAbs were chimerized following the same method. Hence, these anti-MSLN mAbs contain unpaired Cys80, specifically, the motif C-X-X-A. They are herein referred to as xi324O5, xi178F16, xi237N18, xi33O11, and xi383I18.

[0296] After their production, the selected five chimerized mAbs were conjugated to auristatin F (AuF) according to the following methods to generate MSLN-AuF Cys80 conjugated mAbs.

[0297] Antibody purification with “decapping”: Decapping rabbit/human mAbs is a step required for conjugation to Cys80. Using an ÄKTA Explorer (GE Healthcare), a protein A column (GE Healthcare) was equilibrated with 10 CV of 20 mM sodium phosphate, 10 mM EDTA, pH 7.2. The sample was then loaded, followed by washing unbound material with 10 CV of equilibration buffer. The column was washed with 16 CV of 20 mM sodium phosphate, 10 mM EDTA, 5 mM cysteine, pH 7.2 at 0.5 mL/min for 16 h. The column was then washed with 60 CV of 20 mM Tris, pH 7.5 at 0.5 mL/min for 60 h. The sample was eluted using 5 CV of 0.1 M Glycine pH 2.9. The fractions containing the mAb were pooled and dialyzed in DPBS using a MWCO 20K Slide-A-Lyzer (Thermo Fisher Scientific). Protein recovery was determined by BCA assay.

[0298] Auristatin F conjugation: Purified and decapped chimerized MSLN-mAbs containing the C-X-X-A motif were incubated with maleimido-PEG2-auristatin F (mal-PEG2-AuF) (structure shown below), diluted from a 10 mM stock in DMSO (Concortis Biosystems, San Diego, CA) at a 5:1 molar ratio (AuF:MAB) at a final antibody concentration of 5 mg/mL in 1x PBS. Conjugation was performed for 2 hr at 22°C. Unreacted mal-PEG2-AuF was removed by desalting purification on an AKTA FPLC fitted with a 26/10 desalting column (GE Healthcare) using 1x PBS as running buffer. Antibody-containing fractions were pooled and protein concentration determined by BCA assay.

Maleimido-PEG2-auristatin F:



[0299] UPLC/ESI-MS analysis: Samples were reduced by adding DTT to a final concentration of 20 mM, followed by incubation at 60°C for 3 min. Samples were then analyzed using a Waters Acquity UPLC and Q-ToF Premier mass spectrometer. Samples (0.5-2 µg each) were injected onto a MassPrep micro desalting column at 65°C, eluted from the column with a 5 min equilibration in 95% of mobile phase A, a 10 min gradient (5-90% B), and a 10 min re-equilibration in 95% of mobile phase A, at 0.05 mL/min. Mobile phase A was 0.1% formic acid in water. Mobile phase B was 0.1% formic acid in acetonitrile. The Q-ToF mass spectrometer was run in positive ion, V-mode with detection in the range of 500-4000 m/z. The source parameters were as follows: capillary voltage, 2.25 kV (intact antibody)-2.50 kV (reduced antibody); sampling cone voltage, 65.0 V (intact antibody) or 50.0 V (reduced antibody); source temperature, 100°C; desolvation temperature, 250 °C; desolvation gas flow, 550 L/hr. The protein peak was deconvoluted using the MassLynx MaxEnt 1 function. A representative analysis is shown in FIG. 13. Typically, ≥90% of conjugated mAbs had an antibody-to-functional agent ratio of 2, signifying that each chimerized anti-MSLN mAbs carried an AuF molecule conjugated to each of Cys80¹ and Cys80².

In vitro cytotoxicity

[0300] A431-MSLN are cells derived from A431 cells (ATCC[®] CRL-1555[™]) that stably express human MSLN. A431-MSLN cells were sub-cultured and seeded in 96-well plates at 10,000 cells/well/100 µL in RPMI medium containing 10% FBS and incubated at 37°C, 5% CO₂ for 16 hour. MSLN-AuF Cys80 conjugated mAbs were serially diluted 1:4 in 2 mL deep-well dilution plates. Diluted compounds (100 µL) were added to the cell plates, with final concentrations ranging 0.28-75 µg/mL. Mal-PEG2-AuF was used at an equimolar concentration of the conjugated mAbs. For example, 10 µg/mL of MSLN-AuF Cys80 conjugated mAb (DAR=2) equates to 0.14 µg/mL of mal-PEG2-AuF. Plates were incubated at 37°C, 5% CO₂ for an additional 48 hours. Medium was discarded, plates were washed once with 200 µL DPBS, stained with 50 µL of 0.2% crystal violet solution at 22°C for 15 minutes, and then washed extensively with tap water. Plates were air-dried, and crystal violet was dissolved with 200 µL

of 1% SDS solution. Colorimetric optical density was determined at 570 nm. Excel was used to extrapolate cell number from optical densities and GraphPad Prism 6 was used to plot the percent cytotoxicity.

[0301] When MSLN-negative A431 cells were treated with MSLN-AuF Cys80 conjugated mAbs, no significant cytotoxicity was observed, while mal-PEG2-AuF was cytotoxic only at the highest concentration tested (FIG. 14A). When MSLN-positive A431-MSLN cells were treated with MSLN-AuF Cys80 conjugated mAbs, significant cytotoxicity was observed. Based on the dose-response curves (FIG. 14B), MSLN-AuF Cys80 conjugated mAbs could be categorized into 2 groups: *Medium cytotoxicity* – xi324O5-AuF, and xi178F16-AuF; and *High cytotoxicity* – xi237N18-AuF, xi33O11-AuF, and xi383I18-AuF.

In vivo evaluation - Initial selection of MSLN-AuF Cys80 conjugated mAbs

[0302] The *in vivo* efficacy of the MSLN-AuF Cys80 conjugated mAbs was tested against tumor expressing MSLN, with the objective of selecting a few compounds with high efficacy and low toxicity.

[0303] The A431-MSLN cells were propagated in cell culture, suspended in serum-free growth medium, mixed 1:1 with Matrigel™, and 5 million cells/0.2 mL/mouse were implanted subcutaneously (s.c.) into athymic NCr nu/nu mice. Tumor volume was determined by caliper measurements (mm) and using the formula for an ellipsoid sphere: $\text{Length} \times \text{Width}^2 / 2 = \text{Volume (mm}^3\text{)}$. When tumor volume ranged 100-250 mm³, mice were randomized into treatment groups. The animal body weights and tumor size were recorded twice weekly. The overall design of this study is summarized in Table 15. The MSLN-AuF Cys80 conjugated mAbs were administered intravenously (i.v.) Q7D starting on randomization day (day 1), two doses total.

Table 15. Study design

group	# mice	treatment	Dose (mg/kg)	Regimen
1	8	saline	0	Q7D x 2
2	8	xi33O11-AuF	10	Q7D x 2
3	8	xi324O5-AuF	10	Q7D x 2
4	8	xi178F16-AuF	10	Q7D x 2
5	8	xi237N18-AuF	10	Q7D x 2

6	8	xi383I18-AuF	10	Q7D x 2
7	8	mal-PEG2-AuF	10	Q7D x 2

[0304] FIG. 15 shows the average tumor volumes among different treatment groups, up to day 18 post-implantation, when 3 of 8 animals had to be euthanized in the saline-treated group due to high tumor burden and/or tumor ulceration. Day 4 post-implantation corresponds to the day when mice were randomized into different treatment groups, with average tumor volume ranging 157-160 mm³ across all groups. On this day, the first dose of MSLN-AuF Cys80 conjugated mAbs was administered followed by a second dose on day 11.

[0305] All MSLN-AuF Cys80 conjugated mAbs showed anti-tumor response, as evidenced by the fact that the tumor volume on day 18 was 20% or less compared to the saline-treated group (Table 16). In contrast, mal-PEG2-AuF showed no anti-tumor response.

Table 16. Percent of tumor volume vs. saline group

Treatment	% tumor volume vs. saline group	Number of on-study animals on day 18
Saline	100%	8
xi33O11-AuF	4%	8
xi324O5-AuF	11%	6
xi178F16-AuF	7%	6
xi237N18-AuF	6%	6
xi383I18-AuF	7%	6
mal-PEG2-AuF	109%	7

[0306] Toxicity was also evaluated by observing any body weight loss on day 18 (based on average weight of on-study animals in each group) compared to day 4, as well as by observing any dead or moribund animals (Table 17). In the xi324O5-AuF-treated group, a body weight loss of 11% was observed in surviving animals as well as two dead/moribund animals. In the xi178F16-AuF, xi237N18-AuF, and xi383I18-AuF-treated groups, no significant body loss was observed, but one or two dead/moribund animals were observed. All the other treatment groups showed neither body weight loss nor dead/moribund animals.

Table 17. Assessment of gross toxicity

Treatment	% body weight change on day 18 vs. day 4	Number of mice dead or moribund on day 18
Saline	106%	0
xi33O11-AuF	102%	0
xi324O5-AuF	89%	2
xi178F16-AuF	104%	1
xi237N18-AuF	96%	2
xi383I18-AuF	107%	1
mal-PEG2-AuF	111%	0

[0307] Based on the anti-tumor responses as well as the minimal toxicity, mAbs xi33O11-AuF and xi237N18-AuF were chosen for further evaluation.

Assessment of target specificity of anti-tumor activity mediated by MSLN-AuF Cys80 conjugated mAbs

[0308] The method used for this study was the same as described above (*In vivo evaluation - Initial selection of MSLN-AuF Cys80 conjugated mAbs*). In addition to A431-MSLN cells, which were implanted on the left flank of each mouse on Day 4, MSLN-negative A431 cells were implanted in the same mouse on the opposite (right) flank on Day 1. The former cells grow tumors faster than the latter, and hence were implanted 3 days later so that the first dose of test drug were given when the tumor in both flanks were similar in volume. The overall design of this study is summarized in Table 18.

Table 18. Study Design

group	# mice	treatment	Dose (mg/kg)	Regimen
1	8	saline	0	Q7D x 2
2	8	xi33O11-AuF	10	Q7D x 2
3	8	xi237N18-AuF	10	Q7D x 2

4	8	xi1-55-2-AuF	10	Q7D x 2
5	8	mal-PEG2-AuF	0.14	Q7D x 2

[0309] MSLN-positive tumors: FIG. 16A shows the average tumor volumes among different treatment groups, up to day 18 post-implantation, when 4 of 8 animals had to be euthanized in the saline-treated group due to high tumor burden and/or tumor ulceration. Day 4 post-implantation corresponds to the day when mice were randomized into different treatment groups, with average A431-MSLN tumor volume ranging 169-178 mm³ across all groups. On this day, the first dose of MSLN-AuF Cys80 conjugated mAbs was administered followed by a second dose on day 11.

[0310] xi33O11-AuF mediated anti-tumor responses that reduced tumor volume on day 18 to 12% compared to the saline-treated group (Table 19). xi237N18-AuF mediated anti-tumor responses that reduced tumor volume to 24% compared to the saline-treated group (Table 19). An unpaired, two-tailed t test indicated a p value of 0.00039 and 0.00197, respectively, suggesting that these anti-tumor responses versus saline-treated group were statistically significant. In contrast, mal-PEG2-AuF or xi1-55-2-AuF, which targets endosialin/TEM1, showed no significant anti-tumor responses.

Table 19. Percent of A431-MSLN tumor volume vs. saline group

Treatment	% Tumor Volume on Day 18 vs. Saline Group	Number of Mice on Study on Day 18
Saline	100%	8
xi33O11-AuF	12%	8
xi237N18-AuF	24%	8
xi1-55-2-AuF	109%	8
mal-PEG2-AuF	104%	8

[0311] MSLN-negative tumors: FIG. 16B shows the average tumor volumes among different treatment groups, up to day 21 post-implantation; this day corresponds to Day 18 of A431-MSLN post-implantation, as described above, and Day 7 post-implantation corresponds to the day when mice were randomized into different treatment groups, with average A431 tumor volume ranging 174-184 mm³ across all groups. xi33O11-AuF mediated anti-tumor responses that reduced tumor volume on day 21 to 78% compared to the saline-treated group (Table 20). xi237N18-AuF Cys80 conjugated mAb mediated anti-tumor responses that reduced tumor

volume on day 21 to 64% compared to the saline-treated group (Table 20). An unpaired, two-tailed t test indicated a p value of 0.317 and 0.091, respectively, suggesting that these anti-tumor responses versus saline-treated group were not statistically significant. These responses were similar to those observed with mal-PEG2-AuF or xi1-55-2-AuF.

Table 20. Percent of A431 (MSLN-negative) tumor volume vs. saline group

Treatment	% Tumor Volume on Day 21 vs. Saline Group	Number of Mice on Study on Day 21
Saline	100%	6
xi33O11-AuF	78%	5
xi237N18-AuF	64%	6
xi1-55-2-AuF	76%	5
mal-PEG2-AuF	78%	8

[0312] Toxicity was also evaluated by observing any body weight loss on Day 21 post implantation of A431 cells compared to Day 7, as well as by observing any dead or moribund animals (Table 21). No body weight loss $\geq 10\%$ was observed in any of the treatment groups. Two deaths were observed in both xi33O11-AuF and xi237N18-AuF-treated group.

Table 21. Assessment of gross toxicity

Treatment	% Body Weight Change on Day 21 vs. Day 7	Number of Mice Dead or Moribund on Day 21
Saline	100%	0
xi33O11-AuF	97%	2
xi237N18-AuF	92%	2
xi1-55-2-AuF	107%	0
mal-PEG2-AuF	103%	0

Conclusion

[0313] MSLN-AuF Cys80 conjugated mAb were generated and screened based on *in vitro* cytotoxicity and *in vivo* anti-tumor activity. The *in vitro* cytotoxicity analysis indicated that these compounds were targeting and killing MSLN-positive but not MSLN-negative tumor cells.

[0314] All MSLN-AuF Cys80 conjugated mAbs tested had anti-tumor activity, some of which appeared to be potentially more toxic than others. The nature of this toxicity was not further characterized. It was observed that both the MSLN-AuF Cys80 conjugated mAbs tested *in vivo* could target MSLN-positive tumors and inhibit their growth, whereas no significant effect was observed against MSLN-negative tumors in the opposite flank. While the toxicity profile of xi237N18 was similar in both studies, xi33O11-AuF treatment showed no toxicity in the first study but was associated with two deaths in the second study. The nature of this toxicity was not further characterized; however, as xi33O11-AuF-treated mice still carried a large MSLN-negative tumor on the other flank and were therefore sicker than the animals in the first study, these mice may have been more sensitive to the effect of the massive tumor cell lysis against the MSLN-positive tumor.

Example 4 - Generation of antibody-fluorescent dye conjugates

[0315] The xi155D5 mAb containing the C-X-X-A motif was conjugated to the 800CW dye (LI-COR Biotechnology, Lincoln, NE) to generate a xi155D5-800CW Cys80 conjugated mAb having two dye molecules conjugated to Cys80₁ and Cys80₂.

[0316] Conjugation of 800CW onto Cys80 was carried out using maleimide-(CH₂)₂-800CW (LiCor), whereby (CH₂)₂ is an alkyl linker. Briefly, maleimide-(CH₂)₂-800CW was dissolved into 100% DMSO at a final concentration of 10 mM. Maleimide-(CH₂)₂-800CW was added to xi155D5 (5 mg/ml in 1x PBS) at a 5:1 molar ratio of dye:MAB and incubated for 4 hr at room temp. Unincorporated dye was removed by desalting on PD-10 columns (Millipore). xi155D5-800CW was further polished via size-exclusion chromatography on Superdex 75. Void volume material was pooled, aliquotted, and frozen at -80 until use. SDS-PAGE and imaging analyses of reduced xi155D5-800CW indicated that the dye was conjugated only on the light chain but not the heavy chain (FIG. 17).

[0317] NCR-nude female mice were injected with either colo205 or HT-29 human tumor cells subcutaneously to the right hind limbs. Tumor growth was monitored by caliper measurement. When the tumor volume was 200-300 mm³, xi155D5-800CW was injected through the tail veils at 0.1 mg/200μL/mouse. For monitoring xi155D5-800CW distribution via fluorescent living imaging, animals were placed into an anesthesia chamber for approximately 3-4 minutes using isofluorane/O₂ until the animals were unconscious. Animals were imaged using the fluorescence setting of 745 excitation and 840 emission in a IVIS[®] Lumina II-Kinetic

instrument (PerkinElmer, Waltham, MA). Images of the dorsal, right, ventral, and left side were taken at different time points as indicated in FIG. 18. After each successive image the animal were allowed to regain consciousness in a recovery chamber receiving 100% oxygen flush followed by normal air.

[0318] Using the colo205 or HT-29 models, it was observed that xi155D5-800CW efficiently targeted human tumors, as demonstrated by the tumor-specific localization of its fluorescent signal (FIG. 18).

[0319] These results demonstrated that a mAb containing the C-X-X-(not)F, K, or C motif can be conjugated to a dye and that the conjugated mAb can be used to identify and monitor tumor status.

Example 5 - Generation of bivalent/bispecific antigen-binding molecules

[0320] When a mAb containing the C-X-X-(not)F, K, or C motif is digested with papain, or is recombinantly expressed as a Fab fragment, it will contain a single unpaired Cys80 since the Fab contains only one V_K region. Using orthogonal conjugation chemistry, Cys80-containing Fabs can be used to generate chemically-conjugated bivalent/bispecific antigen-binding molecules, such as bivalent/bispecific Fab-Fab, that can be utilized for targeting two independent disease-relevant targets, including two ligands (cytokines, chemokines), two membrane receptors, or ligand/receptor combinations, to name a few.

[0321] As an example, Fabs were generated from xi155D5 and xi1-55-2 using limited papain digestion, followed by protein A chromatography to remove the Fc fragments and undigested mAb. Fabs were shown to be fully decapped using mass spectrometry (data not shown). Subsequently, xi155D5 and xi1-55-2 Fabs were conjugated separately using maleimido-PEG4-dibenzylcyclooctyne (DBCO) and maleimido-PEG4-azide, respectively. Unconjugated compound was removed by desalting chromatography and complete occupancy of the Cys80 sites was confirmed by mass spectrometry (data not shown). Then, xi155D5-maleimido-PEG4-DBCO and xi1-55-2-maleimido-PEG4-azide fragments were conjugated to each other via strain-promoted copper-free click chemistry by incubation in PBS at 22°C for 16 hours. Conjugated products were fractionated by using gel-filtration chromatography (FIG. 19A) and the different species were identified by SDS-PAGE based on their expected molecular size (FIG. 19B). Fractions containing the xi155D5/xi1-55-2 bivalent/bispecific antigen-binding molecule were pooled and the xi155D5/xi1-55-2 bivalent/bispecific antigen-binding molecule identity was confirmed by mass spectrometry based on its expected mass (95,939 Da, FIG. 19C).

[0322] The bispecificity of xi155D5/xi1-55-2 bivalent/bispecific antigen-binding molecule was confirmed via biolayer interferometry (BLI) analysis using an inverse sandwich assay. This analysis demonstrated binding to immobilized human CA9 (bound by the xi155D5 Fab moiety) followed by binding of soluble TEM-1 (bound by xi1-55-2 Fab moiety) (FIG. 20). As expected, xi155D5 mAb, xi155D5 Fab, and the xi155D5/xi1-55-2 bivalent/bispecific antigen-binding molecule bound to immobilized CA9. Only the CA9-immobilized xi155D5/xi1-55-2 bivalent/bispecific antigen-binding molecule was able to bind also human endosialin/TEM-1, as demonstrated by the additional response shift observed (FIG. 20, double arrow). Surface plasmon resonance analysis demonstrated that the affinity of xi155D5/xi1-55-2 for CA9 or TEM-1 was the same or slightly reduced, respectively (Table 22).

[0323] These results demonstrate that: 1) a mAb containing the C-X-X-(not)F can be conjugated to polypeptides, such as an antibody fragment or a Fab; and 2) when two mAbs or Fabs, of different specificity, containing the C-X-X-(not)F are orthogonally conjugated, a bivalent/bispecific compound can be generated.

Table 22. Affinity of xi155D5/xi1-55-2 bivalent/bispecific antigen-binding molecule to CA9 and TEM-1

Antibody	Flowcell	Ligand	k_a (1/Ms)	k_d (1/s)	R_{max} (RU)	KD (M)	χ^2 (RU ²)
xi1-55-2 (Fab)	Fc3	TEM1-Fc	8.62E+05	3.29E-04	37.9	3.82E-10	6.42
	Fc4	TEM1-Fc	8.65E+05	3.26E-04	38	3.76E-10	5.89
bivalent/bispecific	Fc3	TEM1-Fc	8.29E+05	2.57E-04	79.7	3.10E-10	6.65
	Fc4	TEM1-Fc	1.02E+06	1.89E-04	96.8	1.85E-10	31.6
xi155D5 (Fab)	Fc3	CA9	5.72E+05	2.19E-05	65.1	3.83E-11	8.86
	Fc4	CA9	5.19E+05	2.59E-05	63.9	4.99E-11	8.24
bivalent/bispecific	Fc3	CA9	3.22E+05	3.91E-05	130.2	1.21E-10	31.3
	Fc4	CA9	2.94E+05	4.40E-05	131.8	1.50E-10	24.3

Example 6 - Generation of antibody-peptide conjugates

[0324] xi33O11 and xi1-55-2 mAbs containing the C-X-X-A motif were conjugated to azide-modified peptide A β (1-16) (SEQ ID NO:40) (Table 23).

Table 23. A β (1-16) Peptide Sequence

Peptide name	Sequence	mass (Da)	Parent protein
A β (1-16)	NH ₂ -DAEFRHDSGYEVHHQK(PEG8-N ₃)-COOH (SEQ ID NO:40)	2404	human amyloid-beta peptide ACCESSION 1BA6_A

[0325] Conjugation of peptide A β (1-16) onto Cys80 was carried out using a two-step conjugation procedure, whereby Cys80 was first conjugated with maleimido-dibenzylcyclooctyne (mal-DBCO). Azido-modified peptide A β (1-16) was then conjugated to the DBCO-modified mAbs using strain-promoted copper-free click chemistry. Briefly, mAb (20 mgs) was incubated with mal-DBCO (Click Chemistry Tools, cat A108) at a mal-DBCO:MAB molar ratio of 5:1 for 16 hrs at 22°C in 1x DPBS. Unincorporated mal-DBCO was removed from conjugated mAb by desalting chromatography using a HiPrep 26/10 column with 1x DPBS as running buffer. Conjugation efficiency of 100% (no evidence of unconjugated light chain) was confirmed for both mAbs by LC-MS (FIG. 21 and Table 24). Each mAb (50 μ L/each, 95 μ g and 70 μ g of xi1-55-2 and xi33O11, respectively) was incubated with peptide A β (1-16) at peptide:MAB molar ratio of 20:1 in 1X DPBS for 16 hrs at 22°C. Conjugations were analyzed by SDS-PAGE. Samples were run under reducing conditions, with 20 mM DTT as reductant and heating to 75°C for 10 min prior to separation.

[0326] Analysis of the SDS-PAGE indicated retardation of the peptide-conjugated light chain migration accompanied by no detectable unconjugated light chain, indicating efficient conjugation (FIG. 21). No change in heavy chain mobility was observed. Conjugations were then desalted using 0.5 ml Zeba 40k MWCO spin desalting columns (Thermo-Fisher) to remove unconjugated peptide and were analyzed by LC-MS. Mass spectrometry analysis (FIG. 22A-F) indicated that the peptide was conjugated to the light chains of xi1-55-2 and xi33O11 with efficiencies of 85%-100% (Table 24).

[0327] These results demonstrated that a mAb containing the C-X-X-(not)F, K, or C motif can be efficiently conjugated to peptides.

Table 24. Conjugation summary

	DBCO	DBCO-peptide Aβ(1-16)
predicted Δ mass	+ 429 Da	+ 2833 Da
xi1-55-2 measured Δ mass	+ 425 Da	+ 2829 Da
conjugation efficiency	100%	100%
xi33O11 measured Δ mass	+ 432 Da	+ 2834 Da
conjugation efficiency	100%	85%

Table 25. Monoclonal antibodies and corresponding LC and HC

mAb name	LC name	HC name
1-55-2	1-55-2LC	1-55-2HC
xi1-55-2	xi1-55-2LC (SEQ ID NO:108)	xi1-55-2HC (SEQ ID NO:56)
155D5	155D5LC	155D5HC
xi155D5	xi155D5LC (SEQ ID NO:78)	xi155D5HC (SEQ ID NO:52)
zu155D5-1	zu155D5LC-1 (SEQ ID NO:80)	zu155D5HC (SEQ ID NO:54)
zu155D5-2	zu155D5LC-2 (SEQ ID NO:82)	zu155D5HC (SEQ ID NO:54)
zu155D5-3	zu155D5LC-3 (SEQ ID NO:84)	zu155D5HC (SEQ ID NO:54)
zu155D5-4	zu155D5LC-4 (SEQ ID NO:86)	zu155D5HC (SEQ ID NO:54)
zu155D5-5	zu155D5LC-5 (SEQ ID NO:88)	zu155D5HC (SEQ ID NO:54)
zu155D5-6	zu155D5LC-6 (SEQ ID NO:90)	zu155D5HC (SEQ ID NO:54)
zu155D5-7	zu155D5LC-7 (SEQ ID NO:92)	zu155D5HC (SEQ ID NO:54)
zu155D5-huVK1-39	zu155D5LC-huVK1-39 (SEQ ID NO:94)	zu155D5HC (SEQ ID NO:54)
zu155D5-huVK2-40	zu155D5LC-huVK2-40 (SEQ ID NO:96)	zu155D5HC (SEQ ID NO:54)
zu155D5-huVK3-11	zu155D5LC-huVK3-11 (SEQ ID NO:98)	zu155D5HC (SEQ ID NO:54)

zu155D5-huVK4-1	zu155D5LC-huVK4-1 (SEQ ID NO:100)	zu155D5HC (SEQ ID NO:54)
zu155D5-huVK6-21	zu155D5LC-huVK6-21 (SEQ ID NO:102)	zu155D5HC (SEQ ID NO:54)
zu155D5-huVK6D-41	zu155D5LC-huVK6D-41 (SEQ ID NO:104)	zu155D5HC (SEQ ID NO:54)
zu155D5-huVK7-3-Glu81	zu155D5LC-huVK7-3-Glu81 (SEQ ID NO:106)	zu155D5HC (SEQ ID NO:54)
xi1E4	xi1E4LC (SEQ ID NO:110)	xi1E4HC (SEQ ID NO:58)
zu1E4-CXXF	zu1E4LC-CXXF (SEQ ID NO:112)	zu1E4HC (SEQ ID NO:60)
zu1E4-CXXA	zu1E4LC-CXXA (SEQ ID NO:114)	zu1E4HC (SEQ ID NO:60)
xi166B3	xi166B3LC (SEQ ID NO:132)	xi166B3HC (SEQ ID NO:74)
zu166B3-CXXF	zu166B3LC-CXXF (SEQ ID NO:134)	zu166B3HC (SEQ ID NO:76)
zu166B3-CXXA	zu166B3LC-CXXA (SEQ ID NO:136)	zu166B3HC (SEQ ID NO:76)
xi33O11	xi33O11LC (SEQ ID NO:116)	xi33O11HC (SEQ ID NO:62)
zu33O11-CXXF	zu33O11LC-CXXF (SEQ ID NO:118)	zu33O11HC (SEQ ID NO:64)
zu33O11-CXXA	zu33O11LC-CXXA (SEQ ID NO:120)	zu33O11HC (SEQ ID NO:64)
zu33O11-CXXI	zu33O11LC-CXXI (SEQ ID NO:122)	zu33O11HC (SEQ ID NO:64)
xi324O5	xi324O5LC (SEQ ID NO:124)	xi324O5HC (SEQ ID NO:66)
xi178F16	xi178F16LC (SEQ ID NO:126)	xi178F16HC (SEQ ID NO:68)
xi237N18	xi237N18LC (SEQ ID NO:128)	xi237N18HC (SEQ ID NO:70)
xi383I18	xi383I18LC (SEQ ID NO:130)	xi383I18HC (SEQ ID NO:72)

Table 26. Sequences

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
Human TEM- 1/endosomal /CD248	ATGCTGCTGCGCCTGTTGCTGGCTGGCGGCGCCGACGGGCG CACACTGGGCCAGGACCCCTGGGCTGCTGAGCCCCGTGCCG CCTGGGCCCCCAGCAGCTGCTACGCTCTCTTCCACGGCGC CGACCTTCTCTGAGGCTGGCGGCGCTGCCGCGAGCTGGG GGGGACCTGGCCACTCTCGGACCCCGAGGAGGCCCGCAGC GTGTGGACAGCCTGGTGGTGGGCGGCGCCAGCCAGCCGGCTG CTGTGGATCGGGCTGCAGCGGCGGCTTACGTGGACCAACAGGAC GCAGCGCCACTGCGCGGCTTACCAACTGGGCCAGCCAGCTCTGGA AGACACGCTTTCACCAACTGGGCCAGCCAGCTCTGGA GGCCCTGGCCGCGCCAGCGCTGTGTGGCCCTGGAGGCAAG TGGCAGACACCGCTGGCTGGAGGCTCGTGCACGCTGGCTG TCGACGGCTACCTGTGCCAGTTTGGCTCGAGGGCGCCTGC CCGCGCTGCAAGATGAGCGGGCGAGCGCGGCCCGCCAGCCGT GTATACACGCGCTTCCACCTGCTTCCACAGAGTTTGAGT GGCTGCCCTTCGGCTCTGTGGCGCTGTGCAGTGCCAGGCT GGCAGGGGAGCCTCTCTGCTCTGCTGAGGAGCAGCCTGAGGG AGGTGTGGCTGGTCAAGGCTGGCCCTGTGCTGGGGA CTGGCTGCAGCCCTGACAACGGGGCTGCGAACACGAAATGT GTGGAGGAGGTGGATGGTCACTGTCTCTCCGCTGCACCTGA GGGCTTCGGCTGGCAGCAGACGGGCGCAGTTGCGAGGACC CCTGTGCGCCAGGCTCCGTGCGAGCAGCAGTGTGAGCCCGGT GGGCCACAAGGCTACAGTGCCTGCTGCTGCTGGCTGCTTCCG GCCAGCGGAGGATGATCCGCACCGCTGTGTGGACACAGATG AGTGCCAGATTGCCGGTGTGTGCCAGCAGATGTGTCTCAC TACGTTGGTGGCTTCGAGTGTATTGTAGCAGGAGGACATGA GCTGGAGGCTGATGGCATCAGCTGACGCGCTGCAGGGGCCA TGGGTGCCAGGCTTCCAGGACCTCGGAGATGAGTTGCTG	MLLRLLAWAAAGPTLGQD PWAAEPRAACGPSSCYALF PRRRTFLEWRACRELGGD LATPRTPEEAQRVDSLVA GPASRLLWIGLQRARQCQ LQRPLRGFTWTGDDDTAF TNWAQPASGGPCPAQRCA LEASGEHRWLEGSCTLAVD GYLCQFGFEGACPALQDEA QGAGPAVYTPFHLVSTEF EWLPGSVAAVQCQAGRGA SLLCVKQPEGVGWMSRAGP LCLGTGCSPDNGGCEHECV EEVDGHVSCRCCTEGFRLAA DGRSCDEPCAAQAPCEQQCE PGGPQGYSCHCRLLGFRPAE DDPHRCVDTDECQIAGVCQ QMCVNYVGGFECYCSEGHE LEADGISCSPAGAMGAQAS QDLGDELLDDDEDEDEDE AWKAFNGGWTEMPGILWME PTQPPDFALAYRPSFPEDR EPQIPYPEPTWPPPLSAPR VPYHSSVLSVTRPVVVSAT HPTLP SAHQPPVIPATHPA LSRDHQIPVIAANYPDLP AYQPGILSVSHSAQPPAHQ	1-51	NA	NA

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	GATGACGGGAGGATGAGGAAGATGAAGACGAGGCCTGGAA GGCCTTCAACGGTGGCTGGACGGAGATGCCTGGGATCCTGT GGATGGAGCCTACGCAGCGCCTGACTTTGCCCTGGCCTAT AGACCGAGCTTCCCAGAGGACAGAGAGCCACAGATACCCCTA CCCGAGCCCACTGGCCACCCCGCTCAGTGCCCCCAGGG TCCCCACCACTCCTCAGTGCTCTCCGTCACCCGGCCTGTG GTGGTCTCTGCCACGCATCCACACTGCCTTCTGCCCACCA GCCTCCTGTGATCCCTGCCACACACCCAGCTTTGCCCGTG ACCACAGATCCCCGTGATCGCAGCCAACTATCCAGATCTG CCTTCTGCCTACCAACCCGGTATTCTCTGTCTCTCATTC AGCAGAGCCTCCTGCCCCACGAGCCCCCTATGATCTCAACCA AATATCCGGAGCTCTTCCCTGCCACACAGTCCCCCATGTTT CCAGACACCCGGTCTGTGGCACCCAGACCACTCAATTT GCCTGGAATCCCACCTAACCATGCCCTCTGGTCACCACCC TCGGTGCCAGCTACCCCTCAAGCCCCAGATGCCCTTGTG CTCAGAACCCAGGCCACCGAGCTTCCCATTATCCCAACTGC CCAGCCCTCTGTGACCAACCACTCCAGGTCCCCTGTGTCTC CTGCCCATCAAACTCTGTGGCTGCTGCCACCCAGCCCGCA GCCCTCCCCACCCCTCCTGCCCTCTCAGAGCCCCACTAACCA GACCTACCCCATCAGCCCTACACATCCCCATTCCAAAGCCC CCCAATCCCAAGGAAGATGGCCCCAGTCCCAAGTTGGCC CTGTGGCTGCCCTCACCAAGTCCACAGAGCCCCCAACAGC CCTGGGGAGGCTGGTCTTGCCGAGCACAGCCAGAGGGATG ACCGGTGGCTGCTGGTGGCACTCCTGGTGCCAA CGTGTGTC TTTTTTGGTGGTCTGTGCTTGCACTGGGCATCGTGTACTGCACT CCGCTGTGGCCCCCATGCACCCCAACAAAGCGCATCACTGACT GCTATCGCTGGTTCATCCATGCTGGGAGCAAGAGCCCCCAACA GAACCCATGCCCCCCCAGGGGCAAGCCTCACAGGGGTGCAGAC	PPMI STKYPELPFAHQSPM FPDTRVAGTQTTHLPQIP PNHAPLVTTTLGAQLPPQAP DALVLRQTATQLPIIPTAQ PSLTTSRSPVSPAHQISV PAATQPAALPTLLPSQSPT NQTSPISTPHHSKAPQIP REDGSPKLAWLPSAPT AAPTALGEAGLAHRSQRDD RWLLVALLVPTCVFLVLL ALGIYCTRCGPHAPNKRI TDCYRWVIHAGSKSPTEPM PPRGS LTGVQTCRTSV* (SEQ ID NO:42)			

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	CTGCAGAACCCAGCGTGTGA (SEQ ID NO:41)				
human endosialin/T EM1 extracellular r domain fused to mouse IgG2b Fc	ATGCTGTGGCCTGTTGCTGGCTGGCGGGCGCCGACGGCC CACACTGGGCAGGACCCCTGGGTGCTGAGCCCCCGTGCCG CCTGGGCCCCAGCAGCTGCTACGCTCTCTTCCACGGCGC CGACCTTCCTGGAGGCTGGCGGCTGCGGAGGAGGCCAGC GGCGACCTGGCCACTCCTCGGACCCCGAGGAGGCCAGC GTGTGGACAGCCTGGTGGTGGGTGGGCGCCAGCCAGCCGCTG CTGTGGATCGGCTGCAGCGGCTTCACTGGACCCAGGGGACC GCAGCGCCACTGCGCGCTTCACTGGACCCAGGGGACC AGGACACGGCTTTCACCAACTGGGCGCCAGCCAGCTCTGGA GGCCCCTGGCGGCCAGCGCTGTGTGGCCCTGGAGGCAAG TGGCGAGCACCGCTGGCTGGAGGCTCGTGACGCTGGCTG TCGACGGCTACCTGTGCCAGTTTGGCTTCGAGGGCGCCTGC CCGGCGTGCAGATGAGGGGGCGCAGGCCGCCAGCCGCT GTATACACGCCCCTTCCACCTGTCTCCACAGATTGAGT GGTGCCCTTCGGCTCTGTGGCCGCTGTGCAAGTCCAGGCT GGCAGGGAGCCCTCTCTGCTCTGCTGAAGCAGCCTGAGGG AGGTGTGGGCTGGTCAAGGCTGGGCGCTGTGCTGGGGA CTGGCTGCAGCCCCTGACCAACGGGGCTGCGAACACGAATGT GTGGAGGAGGTGGATGGTCACTGTCTGCGGCTGCACTGA GGCTTCCGGCTGGCAGCAGACGGGCGCAGTTGCGAGGACC CCTGTGCCAGGCTCCGTGCGAGCAGCAGTGTGAGCCCGGT GGCCACAAAGGCTACAGCTGCCACTGTGCGCTGGGTTCCG GCCAGCGGAGGATGATCCGACCGCTGTGTGGACACAGATG	MLRLLLAWAAAGPTLGQD PWAAPRAACGPSSCYALF PRRTFFLEAWRACRELGGD LATPRTPEEAQRVDSLVA GPASRLLIWIGLQARQCQ LQRLRGFTTWTGQDTAF TNWAQPASGGPCPAQRCA LEASGEHRWLEGSCTLAVD GYLCQFFEGACPALQDEA GQAGPAVYTTTFFHLVSTEF EWLPFGSVAAVQCQAGRA SLLCVKQPEGGVGWSRAGP LCLGTGCSPDNGGCEHECV EEVDGHVSCRCTEGFRLLA DGRSCEDPCAQAPCEQQCE PGGPQGYSCHRLGFRPAE DDPHRCVDTDECQLAGVCQ QMCVNYVGGFECYCSEGHE LEADGISCSPPAGAMGAQAS QDLGDELLDDGDEDEDE AWKAFNGGWTMPGIIWME PTQPPDFALAYRSPFPEDR EPQIPYPEPTWPPPLSAPR	1-51	NA	NA

Clone Name (Species - Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain	
	AGTGCCAGATTGCCGGTGTGTGCCAGCAGATGTGTCTCAAC TACGTTGGTGGCTTCGAGTGTATTGTAGCGAGGGACATGA GCTGGAGGCTGATGGCATCAGCTGCAGCCCTGCAGGGGCCA TGGGTGCCCCAGGCTTCCAGGACCTCGAGATGAGTTGCTG GATGACGGGAGGATGAGGAAGATGAAGACGAGGCCCTGGAA GGCCTTCAACGGTGGCTGGACGGAGATGCCTGGATCCTGT GGATGGAGCCTACGCAGCGCCTGACTTTGCCCTGGCCTAT AGACCGAGCTTCCCAGAGGACAGAGGCCACAGATACCCCTA CCGGAGCCCACTGGCCACCCCGCTCAGTCCCCCAGGG TCCCCACCACTCCTCAGTGTCTCCGTACCCGGCCTGTG GTGGTCTCTGCCACGCATCCACACTGCCCTTCTGCCACCA GCCTCCTGTGATCCCTGCCACACACCCAGCTTTGTCCCGTG ACCACAGATCCCCGTGATCGAGCCAACTATCCAGATCTG CCTTCTGCCTACCAACCGGTATTCTCTGTCTCTCATTC AGCACAGCTCCTGCCCCACAGCCCCCTATGATCTCAACCA AATATCCGGAGCTCTTCCCTGCCACCCAGTCCCCCATGTTT CCAGACACCCGGTCCGTGGCACCCAGACCACTCATTT GCCTGGAATCCCACCTAACCATGCCCTCTGGTCAACACCC TCGGTGCCCACTACCCCTCAAGCCCCAGATGCCCTTGTCTC CTCAGAACCCAGGCCACCACTTCCCATTATCCCAACTGC CCAGCCCTCTCTGACCAACCACTCCAGGTCCCCTGTGTCTC CTGCCCCATCAAACTCTGTGCTGTGCTGCCACCCAGCCCGCA GCCCTCCCCACCCCTCCTGCCCTCTCAGAGCCCCCACTAACCA GACCTCACCCATCAGCCCTTACACATCCCCATTCCAAAAGCCC CCCAAA'TCCCAAGGAAAGATGGCCCCCAGTCCCCAAGTTGGCC CTGTGGCTGCCCTCACCACTCCACAGCAGCCCCCAACAGC CCTGGGGGAGGCTGGTCTTGCCCGAGCACAGCCAGAGGGATG ACCGGGTTAA CGACGACGACGACAAAAGAGCCCCAGCGGACCA	VPYHSSVLVTRPVVVSAT HPTLPSAHQPPVIPATHPA LSRDHQIPVIAANYPDLPS AYQPGILSVSHSAQPPAHQ PPMI STKYPELFFAHQSPM FPDTRVAGTQTTHLP PNHAPLVTTLGAQLPPQAP DALVLRQTATQLPIIPTAQ PSLTTSRSPVSAHQISV PAATQPAALPTLLPSQSPT NQTSPISTPHPSKAPQIP REDGSPKALWLPSPAPT AAPTALGEAGLAEHSQRDD RVNDDDDKEPSGPISTINP SPPSKESHKSPAPNLEGGP SVFIFPPNLIKDVLMISLTP KVTCVVVDVSEDDPDVQIS WVFNNEVHTAQQTTHRED YNSTIRVVSSTLPIQHDDWM SGKEFKCKVNNKDLPSPIE RTISKIKGLVRAPOVYILP PPAEQLSRKDVSLTCLVVG ENPGDISVEWTSNGHTEEN YKDTAPVLDSGSGYFIYSK LNMKTSKWEKTDTSFSCNVR HEGLKNYYLKKTTISRSPGK * (SEQ ID NO:44)				

Clone Name (Species - Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	ATTTCAACAATCAACCCCTCTCTCCATCCAAGGAGTCTCA CAAAAAGCCAGCTCCTAACCTCGAGGGTGGACCATCCGTCT TCATCTTCCCTCCAAATATCAAGGATGTACTCATGATCTCC CTGACACCCAAAGGTACGTTGTGGTGGTGGATGTGAGCGA GGATGACCCAGACGTCCAGATCAGCTGGTTTGTGAACAACG TGGAAGTACACACAGCYCAGACACAAACCCATAGAGAGGAT TACAACAGTACTATCCGGTGGTCAGCACCCCTCCCCATCCA GCACCGAGCTGGATGAGTGGCAAGGAGTTCAAATGCAAGG TCAACAACAAGACCTCCCATCACCCTCGAGAGAACCATC TCAAAAAATTAAAGGGCTAGTCAGAGCTCCACAAGTATACAT CTTGCCGCCACAGCAGAGAGAGTTGTCCAGGAAAGATGTCA GTCTCACTTGCCTGGTCTGGGCTTCAACCTGGAGACATC AGTGTGGAGTGGACCAAGCAATGGGCATACAGAGGAGAACTA CAAGGACACCGCACCAAGTCTTGACTCTGACGGTCTTACT TCATATATAGCAAGCTCAATATGAAAAACAAGCAAGTGGGAG AAAAACAGATTCTTCTCATGCAACGTGAGACACAGGGTCT GAAAAATTACTACCTGAAGAAAGACCATCTCCCGGTCTCCGG GTAAATGA (SEQ ID NO:43)	MAPLCPSWLPPLIPAPAP GLTVQLLLSLLLLVPVHPQ RLPRMQEDSPGGSSGED DPLGEEDLPSEEDSPREED PPGEEDLPGEEDLPGEEDL PEVKPKSEEEGSLKLEDLP TVEAPGDPQEPQNNAHARDK EGDDQSHWRYGGDPPWPRV SPACAGRFQSPVDIRPQLA	1-111	NA	NA
Human CA9	ATGGCACCACTGTGCCCCAAGCCCATGGCTGCCACTGCTGAT CCGACGACCAAGCACCAGGACTGACCGTGCAGCTGCTGCTGA GCTGCTGCTGCTGTTGCCGTGCACCCCCAGCGGTGCC CGGATGCAGGAGGACAGCCCCCTGGCGGGCGGCAGCAGCGG CGAGGACGACCCCTGGCGGAGGAGGACCTGCCCCAGCGAGG AGGACAGCCCCCGGAGGAGGACCCCCCGGAGAGAGAGGAC CTGCCCCGCGAGGAGGACCTGCCAGGAGAGGAGGACCTGCC AGAGGTGAAGCCAAAGAGCGAGGAGGAGGAGGAGGAGGAGG TGGAGGACCTGCCAACCGTGGAGGACCCAGGCGACCCACAG				

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	GAGCCCGAGAACAAACGCCCCACCGGGACAAGAGGGCGACGA CCAGAGCCACTGGAGATACGGAGGGGACCCACCATGGCCAC GGGTAGCCAGCATGCGCAGGACGGTTCAGAGCCCGCTG GACATCCGGCCCCAGCTGGCCGCTTCCAGCCCGCCCTGCG GCCCCTGAGCTGCTGGCTTCCAGCTGCCCCCCTGCCCG AGCTGCGGTGCGGAACAACGGCCACAGCTGCAGTGACC CTGCCCCCGGCTGGAGATGGCCCTGGCCCCCGCCGGGA GTACCGGGCCCTGCAGCTGCACCTGCAGTGGGGCGCCCG GCCGGCCCGCAGCAGCACACCGTGGAGGACACAGGTTT CCAGCAGATCCACGTGCTGCTGACCTGAGCACCGCATTCGC AAGGTGGACGAGGCACTGGGAAGGCCAGGAGGACTGGCAG TGCTGGCAGCCTTCCTGGAGGAGGACCAAGAGAGACAGC GCATACGAGCAGCTGCTGAGCCGGCTGGAGGAGATCGCAGA GGAGGAAAGCAGACCCAGGTGCCAGGCCCTGGACATCAGCG CACTGCTGCCAAGCGACTTCAGCCGGTACTTCCAGTACGAG GGCAGCCTGACCAACCCCGCTGCGCCAGGGCGTGATCTG GACCGTGTCAACCAAGACCGTGATGCTGAGCGCAAGCAGC TGACACCCCTGAGCGACACCCCTGTGGGACCAAGCGACAGC CGGCTGCAGTGAACTTCAGGGCAACCCAGCCCCCTGAACGG AAGAGTGATCAGGCAAGCTTCCAGCAGGAGTGGACAGCA GCCCCAGGCGCAGAGCCAGTCCAGCAGGAGTGGACAGCA GCAGCAGCGACATCCTGGCAGTGGTTCGGACTGCTGTT CGCAGTGACAGCGTGGCTTCCCTGGTGCAGATCGGGCGGC AGCACCGGGGGGACCAAGGGGGCGGTGAGTACCGGCCC GCCGAGGTGGCCGAGACCGGGCCCTGA (SEQ ID NO: 45)	AFCPALRPLELLGFQLPPL PELRLRNNHGSVQLTLPPG LEMALGPGREYRALQLHLH WGAAGRPGSEHTVEGHRFP AEIHVVHLSTAFARVDEAL GRPGGLAVLAFLLEGPPEE NSAYEQLLSRLEEAEEGS ETQVPGLDISALLPSDFSR YQYEGSLTTPPCAQGVIV TVFNQTVMLSAKQLHTLSD TLWGPGRDLQLNFRATQP LNGRVIEASFAGVDSPPR AAEPVQLNSCLAAGDILAL VFGLLFAVTSVAFVQMRR QHRRGTGGVSYRPAEVAE TGA* (SEQ ID NO: 46)			

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
Human CA9 extracellular r domain	ATGGGCTGGAGCTGCATCATCTCTGTTCTCTGGTGCAACCGC AACCGGAGTGCACAGCCAGAGGCTGCCACGGATGCAGGAGG ACAGCCCCCTGGCGGGCGGACAGCGCGGAGGACGACCC CTGGCGGAGGAGGACCTGCCCCAGCAGGAGGACAGCCCCAAG GGAGGAGGACCCACAGGAGGAGGACCTGCTGGCGGAGG AGGACCTGCTGGGAGGAGGACCTGCCAGAGGTGAAGCCA AAGAGCAAGAGGAGGAGGAGCCCTGAAGCTGGAGACCTGCC AACCTGGAGGACACAGGCGACCCACAGGAGCCCCAGAAC ACGCCACCGGACAAAGAGGGGACGACAGAGCCACTGG AGATACGAGGACCCACCATGGCCACGGGTGAGCCACG ATGCGCAGGACGGTTCAGAGCCCCGTGGACATCCGGCCCC AGCTGGCGCCTTCTGCCCCCCTGCGGCCCCCTGGAGCTG CTGGGCTTCAGCTGCCCCCCTGCCCCGAGCTGCGGCTGCG GAACAACGGCCACAGCGTGACGTGACCTGCCCCCCTGGC TGGAGATGGCCCTGGGCCCCCGCGGAGTACCGGGCCCTG CAGCTGCACCTGCACTGGGGCGCCGCGGCGGCCCCGCGCAG CGAGCACACCGTGGAGGACACAGGTTCCAGCAGAGATCC ACGTGGTGCACTGAGCACCGCATTCGCAAGGCTGGACGAG GCACTGGGAAGGCCAGGAGGACTGGCAGTCTGGCAGCCTT CCTGGAGGAGGACACAGGAGAAACAGCGCATACGAGCAGC TGCTGAGCGGCTGGAGGAGATCGCAGAGGAGGAAGCGAG ACCCAGGTGCCAGGCTGGACATCAGCGCACTGCTGCCAAG CGACTTCAGCCGGTACTTCCAGTACGAGGGCAGCCTGACCA CCCCCCCCCTGGCCCCAGGGCTGATCTGGACCGTGTTCAC CAGACCGTGATGCTGAGCGCAAGCAGCTGCACACCCCTGAG CGACACCCCTGTGGGGACAGGCGACAGCCCGCTGCAGCTGA ACTTCAGGGCAACCCAGCCCCCTGAACGGGAAGATGATCGAG GCAAGCTTCCAGCAGGAGTGGACAGCAGCCCCCAAGGCGAGC	MGWSCIIILFLVATATGVHS QRLPRMQEDSPLGGSSGE DDPLGEEDLPSEEDSPREE DPPGEEDLPGEEDLPGEED LPEVKPKSEEEGSLKLEDL PTVEAPGDPQEPQNNARD KEGDDQSHWRYGGDPPWPR VSPACAGRFQSPVDIRPQL AAFCALRPLELLGFQLPP LPELRLRNNHGSVQLTLPP GLEMALGPGREYRALQLHL HWGAAGRPGEHTVEGHRF PAEIHVHLSTAFARVDEA LGRPGGLAVLAFLLEEGPE ENSAYEQLLSRLEELAEEG SETQVPGLDISALLPSDFS RYFQYEGSLTTPPCAQGV WTVFNQTVMLSAKQLHTLS DTLWGPDSRLQLNFRATQ PLNGRVIEASFPAGVDSPP RAAEPVQLNSCLAGHHHH H* (SEQ ID NO:48)	1-57	NA	NA

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	AGAGCCAGTGCAGCTGAACAGCTGCTGGCCGGCCACCACC ACCACCACCTGA (SEQ ID NO:47)				
MSLN	ATGGGATGGAGCTGTATCATCTCTTCTTGGTAGCAACAGC TACAGGTGTACACAGCGAAGTGGAGAAGACAGCCTGTCTT CAGGCAAGAAAGCCCGGAGATAGACGAGAGCCTCATCTTC TACAAGAAAGTGGAGCTGGAAGCCTGCGTGGATCGGCCCT GCTGGCCACCCAGATGGACCGCGTGAAAGCCATCCCTTCA CCTACGAGCAGCTGGACGCTCTAAGCATAAACTGGATGAG CTCTACCCACAAGGTTACCCGAGTCTGTATCCAGCACCT GGGTACCTCTTCTCAAGATGAGCCCTGAGGACATTCGCA AGTGGAAATGTACGTCCCTGGAGACCTGAAGGCTTTGCTT GAAGTCAACAAAGGACACGAAATGAGTCTCAGGTGGCCAC CCTGATCGACCGCTTTGTGAAGGAAGGGCCAGCTAGACA AAGACACCTAGACACCTGACCGCTTCTACCCCTGGGTAC CTGTGCTCCCTCAGCCCCGAGGAGCTGAGCTCCGTGCCCC CAGCAGCATCTGGGCGGTGAGCGTCCCTCTATCCCAAGGCCGC GTGACCCAAAGCAGCTGGACGTCCTCTGTCCTGAGGAGGAG CTTGCTTCCAGAACATGAACGGGTCCGAATACTTCGTGAA GATCCAGTCTTCTGCGTGGGCCCCCAGGAGGATTTGA AGGCGCTCAGTCAGCAGAAATGTGAGCATGGACTTGGCCACG TTTCATGAAGCTGCGGACGATGCGGTGCTGCCGTGACTGT GGCTGAGGTGCAGAAACTTCTGGGACCCACGTTGGAGGGCC TGAAGGGAGGAGCGGACCGCCCGGTGCGGAGCTGGATC CTACGGCAGCGGAGGAGCAGCCTGGACACGCTGGGGCTGGG GCTACAGGGGGGATCCCCAACGGCTACCTGGTCTTAGACC TCAGCATGCAAGAGGCGCTCTCGGGGACGCCCTGCCTCTA GGACCTGGACCTGTTCTCTACCGTCTCTGGCACTGCTCCTAGC	MGWSCIIILFLVATATGVHS EVEKTACPSGKKAPRIDES LI FYKKWELEACVDAALLA TQMDRVNAIPFTYEQLDVL KHKLDELYPQGYPESVIOH LGYLFLKMSPEDIRKWNVT SLETLKALLEVNKGHEMSP QVATLI DRFVKGRQLDKD TLDTLTAFYPGYLCSLSPE ELSSVPPSSIWAVRPQDLID TCDPRQLDVLYPKARLAFQ NMNGSEYFVKIQSFLGGAP TEDLKALSQQNVSMDLATF MKLRTDVAVLPLTVAEVQKL LGPHVEGLKAEERHRPVRD WILRQRQDDLDLTLGLGLQG GIPNGYLVLDLSMQEALSG TPCLLGGPVLTVLALLIA STLA* (SEQ ID NO:50)	1-57	NA	NA

Clone Name (Species- Isotype) (antigen)	DNA Sequence (SEQ ID NO:49)	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
xi155D5HC (rabbit Vh- human IgG1) (human CA9)	ATGGGCTGGTCTCTGCATCATCTCTTTCTGGTGGCCACCGC CACCGGCTGCACAGCCAGTCGGTGAAGAGTCCGGGGGTC GCCTGGTCACGCCTGGACACCCCTGACACTCACCTGCACA GTCTCTGGATTCTCCCTCAATAGCTATGCGATGATCTGGGT CCGCCAGGCTCCAGGGAGGGCTGGAATACATCGGATTCA TTACTACTGGTGTACCACTACTACGCGAGCTGGGCAAAA GGCCGATTCAACATCTCCAGAACCTCGACACCGGTGGATCT GAAGCTCACCCGTCCGACAAACCGAGGACACGGCCACCTATT TCTGTGCCAGAGATCGGTTAAAGTACGATGACTATGGT GATTTAGATGCTTTTCGAGCCCTGGGCCCCAGGCACCCCTGGT CACCATCTCCTCAGCATCCACCAAGGACCTCTGGGGGCACAGCG CCCTGGCACCCCTCCTCCAGAGACCTCTGGGGGCACAGCG GCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCCGT GACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGC ACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCC CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCAC CCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACA CCAAGGTGGACAAGAAAGTTGAGCCCAATCTTGTGACAAA ACTCACACATGCCACCGTGCCAGCACCTGAATCCTCTGGG GGGACCGTCAGTCTTCTCTTCCCCCCCCAACCCAGGACA CCCTCATGATCTCCCGGACCCCTGAGGTCACATCGGTGGTG GTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTG GTACGTGGACGGCGTGGAGGTGCAATATGCCAAGACAAAGC CGCGGGAGGAGCAGTACAACAGCAGTACCGTGTGGTCAGC GTCTCTACCGTCTCTGCACCCAGGAGTGGCTGAATGGCAAGGA	MGWSCIIILFLVATATGVHS QSVKESGGRLVTPGTPLTL TCTVSGFSLNSYAMIWVRQ APGEGLEYIGFITTGTTY YASWAKGRFTISRSTTVD LKLTRPTTDTATYFCARD RVKSYDDYGDLDAPFPWGP GTLVTISSASTKGPSPFPL APSSKSTSGGTAALGCLVK DYFPEPVTVMNSGALTSG VHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKT HTCPPCPAPELGGPSVFL FPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTT PPVLDSDSGSFFLYSKLTVD KSRWQQGNVFCSSVMHEAL HNHYTQKSLSLSPGK*	1-57	58-423	424-1416

Clone Name (Species- Isotype) (antigen)	DNA Sequence (SEQ ID NO:51)	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	GTACAAAGTGCAAGGTCTCCAAACAAAGCCCTCCAGCCCCCA TCGAGAAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA CCACAGGTGTACACCTGCCCCCATCCCGGATGAGCTGAC CAAGAACCAAGGTACGCTGACCTGCCTGGTCAAAGGCTTCT ATCCCAGGCACATCGCCGTGGAGTGGGAGAGCAATGGGCAG CCGAGAACAACTACAAGACCACGCCCTCCCGTGTGGACTC CGACGGCTCCTTCTTATATTCAAAGCTCACCGTGGACA AGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG ATGCATGAGGCTCTGCACAACCACTACACGCAGAGAGCCT CTCCCTGTCTCCCGGAAATGA	(SEQ ID NO:52)			
zu155D5HC (human Vh- human IgG1) (human CA9)	ATGGGTGGAGTTGCATCATCTGTTCCTGGTGCCACAGC TACTGGCGTGCACTCACAGGTGCAGCTGGTGGAGTCCGGAG GAGGACTGGTGCAGCCAGGTGGCTCTCTGCGACTGTCTTGT AGTGCTTCAGGCTTTTCCCTGAACAGCTACGCTATGATCTG GGTCAGGCAGGCACCTGGCAAGGGCCTGGAATATATATCGGAT TCATTACACAGAGGAGCTACCTACTATGCCGACTCCGTG AAGGGAGATTCACTATCTCTCGGATAACAGTAAGAATAC CCTGTACCTGCAGATGAATAGCCTGAGAGCAGAGGACACAG CCGTGTACTATTGCGCCAGGGATCGGGTGAATCTTTACGAC GATTATGGAGACCTGGATGCTTTTCAAACCATGGGACAGGG GACCTGGTGACAGTGTCCAAGCATCCACCAAGGGCCCAT CGGTCTTCCCCCTGGCACCCCTCTCCAAGAGCACCTCTGGG GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCC CGAACCGGTGACGGTGTCTGGAACTCAGGCGCCCTGACCA GCGGCGTGCACACCTTCCCGGCTGTCTACAGTCTCTCAGGA CTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAG CTTGGGCACCCAGACCTACATCTGCAACGCTGAATCACAAAGC	MGWSCIIFLVATATGVHS QVQLVESGGGLVQPGGSLR LSCSASGFSLSNYAMIVWR QAPGKGLYIGFTTGGTT YYADSVKGRFTISRDNKSN TLYLQMNSLRAEDTAVYVC ARDRVKSYDDYGDLDLAFEP WGQGTLLTVSSASTKGPSV FPLAPSSKSTSGGTAAALGC LVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSL SSVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSC DKHTTCPPCPAPELLGGPS VFLFPPKPKDITLMSRTPE VTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQY	1-57 58-432 433-1425		

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	CCAGCAACACCAAGGTGGACAAAGAAAAGTTGAGCCCAAAATCT TGTGACAAAAACTCACACATGCCACCGTGCCCGAGCACCTGA ACTCCTGGGGACCGTCAGTCTTCTCTTCCCGCCCAAAAC CCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTCAA TGCCTGCTGGTGGACGTGAGCCAGGAGACCCCTGAGGTCAA GTTCAACTGGTACGTGGACCGCTGGAGGTGCATAATGCCA AGACAAAGCCCGGGAGGAGCAGTACAAACAGCACGTACCGT GTGTCAGCGTCTCTCACCGTCTTGACACGAGACTGGCTGAA TGGCAAGGAGTACAAAGTGCAAGGTCTCAACAAAGCCCTCC CAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAG CCCCGAGAACACAGGTGTACACCTGCCCGCCATCCCGGGA TGAGCTGACCAAGAACAGGTGAGCTGACCTGCCTGTCTCA AAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGC AATGGGAGCCGGAGAACAACTACAAGACACAGCCTCCCGT GCTGGACTCCGACGGCTCCTTCTTCTATATTCAAAAGCTCA CCGTGGACAAAGAGCAGGTGGCAGAGGGGAAACGTCTTCTCA TGCTCCGTGATGATGAGGCTCTGCACAAACCACTACACGCA GAAGAGCCTCTCCCTGTCTCCCGGAAATGA (SEQ ID NO: 53)	NSTYRVSVLTVLHQDWLNL GKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNY KTTTPVLDSDSGEFFLYSKL TVDKSRWQQGNVFSCVMH EALHNHYTQKSLSLSPGK* (SEQ ID NO: 54)			
xil-55-2HC (rabbit Vh- human IgG1) (human TEM1)	ATGGGCTGGTCTGTCATCATCTCTTTCTGGTGGCCACCGC CACCGCGTGCACTCCAGTCGTGGAGGAGTCCGGGGGAG ACCTGGTCAAGCCTGAGGGATCCCTGACACTCACCTGCACA GCCTCTGGATTCTCCTTCAGTAGAGCTACTGGGATGCTG GGTCCGCCAGGCTCCAGGGAAGGGCCCTGAGTGGATCGCAT GCATTTATGGTGGTAGTAGTGTGACCACTATTACCCGGAAC TGGGCGAAAGGCCGATTCTCCATCTCCAAACCTCGTCGAC CAGGCTGACTCTGCAAAATGGCCAGTCTGACAGCCCGGACA CGGCCACCTATTCTGTGCGAGAGTGACTAATGGTGGTGTAT GAAGAGCCTCTCCCTGTCTCCCGGAAATGA (SEQ ID NO: 53)	MGWSCIIILFLVATATGVHS QSVESGGDLVKPEGLTL TCTASGFSFSSYWGCWVR QAPGKGPWIAIYGGSSG TTYYPNWKGRFSISKTS TTVTLQMASLTAADTATYF CARVTNGGDWDFKLWGPQT LVTI SSASTKGPVFPLAP SSKSTSGGTAALGCLVKDY	1-57	58-417	418-1410

Clone Name (Species - Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	TGGGATTTTAAATTGTGGGCCCAGGCACCCCTGGTCACCAT CTCCTCAGCATCCACCAAGGCCCATCGGTCTTCCCCCTGG CACCCTCCTCAAGAGCACCTCTGGGGGCACAGCGGCCCTG GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGT GTCGTGGAATCAGGCGCCCTGACCAGCGGCGTGCACACCT TCCCCGGCTGTCTACAGTCTCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGAC CTACATCTGCAACGTGAATCACAAAGCCAGCAACACCAAGG TGGACAAGAAAGTTGAGCCCAATCTTGTGACAAAACCTCAC ACATGCCACCGTGCCCGAGCACCTGAACTCCTGGGGGGACC GTCAGTCTTCTCTTCCCCCAGAAACCCAGGACACCCCTCA TGATCTCCGGACCCCTGAGGTCACTGCGTGGTGGTGGAC GTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGT GGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGGGGG AGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTC ACCGTCTCGCACCCAGGACTGGCTGAATGGCAAGGAGTACAA GTGCAAGGTCTCCAACAAAAGCCCTCCCAGCCCCCATCGAGA AAACCATCTCCAAGCCAAAAGGCAGCCCCCGAGAACCCACAG GTGTACACCCCTGCCCCCATCCCGGGATGAGCTGACCAAGAA CCAGGTCAAGCTGACCTGCCTGGTCAAAGGCTTCTATCCCA GCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCCTCCCGTGTGGACTCCGACGG CTCCTTCTTCTTATATTCAAAGCTCACCGTGGACAAGAGCA GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT GAGGCTCTGCACAACCACTACAGCAGAGAGCCTCTCCCT GTCTCCCGGGAATGA (SEQ ID NO:55)	FPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSVVT VPSSSLGTQYICNVNHP SNTKVDKKVEPKSCDKTHT CPPCPAPPELLGGPSVFLFP PKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYR VSVLTVLHQDWLNKEYK CKVSNKALPAPIEKTI SKA KGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTP VLDSGSEFFLYSKLTVDKS RWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK* (SEQ ID NO:56)			

[illegible]

Clone Name (Species- Isotype) (antigen)	DNA Sequence (SEQ ID NO: 57)	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	GGTCAGCCTGACCTGCCTGGTCAAGGCTTCTATCCCAGCG ACATCGCCGTGGAGTGGAGAGCAATGGCAGCCGGAGAAC AACTACAGACCAAGCCCTCCCGTCTGGACTCCGACGGCTC CTTCTTTATATTCAAAGCTCACCGTGGACAAGACAGGT GGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAG GCTCTGCACAACCACTACACGCAGAAAGAGCCTCTCCCTGTC TCCCCGGGAAATGA				
zu1E4HC (human Vh- human IgG1) (human CA9)	ATGGGCTGGTCCCTGTCATCATCTGTTTCTGGTGGCCACCGC CACCGCGTGACACAGCGAGGTCCAGCTGCTGGAATCAGGGG GAGGACTGGTGCAGCCCCGAGGGTCACTGCGACTGTCTTGT GCCGCTTCAGGCATTGATCTGTCTAACTACGCTATGACTTG GGTGAGGAGGCACCCGGCAAGGACTGGAGTGGTGGTCCGAA TCATTAGCTCCAATGACAAGACCTGGTACGCCGATTTCAGTG AAAGGCCGGTTCACCATCTCTAGAGACAAACAGTAAGAATAC ACTGTATCTGCAGATGAACAGCCTGCGGGCAGAAAGATACAG CCGTCTACTATTGGCTAAAGCCGCTATGCCCTGGCGGACTG AAGAACGCATTGATCCTTGGGGACAGGAACTCTGCTCAC CGTCTCATCTGCATCCACCAAGGGCCCATCGGTCTTCCCCC TGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCC CTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGAC GGTGTCTGGAACTCAGGGCCCTGACCAAGCGGCGTGCACA CCTTCCCGGCTGTCTACAGTCCCTCAGGACTCTACTCCCTC AGCAGCGTGGTGACCGTGCCTCCAGCAGCTTGGGCACCCCA GACCTACATCTGCAACGTGAATCAAGCCCAAGCAACACCA AGGTGGACAAGAAAGTTGAGCCCAATCTTGTGACAAAAT CACACATGCCCAACCGTGCCCAAGCACTGAACTCCTGGGGGG ACCGTCACTCTTCTCTTCCCCCCCCAAAACCCAAAGGACACCC	MGWSCIIILFLVATATGVHS EVQLLESGGGLVQPGGSLR LSCAASGIDLSNYAMTWVR QAPKGLEWVGIISNDKT WYADSVKGRFTISRDN SKN TLYLQMNSLRAEDTAVYYC AKAAMP GGLKNAFDPWGQG TLVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSVV TVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLF PPKPKDTLMI SRTPEVTCV VVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSRDE	1-57	58-420	421-1413

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	TCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTG GACGTGAGCCACGAAGACCCCTGAGGTCAAAGTTCAACTGGTA CGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGC GGGAGGAGCAGTACAAAGCAGCAGTACCGTGTGGTCAGCGTC CTCACCGTCTGCACCAAGGACTGGCTGAATGGCAAGGAGTA CAAGTGCAAGGTCTCCAAACAAAGCCCTCCAGCCCCCATCG AGAAACCATCTCCAAAGCCAAAGGCGAGCCCCGAGAACCA CAGGTGTACACCCCTGCCCCATCCCGGATGAGTGAACAA GAACAGGTGAGCTGACCTGCCTGGTCAAAGGCTTCTATC CCAGCGACATCGCCGTGGAGTGGAGAGCAATGGGACGCCG GAGAACAACTACAAAGACCAAGCCCTCCCGTCTGGACTCCGA CGGCTCCTTCTTCTTATATTCAAAGCTCACCGTGGACAAAG GCAGGTGGAGCAGGGGAAAGCTCTTCTCATGCTCCGTGATG CATGAGGCTCTGCACAAACCACTACACGAGAGAGCCCTCTC CCTGTCTCCCGGAAATGA (SEQ ID NO: 59)	LTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTP PVLDSDSGFFLYSKLTVDK SRWQQGNVFSCSVMEALH NHYTQKSLSLSPGK* (SEQ ID NO: 60)			
xi33011HC (rabbitVh- human IgG1) (human MSLN)	ATGGGCTGGTCTCTGCATCATCTCTGTTCTGGTGGCCACCGC CACCGCGTGCACCTCCAGTCCGTGGAGGAGTCCGGGGTC GCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACC GTCTCTGGAATCTCCCTCAGTAGCGATGCAATAAGCTGGT CCGCCAGGCTCCAGGAAGGGGCTCGAATACATCGGAATCA TTAATGGTGGTGTAAACATATACCGGAGCTGGGCGGAA GGCCGATTACCATCTCCAAACCTCGACCACCGTGGATCT GAAATCACCAAGTCCGACACCCAGGACACGGCCACCTATT TCTGTGCCAGAGGCATTCAACATGGTGGTGGTAATAGTAT TATTATTATTACGGCATGGACCTCTGGGGCCCCAGGCACCT GGTCACTGTCTCTTTCAGCATCCACCAAGGGGCCCATCGGTCT TCCCCCTGGCACCCCTCTCTCCAAAGAGACCTCTGGGGGCACA	MGWSCIIILFLVATATGVHS QSVESGGRLVTPETPLTL TCTVSGISLSDAISWVRQ APKGLIYIGIINGGNTY YASWAKGRFTISKSTTVD LKITSPTTETATYFCARG IQHGGNSDYIYGMDLWG PGLTVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLS VVTVPSSSLGTQTYICNVN	1-57	58-426	427-1419

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	GCGGCCCTGGCTGCCTGGTCAAGGACTACTTCCCCGAACC GGTGACGGGTGCTGGAACTCAGGGGCCCTGACCAGCGGG TGCACACCTTCCCGGCTGTCTACAGTCTTCAGGACTCTAC TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGG CACCAGACCTACATCTGCAACGTGAATCACAAGCCCAAGCA ACACCAAGGTGGACAAGAAAGTTGAGCCCCAAATCTTGTGAC AAAATCACACATGCCCCACCGTGCCCGCAGCACCTGAATCCT GGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAAAG ACACCTCATGATCTCCCGGACCCCTGAGGTCAATGCGTG GTGGTGACGTGAGCCACGAGACCCCTGAGGTCAAGTTCAA CTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAA AGCCGGGAGGAGCAGTACAAAGCAGTACCGTGTGCTC AGCGTCTCACCGTCTCTGCAACGAGTGGCTGAATGGCAA GGAGTACAAGTGCAAGGTCTCCAAACAAAGCCCTCCCAGCCC CCATCGAGAAAAACCATCTCCAAAGCCAAAGGCGAGCCCCGA GAACACAGGTGTACACCCCTGCCCCCATCCCGGATGAGCT GACCAAGAACCAAGGTGAGCTGAGCTGAGTGGTCAAAAGGCT TCTATCCAGCGACATCGCGTGGAGTGGGAGAGCAATGGG CAGCCGGAGAACAACTACAAGACACGCTCCCGTGTGGA CTCCGACGGCTCTCTCTTATATTCAAAGCTCACCGTGG ACAAGAGCAGGTGGCAGCAGGGGAACTCTTCTCATGCTCC GTGATGCATGAGGCTCTGCACAACCACTACACGAGAAAGAG CCTCTCCCTGTCTCCCGGAAATGA (SEQ ID NO: 61)	HKPSNTKVDKKVEPKSCDK THTCPPCPAPELGGPSVFF LFPPKPKDTLMISRTPEVT CVAVDVSHEDPEVKFNWYV DGEVEHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKT TPPVLDSGSEFFLYSKLTIV DKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK* (SEQ ID NO: 62)			
zu33011HC (humanVh- human IgG1)	ATGGGCTGGTCCCTGCATCATCTCTGTTTCTGGTGGCCACCCG CAGCGGCTGCACAGCGAAGTCCAACTGGTGGAAAGCGGGG GAGGACTGGTGCAGCGCGGGGATCCCTCCGGCTGTCTATGT GCTGCATCGGGAATTTCCCTCTCTCCGACGCGATTAGCTG CCTCTCCCTGTCTCCCGGAAATGA (SEQ ID NO: 61)	MGWSCIIILFLVATATGVHS EVQLVESGGGLVQPGGSLR LSCAASGISLSDAISWVR QAPGKLEYIGIINGGNT	1-57	58-435	436-1428

Clone Name (Species - Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain	
(human MS1N)	GGTCAGACAGGCCCCCGGAAAGGGCTGGAGTACATCGGTA TCATCAACGGCGGGGAAACACCTACTACGCCCTCCTGGGCC AAGGCCGCTTCACCATCTCGCGGCATAATTCGAAGAACAC TCTGTACTTGCAAATGAACCTCCCTGAGGGCCGAGGACACCG CCGTGTACTACTGCGCGCGGCATCCAGCACGGTGGTGA AACAGCGACTACTACTACTATGGGATGGATCTGTGGGGCCA GGGAACCTTGTGACCGTGTCTGTCAGCATCCACCAAGGGCC CATCGGTCTTCCCCCTGGCACCTCTCTCAAGAGCACCTCT GGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTT CCCCGAACCGGTGACGGTGTCTGGAACTCAGGCGCCCTGA CCAGCGCGTGCACACCTTCCCGGTGTCTACAGTCTCTCA GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG CAGCTTGGCACCCAGACCTACATCTGCAACGTGAATCACA AGCCAGCAACACCAAGTGGACAAGAAAGTTGAGCCCAAA TCTTGTGACAAAACCTCACACATGCCCCACCGTGCCCGACAC TGAACCTCCTGGGGGACCGTCAGTCTTCTCTTCCCCCCAA AACCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTC ACATGCGTGGTGGCTGACCTGAGCCACGAAGACCCCTGAGGT CAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG CCAAGACAAAGCCGGGAGGAGCAGTACAAACAGCACGTAC CGTGTGGT'CAGCGT'CCT'CA'CCGT'CCT'GCACCCAGGACTGGCT GAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA'CAAAAGCCC TCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGG CAGCCCCGAGAACACAGGTGTACACCCCTGCCCCCATCCCG GGATGAGCTGACCAAGAACAGGTGAGCCTGACCTGCCCTGG TCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAG AGCAATGGGACCGCGAGAACAACTACAAGACCAAGCCTCC CGTGTGGACTCCGACGGCTCCTTCTTCTTATATTCAAAGC	YYASWAKGRFTISRHSKN TLYLQMNSLRAEDTAVYIC ARGIQHGGGNSDYIYGMD LWGQGTLLTVSSASTKGPS VFPLAPSSKSTSGGTAALG CLVKDYFPEPTVSWNSGA LTSGVHTFPAVLQSSGLYS LSSVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKS CDKHTCPCPAPELLGGP SVFLFPPKPKDTLMISRT P EVTCAVVDVSHEDPEVKFN WYVDGVEVHNAKTPREEQ YNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIE KTIISKAGQPREPQVYTL P PSRDELTKNQVSLTCLVK G FYPSDIAVEWESNGQPENN YKTTFPVLDSGSFFLYSK LTVDKSRWQQGNVFC SVM HEALHNHYTQKSLSLSPGK * (SEQ ID NO:64)				

Clone Name (Species - Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
xi32405HC (rabbitVh- human IgG1)	TCACCGTGGACAAGACGAGGTGGCAGCAGGGGAACGTCTTC TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACAC GCAGAAAGAGCCTCTCCCTGTCTCCCGGGAATGA (SEQ ID NO:63)				
	ATGGCGTGTCTCTGCATCATCTCTGTTTCTGGTGGCCACCGC CACCGCGGTGCACTCCCACTCGCTGGAGAGTCCGGGGGTC GCCTGGTCACGCCCTGGGACACCCCTGACACTCACCTGCACA GCCTCTGGATTCTCCCTCAGTAACTATGCAATGACCTGGGT CCGCCAGGCTCCAGGGAAGGGCTAGAAATACATCGGAATCA TTAGTACTGGCGGTATCACATACTATATGACTCGGCAAAA GGCCGATTCACCATCTCCAGAACTCGACCACGGTGGATCT GAAATGACCAGTCTGACAAACGAGGACACGGCCACCTATT TCTGTGGCAGAAATGCTGGTGGTAGTTATATTTTCTATTAT TTTGACTTGTGGGCCAAGGCACTTCTCCCTGGTCACTGTCTCTC AGCATCCACCAAGGCCCATCGGTCTTCCCCCTGGCACCT CCTCAAAGACACCTCTGGGGCACAGCGGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCGAACCGGTGACGGTGTCTGTG GAACTCAGGCGCCCTGACACAGCGCGTGCACACCTTCCCGG CTGTCTCTACAGTCTCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT CTGCAACGTGAATCACAAAGCCAGCAACACCAAGGTGGACA AGAAAGTTGAGCCCAATCTTGTGACAAAACTCACACATGC CCACCGTCCCCAGCACCTGAATCTCTGGGGGACCGTCACT CTTCTCTTCCCCCAAAACCAAGGACACCCCTCATGATCT CCCGGACCCCTGAGGTCAATGCGTGGTGGACGTGAGC CACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGG CGTGGAGGTGCATAATGCCAAGACAAAGCCCGGGGAGGAGC AGTACAACAGCAGCATCCGTGTGGTCAGCGTCTCTCACCGTCTC	MGWSCIILFLVATATGVHS QSLEESGGRLVTPGTPLTL TCTASGFSLSNYAMTWVRQ APKGLEYIGIISTGGITY YMDSAKGRFTISRTSTVVD LKMTSLTTEDTATYFCGRN AGGSYIFYFEDLWGQTLV TVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSVVTVFP SSSLGTQTYICNVNHHKPSN TKVDKKVEPKSCDKHTHTCP PCPAPELLGGPSVFLFPPPK PKDTLMIISRTPEVTCVVVD VSHEDPEVKENWYVDGVEV HNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYCKK VSNKALPAPIEKTIISKAGG QPREPQVYITLPPSRDELTK NQVSLTCLVKGFYPSDIAV EWESNGQPENNYKITPPVL DSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHY	1-57	58-411	412-1404
(human MSLN)					

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	CTGCACCAAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAA GGTCTCCAAACAAAGCCCTCCAGCCGCCCATCGAGAAACCA TCTCCAAAGCCAAAGGGCAGCCCGAGAACACACAGGTGTAC ACCTGCCCCCATCCCGGATGAGCTGACCAAGAACCAGGT CAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCACGGACA TCGCCGTGGAGTGGAGAGCAATGGCGAGCCCGAGAACCAAC TACAAGACACGCCCTCCCGTGTGGACTCCGACGGCTCCTT CTTCTTATATTCAAAGCTCACCGTGGACAAGACAGGTGGC AGCAGGGGAAGTCTTCTCATGTCCGTGATGCATGAGGCT CTGCACAAACCACTACACGACGAGAGAGCCTCTCCCTGTCTCC CGGAAATGA (SEQ ID NO: 65)	TQKSLSLSPGK* (SEQ ID NO: 66)			
xil78F16HC (rabbitVh- human IgG1) (human MSLN)	ATGGGCTGCTCTGCATCATCTCTGTTTCTGGTGCCACCGC CACCGCGTGCATCCAGTCCGTGGAGGAGTCCGGGGTTC GCCTGGTCACGCTGGGACACCCCTGACACTCACCTGCACA GCCTCTGGATTCTCCCTCAGTAACTATGCAATGACCTGGGT CCGCCAGGCTCCAGGGAAGGGGCTAGAATACATCGGAATCA TTAGTACTGGCGGTATCACATACATATATGGACTCGGCAAAA GGCCGATTCAACCATCTCCAGAACCTCGACCACGGTGGATCT GAAAATGACCAAGTCTGACAAACCGAGGACACGGCCACCTATT TCTGTGGCAGAAATGCTGGTGGTAGTTATATTTCTATTAT TTCCGACTTGTGGGGCCAAGGACCTCTGCTCACTGTCTCTTC AGCATCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT CCTCCAGAGACACTCTGGGGGACAGCGGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAAACCGGTGACGGTGTCTGTG GAATCAGGCGCCCTGACCAAGCGGCTGCACACCTTCCCGG CTGTCTCAGTCTCAGGACTCTACTCTCCCTCAGCAGCGGTG GTGACCGTGGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT	MGWSCIILFLVATATGVHS QSLEESGGRLVTPGPTLTL TCTASGFSLSNYAMTWVRQ APGKGL EYIGIISTGGITY YMDSAKGRFTISRSTTVD LKMTSLTTEDTATYFCGRN AGGSYIFYYFDLWGGTLV TVSSASTKGPSPVFPLAPSS KSTSGGTAALGCLVKDYFP EPVTVSNWNSGALTSVHTF PAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHPN TKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLPFPK PKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEV	1-57	58-411	412-1404

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	CTGCAACGCTGAATCACAAAGCCAGCAACACCAAGGTGGACA AGAAAGTTGAGCCCAAAATCTTGACAAAACTCACACATGC CCACCGTGCCAGCACCTGAACCTCTGGGGGACCGTCAGT CTTCCTCTTCCCCCAAAACCAAGGACACCCCTCATGATCT CCCGGACCCCTGAGGTACATGCGTGGTGGACGTGAGC CACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGG CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGAGGAGC AGTACAAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC CTGCACCAAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAA GGTCTCCAACAAAGCCCTCCAGGCCCTCATCGAGAAACCA TCTCCAAAGCCAAAGGGCAGCCCGAGAACACACAGGTGTAC ACCTGCCCCCATCCCGGATGAGTGAACCAAGAACAGGT CAGCCTGACCTGCTGCTGCTCAAGGCTTCTATCCCAGCGACA TCGCCGTGAGTGGAGAGCAATGGCAGCCCGAGAACAAAC TACAAGACACGCTCCCGTGTGGACTCCGACGGCTCCCTT CTTCTTATATCAAAGCTCACCGTGGACAAAGACAGGTGGC AGCAGGGGAACGTCTTCTCATGCTCCGTGATGATGAGGCT CTGCACAAACCACTACACGACAGAGAGCCTCTCCCTGTCTCC CGGGAATGA (SEQ ID NO: 67)	HNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTIISKAGG QPREPQVYITLPPSRDELTK NQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPVVL DSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHY TQKSLSLSPGK* (SEQ ID NO: 68)			
xi237N18HC (rabbitVh- human IgG1) (human MSLN)	ATGGGCTGGTCTGCATCATCTCTTTCTGGTGGCCACCGC CACCGGCTGCACTCCAGTCGCTGGAGGAGTCCGGGGGTC GCCTGGTCGGCCCTGGACACCCCTGACACTCACCTGCACA GTCTCTGGATTCTCCCTCAGTAGTATACCATGAGCTGGGT CCGCCAGGCTCCAGGGGAGGGCTGGAATGGATCGGAGGCA TTACTGCTATGAGTCGCACATACGCGAGCTGGCGCGAAA GGCCGATTCAACCATCTCCAAAACCTCGACACCGTCCATCT GAAAATCACCAAGTCCGACAAACCGAGACACAGGCCACCTATT CGGGAATGA (SEQ ID NO: 67)	MGWSCIIILFLVATATGVHS QSLSESGGRLVAPGTPLTL TCTVSGFSLSSYHMSWVRQ APGEGLEWIGGITAMSRTY YASWAKGRFTISKSTTVH LKITSPTTEDTATYFCARE PGFVSNIWGPGLVTVSLA STKGPSVFPLAPSSKSTSG	1-57	58-396	397-1389

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	TCTGTGCCAGGGAACCTGGTTTGTGTAGTAACATCTGGGGC CCAGGCACCTGGTCAACCGTCTCCTTAGCATCCACCAAGG CCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCT CTGGGGCACAGCGGCCCTGGGCTGCTGGTCAAGGACTAC TTCCCCGAACCGGTGACGGTGTCTGGAACTCAGGCGCCCT GACCAGCGGTGCACACCTTCCCCGGCTGTCTACAGTCTCT CAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTCCCTCC AGCAGCTTGGCACCCAGACCTACATCTGCAACGTGAATCA CAAGCCAGCAACACCAAGGTGGACAAGAAATTGAGCCCA AATCTTGTGACAAAACCTCACATGCCCCACCGTGCCAGCA CCTGAATCCTGGGGGACCGTCACTTCTCTTCCCCC AAACCCAGGACACCTCATGATCTCCGGACCCCTGAGG TCACATGGTGGTGGACGTGACGACGACGAAGACCCCTGAG GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAAGACAAAAGCCGCGGAGGAGCAGTACAACAGCACGT ACCGTGTGGTCAAGCTCCTCACCGTCTGCACCAAGGACTGG CTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC CCTCCAGCCCCCATCGAGAAAACCATCTCCAAGGCCAAG GGCAGCCCGAGAACACACAGGTGTACACCTGCCCCCATCC CGGGATGAGCTGACCAAGAACCCAGGTGACCGTGACCTGCCT GGTCAAGGCTTCTATCCACGACATGCCGTGGAGTGGG AGAGCAATGGCAGCCGGAGAACAACTACAAGACCACGCT CCCGTGTGGACTCCGACGGCTCTTCTTATATTTCAA GCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGACGCT TCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCATAC ACGCAGAGAGCCTCTCCCTGTCTCCCGGGAATGA (SEQ ID NO: 69)	GTAALGCLVKDYFPEPVT SWNSGALTSGVHTFPAVLQ SSGLYSLSVVTVPSSSLG TQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLT LHQDWLNGKEYCKVSNKA LPAPIEKTIISKAGQPREP QVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNV FSCSVMHREALHNYHTQKSL SLSPGK* (SEQ ID NO: 70)			

Clone Name (Species - Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain	
xi383I18HC (rabbitVh- human IgG1) (human MSLN)	ATGGGCTGGTCTCTGCAATCATCCTGTTTCTGGTGGCCACCGC CACCGGCGTGCACTCCCACTCGGTGGAGGAGTCCGGGGGTC GCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACA GTCTCTGGATTCTCCCTCAGTAGCTATGCAATGGGCTGGGT CCGCCAGGCTCCAGGGAGGGGCTGGAATGGATCGGAACCA TTAGTACTGGTGTATTACATACTACGCGAGCTGGGCGAAA GGCCGATTCAACCATCTCCAAAACCTCGACCACGGTGGATCT GAAAATCACCACTCCGACAAACCGAGGACACGGCCACCTATT TCTGTGCCAGAGTGGTAGTAGTGGTTATCTTTTCTACTTC TTTAACTTGTGGGGCCAAAGCACCCCTCGTCACTGTCTCCTC AGCATCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT CCTCAAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTG GAACTCAGGCGCCTGCACAGCGCGTGCACACCTTCCCGG CTGTCTTACAGTCTCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT CTGCAACGTGAATCACAAAGCCAGCAACACCAAGGTGGACA AGAAAGTTGAGCCCAATCTTGTGACAAAACCTCACACATGC CCACCGTGCCCAAGCACCTGAACTCTCTGGGGGACCGTCAGT CTTCTCTTCCCCCAAAACCCAGGACACCCCTCATGATCT CCCGGACCCCTGAGGTCAATGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCTGAGGTCAAAGTTCAAAGTACGTGGACGG CGTGGAGGTGCATAATGCCAAGACAAAGCCCGGGAGGAGC AGTACAAACAGCAGTACCGTGTGGTACGCTCTCTACCGTCT CTGCACCAAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAA GGTCTCCAAACAAAGCCCTCCAGCCCCCATCGAGAAAACCA TCTCCAAAGCCAAAGGGCAGCCCCGAGAAACACAGGTGTAC ACCTTGCCCCCATCCCGGGATGAGCTGACCAAGAACACAGGT	MGWSCIILFLVATATGVHS QSVESGGRLVTPGTPLTL TCTVSGFSLSSYAMGWVRQ APGEGLEWIGTISTGGITY YASWAKGRFTISKSTTVD LKITSPTTEDTATYFCARV GSSGYLFYFFNLWGQGLV TVSSASTKGPSPFLAPSS KSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSVHTF PAVLQSSGLYSLSSVTVP SSSLGTQTYICNVNHPSPN TKVDKKVEPKSCDKHTCTP PCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTIISKAGG QPREPQVYITLPPSRDELTK NQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRW QQGNVFCSCVMHEALHNNHY TQKSLSLSPGK* (SEQ ID NO:72)	1-57 58-411 412-1404			

Clone Name (Species- Isotype) (antigen)	DNA Sequence (SEQ ID NO: 71)	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	CAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCACGCGACA TCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAC TACAAGACACGCCCTCCGCTGCTGACTCCGACGGCTCCTT CTTCTTATATTCAAAGCTCACCGTGGACAAGAGCAGGTGGC AGCAGGGGAACGCTCTTCTCATGCTCCGTGATGCATGAGGCT CTGCACAACCACTACACGCAGAGAAGCCTCTCCCTGTCTCC CGGGAATGA				
xi166B3HC (rabbitVh- human IgG1) (human CA9)	ATGGGCTGGTCCCTGCATCATCCTGTTTCTGGTGGCCACCGC CACCGCGTGACACAGCCAGTCGGTGAAGGAGTCCAGGGGTC GCCTGGTACGCCCTGGGACACCCCTGACACTCACCTGCACA GTCTCTGGATTCTCCCTCAGTAGGTATACATTGATCTGGGT CCGCCAGGCTCCAGGGAAGGGGTGGAATGGATCGGAATCA TAGATAGTAGTAGTAGTGATGCTACTACGCGAGGTGGCGGAAA GGCCGATTCAACCATCTCCAAAACCTCGACCACGCGTGATCT GAAAATCACCAAGTCCGACAAACCGAGGACACGGCCACCTATT TCTGTGCCAGAGACAGAGTCTTAAGCTACGATGACTATGCT GATTTGCCCGATGTTTCGATCCCTGGGGCCAGGCACCCCT GGTCACCGTCTCCTCAGCATCCACCAAGGGCCCATCGGTCT TCCCCCTGGCACCCCTCCTCCAAGACACCTCTGGGGGCACA GCGGCCCTGGGCTGCCCTGGTCAAGGACTACTTCCCCGAACC GGTGACGGTGTGCTGGAACCTCAGGGCCCTGACACGCGCG TGACACCTTCCCCGCTGTCTACAGTCTCAGGACTCTAC TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGG CACCCAGACCTACATCTGCAACGTGAATCACAAAGCCACGCA ACACCAAGGTGGACAAGAAAGTTGAGCCCAAACTTTGTGAC AAAATCACACATGCCCCACCGTGCCAGCACCTGAACCTCCT GGGGGACCGTCACTCTTCTCTTCCCCCCCCAAAACCCAAAGG	MGWSCIIILFLVATATGVHS QSVKESRGLVTPGTPLTL TCTVSGFSLRYTLIWRQ APGKLEWIGIIDSSSSAY YARWAKGRFTISKSTTVD LKITSPTTETATYFCARD RVLSYDDYGDLPDGFDPWG PGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALT GVHTFPAVLQSSGLYSLSS VTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDK THTCPPCPAPPELLGGPSVF LFPPKPKDTLMI SRTPEVT CVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNS TYRVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSR	1-57	58-426	427-1419

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	ACACCCTCATGATCTCCGGACCCCTGAGGTACATGCGTGTG GTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAAGTCAA CTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAA AGCCGGGGAGGAGCAGTACAACAGCACGTACCGTGTGCTC AGCGTCTCACCGTCTTGCAACAGGACTGGCTGAATGGCAA GGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCACGCC CCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAACCAAGGTGTACACCTGCCCCCATCCCGGGATGAGCT GACCAAGAACAGGTACGCTGACCTGCCTGGTCAAAGGCT TCTATCCAGCGACATCGCCGTGGAGTGGAGAGCAATGGG CAGCCGGAGAACAACATAAAGACCACGCCCTCCCGTGTGGA CTCCGACGGCTCCTTCTTTATATTCAAAGCTCACCGTGG ACAAAGACAGGTGGCAGAGGGGAACGTCTTCTCATGCTCC GTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAAGAG CCTCTCCCTGTCTCCCGGGAATGA (SEQ ID NO: 73)	DELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTIV DKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK* (SEQ ID NO: 74)			
zul66B3HC (humanVh- human IgG1) (human CA9)	ATGGGCTGGTCCTGCATCATCTCTGTTCTGGTGGCCACCGC CACCGGCGTGCAAGCGAAAGTGACAGCTGGTCGAATCTGGAG GAGGACTGGTGCAGCCTGGAGGGAGCCTGAGACTGAGTTGC GCAGCAAGCGGGTTTAGCCTGTCCCGATACACCCCTGATCTG GGTGAGACAGGCCCCCGCAAGGACTGGAGTGGGTCTCTA TCATTGACAGCTCCTCTAGTGCCTACTATGCTGATAGTGTG AAGGCGAGGTTCAACATTTACGCGACAAACGCTAAAATAAG CCTGTATCTGCAGATGAACCTCCCTCGGGCAGAAAGACACAG CCGTGTACTATTGCGCACGGGATAGAGTCTCTGAGCTACGAC GATTATGGGACCTGCCTGACGGCTTTGATCTCTTGGGGACA GGGAACTCTGGTGACAGTGAGCAGCGCATCCACCAAGGGCC CATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCT	MGWSCIILFLVATATGVHS EVQLVESGGGLVQPGGSLR LSCAASGFSLSRYTLIWR QAPKGLEWVSIIDSSSA YYADSVKGRFTISRDAKN SLYLQMNSLRRAEDTAVYIC ARDRVLSYDDYGDLPDGF PWGQGTLLTVSSASTKGPS VFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYIC	1-57 58-435 436-1428		

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	GGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTT CCCCGAACCGGTGACGGTGTCTGGAACTCAGGCGCCCTGA CCAGCGCGTGCACACCTTCCCGGCTGTCTACAGTCTCTCA GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG CAGCTTGGCACCCAGACCTACATCTGCAACGTGAATCACA AGCCAGCAACACCAAGGTGGACAAGAAATTGAGCCCAAA TCTTGTGACAAAACCTCACACATGCCACCGTGCCCGACACC TGAACCTCCTGGGGGACCGTCAGTCTTCTCTTCCCCCAA AACCCAGGACACCCCTCATGATCTCCCGACCCCTGAGGTC ACATGCGTGGTGGCTGGACGTGAGCCACGAAGACCCCTGAGGT CAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG CCAAAGACAAAGCCCGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCTCTACCGTCTGCAACCAAGGACTGGCT GAATGGCAAGGATCAAGTGCAAGGTCTCCAACAAAGCCC TCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCCAAGGG CAGCCCCGAGAACCAAGGTGTACACCTGCCCCCATCCCG GGATGAGCTGACCAAGAACCAAGGTGACCGTGACCTGCCTGG TCAAAGGCTTCTATCCCAAGCACATCGCCGTGGAGTGGGAG AGCAATGGCAGCGGGAGAAACAATAACAAGACCAAGCCTCC CGTGCTGGACTCCGACGGCTCTTCTTCTTATATTCAAAGC TCACCGTGGACAAGAGCAAGGTGGCAGCAGGGGAACGTCTTC TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACAC GCAGAAAGACCTCTCCCTGTCTCCCGGGAATGA (SEQ ID NO:75)	NVNHKPSNTKVDKKVEPKS CDKHTTCPCPAPELLGGP SVFLFPKPKDITLMISRT EVTCAVVDVSHEDPEVKFN WYVDGVEVHNATKPREEQ YNSTYRVVSVTLVHQDWL NGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTL PSRDELTKNQVSLTCLVKG FYPSDIAVEWESNGQENN YKTTPLVLDSDGSFFLYSK LTVDKSRWQQGNVFCSVM HEALHNHYTQKSLSLSPGK * (SEQ ID NO:76)			
xi155D5LC (rabbitVk-	ATGGGCTGGTCTCTGCATCATCTGTTTCTGGTGGCCACCGC CACC GGCTGCACAGCGAGCTCTGTGATGACCCAGACTCCAT CCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAAT TGCCAGGCCAGTCAGAGCAFTAGTAGCTACTTAGCCTGGTA GCAGAAAGACCTCTCCCTGTCTCCCGGGAATGA (SEQ ID NO:75)	MGWSCIIILFLVATATGVHS ELVMTQTTPSSVSAAVGGTV TINCQASQSISSYLAWYQQ KPGQPPKLLIYYASTLASG	1-57	58-390	391-714

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
human kappa) (human CA9)	TCAGCAGAAAACAGGGCAGCCTCCAAAGCTCCTGATCTATT ATGCATCCACTCTGGCGTCTGGGTCCCATCGCGTTCAA GGCAGTGGCTCTGGGACAGAGTTCACTCTCACCATCACCG CGTGCACTGTGACGATGCTGCCACTTACTACTGTCTAGGTG TTTATGTTATAGTTTGTGATGATGGTATTGCTTTCGGCGGA GGACCGAGCTGGAGATCAAAACGAACCTGTGGTGCACCATC TGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTG GAACCTGCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAGGTGGATAACGCCCTCCA ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCA AGACAGACCTACAGCCTCAGCAGCAGCCTGACGCTGAGC AAAGCACTACGAGAAACACAAAGTCTACGCCCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCGCTCACAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO: 77)	VPSRFKSGSGTEFTLTIT GVQ CDDA ATYYCLGVYGYS FDDGIAFGGTELEIKRTV AAPSVFIFFPPDEQLKSGT ASVVCLLNMFYPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSTLTLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO: 78)			
zu155D5LC-1 (humanVk- human kappa) (human)	ATGGGGTGGTCTTGTCATCATCTCTGTTCTGTTGGTGCAACCGC CACAGGTGTGCACTCCGACATCCAGATGACTCAGAGTCCAT CAACCCCTGTCGGCTAGCGTGGGAGATAGAGTACTATCACC TGTCAGGCCCTCTCAGAGTATTTCCAGCTACCTGGCTTGGTA TCAGCAGAAAGCCAGGCAAGCAAGCAGCAGCTGCTGATCTACT ATGCTAGTACACTGGCATCAGGAGTGCCTTCCCGCTTCTCA GGTTCGGCAGCGGAACCTGAGTTTACACTGACTATTTCTAG TCTGCAGTGGACGATTTCCGTAACCTACTATTGCCTGGGGG TGTAACGTTATTCTTTCCAGCATGGCATCGCATTTGGCGGA GGGACAAAAGTGGAGATTAAAGAGACTGTGGCCGCTCCACAG TGTGTTCAATTTTCCCCCTAGCGACGACAGCTGAAAGCG GGACAGCCTCTGTGGTGTGTCTGCTGAACAATTTTACCCCT CGGAGGCCAAAGTGCAGTGGAGGTGGATAACGCTCTGCA	MGWSCIIILFLVATATGVHS DIQMTQSPSTLSASVGRV TITCQASQSISSYLAWYQ KPGKAPKLLIYYASTLASG VPSRFSGSGSGTEFTLTIS SLQ CDD EATYYCLGVYGYS FDDGIAFGGTELEIKRTV AAPSVFIFFPPDEQLKSGT ASVVCLLNMFYPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSTLTLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC*	1-57	58-390	391-714

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	GTCTGGCAATAGTCAGGAGTCAGTGACCGAACAAGGACTCCA AAGATAGCACATATCTCTGTCTATCCACCTGACACTGTCC AAGGCAGACTACGAGAAGCACAAAGTGATGCTGCGAAGT GACTCATCAGGGCCTGAGCTCTCCGCTGACCAAGAGCTTTA ACAGGGGAGAAATGTTGA (SEQ ID NO: 79)	(SEQ ID NO: 80)			
zu155D5LC-2 (humanVk- human kappa) (human CA9)	ATGGGGTGGTCTTGCAATCTCTGTTCTCTGGTGGCAACCGC CACAGGTGTGCACTCCGACATCCAGATGACTCAGAGTCCAT CAACCTGTCCGCTAGCGTGGGAGATAGAGTACTATCAC TGTCAGGCCTCTCAGAGTATTTCCAGCTACCTGGCTTGGA TCAGCAGAAAGCCAGGCAAGCACCAAGCTGCTGATCTACT ATGCTAGTACACTGGCATCAGGAGTGCCTTCCCGCTTCTCA GGTCCGGCAGCGGAACCTGAGTTTACACTGACTATTACTGG TGTGCAGTGCGACGATTTCCGCTACCTACTATTGCCTGGGG TGTAACGCTTATCTTTCGACGATGGCATCGCATTTGGCGGA GGACAAAAGTGGAGATTAAAGAGGACTGTGGCCGCTCCACG TGTGTTCAATTTTCCCCCTAGCGACGAAACAGCTGAAAAGCG GGACAGCCTCTGTGGTGTCTGCTGAACAATTTTACCTT CGGGAGGCCAAAGTGCAGTGGAAAGTGGATAACGCTCTGCA GTCTGGCAATAGTCAGGAGTCAGTGACCGAACAAGGACTCCA AAGATAGCACATATCTCTGTCTATCCACCTGACACTGTCC AAGGCAGACTACGAGAAGCACAAAGTGATGCTGCGAAGT GACTCATCAGGGCCTGAGCTCTCCGCTGACCAAGAGCTTTA ACAGGGGAGAAATGTTGA (SEQ ID NO: 81)	MGWSCIIILFLVATATGVHS DIQMTQSPSTLSASVGDV TITCQASQSISSYLAWYQQ KPGKAPKLLIYYASTLASG VPSRFGSGSGTEFTLTIT GVQCDD EF ATYYCLGVYGS FDDGIAFGGKVEIKRTV AAPSVFIFPPSDEQLKSGT ASWCLLNNFYPPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSSLTLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO: 82)	1-57 58-390 391-714		

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
zu155D5LC-3 (human V κ - human kappa) (human CA9)	ATGGGGTGGTCTTGTCATCATCTCTGTTCTCTGGTGCAACCGC CACAGGTGTGCACTCCGACATCCAGATGACTCAGAGTCCAT CAACCCCTGTCGGCTAGCGTGGGAGATAGAGTGACTATCACC TGTCAGGCCCTCTCAGAGTATTTCCAGCTACCTGGCTTGGTA TCAGCAGAAGCCAGGCAAGCAACCAAGCTGCTGATCTACT ATGCTAGTACACTGGCATCAGGAGTGCCTTCCCGCTTCTCA GGTTCGGCAGCGGAACCTGAGTTTACACTGACTATTTCTAG TCTGCAGTGGACGATGCCGCTACCTACTATTCCTGGGG TGTAACGTTATTTCTTTCCAGCATGGCATCGCATTTGGCGGA GGGACAAAAGTGGAGATTAAAGGACTGTGGCCGCTCCAG TGTGTTCAATTTTCCCGCTAGCGACGAACAGCTGAAAAGCG GGACAGCCTCTGTGGTGTGTCTGTGTAACAATTTTACCCCT CGGAGGCCAAAGTGCAGTGGAAAGGTGGATAACGCTCTGCA GTCTGGCAATAGTCAGGAGTCACTGACCCGAACAGGACTCCA AAGATAGCACATATTTCTGTCTATCCACCCCTGACACTGTCC AAGGCAGACTACGAGAAAGCACAAAGTGTATGCCTGCGAAGT GACTCATCAGGGCCTGAGCTCTCCGCTGACCAAGAGCTTTA ACAGGGGAGAAATGTTGA (SEQ ID NO: 83)	MGWSCIIILFLVATATGVHS DIQMTQSPSTLSASVGD TITCQASQSISSYLAWYQ KPGKAPKLLIYYASTLASG VPSRFGSGSGTEETLTIS SLQ CDDA ATYYCLGVYGS FDDGIAFGGGTKVEIKRTV AAPSVFIFFPPSDEQLKSGT ASVVCLLNNFYPRKAVQW KVDNALQSGNSQESVTEQD SKDSTYSLSTLTLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO: 84)	1-57	58-390	391-714
zu155D5LC-4 (human V κ - human kappa) (human CA9)	ATGGGGTGGTCTTGTCATCATCTCTGTTCTCTGGTGCAACCGC CACAGGTGTGCACTCCGACATCCAGATGACTCAGAGTCCAT CAACCCCTGTCGGCTAGCGTGGGAGATAGAGTGACTATCACC TGTCAGGCCCTCTCAGAGTATTTCCAGCTACCTGGCTTGGTA TCAGCAGAAGCCAGGCAAGCAACCAAGCTGCTGATCTACT ATGCTAGTACACTGGCATCAGGAGTGCCTTCCCGCTTCTCA GGTTCGGCAGCGGAACCTGAGTTTACACTGACTATTTACTGG TGTGCAGTGGACGATGCCGCTACCTACTATTCCTGGGG TGTAACGTTATTTCTTTCCAGCATGGCATCGCATTTGGCGGA (SEQ ID NO: 83)	MGWSCIIILFLVATATGVHS DIQMTQSPSTLSASVGD TITCQASQSISSYLAWYQ KPGKAPKLLIYYASTLASG VPSRFGSGSGTEETLTIT GVQ CDDA ATYYCLGVYGS FDDGIAFGGGTKVEIKRTV AAPSVFIFFPPSDEQLKSGT ASVVCLLNNFYPRKAVQW	1-57	58-390	391-714

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	GGGACAAAAGTGGAGATTAAAGAGGACTGTGGCGCTCCAG TGTGTTCAATTTTCCCCCTAGCGACGAACAGCTGAAAAGCG GGACAGCCTCTGTGGTGTGCTGCTGAACAAATTTTACCCT CGGAGGCCAAAAGTGCAGTGGAAAGGTGGATAACGCTCTGCA GTCTGGCAATAGTCAGGAGTCAGTGACCGAACAGGACTCCA AAGATAGCACATATTTCTGTCTCATCCACCTGACACTGTCC AAGGCAGACTACGAGAAGCAAAAAGTGATGCTGCGGAAGT GACTCATCAGGGCCTGAGCTCTCCCGTGACCAAGAGCTTTA ACAGGGGAGAATGTTGA (SEQ ID NO: 85)	KVDNALQSGNSQESVTEQD SKDSTYSLSSITLTSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO: 86)			
zu155D5LC-5 (humanV κ - human κ appa) (human CA9)	ATGGGGTGGTCTTGCATCATTTCTGTTCTCTGGTGGCAACCGC CACAGGTGTGCACTCCGACATCCAGATGACTCAGAGTCCAT CAACCCCTGTCCGCTAGCGTGGGAGATAGAGTACATATCACC TGTACGGCCTCTCAGAGTATTTCCAGCTACCTGGCTTGGA TCAGCAGAAGCCAGGCAAAAGCAACCAAGCTGCTGATCTACT ATGCTAGTACACTGGCATCAGGAGTGCCCTTCCCGCTTCAAA GGTTCGGCAGCGGAACGTAGTTTACACTGACTATTAATCTGG TGTGCAGTGGCAGATGCCGCTACCTACTATTGCTGCTGGGG TGTAACGGTTATTCTTTCCAGATGGCATCGCATTTGGCGGA GGGACAAAAGTGGAGATTAAAGGACTGTGGCCGCTCCCAG TGTGTTCAATTTTCCCCCTAGCGACGAACAGCTGAAAAGCG GGACAGCCTCTGTGGTGTGCTGCTGAACAAATTTTACCCT CGGAGGCCAAAAGTGCAGTGGAAAGGTGGATAACGCTCTGCA GTCTGGCAATAGTCAGGAGTCAGTGACCGAACAGGACTCCA AAGATAGCACATATTTCTGTCTCATCCACCTGACACTGTCC AAGGCAGACTACGAGAAGCAAAAAGTGATGCTGCGGAAGT GACTCATCAGGGCCTGAGCTCTCCCGTGACCAAGAGCTTTA ACAGGGGAGAATGTTGA	MGWSCIIILFLVATATGVHS DIQMTQSPSTLSASVGDRV TITCQASQSISSYLAWYQQ KPGKAPKLLIYYASTLASG VPSRFKSGSGTEFTLTIT GVQ CDDA ATYYCLGVYGS FDDGIAFGGGTKVEIKRTV AAPSVFIFPPSDEQLKSGT ASVCLLNNFYDPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSSITLTSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO: 88)	1-57 58-390 391-714		

Clone Name (Species- Isotype) (antigen)	DNA Sequence (SEQ ID NO: 87)	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
zu155D5LC-6 (humanVk- human kappa) (human CA9)	ATGGGGTGGTCTTGCAATCATCTGTTCTCTGGTGGCAACCGC CACAGGTGTGCACTCCGACATCCAGATGACTCAGAGTCCAT CAACCGTGTCCGCTGCAGTGGAGGTACAGTGACTATCACC TGTCAGGCTCTCAGAGTATTTCCAGCTACCTGGCTTGGA TCAGCAGAAAGCCAGGCAAGCAACCAAGCTGCTGATCTACT ATGCTAGTACACTGGCATCAGGAGTGCCTTCCCGCTTCTCA GGTCCGGCAGCGGAACCTGAGTTTACACTGACTATTACTGG TGTGCACTGCAGCATGCCGCTACCTACTATTGCCCTGGGG TGTAACGCTATTCTTTTCGACGATGGCATCGCATTTGGCGGA GGGACAAAAGTGGAGATTAAAGAGGACTGTGGCCGCTCCAG TGTGTTCAATTTTCCCCCTAGCGACGAACAGCTGAAAAGCG GGACAGCCTCTGTGGTGTCTGTCTGCTGAACAATTTTACCCCT CGGAGGCCAAAGTGCAGTGGAGGTGGATAACGCTCTGCA GTCTGGCAATAGTCAGGAGTCACTGACCCGACAGGACTCCA AAGTAGCACATATTCTCTGTCAATCCACCTGACACTGTCC AAGGCAGACTACGAGAAGCAAAAGTGTATGCCCTGCGAAGT GACTCATCAGGGCTGAGCTCTCCCGTGACCAAGAGCTTTA ACAGGGGAGAAATGTTGA (SEQ ID NO: 89)	MGWSCIIILFLVATATGVHS DIQMTQSPSTVSAAVGGTV TITCQASQSISSYLAWYQQ KPGKAPKLLIYYASTLASG VPSRFSGSGSGTEFTLTIT GVQ CDDA ATYYCLGVYGS FDDGIAFGGGTKVEIKRTV AAPSVFIFFPPSDEQLKSGT ASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSSLTILSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO: 90)	1-57	58-390	391-714
zu155D5LC-7 (humanVk- human kappa) (human CA9)	ATGGGGTGGTCTTGCAATCATCTGTTCTCTGGTGGCAACCGC CACAGGTGTGCACTCCGACATCCAGATGACTCAGAGTCCAT CAACCGTGTCCGCTAGCGTGGAGATAGAGTGACTATCACC TGTCAGGCTCTCAGAGTATTTCCAGCTACCTGGCTTGGA TCAGCAGAAAGCCAGGCAAGCAACCAAGCTGCTGATCTACT ATGCTAGTACACTGGCATCAGGAGTGCCTTCCCGCTTCTCA GGTCCGGCAGCGGAACCTGAGTTTACACTGACTATTACTGG TGTGCACTGCAGCATGCCGCTACCTACTATTGCCCTGGGG TGTAACGCTATTCTTTTCGACGATGGCATCGCATTTGGCGGA GGGACAAAAGTGGAGATTAAAGAGGACTGTGGCCGCTCCAG TGTGTTCAATTTTCCCCCTAGCGACGAACAGCTGAAAAGCG GGACAGCCTCTGTGGTGTCTGTCTGCTGAACAATTTTACCCCT CGGAGGCCAAAGTGCAGTGGAGGTGGATAACGCTCTGCA GTCTGGCAATAGTCAGGAGTCACTGACCCGACAGGACTCCA AAGTAGCACATATTCTCTGTCAATCCACCTGACACTGTCC AAGGCAGACTACGAGAAGCAAAAGTGTATGCCCTGCGAAGT GACTCATCAGGGCTGAGCTCTCCCGTGACCAAGAGCTTTA ACAGGGGAGAAATGTTGA (SEQ ID NO: 89)	MGWSCIIILFLVATATGVHS DIQMTQSPSTVSAVGDV TITCQASQSISSYLAWYQQ KPGKAPKLLIYYASTLASG VPSRFSGSGSGTEFTLTIT GVQ CDDA ATYYCLGVYGS FDDGIAFGGGTELEIKRTV	1-57	58-390	391-714

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	TGTGACGTGGACGATGCCGCTACCTACTATTGCTGCGGG TGTAACGGTATTCTTTGACGATGGCATCGCATTTGGCGGA GGGACAGAGCTGGAGATTAAAGAGACTGTGGCCGCTCCAG TGTGTTCAATTTTCCCCCTAGCGACGAACAGCTGAAAAGCG GGACAGCCTCTGTGGTGTCTGCTGAACAATTTTACCT CGGAGGCCAAAGTGCAGTGGAGGTGGATAACGCTCTGCA GTCTGGCAATAGTCAGGAGTCAGTGACCGAACAGGACTCCA AAGATAGCACATATTCTCTGTCTATCCACCCTGACACTGTCC AAGGACACTACGAGAAGACAAAGTGTATGCCTGCGAAGT GACTCATCAGGGCCTGAGCTCTCCCGTGACCAAGAGCTTTA ACAGGGAGAAATGTTGA (SEQ ID NO: 91)	AAPSVFIFPPPSDEQLKSGT ASVVCLLNFPYPRPAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSTLTLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO: 92)			
zu155D5LC- huVK1-39 (humanVk- human kappa) (human CA9)	ATGGGCTGTCCTGCATCATCTGTTTCTGGTGCCACCGC CACCGGCTGCACAGCGACATTACAGATGACTCAGTCCCCCT CCTCCCTTTCGGCTCCGTCGGCGACCGCGTGACCATTAAC TGTCAGCCAGCCAGTCCATCTCCTCCTACTTGGCCTGGTA CCAACAGAAAGCCAGGAAAGCTCCTAAGCTGCTCATCTACT ACGCCCTCCACTCTGGCGTCTGGTGTCCCGTCACGGTTCAGC GGTCCGGATCAGGAACCTGACTTCAACCTGACGATCAGCAG CCTCCAGTCGAGGATTTTGGGACCTACTACTGCTGGGG TGTAATGTTACTCGTTCCAGCATGGAATCGCATTCGGCTCG GGACCAAGGTGGAATCAACAGCACTGGCTGCACCATC TGCTTTCATCTTCCCGCATCTGATGAGCAGTTGAAATCTG GAAGTCCCTCTGTTGTGTGCTGCTGAATACTTCTATCCC AGAGAGCCAAAGTACAGTGGAGGTGGATAACGCCCTCCA ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGACCTACAGCCTCAGCAAGCAACCTGACGCTGAGC AAAGCAGACTACGAGAAACAAAGTCTACGCCCTGCGAAGT	MGWSCIILFLVATATGVHS DIQMTQSPSSLSASVGRV TITCQASQSISSYLAWYQ KPGKAPKLLIYYASTLASG VPSRFGSGSGTDFLTIS SLQCEDFATYYCLGVYGS FDDGIAFGSGTKVEIKRTV AAPSVFIFPPPSDEQLKSGT ASVVCLLNFPYPRPAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSTLTLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO: 94)	1-57	58-390	391-714

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Leader Position	cDNA Variable Domain	cDNA Constant Domain
	CACCCATCAGGGCCTGAGCTCGCCGCTCACAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO:93)				
zu155D5LC- huVK2-40 (humanVk- human kappa) (human CA9)	ATGGGCTGGTCCTGTCATCATCTGTTTCTGGTGCCACCGC CACCGGCTGCACAGCGACATTTGTGATGACCCAGACTCCTC TCTCCCTGCCCGTGAATCTCTGGAGAACCCGCTCGATCTCA TGTCAGGCTGCAGAGCATCTCCTCATACTTGGCTTGGTA CCTCCAAAAGCCGGCCAGAGCCACAGCTTCTGATCTATT ACGCTCCACCTGGCTCGGCTCGGCTCGGATCGGTTTCC GGTCTGGAAGCGGAACCGACTTACCCCTGAAATCTCCCG CGTGGAGTCGAGGACGTGGCGTGTACTACTGCCTGGGAG TCTACGGGTACTCTTCTGATGACGCGATTCGATTCGGTCC GGTACCAAGTCGAAATCAACGAACTGGCTGCACCATC TGTCTTCACTCTCCCGCATCTGATGAGCAGTTGAAATCTG GAATGCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAAAGTGGATAACGCCCTCCA ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGCCTACAGCCTCAGCAGCACCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCGCTCACAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO:95)	MGWSCIIILFLVATATGVHS DIVMTQTPLSLPVPPEPA SISQASQSISSYLAWYLQ KPGQSPQLLIYYASTLASG VPDRFSGSGGTDFTLKIS RVECEDVGVVYCLGVYGS FDDGIAFGSGTKVEIKRTV AAPSVFIFFPSDEQLKSGT ASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSSLTLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO:96)	1-57	58-390	391-714
zu155D5LC- huVK3-11 (humanVk- human kappa)	ATGGGCTGGTCCTGTCATCATCTGTTTCTGGTGCCACCGC CACCGGCTGCACAGCGAAATTTGTCTCACTCAATCCCCTG CCACCCCTTCTCTGTCCCCCGGCGAAAGAGCCACTCTGTCA TGTCAGGCGAGCCAGTCGATCTCTCTTACCTGGCTTGGTA CCAGCAAGAGCCAGGACAGGACCCGCGCTGCTGATCTACT	MGWSCIIILFLVATATGVHS EIVLTQSPATLSLSPGERA TLSCQASQSISSYLAWYQO KPGQAPRLLIYYASTLASG IPARFSGSGGTDFTLTIS	1-57	58-390	391-714

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
(human CA9)	ACGGCTCGACCCCTCGCCTCGGGAATCCCGCCCGGTTGAGC GGATCAGGCTCCGGTACCGACTTCACTCTGACCATTAGCTC CCTGGAGTCGAGGACTTCGGGTGTATTACTGCTGCGGGG TGTAACGGCTACTCTTCTCGATGACGGAATCGCCTTTGGGAGC GGTACCAAGGTCGAGATCAAAACGAACTGTGGCTGCACCATC TGCTTTCACTCTTCCCGCCATCTGATGAGCAGTTGAAATCTG GAACTGCCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAAAGTGGATAACGCCCTCCA ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCA AGACAGCAGCTACAGCCTCAGCAGCAGCCTGACGCTGAGC AAAGCAGACTACGAGAAACAAAGTCTACGCCCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO: 97)	SLECEDFAVYYCLGVYGYGYS FDDGIAFGSGTKVEIKRTV AAPSVFIFFPPSDEQLKSGT ASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSSLTLLSKADY EKHKVYACEVTHQGLSPV TKSFNRGEC* (SEQ ID NO: 98)			
zu155D5LC- huVK4-1 (human Vκ- human kappa) (human CA9)	ATGGGCTGGTCTGTCATCATCTCTGTTCTGGTGCCACCGC CACCGGCGTGACAGCGATATCGTGATGACCCAAAGCCCTG ACTCCCTTGCCGTGTCACTCGGAGAACGCGCCACCATCAAC TGTCAGGCTCGCAGTCCATCTCCTCATACCTGGCCTGGTA TCAGCAGAAACCGGGGACGCGCCAAAGCTGCTGATCTACT ACGCTTCCACTCTGGCCTCCGGCGTCCCGATCGGTTCTCC GGATCGGGCTCCGGCACCGACTTTACTCTGACCATTAGCAG CCTCCAGTCGAGGACGTGGCGGTGTAATACTGCTTGGGTG TCTACGGATACTCTTTCGACGACGGGATCGCATTCGGTTCC GGAACCAAGGTCGAGATTAAACGAACTGTGGCTGCACCATC TGCTTTCACTCTTCCCGCCATCTGATGAGCAGTTGAAATCTG GAACTGCCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAAAGTGGATAACGCCCTCCA ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCA (SEQ ID NO: 100)	MGWSCIIILFLVATATGVHS DIVMTQSPDLSAVSLGERA TINCQASQSISSYLAWYQQ KPGQPPKLLIYYASTLASG VPDRFSGSGGTDFLTIS SLQCEDEVAVYYCLGVYGYGYS FDDGIAFGSGTKVEIKRTV AAPSVFIFFPPSDEQLKSGT ASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSSLTLLSKADY EKHKVYACEVTHQGLSPV TKSFNRGEC* (SEQ ID NO: 100)	1-57	58-390	391-714

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	AGGACAGCACCTACAGCCTCAGCAGCAGCACCCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAAGTCTACGCCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO: 99)				
zu155D5LC- huVK6-21 (humanVk- human kappa) (human CA9)	ATGGGCTGGTCTGCATCATCTCTGTTCTGGTGGCCACCGC CACCGCGTGACACAGCGAAATCGTCTTGACCCAAAGCCCTG ACTTCCAATCCGTGACCCCAAGGAAAAGGTACCATCAG TGTCAGGCTCCAGTCAATTTCTCGTACCTTGCCTGGTA CCAGCAAGCCAGACCAAGTCCCGAAGTCTCTGATTAAAT ACGCATCACCCCTGGCTAGCGGAGTCCGAGCCGGTCTCG GGATCCGGCTCTGGAATGACTTCACTCTGACCATCAACTC GCTCGAGTGGAAAGATGCCGCCACTTACTATTGCTGGGG TGACGGGTACTCATTTGACGATGGCATCGCCTTCGGCTCC GGTACCAAAAGTGGAGATCAAAACGAATGTGGCTGCACCAATC TGCTTTCATCTTCCCGCATCTGATGAGCAGTTGAAATCTG GAACTGCCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAAAGTGGATAACGCCCTCCA ATCGGGTAACCTCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAAGTCTACGCCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO: 101)	MGWSCIIILFLVATATGVHS EIVLTQSPDFQSVTPKEKV TITCQASQSISSYLAWYQQ KPDQSPKLLIKYASTLASG VPSRFSGSGSDFTLTIN SLECEDAATYYCLGVYGS FDDGIAFGSGTKVEIKRTV AAPSVFIFFPSDEQLKSGT ASWCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSSLTLLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO: 102)	1-57 58-390 391-714	58-390	391-714
zu155D5LC- huVK6D-41 (humanVk-)	ATGGGCTGGTCTGCATCATCTCTGTTCTGGTGGCCACCGC CACCGCGTGACACAGCGAGCTCGTGTGATGACTCAAAGCCCGG CATTCCTTTCGGTGAATCTCTGGAGAAAAGGTACCATCACC TGTCAGGCTCCAGTCAATTTCTCGTACCTTGCCTGGTA CCAGCAAGCCAGACCAAGTCCCGAAGTCTCTGATTAAAT ACGCATCACCCCTGGCTAGCGGAGTCCGAGCCGGTCTCG GGATCCGGCTCTGGAATGACTTCACTCTGACCATCAACTC GCTCGAGTGGAAAGATGCCGCCACTTACTATTGCTGGGG TGACGGGTACTCATTTGACGATGGCATCGCCTTCGGCTCC GGTACCAAAAGTGGAGATCAAAACGAATGTGGCTGCACCAATC TGCTTTCATCTTCCCGCATCTGATGAGCAGTTGAAATCTG GAACTGCCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAAAGTGGATAACGCCCTCCA ATCGGGTAACCTCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAAGTCTACGCCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO: 101)	MGWSCIIILFLVATATGVHS DVVMTQSPAFLSVTPGEKV TITCQASQSISSYLAWYQQ KPDQAPKLLIKYASTLASG	1-57 58-390 391-714	58-390	391-714

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
human kappa) (human CA9)	TCAGCAGAAAGCCAGACAGGCCCGGAGCTGCTGATTAAGT ACGCCTCCACCCCTGGCCAGCGGAGTCCCGTACGGTTCTCC GGGTCCGGCTCAGGAACCGACTTCACGTTACCATCTCGTC CCTCGAGTGCAGAGATGCTGCGACTTACTACTGCTGCGGCG TGTAACGTTACTCGTTTGATGACGGCATCGCTTCGGGTCT GGAACCAAGTGGAGATCAACGAACTGTGGCTGCACCATC TGCTTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTG GAATGCCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAGGTGGATAACGCCCTCCA ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCA AGACAGACCTACAGCCTCAGCAGCAGCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCGCTCAGAAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO:103)	VPSRFSGSGSGTDFTFITIS SLECEDAATYYCLGVYGYGYS FDDGIAFGSGTKVEIKRTV AAPSVFIFFPPSDEQLKSGT ASVVCLLNNFYBPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSSLTLLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO:104)			
zu155D5LC- huVK7-3- Glu81 (humanVk- human kappa) (human CA9)	ATGGGCTGGTCTCGCATCATCTCTGTTCTGGTGCCACCGC CACCGGCTGCACAGCGATATCGTCTCACTCAATCCCCCG CTTCACCTCGCGGTGCCCTGCTCCCTGTTCAACGCGCCACCATTAAG TGTCAGGGCTCCAGTCCATTTGAGCTACCTTGCAATGGTA CCAGCAGAAAGCCTGGACAGCCCCCGAAACTGCTGATCTATT ACGCCCTCCACCTTGGCCTCGGGAGTGCCAGCGCGTTTAGC GGTTCGGGCTCCGGCACTGACTTCACTCTGACCATCAACCC GGTGGAGTGGAGATACCGCCCACTACTACTGCTGCGGGGG TGTAACGATACTCATTCGACGACGGGATCGCTTCGGGAAGC GGCACCAAGTCCGAAATCAACAGAACTGTGGCTGCACCATC TGCTTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTG GAATGCCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAGGTGGATAACGCCCTCCA	MGWSCIIILFLVATATGVHS DIVLTQSPASLAVSPGQRA TITCQASQSISSYLAWYQO KPGQPPKLLIYYASTLASG VPARFSGSGSGTDFLTIN PVECEDTANYCYCLGVYGYGYS FDDGIAFGSGTKVEIKRTV AAPSVFIFFPPSDEQLKSGT ASVVCLLNNFYBPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSSLTLLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC*	1-57	58-390	391-714

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO:105)	(SEQ ID NO:106)			
xil-55-2LC (rabbitVκ- human kappa) (human TEM1)	ATGGGCTGGTCCCTGCATCATCTCTGTTTCTGGTGGCCACCGC CACCGCGTGCACTCCGAGCTCGTGATGACCCAGACTCCAG CCTCTGTGGAGGTAGCTGTGGGAGGACAGTCACCATCAAG TGCCAGGCCAGTCAGAGCATTAATACCTACTTAGCCTGGTA TCAGCAGAAACAGGGCAGCCTCCAAAGCTCCTGATCTACA GGGCATCCACTCTGGCATCTGGGTCCCATCGCGTTCAA GGCAGTGGATCTGGACAGAGTTCACTCTCACCATTAGCGA CCTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAACAGA GTGTTCCGTATTATGATGTTGATAATACTTTCGGCGGAGGG ACCGAGTGGTGGTCAAAACGAACCTGTGGCTGCACCATCTGT CTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAA CTGCCCTCTGTTGTGCTGCTGCTGAATAACTTCTATCCAGA GAGGCCAAAGTACAGTGGAGGTGGATAACGCCCTCCAAATC GGTAACCTCCAGGAGAGTGTACAGAGCAGGACAGCAAGG ACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAA GCAGACTACGAGAAACAAAAGTCTACGCCTGCGAAGTCAC CCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACA GGGAGAGTGTGA (SEQ ID NO:107)	MGWSCIIILFLVATATGVHS ELVMTQTPASVEVAVGGTV TIKCQASQSINTYLAHYQQ KPGQPPKLLIYRASTLASG VPSRFKSGSGTEFTLTIS DLE CADA AATYYCQOSVRVI DVDNTFGGTEVVVKRTVA APSVFIFFPSDEQLKSGTA SWVCLLNNFYPPREAKVQWK VDNALQSGNSQESVTEQDS KDSYISLSSTLTLSKADYE KHKVYACEVTHQGLSSPVT KSFNRGEC* (SEQ ID NO:108)	1-57 58-387 388-711		

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
xi1E4LC (rabbitVk- human kappa) (human CA9)	ATGGGCTGGTCTGCATCATCTCTGTTTCTGGTGCCACCGC CACCGGCGTGACAGCGAGCTCGTGATGACCCAGACTCCAT CCTACACGCTGTCAGCTGTGGGAGACACAGTCAACATCAAG TGCCAGGCCAGTCAGACCATTTGGTAGCGACTTATCCTG GTATCAGCAGAAACAGGGCAGCCTCCAAACTCCTGATCT GGTATGCAACCAATCTGCCATCTGGGGTCCCATCGCGGTC AGTGGCAGTAGATCTGGACAGAGTACACTCTCACCATCAG CGCGTGCAAGTGTGAGGATGCTGCCACCTACTACTGTCTAG GTGGTTATGCTGCTGCTTCTTACAGAACTGCTTTCGGCGGA GGACCGAGGTGGTCTGTCAAACGAACTGTGGCTGCACCATC TGTCTTCATCTTCCCGCATCTGATGAGCAGTTGAAATCTG GAATGCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAAAGTGGATAACGCCCTCCA ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGACAGCA AGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCCCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO:109)	MGWSCIIILFLVATATGVHS ELVMTQTPSYTSAAVGDTV TIKCQASQTIGGSDLWSYQ QKPGQPKLLIWIYATNLP GVPSRFSGSRSGTEYTLTI SGVQ CEDA ATYYCIGGYAA ASYRTAFGGGTEVVVKRTV AAPSVFIFFPSDEQLKSGT ASVVCLLNNFYPRKAVQW KVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO:110)	1-57	58-390	391-714
	ATGGGCTGGTCTGCATCATCTCTGTTTCTGGTGCCACCGC CACCGGCGTGACAGCGATATTCAGATGACCCAGAGCCCTT CCTTCTCTGTCAGCCAGCGTTCGGGATAGAGTCAATCACT TGCCAGGCCAGCAGACCATTTGGCGGAGCGACCTGCTCTG GTACCCAGAGAAGCCCGGAAAAGCCCTAAGCTGCTGATCT ACTATGCTACAAACCTGCCATCTGGCGTGCCAGCCGGTTC TCTGGAAGTGGCTCAGGAGACTGACTTACCCTGACAAATAG CTCCCTGCAGTGCAGGAGATTTCCGACCTACTATTGTCTGG GGGCTATGCGCGCCGCAAGCTACCGACCCGCTTCGGAGGA	MGWSCIIILFLVATATGVHS DIQMTQSPSFLSASVGDV TITCQASQTIGGSDLWSYQ QKPGKAPKLLIYATNLP GVPSRFSGSGGTDFTLTI SSLQCEDEFATYYCIGGYAA ASYRTAFGGGTEVVKRTV AAPSVFIFFPSDEQLKSGT ASVVCLLNNFYPRKAVQW	1-57	58-390	391-714
	zule4LC-CXXF (humanVk- human kappa) (human CA9)				

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	GGAACATAAAGTGGAATCAAAACGAACTGTGGCTGCACCAATC TGTCTTCATCTTCCCGCATCTGATGAGCAGTTGAAATCTG GAACTGCCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAGGTGGATAACGCCCTCCA ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACTACAGCTCAGCCTCAGCAGCACCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAAGTCTACGCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCCGTCAAAAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO:111)	KVDNALQSGNSQESVTEQD SKDSTYSLSSLTLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO:112)			
zule4LC-CXXA (human Vκ- human kappa) (human CA9)	ATGGGCTGGTCTGTCATCATCTGTTTCTGGTGGCCACCGC CACCGGCTGCACAGCATATTACATGACTCAGAGCCCTT CCTTCTGTCGGCTTCCGTCGGGATCGGGTCAAAATCACT TGCCAGGCCTCACAGACTATTGGGGAGCGACCTGTCCTG GTACCAAGCAGAAAGCCCGGAAAGCACCTAAGCTGCTGATCT ACTATGCCACAAACCTGCCATCTGGCGTGCCAGCCGGTTC TCTGGAAGTGGCTCAGGACTGACTTTACCTGACAAATTAG CTCCCTGCAGTGGAGGATGCCGTACTACTATTGTCCTGG GGGCTACGCCCGCTTTCATACAGGACCGCTTCGGAGGA GGAACATAAAGTGGAATCAAAACGAACTGTGGCTGCACCATC TGTCTTCATCTTCCCGCATCTGATGAGCAGTTGAAATCTG GAACTGCCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAGGTGGATAACGCCCTCCA ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACTACAGCCTCAGCAGCACCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAAGTCTACGCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCCGTCAAAAAGAGCTTCA ACAGGGGAGAGTGTGA	MGWSCIILFLVATATGVHS DIQMTQSPSFLSASVGDV TITCQASQTIGGSDLSWYQ QKPGKAPKLLIYYATNLP GVPSRFSGSGSGTDFTLT SSLQCEDAATYYCLGGYAA ASYRTAFGGGTKEIKRTV AAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPRFAKQW KVDNALQSGNSQESVTEQD SKDSTYSLSSLTLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO:114)	1-57	58-390	391-714

Clone Name (Species- Isotype) (antigen)	DNA Sequence (SEQ ID NO:113)	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
xi33011LC (rabbitVk- human kappa) (human MSLN)	ATGGGCTGGTCTGTCATCATCTGTTTCTGGTGCCACCGC CACCGCGTGCACTCCGAAGTGTGATGACCCAGACTCCAT CCTCCGTGCTGCACTGTGGGAGACACAGTCAACATCAAG TGCCAGGCCAGTCAGAGCATTAGTAGTGTCTTGCTCCTGGTA TCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATC TGGCATCCACTCTGGCATCTGGGTCCTCCATCGCGGTTGAG GGCAGTAGATCTGGGACAGAGTTCACCTCACCATCAGCGA CCTGGAGTGACGATGCTGCCACTTACTACTGTCAAACCA ATTATGGTACTAGTAGTAGTAATATGTTTGTCTTTCGGC GGAGGGACCGAGGTGTCGTCAAACGAACGTGGCTGCACC ATCTGTCTCATCTTCCCGCCACTGTGATGAGCAGTTGAAT CTGGAACCTGCCTCTGTTGTGCTGCTGTAATACTTCTAT CCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCT CCAATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACA GCAAGGACAGACCTACAGCCTCAGCAGCACCCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCCTGCCA AGTCACCCATCAGGGCCTGAGCTCGCCCGTCAACAAAGAGCT TCAACAGGGGAGAGTGTGA (SEQ ID NO:115)	MGWSCIIILFLVATATGVHS EVLMTQTPSSVSAAVGDTV TIKCQASQSISSVLSWYQQ KPGQPPKLLIYLASTLASG VPSRFSGSRSGTEFTLTIS DLECDAAATYYCQINYGTS SSNYGFAFGGGTEVVVKRT VAAPSVFI FPPSDEQLKSG TASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQ DSKDSTYSLSTLTLSKAD YEKHKVYACEVTHQGLSSP VTKSFNRGEC* (SEQ ID NO:116)	1-57	58-393	394-717
zu33011LC- CXXF (humanVk- human kappa)	ATGGGCTGGTCTGTCATCATCTGTTTCTGGTGCCACCGC CACCGCGTGCACTCCGAAGTGTGATGACCCAGACTCCCA GCTCGCTGCTCGCCTCCGTGGGCGACCGCGTGACCATCACG TGCCAGGGCTCCAGTCAATTAGACGGTGCTCTCCTGGTA CCAACAGAAAGCCGGGAAAGCACCAAGCTGCTGATCTACT TGGCCCTCCACTCTGGCCTCGGGAGTGCCTTCACGGTTCTCC (SEQ ID NO:115)	MGWSCIIILFLVATATGVHS DIQMTQSPSSLSASVGRV TITCQASQSISSVLSWYQQ KPGKAPKLLIYLASTLASG VPSRFSGSGSGTDFLTIS SLQCEDEATYYCQINYGTS	1-57	58-393	394-717

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
(human MSLN)	GGATCGGGATCTGGTACTGATTTACCCCTCACCATCTCGAG CCTTCAGTGGGAGGACTTCGCTACTTACTATTGTCAAAACCA ACTACGGAACCTCCAGCTCCAACCTACGGCTTTGCCCTTCGGT GGCGGGACCAAGGTCGAAATCAAAAGAACTGTGGCTGCACC ATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAAT CTGGAACCTGCCTCTGTTGTGTGCTGCTGAATAACTTCTAT CCCAGAGAGGCCAAAGTACAGTGAAGGTGGATAACGCCCT CCAATCGGTAACTCCAGGAGAGTGTACAGAGCAGGACA GCAAGGACAGACCTACAGCCTCAGCAGCACCTTGACGCTG AGCAAAGCAGACTACGAGAAACAAAGTCTACGCCCTGCCA AGTCACCCATCAGGGCCTGAGCTCGCCCGTCAAAAAGAGCT TCAACAGGGGAGAGTGTGA (SEQ ID NO:117)	SSNYGFAFGGKVEIKRT VAAPSVFIFFPPSDEQLKSG TASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQ DSKDYSLSSLTLSKAD YEKHVYACEVTHQGLSSP VTKSFNRGEC* (SEQ ID NO:118)			
zu33011LC- CXXA (human V κ - human kappa) (human MSLN)	ATGGGCTGGTCTCTGCATCATCTGTTCTGGTGGCCACCGC CACCGCGTGCACAGCGACATTTCAGATGACCCAGTCCCCAA GCTCGCTGCGCCTCCGTGGCGACCGCGTGACCATCACG TGCCAGGCTCCAGTCAATTAGCAGCGTGCTCTCCTGGTA CCAACAGAAAGCGGGAAAGCACCAAGCTGCTGATCTACT TGGCCTCCACTCTGGCCTCGGGAGTGCCTTCACGGTTCTCC GGATCGGGATCTGGTACTGATTTACCCCTCACCATCTCGAG CCTTCAGTGGGAGGACGCCGCTACTTACTATTGTCAAACCA ACTACGGAACCTCCAGCTCCAACCTACGGCTTTGCCCTTCGGT GGCGGGACCAAGGTCGAAATCAAAAGAACTGTGGCTGCACC ATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAAT CTGGAACCTGCCTCTGTTGTGTGCTGCTGAATAACTTCTAT CCCAGAGAGGCCAAAGTACAGTGAAGGTGGATAACGCCCT CCAATCGGTAACTCCAGGAGAGTGTACAGAGCAGGACA GCAAGGACAGACCTACAGCCTCAGCAGCACCTTGACGCTG	MGWSCIIILFLVATATGVHS DIQMTQSPSSLSASVGRV TITCQASQSISSVLSWYQ KPGKAPKLLIYLASTLASG VPSRFSGSGSGTDFTLTIS SLQCEDAATYYCQINYGTS SSNYGFAFGGKVEIKRT VAAPSVFIFFPPSDEQLKSG TASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQ DSKDYSLSSLTLSKAD YEKHVYACEVTHQGLSSP VTKSFNRGEC* (SEQ ID NO:120)	1-57	58-393	394-717

[illegible]

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
(human MSLN)	CTGCATCCACTCTGGCATCTGGGGTCTCATCGCGGTTCAGA GGCAGTGGATCTGGGACAGAAATACACTCTCACCATCAGCGA CCTGGAGTGTCCGATGCTGCCACTTACTACTGTCAAACCT ATTATGATATTGTTACTACTACTTTCGGCGGAGGACCGAG GTGGTCGTCAACGAACTGTGGCTGCACCATCTGTCTTCAT CTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCT CTGTTGTGTGCCCTGCTGAATAACTTCTATCCCAGAGAGGCC AAAGTACAGTGGAAAGGTGGATAACGCCCTCCAATCGGGTAA CTCCAGGAGAGTGTCAAGAGCAGGACAGCAAGGACAGCA CCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGAC TACGAGAAACACAAAGTCTACGCCCTGCGAAGTCACCCATCA GGCCCTGAGCTCGCCCGCTCACAAGAGCTTCAACAGGGGAG AGTGTGA (SEQ ID NO:123)	DLEADAATYYCQYYDIV TSTFGGGTEVVVKPTVAAP SVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKD STYLSLSTLTLSKADYEKH KVYACEVTHQGLSSPVTKS FNRGEC* (SEQ ID NO:124)			
xi178F16LC (rabbit/k- human kappa) (human MSLN)	ATGGGCTGGTCCTGCATCATCTCTGTTTCTGGTGGCCACCCG CACCGGCTGCACCTCCGATTCGAATTGACCCAGACTCCAT CCTCCGTGGAGGAGCTGTGGAGGCACAGTCACCAATCAAG TGCCAGGCCAGTCAGAGCATTTACAGTTATTTAGCCTGGTA TCAGCAGAAAACGAGGACGCTCCCAAGCTCCTGATCTATT CTGCATCCACTCTGGCATCTGGGGTCTCATCGCGGTTCAGA GGCAGTGGATCTGGGACAGAAATACATTCTCAACATCAGCGA CCTGGAGTGTCCGATGCTGCCACTTACTACTGTCAAACCT ATTATGATATTGTTACTACTACTTTCGGCGGAGGACCGAG GTGGTGGTCAACCGAACTGTGGCTGCACCATCTGTCTTCAT CTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCT CTGTTGTGTGCCCTGCTGAATAACTTCTATCCCAGAGAGGCC AAAGTACAGTGGAAAGGTGGATAACGCCCTCCAATCGGGTAA CTCCAGGAGAGTGTCAAGAGCAGGACAGCAAGGACAGCA (SEQ ID NO:126)	MGWSCIIFLVATATGVHS AFELTQTPSSVEAAVGGTV TIKQASQSIYSLAWYQQ KPGQPPKLLIYSASTLASG VSSRFRGSGSTEYILTIS DLEADAATYYCQYYDIV TSTFGGGTEVVVKPTVAAP SVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKD STYLSLSTLTLSKADYEKH KVYACEVTHQGLSSPVTKS FNRGEC* (SEQ ID NO:126)	1-57	58-381	382-705

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	CCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAGCAGAC TACGAGAAACACAAAGTCTACGCCCTGCGAAGTCACCCATCA GGCCCTGAGCTCGCCCGTCAAAAGAGCTTCAACAGGGGAG AGTGTGA (SEQ ID NO: 125)				
xi237N18LC (rabbitVk- human kappa) (human MS1N)	ATGGGCTGGTCTGCATCATCTGTTTCTGGTGGCCACCGC CACCGCGTGCACTCCGCCGTCGTGCTGACCCAGACTGCAT CCCCGTGCTGGAGTTGTGGGAGGCACAGTCACCATCAAG TGCCAGGCCAGTCAGAACATTTACAGCAATTTAGCCTGGTA TCAGCAGAAACAGGGCAGCGTCCAAAGCTCCTGATGTATG ATGCATCCACTCTGGCATCTGGGTCCTCATCGCGTTCAA GGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGA CCTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAAAGTA TTAGTAGTGTGACAATAATGTTTTCGGCGGAGGACCGAG GTGGTGTCAAAACGAACTGTGGCTGCACCATCTGTCTTCAT CTTCCCGCATCTGATGAGCAGTTGAAATCTGGAACCTGCCT CTGTTGTGCTGCTGCTGAATAACTTCTATCCAGAGAGGCC AAAGTACAGTGAAGGTGGATAACGCCCTCCAATCGGTTAA CTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCA CCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAAGCAGAC TACGAGAAACACAAAGTCTACGCCCTGCGAAGTCACCCATCA GGCCCTGAGCTCGCCCGTCAAAAGAGCTTCAACAGGGGAG AGTGTGA (SEQ ID NO: 127)	MGWSCIIILFLVATATGVHS AVLTQTASPVSQVVGTV TIKQASQNIYSNLAWYQQ KPGQRPKLLMYDASTLASG VPSRFKSGSGTQFTLTIS DLECAADAATYYCQSISSVD NNVFGGTEVVVKRTVAAP SVFI FPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKD STYSLSSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKS ENRGECA* (SEQ ID NO: 128)	1-57 58-381 382-705		
xi383I18LC (rabbitVk- human kappa)	ATGGGCTGGTCTGCATCATCTGTTTCTGGTGGCCACCGC CACCGCGTGCACTCCGTATTCGAAATTGACCCAGACTCCAT CCCCGTGCTGCACTCCGTATTCGAAATTGACCCAGACTCCAT TGCCAGGCCAGTCAGAACATTTAGCCTGGTA TCAGCAGAAACAGGGCAGCGTCCAAAGCTCCTGATGTATG ATGCATCCACTCTGGCATCTGGGTCCTCATCGCGTTCAA GGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGA CCTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAAAGTA TTAGTAGTGTGACAATAATGTTTTCGGCGGAGGACCGAG GTGGTGTCAAAACGAACTGTGGCTGCACCATCTGTCTTCAT CTTCCCGCATCTGATGAGCAGTTGAAATCTGGAACCTGCCT CTGTTGTGCTGCTGCTGAATAACTTCTATCCAGAGAGGCC AAAGTACAGTGAAGGTGGATAACGCCCTCCAATCGGTTAA CTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCA CCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAAGCAGAC TACGAGAAACACAAAGTCTACGCCCTGCGAAGTCACCCATCA GGCCCTGAGCTCGCCCGTCAAAAGAGCTTCAACAGGGGAG AGTGTGA (SEQ ID NO: 127)	MGWSCIIILFLVATATGVHS VFELTQTPSPVSAAVGGTV TIRCQASQNIKSYLAWYQQ KPGQPPKLLIYEASILLASG	1-57 58-381 382-705		

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
(human MS1N)	TCAGCAGAAACACGAGGAGCCTCCAAAACCTCCTGATCTATG AAGCATCCATCTTGGCATCTGGGCTCTCATCGCGGTTCAA GGCAGTGGATCTGGGACAGAGTTCACTCTCACCATCAGCGA CCTGGAGTGTCCGATGCTGCCACTTACTACTGTCAAAGCT ATTATGCTGTAGTAGTAATGCTTTCGGCGGAGGACCGAG GTGGTGTCAACGAACTGTGGCTGCACCATCTGTCTTCAT CTTCCCGCATCTGATGAGCAGTTGAAATCTGGAACGCT CTGTTGTGCTGCTGCTGAATACTTCTATCCAGAGAGGCC AAGTACAGTGAAGGTGGATAACGCCCTCCAATCGGGTAA CTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCA CCTACAGCTCAGCAGCACCTGACGCTGAGCAAAGCAGAC TACGAGAAACACAAAGTCTACGCTGCGAAAGTCACTCATCA GGGCTGAGCTCGCCCTCACAAAGAGCTTCAACAGGGGAG AGTGTTGA (SEQ ID NO:129)	VSSRFKSGSGTEFTLTIS DLECADAAATYYCQSYAAS SNAFGGTEVVVKRTVAAP SVFIFFPSDEQLKSGTASV VCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKD STYLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKS FNRGEC* (SEQ ID NO:130)			
xi166B3LC (rabbitVκ- human kappa) (human CA9)	ATGGGCTGGTCTCGCATCATCCTGTTTCTGGTGCCACCGC CACCGGCTGCACAGCAGCTCGTGATGACCCAGACTCCAT CCTCCGTGCTGCAGCTGTGGGAGGCACAGTCACCATCAAT TGCCAGGCCAGTCAGAGCATTAGTAACACTTATCCTGGTA TCAGCAGAAACACGAGCAGCCTCCCAAGCTCCTGATCTTTG CTGCATCCAAACTGGCATCTTGGGTCCCAAGCGGTTCAAT GGCAGCAGATCTGGGATAGAAATACACTCTCACCATTAGCGG CGTGCACTGTGACGATGCTGCCACTTACTTCTGTCTAGGAG TTTATAGTATTAGTACTGATGATGAGCTGCTTTCGGCGGA GGACCCGAGGTGGTCTGTAACAGAACTGTGGCTGCACCATC TGTCTTCACTCTCCCGCATCTGATGAGCAGTTGAAATCTG GAATGCCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAGAGGTGGATAACGCCCTCCA	MGWSCIIILFLVATATGVHS ELVMTQTPSSVSAAVGGTV TINCQASQSI SNYLSWYQQ KPGQPPKLLIFAAASKLASW VPKRFSGRSRGIEYTLTIS GVQCDDAATYFCLGVYSIS TDDGAAFGGTEVVVKRTV AAPSVFIFFPSDEQLKSGT ASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSTLTLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC*	1-57	58-390	391-714

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO:132)	(SEQ ID NO:132)			
zu166B3LC- CXXF (humanVk- human kappa) (human CA9)	ATGGGCTGGTCTCGCATCATCTCTGTTTCTGGTGGCCACCGC CACGGCGTGACACAGCGATATTCAGATGACCCAGTCTCCTT CCTCCCTGTCGGCTTCCGTGGCGATAGAGTCACAATCACT TGCCAGGCTTCCAGAGCATCAGCAACTACCTGTCTCTGGTA TCAGCAGAAAGCCCGGCAAGCAGCACTAAAGCTGCTGATCTACG CCGCTTCTAAACTGGCAAGCGGAGTGCCAAAGCCGGTCTCTCT GGAAGTGGGTGAGGAACTGACTTTACCCCTGACAAATTAGTCTC CCTGCAAGTGGGAGGATTTCCGCTACCTACTATTGTCTGGCGG TCTATTCAATCTCAACTGACGACGAGCGGCGCATTCGGAGGG GGCACCAAGTGGAAATCAAAACGAACTGTGGCTGCACCAATC TGCTCTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTG GAACTGCCCTCTGTGTGTGCTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAAAGTGGATAACGCCCTCCA ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO:133)	MGWSCIIILFLVATATGVHS DIQMTQSPSSLSASVGDV TITCQASQSI SNYLSWYQQ KPGKAPKLLIYAA SKLASG VPSRFSGSGGTDFTLTIS SLQCEDEFATYYCLGVYSIS TDDGAAFGGGTKVEIKRTV AAPSVFIFPPSDEQLKSGT ASWCLLNNFYPPREARVQW KVDNALQSGNSQESVTEQD SKDSTYSLSSLTLLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO:134)	1-57 58-390 391-714		

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
z166B3LC- CXXA (human V κ - human kappa) (human CA9)	ATGGGCTGGTCTGCATCATCTCTGTTTCTGGTGCCACCGC CACCGGCTGCACAGCGATATTACAGATGACCCAGAGCCCTT CCTCCCTGTCGGCTAGTGTGGGGATAGAGTGACCATTTACT TGCCAGGCCAGCCAGAGCATTAGCAACTACCTGTCTCTGGTA TCAGCAGAAAGCCCGGCAAGCTCCTAAGCTGCTGATCTACG CCGCTTCTAACTGGCAAGCGGAGTGCCAAAGCCGGTTCTCT GGAAGTGGTCAAGAACTGACTTTACCTGACAATTAGCTC CCTGCAGTGGAGGATGCAGCCACTACTATTGTCTGGCGG TCTACTCAATCTCAACCGACGAGAGCTGCTTTTGGAGGG GGCACTAAGGTGGAATCAACGAACTGTGGCTGCACCATC TGTCTTCACTCTCCCGCATCTGATGAGCAGTTGAAATCTG GAACGCTCTGTTGTGTGCTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAAAGTGGATTAACGCCCTCCA ATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCCCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCGCTCACAAAGAGCTTCA ACAGGGGAGAGTGTGA (SEQ ID NO:135)	MGWSCIIILFLVATATGVHS DIQMTQSPSSLSASVGRV TITCQASQSIISNYLSWYQQ KPGKAPKLLIYAASKLASG VPSRFGSGSGTDTLTITIS SLQCEDAATYYCLGVYSIS TDDGAAFGGGTKVEIKRTV AAPSVFIFFPPSDEQLKSGT ASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQD SKDSTYSLSTLTLSKADY EKHKVYACEVTHQGLSSPV TKSFNRGEC* (SEQ ID NO:136)	1-57	58-390	391-714
human gamma chain constant region	GCATCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTC CTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC TGGTCAAGGACTACTTCCCGCAACCGGTGACGGTGTCTGCG AACTCAGGCGCCCTGACCAGCGGGGTGCACACCTTCCCGGC TGTCTTACAGTCTCTCAGGACTCTACTCCCTCAGCAGCGTGG TGACCGTGCCCTCCAGCAGCTTGGGACCCAGACCTACATC TGCAACGTGAATCACAAAGCCGCAACACCAAGGTGGACAA GAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCC CACCGTGCCAGCAGCACCTGAACCTCTGGGGGGACCGTCACTC	ASTKGPSVFPLAPSSKSTIS GGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVL QSSGLYSLSVVTVFSSSL GTQTYICNVNHKPSNTKVD KKVEPKSCDKTHCTCPCPA PELLGGPSVFLFPPPKKDT LMISRTPEVTCVVVDVSH DPEVKFNWYVDGVEVHNAK	NA	NA	1-993

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	TTCTCTTCCCCCAAAACCCAAAGACACCCCTCATGATCTC CCGGACCCCTGAGGTCACATGCGTGGTGGTGACGTGAGCC ACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC GTGGAGTGCATAATGCCAAGACAAAGCCCGGGAGGAGCA GTACAACAGCACGTACCGTGTGTGTCAGCGTCTCACCGTCC TGCAACAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG GTCTCCAAACAAGCCCTCCAGCCCCCATCGAGAAAACCAT CTCCAAAGCCAAAGGGCAGCCCCGAGAACACACAGGTGTACA CCCTGCCCCCATCCCGGATGAGCTGACCAAGAACCCAGGTC AGCTGACCTGCCTGGTCAAGGCTTCTATCCCAGCGACAT CGCGTGGAGTGGGAGAGCAATGGCAGCCCGGAGAACAACT ACAAGACAGCCCTCCCGTGTGGAATCCGACGGCTCCTTC TTCTTATATTCAAAGCTCACCGTGGACAAGACAGGTGGCA GCAGGGAAAGCTTCTTCATGCTCCGTGATGATGATGAGGTC TGCACAACCACTACACGACGAGAGAGCCTCTCCCTGTCTCCC GGAAATGA (SEQ ID NO:137)	TKPREEQYNSTYRVSVLT VLHQDWLNGKEYCKVSNK ALPAPIEKTIISKAKGQPRE PQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGN VFSCVMHEALHNHYTQKS LSLSPGK* (SEQ ID NO:138)			
human kappa chain constant region	CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATC TGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGCGC TGCTGAATAACTTCTATCCCAAGAGAGGCCCAAGTACAGTGG AAGGTGGATAACGCCCTCCCAATCGGGTAACTCCAGGAGAG TGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCA GCAGCACCTGACGCTGAGCAAGACGAGACTACGAGAAACAC AAAGTCTACGCTGCCAAGTCAACCATCAGGGCCTGAGCTC GCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTGA (SEQ ID NO:139)	RTVAAPSVFIFPPSDEQLK SGTASVVCCLLNNFYPREAK VQWKVDNALQSGNSQESVT EQDSKDSITYSLSTLTLSK ADYEKHKVYACEVTHQGLS SPVTKSFNRGEC* (SEQ ID NO:140)	NA	NA	1-324
rabbit gamma chain	GGCAACCTAAGGCTCCATCATGCTTCCCACCTGGCCCCCTG CTCGGGGACACACCCAGCTCCACGGTGACCCCTGGGCTGCC	GQPKAPSVFPLAPCCGDTF SSTVTLGCLVKGYLPEPVT	NA	NA	1-972

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
constant region	TGGTCAAAAGGCTACCTCCCGAGCCAGTGACCGTGACCTGG AACTCGGGCACCCCTCACCAATGGGTACGCACCTTCCCGTC CGTCCGGCAGTCTCAGGCCTCTACTCGCTGAGCAGCGTGG TGAGCGTGACCTCAAGCAGCAGCCCGTCACTGCAACGTG GCCACCCAGCCACCAACCAAAAGTGACAAGACCGTTGC ACCTCGACATGCAGCAAGCCCGACGTGCCCCACCCCTGAAC TCCTGGGGGACCGTCTGTCTTCACTTCCCCCAAAACCC AAGACACCCCTCATGATCTACGCACCCCGAGGTCAATG CGTGGTGGTGACGTGAGCCAGGATGACCCCGAGGTGCAGT TCACATGGTACATAAACAACGAGCAGGTGCGCACCGCCCG CGCCCGTACGGGAGCAGCAGTTCAACAGCACGATCCGCGT GGTCAGCACCCCTCCCCATCAGCACAGGACTGGCTGAGGG GCAAGGAGTTCAAGTGCAAGTCCACAACAAGGCACTCCCG GCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCC CCTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAGG AGCTGAGCAGCAGGTGCGTCAGCCTGACCTGCATGATCAAC GGCTTCTACCCTTCCGACATCTCGGTGGAGTGGGAGAAGAA CGGAAGGCAGAGGACAACATAAGACCCAGCCCGGCCGTGC TGGACAGCGACGGCTCTACTTCTCTACAAACAAGCTCTCA GTGCCCCAGAGTGAGTGGCAGCGGGCGCAGCTTTCACCTG CTCCGTGATGCACGAGGCCCTTGCAACAACCACTACACGAGA AGTCCATCTCCCGCTCTCCGGGTAATGA (SEQ ID NO:141)	VTWNSGTLTNGVRTFPFSVR QSSGLYSLSSVVSVTSSSQ PVTNCVAHPATNTKVDKTV APSTCSKPTCPPPELLGGP SVFIFFPKPKDTLMSRTP EVTCVVVDVSQDDPEVQFT WYINNEQVRTARPELREQQ ENSTIRVSTLPITHQDWL RGKEFKCKVHNKALPAPIE KTISKARGQPLEPKVYTMG PPREELSSRSVSLTCMING FYPSDI SVEWEKNGKAEDN YKTTPAVLDSGDSYFLYNK LSVPTSEWQRGDVFTCSVM HEALHNHYTQKSI SRSPGK * (SEQ ID NO:142)			
rabbit kappa chain constant region	GGTGATCCAGTTGCACCTACTGTCTCTCATCTTCCCACCATC TGCTGATCTTGTGGCAACTGGAAAGATCACCATCGTGTGTG TGGCGAATAAATACTTTCCCGATGTCAACCGTCACCTGGGAG GTGGATGGCACCAACCCAAACAATGGCATCGAGAACAGTAA AACACCGCAGAATTCTGCAGATTGTACCTACAAACCTCAGCA AGTCCATCTCCCGCTCTCCGGGTAATGA (SEQ ID NO:141)	GDPVAPTVLIFPFSADLVA TGTVTIVCVANKYFPDVTV TWEVDGTTQTGTGIENSKTP QNSADCTYNLSLTLTST QYNSHKEYTCKVTQGTTSV	NA	NA	1-315

Clone Name (Species- Isotype) (antigen)	DNA Sequence	Amino Acid Sequence	cDNA Position Leader	cDNA Position Variable Domain	cDNA Position Constant Domain
	GCACCTGTGACACTGACCCAGCACACAGTACAAACAGCCACAAA GAGTACACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGT CCAGAGCTTCAATAGGGGTGACTGTTAG (SEQ ID NO:143)	VQSFNRGDC* (SEQ ID NO:144)			

Table 27. CDR sequences

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
xi155D5HC (rabbitVh- human IgG1) (human CA9)	GGATTCTCCCTCAATAG CTATGCG (SEQ ID NO:145)	GFSLNSYA (SEQ ID NO:146)	ATTACTACTGGTGGT ACCACA (SEQ ID NO:147)	ITTGGTT (SEQ ID NO:148)	GATCGGTTAAAAGCTA CGATGACTATGGTGATT TAGATGCTTTCGAGCCC (SEQ ID NO:149)	DRVKSYYDDYGDLDAFE PWGP (SEQ ID NO:150)
zul55D5HC (humanVh- human IgG1) (human CA9)	GGCTTTTCCCTGAACAG CTACGCT (SEQ ID NO:151)	GFSLNSYA (SEQ ID NO:152)	ATTACCACAGGAGGG ACTACC (SEQ ID NO:153)	ITTGGTT (SEQ ID NO:154)	GATCGGTTGAAATCTTA CGACGATTATGGAGACC TGGATGCTTTCGAACCA (SEQ ID NO:155)	DRVKSYYDDYGDLDAFE PWGP (SEQ ID NO:156)
xi1-55-2HC (rabbitVh- human IgG1) (human TEM1)	GGATTCTCCTTCAGTAG CAGCTAC (SEQ ID NO:157)	GFSSFSSY (SEQ ID NO:158)	ATTATGGTGGTAGT AGTGTACCACT (SEQ ID NO:159)	IYGGSSGTT (SEQ ID NO:160)	GTGACTAATGGTGGTGA TTGGGATTTTAAATTG (SEQ ID NO:161)	VTNGGDWDFKL (SEQ ID NO:162)

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
x11E4HC (rabbitVh- human IgG1) (human CA9)	GGAAATCGACCTCAGTAA TTATGCA (SEQ ID NO:163)	GIDLSNYA (SEQ ID NO:164)	ATTAGTAGTAATGAT AAGACA (SEQ ID NO:165)	ISSNDKT (SEQ ID NO:166)	GCTGCTATGCCTGGTGG TTTAAAGAATGCTTTTCG ATCCC (SEQ ID NO:167)	AAMPGGLKNAFDP (SEQ ID NO:168)
zu1E4HC (humanVh- human IgG1) (human CA9)	GGCAATTGATCTGTCTAA CTACGCT (SEQ ID NO:169)	GIDLSNYA (SEQ ID NO:170)	ATTAGCTCCAATGAC AAGACC (SEQ ID NO:171)	ISSNDKT (SEQ ID NO:172)	GCCGCTATGCCTGGCGG ACTGAAAGAACGCATTTG ATCCT (SEQ ID NO:173)	AAMPGGLKNAFDP (SEQ ID NO:174)
xi33O11HC (rabbitVh- human IgG1) (human MSLN)	GGAAATCTCCCTCAGTAG CGATGCA (SEQ ID NO:175)	GISLSSDA (SEQ ID NO:176)	ATTAATGGTGGTGGT AACACA (SEQ ID NO:177)	INGGGNT (SEQ ID NO:178)	GGCATTCAACATGGTGG TGCTAATAGTGATTATT ATTATTACGGCATGGAC CTC (SEQ ID NO:179)	GIQHGGNSDYYYGGM DL (SEQ ID NO:180)

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
zu33011HC (humanVh- human IgG1) (human MSLN)	GGAAATTCCCTCTCCTC CGACGG (SEQ ID NO:181)	GISLSSDA (SEQ ID NO:182)	ATCAACGGCGCGGA AACACC (SEQ ID NO:183)	INGGNT (SEQ ID NO:184)	GGCATCCAGCACGGTGG TGGAAACAGCGACTACT ACTACTATGGGATGGAT CTG (SEQ ID NO:185)	GIQHGGNSDYIYYGMDL (SEQ ID NO:186)
xi32405HC (rabbitVh- human IgG1) (human MSLN)	GGATTCTCCCTCAGTAA CTATGCA (SEQ ID NO:187)	GFSLSNYA (SEQ ID NO:188)	ATTAGTACTGGCGGT ATCACA (SEQ ID NO:189)	ISTGGIT (SEQ ID NO:190)	AATGCTGGTGGTAGTTA TATTCTCTATATTTTG ACTTG (SEQ ID NO:191)	NAGGSYIFYYFDL (SEQ ID NO:192)
xi178F16HC (rabbitVh- human IgG1) (human MSLN)	GGATTCTCCCTCAGTAA CTATGCA (SEQ ID NO:193)	GFSLSNYA (SEQ ID NO:194)	ATTAGTACTGGCGGT ATCACA (SEQ ID NO:195)	ISTGGIT (SEQ ID NO:196)	AATGCTGGTGGTAGTTA TATTCTCTATATTTTG ACTTG (SEQ ID NO:197)	NAGGSYIFYYFDL (SEQ ID NO:198)
xi237N18HC (rabbitVh- human IgG1) (human MSLN)	GGATTCTCCCTCAGTAG TTACCAC (SEQ ID NO:199)	GFSLSSYH (SEQ ID NO:200)	ATTACTGCTATGAGT CGCACA (SEQ ID NO:201)	ITAMSPRT (SEQ ID NO:202)	GAACCTGGTTTTGTTAG TAACATC (SEQ ID NO:203)	EPGFVSNI (SEQ ID NO:204)

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
human IgG1)						
(human MSLN)						
xi383I18HC	GGATTCTCCCTCAGTAG CTATGCA (SEQ ID NO:205)	GFSLSSYA (SEQ ID NO:206)	ATTAGTACTGGTGGT ATTACA (SEQ ID NO:207)	ISTGGIT (SEQ ID NO:208)	GTGGGTAGTAGTGGTTA TCTTTTCTACTTCTTTA ACTTG (SEQ ID NO:209)	VGSSGYLFYFFNL (SEQ ID NO:210)
(rabbitVh- human IgG1)						
(human MSLN)						
xi166B3HC	GGATTCTCCCTCAGTAG GTATACA (SEQ ID NO:211)	GFSLSRYT (SEQ ID NO:212)	ATAGATAGTAGTAGT AGTGCA (SEQ ID NO:213)	IDSSSSA (SEQ ID NO:214)	GACAGAGTCCTAAGCTA CGATGACTATGGTGATT TGCCCGATGGTTTCGAT CCC (SEQ ID NO:215)	DRVLSYDDYGDLPDGF DP (SEQ ID NO:216)
(rabbitVh- human IgG1)						
(human CA9)						
zul66B3HC	GGGTTTAGCCTGTCCCG ATACACC (SEQ ID NO:217)	GFSLSRYT (SEQ ID NO:218)	ATTGACAGCTCCTCT AGTGCC (SEQ ID NO:219)	IDSSSSA (SEQ ID NO:220)	GATAGAGTCCTGAGCTA CGACGATTATGGGACC TGCCTGACGGCTTTGAT CCT (SEQ ID NO:221)	DRVLSYDDYGDLPDGF DP (SEQ ID NO:222)
(humanVh- human IgG1)						

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
(human CA9)						
xi155D5LC (rabbitVk- human kappa) (human CA9)	CAGAGCATTAGTAGCTA C (SEQ ID NO:223)	QSISSY (SEQ ID NO:224)	TATGCATCC (SEQ ID NO:225)	YAS (SEQ ID NO:226)	CTAGGTGTTTATGGTTA TAGTTTGTGATGATGGTA TTGCT (SEQ ID NO:227)	LGVYGYSFDDGIA (SEQ ID NO:228)
zu155D5LC- 1 (humanVk- human kappa) (human CA9)	CAGAGTATTTCAGCTA C (SEQ ID NO:229)	QSISSY (SEQ ID NO:230)	TATGCTAGT (SEQ ID NO:231)	YAS (SEQ ID NO:232)	CTGGGGGTGTACGGTTA TTCTTTCGACGATGGCA TCGCA (SEQ ID NO:233)	LGVYGYSFDDGIA (SEQ ID NO:234)
zu155D5LC- 2 (humanVk- human kappa) (human CA9)	CAGAGTATTTCAGCTA C (SEQ ID NO:235)	QSISSY (SEQ ID NO:236)	TATGCTAGT (SEQ ID NO:237)	YAS (SEQ ID NO:238)	CTGGGGGTGTACGGTTA TTCTTTCGACGATGGCA TCGCA (SEQ ID NO:239)	LGVYGYSFDDGIA (SEQ ID NO:240)

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
CA9)						
zu155D5LC- 3 (humanV/k- human kappa) (human CA9)	CAGAGTATTTCAGCTA C (SEQ ID NO:241)	QSISSY (SEQ ID NO:242)	TATGCTAGT (SEQ ID NO:243)	YAS (SEQ ID NO:244)	CTGGGGGTGTACGGTTA TTCTTTTCGACGATGGCA TCGCA (SEQ ID NO:245)	LGVYGYSFDDGLA (SEQ ID NO:246)
zu155D5LC- 4 (humanV/k- human kappa) (human CA9)	CAGAGTATTTCAGCTA C (SEQ ID NO:247)	QSISSY (SEQ ID NO:248)	TATGCTAGT (SEQ ID NO:249)	YAS (SEQ ID NO:250)	CTGGGGGTGTACGGTTA TTCTTTTCGACGATGGCA TCGCA (SEQ ID NO:251)	LGVYGYSFDDGLA (SEQ ID NO:252)
zu155D5LC- 5 (humanV/k- human kappa)	CAGAGTATTTCAGCTA C (SEQ ID NO:253)	QSISSY (SEQ ID NO:254)	TATGCTAGT (SEQ ID NO:255)	YAS (SEQ ID NO:256)	CTGGGGGTGTACGGTTA TTCTTTTCGACGATGGCA TCGCA (SEQ ID NO:257)	LGVYGYSFDDGLA (SEQ ID NO:258)

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
(human CA9)						
zu155D5LC- 6						
(humanV/k- human kappa)	CAGAGTATTTCAGCTA C (SEQ ID NO:259)	QSISSY (SEQ ID NO:260)	TATGCTAGT (SEQ ID NO:261)	YAS (SEQ ID NO:262)	CTGGGGGTGTACGGTTA TTCTTTCGACGATGGCA TCGCA (SEQ ID NO:263)	LGVYGYSFDDGLA (SEQ ID NO:264)
(human CA9)						
zu155D5LC- 7						
(humanV/k- human kappa)	CAGAGTATTTCAGCTA C (SEQ ID NO:265)	QSISSY (SEQ ID NO:266)	TATGCTAGT (SEQ ID NO:267)	YAS (SEQ ID NO:268)	CTGGGGGTGTACGGTTA TTCTTTCGACGATGGCA TCGCA (SEQ ID NO:269)	LGVYGYSFDDGLA (SEQ ID NO:270)
(human CA9)						
zu155D5LC- huVK1-39						
(humanV/k- human kappa)	CAGTCCATCTCCTCCTA C (SEQ ID NO:271)	QSISSY (SEQ ID NO:272)	TACGCCTCC (SEQ ID NO:273)	YAS (SEQ ID NO:274)	CTGGGGGTGTATGGTTA CTCGTTCGACGATGGAA TCGCA (SEQ ID NO:275)	LGVYGYSFDDGLA (SEQ ID NO:276)

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
(human CA9)						
zu155D5LC- huVK2-40 (humanV/k- human kappa)	CAGAGCATCTCCTCATA C (SEQ ID NO:277)	QSISSY (SEQ ID NO:278)	TACGCCTCC (SEQ ID NO:279)	YAS (SEQ ID NO:280)	CTGGGAGTCTACGGTA CTCCTTCGATGACGGCA TTGCA (SEQ ID NO:281)	LGVYGYSFDDGIA (SEQ ID NO:282)
(human CA9)						
zu155D5LC- huVK3-11 (humanV/k- human kappa)	CAGTCGATCTCCTCTTA C (SEQ ID NO:283)	QSISSY (SEQ ID NO:284)	TACGCCTCG (SEQ ID NO:285)	YAS (SEQ ID NO:286)	CTGGGGGTGTACGGCTA CTCCTTCGATGACGGAA TCGCC (SEQ ID NO:287)	LGVYGYSFDDGIA (SEQ ID NO:288)
(human CA9)						
zu155D5LC- huVK4-1 (humanV/k- human kappa)	CAGTCCATCTCCTCATA C (SEQ ID NO:289)	QSISSY (SEQ ID NO:290)	TACGCCTCC (SEQ ID NO:291)	YAS (SEQ ID NO:292)	TTGGGTGTCTACGGATA CTCCTTCGACGACGGGA TCGCA (SEQ ID NO:293)	LGVYGYSFDDGIA (SEQ ID NO:294)
(human CA9)						

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
zu155D5LC- huVK6-21 (humanVk- human kappa) (human CA9)	CAGTCAATTTCCTCGTA C (SEQ ID NO:295)	QSISSY (SEQ ID NO:296)	TACGCATCC (SEQ ID NO:297)	YAS (SEQ ID NO:298)	CTGGGGGTGTACGGGTA CTCATTTCACGATGGCA TCGCC (SEQ ID NO:299)	LGVIYGYSFDDGIA (SEQ ID NO:300)
zu155D5LC- huVK6D-41 (humanVk- human kappa) (human CA9)	CAGTCCATTTCGTCCTA C (SEQ ID NO:301)	QSISSY (SEQ ID NO:302)	TACGCCTCC (SEQ ID NO:303)	YAS (SEQ ID NO:304)	CTGGGCGTGTACGGTTA CTCGTTTGATGACGGCA TCGCG (SEQ ID NO:305)	LGVIYGYSFDDGIA (SEQ ID NO:306)
zu155D5LC- huVK7-3- Glu81 (humanVk- human kappa) (human CA9)	CAGTCCATTTCGAGCTA C (SEQ ID NO:307)	QSISSY (SEQ ID NO:308)	TACGCCTCC (SEQ ID NO:309)	YAS (SEQ ID NO:310)	CTGGGGGTGTACGGGTA CTCATTCGACGACGGGA TCGCC (SEQ ID NO:311)	LGVIYGYSFDDGIA (SEQ ID NO:312)
xi1-55-2LC	CAGAGCATTAATACCTA C	QSINTY (SEQ ID NO:314)	AGGGCATCC (SEQ ID NO:315)	RAS (SEQ ID NO:316)	CAACAGAGTGTTCGTGT TATTGATGTTGATAATA	QQSVRVIDVDNT (SEQ ID NO:318)

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
(rabbitVκ- human kappa) (human TEM1)	(SEQ ID NO:313)				CT (SEQ ID NO:317)	
xilE4LC (rabbitVκ- human kappa) (human CA9)	CAGACCATTGGTGGTAG CGAC (SEQ ID NO:319)	QTIGGSD (SEQ ID NO:320)	TATGCAACC (SEQ ID NO:321)	YAT (SEQ ID NO:322)	CTAGTGGTTATGCTGC TGCTTCTTACAGAACTG CT (SEQ ID NO:323)	LGGYAAASYRTA (SEQ ID NO:324)
zulE4LC- CXXF (humanVκ- human kappa) (human CA9)	CAGACCATTGGCGGGAG CGAC (SEQ ID NO:325)	QTIGGSD (SEQ ID NO:326)	TATGCTACA (SEQ ID NO:327)	YAT (SEQ ID NO:328)	CTGGGGGGCTATGCCGC CGCAAGCTACCGCACCG CC (SEQ ID NO:329)	LGGYAAASYRTA (SEQ ID NO:330)

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
zu1E4LC- CXXA (humanVk- human kappa) (human CA9)	CAGACCATTGGCGGGAG CGAC (SEQ ID NO:331)	QTIGGSD (SEQ ID NO:332)	TATGCTACA (SEQ ID NO:333)	YAT (SEQ ID NO:334)	CTGGGGGGCTATGCCGC CGCAAGCTACCGCACCG CC (SEQ ID NO:335)	LGGYAAASYRTA (SEQ ID NO:336)
xi33011LC (rabbitVk- human kappa) (human MSLN)	CAGAGCATTAGTAGTGT C (SEQ ID NO:337)	QSISSV (SEQ ID NO:338)	CTGGCATCC (SEQ ID NO:339)	LAS (SEQ ID NO:340)	CAAACCAATTATGGTAC TAGTAGTAGTAATTATG GTTTGTCT (SEQ ID NO:341)	QTNYGTSNNYGEA (SEQ ID NO:342)
zu33011LC- CXXF (humanVk- human kappa) (human MSLN)	CAGTCAATTAGCAGCGT G (SEQ ID NO:343)	QSISSV (SEQ ID NO:344)	TTGGCCTCC (SEQ ID NO:345)	LAS (SEQ ID NO:346)	CAAACCAACTACGGAAC CTCCAGCTCCAACACTACG GCTTTGCC (SEQ ID NO:347)	QTNYGTSNNYGEA (SEQ ID NO:348)

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
zu33011LC- CXXA (humanVk- human kappa) (human MSLN)	CAGTCAATTAGCAGCGT G (SEQ ID NO:349)	QSISSV (SEQ ID NO:350)	TTGGCCTCC (SEQ ID NO:351)	LAS (SEQ ID NO:352)	CAAAACCAACTACGGAAC CTCCAGCTCCAACCTACG GCTTTGCC (SEQ ID NO:353)	QTNYGTSNNYGF (SEQ ID NO:354)
zu33011LC- CXXI (humanVk- human kappa) (human MSLN)	CAGTCAATTAGCAGCGT G (SEQ ID NO:355)	QSISSV (SEQ ID NO:356)	TTGGCCTCC (SEQ ID NO:357)	LAS (SEQ ID NO:358)	CAAAACCAACTACGGAAC CTCCAGCTCCAACCTACG GCTTTGCC (SEQ ID NO:359)	QTNYGTSNNYGF (SEQ ID NO:360)
xi32405LC (rabbitVk- human kappa) (human MSLN)	CAGAGCATTTACAGTTA T (SEQ ID NO:361)	QSIYSY (SEQ ID NO:362)	TCTGCATCC (SEQ ID NO:363)	SAS (SEQ ID NO:364)	CAAACTATTATGATAT TGTTACTAGTACT (SEQ ID NO:365)	QTYVDIVTST (SEQ ID NO:366)

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
xi178F16LC (rabbitVk- human kappa) (human MSLN)	CAGAGCATTACAGTTA T (SEQ ID NO:367)	QSIYSY (SEQ ID NO:368)	TCTGCATCC (SEQ ID NO:369)	SAS (SEQ ID NO:370)	CAAACTTATTATGATAT TGTACTAGTACT (SEQ ID NO:371)	QTYDVTST (SEQ ID NO:372)
xi237N18LC (rabbitVk- human kappa) (human MSLN)	CAGAACATTACAGCAA T (SEQ ID NO:373)	QNIYSN (SEQ ID NO:374)	GATGCATCC (SEQ ID NO:375)	DAS (SEQ ID NO:376)	CAAGTATTAGTAGTGT TGACAATAATGTT (SEQ ID NO:377)	QSISSVDNNV (SEQ ID NO:378)
xi383I18LC (rabbitVk- human kappa) (human MSLN)	CAGAACATTAAAGAGCTA C (SEQ ID NO:379)	QNIKSY (SEQ ID NO:380)	GAAGCATCC (SEQ ID NO:381)	EAS (SEQ ID NO:382)	CAAACTTATTATGCTGC TAGTAGTAATGCT (SEQ ID NO:383)	QSYAAASSNA (SEQ ID NO:384)
xi166B3LC (rabbitVk- human kappa) (human MSLN)	CAGAGCATTAGTAACCTA C (SEQ ID NO:385)	QSI SNY (SEQ ID NO:386)	GCTGCATCC (SEQ ID NO:387)	AAS (SEQ ID NO:388)	CTAGAGTTTATAGTAT TAGTACTGATGATGGAG CTGCT	LGVYSISTDDGAA (SEQ ID NO:390)

Clone Name (Species- Isotype) (antigen)	CDR1 DNA Sequence	CDR1 Amino Acid Sequence	CDR2 DNA Sequence	CDR2 Amino Acid Sequence	CDR3 DNA Sequence	CDR3 Amino Acid Sequence
human kappa) (human CA9)					(SEQ ID NO:389)	
zu166B3LC- CXXF (humanV/k- human IgG1) (human CA9)	CAGAGCATCAGCAACTA C (SEQ ID NO:391)	Q S I S N Y (SEQ ID NO:392)	G C C G C T T C T (SEQ ID NO:393)	A A S (SEQ ID NO:394)	CTGGGCGTCTATTCAAT CTCAACTGACGACGGAG CCGCA (SEQ ID NO:395)	LGVYSISTDDGAA (SEQ ID NO:396)
zu166B3LC- CXXA (humanV/k- human IgG1) (human CA9)	CAGAGCATCAGCAACTA C (SEQ ID NO:397)	Q S I S N Y (SEQ ID NO:398)	G C C G C T T C T (SEQ ID NO:399)	A A S (SEQ ID NO:400)	CTGGGCGTCTATTCAAT CTCAACTGACGACGGAG CCGCA (SEQ ID NO:401)	LGVYSISTDDGAA (SEQ ID NO:402)

[0328] Those skilled in the art will appreciate that numerous changes and modifications can be made to the preferred embodiments of the invention and that such changes and modifications can be made without departing from the spirit of the invention. It is, therefore, intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

EMBODIMENTS

The following list of embodiments is intended to complement, rather than displace or supersede, the previous descriptions.

- Embodiment 1. A method for generating a conjugated immunoglobulin, the method comprising:
- decapping a cysteine at amino acid position 80 (“Cys80”) in a light chain variable region of an immunoglobulin derived from rabbit, wherein the immunoglobulin comprises a heavy chain variable region and the light chain variable region; and
 - conjugating a thiol-reactive compound to the Cys80, wherein the thiol-reactive compound comprises a thiol-reactive group.
- Embodiment 2. The method of embodiment 1, wherein the light chain variable region is a kappa light chain variable region.
- Embodiment 3. The method of embodiment 1 or 2, wherein the light chain variable region is derived from *Oryctolagus cuniculus*.
- Embodiment 4. The method of embodiment 1 or 2, wherein the light chain variable region is a human kappa light chain variable region of family IGKV-1.
- Embodiment 5. The method of any one of the previous embodiments, wherein the decapping comprises incubating the immunoglobulin with a reducing buffer followed by incubating the immunoglobulin with an oxidizing buffer.
- Embodiment 6. The method of embodiment 5, further comprising immobilizing the immunoglobulin on a matrix prior to the incubating with the reducing buffer and eluting the immunoglobulin from the matrix following the incubating with the oxidizing buffer.
- Embodiment 7. The method of embodiment 6, wherein the matrix comprises Protein A.
- Embodiment 8. The method of any one of the previous embodiments, wherein the thiol-reactive group is maleimide or haloacetyl.

- Embodiment 9. The method of any one of the previous embodiments, wherein the thiol-reactive group is appended to a linker.
- Embodiment 10. The method of embodiment 9, wherein the linker is a non-cleavable linker or a cleavable linker.
- Embodiment 11. The method of embodiment 10, wherein the linker is a disulfide-containing linker, an acetal-based linker or a ketal-based linker.
- Embodiment 12. The method of any one of the previous embodiments, wherein the thiol-reactive compound is attached to a functional agent.
- Embodiment 13. The method of embodiment 12, wherein the functional agent comprises a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug.
- Embodiment 14. The method of any one of the previous embodiments, wherein the thiol-reactive compound is bound to a second thiol-reactive compound, the second thiol-reactive compound being bound to a second immunoglobulin having a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at amino acid position 80 ("Cys80²"), wherein the second thiol-reactive compound comprises a second thiol-reactive group bound to the Cys80².
- Embodiment 15. The method of embodiment 14, wherein the thiol-reactive compound is bound to the second thiol-reactive compound by click chemistry.
- Embodiment 16. The method of any one of the previous embodiments, wherein Cys80 is unpaired.
- Embodiment 17. The method of any one of the previous embodiments, wherein the immunoglobulin is a chimerized immunoglobulin.
- Embodiment 18. The method of any one of embodiments 1-16, further comprising chimerizing the immunoglobulin prior to the decapping.

- Embodiment 19. The method of any one of embodiments 1-16, wherein the immunoglobulin is a humanized immunoglobulin.
- Embodiment 20. The method of any one of embodiments 1-16, further comprising humanizing the immunoglobulin.
- Embodiment 21. The method of any one of the previous embodiments, further comprising substituting an amino acid at position 83 with an amino acid residue other than Phe, Lys, or Cys.
- Embodiment 22. The method of embodiment 21, wherein the amino acid residue other than Phe, Lys, or Cys is a polar or hydrophobic residue.
- Embodiment 23. A method for generating an antigen-binding molecule, the method comprising incubating a first conjugated immunoglobulin with a second conjugated immunoglobulin to generate the antigen-binding molecule, wherein:
the first conjugated immunoglobulin comprises a first heavy chain variable region and a first light chain variable region, the first light chain variable region having a cysteine at position 80 ("Cys80¹") wherein the Cys80¹ is conjugated to a first thiol-reactive compound comprising a first thiol-reactive group; and
the second conjugated immunoglobulin comprises a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at position 80 ("Cys80²") wherein the Cys80² is conjugated to a second thiol-reactive compound comprising a second thiol-reactive group.
- Embodiment 24. The method of embodiment 23, wherein the Cys80¹, the Cys80², or both, is unpaired.
- Embodiment 25. The method of embodiment 23 or 24, further comprising, prior to the incubating step,
decapping the Cys80¹, Cys80², or both; and
conjugating a first thiol-reactive compound to the Cys80¹, a second thiol-reactive compound to the Cys80², or both, wherein the first thiol-reactive compound comprises a

first thiol-reactive group and the second thiol-reactive compound comprises a second thiol-reactive group.

- Embodiment 26. The method of any one of embodiments 23-25, wherein the decapping comprises incubating the first immunoglobulin, the second immunoglobulin, or both, with a reducing buffer followed by incubating the first immunoglobulin, the second immunoglobulin, or both, with an oxidizing buffer.
- Embodiment 27. The method of embodiment 26, further comprising immobilizing the first immunoglobulin, the second immunoglobulin, or both on a matrix prior to the incubating with the reducing buffer and eluting the first immunoglobulin, the second immunoglobulin, or both from the matrix following the incubating with the oxidizing buffer.
- Embodiment 28. The method of embodiment 27, wherein the matrix comprises Protein A.
- Embodiment 29. The method of any one of embodiments 23-28, wherein the first immunoglobulin, the second immunoglobulin, or both, is chimerized.
- Embodiment 30. The method of any one of embodiments 23-28, further comprising chimerizing the first immunoglobulin, the second immunoglobulin, or both, prior to the decapping.
- Embodiment 31. The method of any one of embodiments 23-28, wherein the first immunoglobulin, the second immunoglobulin, or both, is humanized.
- Embodiment 32. The method of any one of embodiments 23-28, further comprising humanizing the first immunoglobulin, the second immunoglobulin, or both.
- Embodiment 33. The method of any one of embodiments 23-32, wherein the first thiol-reactive group, the second thiol-reactive group, or both, is maleimide or haloacetyl.
- Embodiment 34. The method of any one of embodiments 23-33, wherein the first thiol-reactive compound is appended to a first linker, the second thiol-reactive compound is appended to a second linker, or both.

- Embodiment 35. The method of embodiment 34, wherein the first linker, the second linker, or both, is a non-cleavable linker or a cleavable linker.
- Embodiment 36. The method of embodiment 35, wherein the first linker, the second linker, or both, is a disulfide-containing linker, an acetal-based linker or a ketal-based linker.
- Embodiment 37. The method of any one of embodiments 23-36, wherein the first thiol-reactive compound further comprises a first functional agent, the second thiol-reactive compound further comprises a second functional agent, or both.
- Embodiment 38. The method of embodiment 37, wherein the first functional agent, the second functional agent, or both, is a chemical linker.
- Embodiment 39. The method of embodiment 38, wherein the first thiol-reactive compound is maleimido-PEG4-azide or maleimido-PEG4-dibenzocyclooctyne.
- Embodiment 40. The method of embodiment 38 or 39, wherein the second thiol-reactive compound is maleimido-PEG4-dibenzocyclooctyne or maleimido-PEG4-azide.
- Embodiment 41. The method of any one of embodiments 23-40, wherein the first immunoglobulin and the second immunoglobulin are conjugated to each other by click chemistry.
- Embodiment 42. The method of any one of embodiments 23-41, wherein the first immunoglobulin is a first Fab, the second immunoglobulin is a second Fab, or both.
- Embodiment 43. The method of any one of embodiments 23-42, further comprising substituting an amino acid at position 83 of the first light chain variable region with an amino acid residue other than Phe, Lys, or Cys, substituting an amino acid at position 83 of the second light chain variable region with an amino acid residue other than Phe, Lys, or Cys, or both.
- Embodiment 44. The method of embodiment 43, wherein amino acid residue other than Phe, Lys, or Cys is a polar or hydrophobic residue.

- Embodiment 45. The method of any one of embodiments 23-44, wherein the first light chain variable region, the second light chain variable region, or both, is derived from *Oryctolagus cuniculus*.
- Embodiment 46. The antigen-binding molecule produced according to the method of any one of embodiments 23-45.
- Embodiment 47. An immunoglobulin comprising a heavy chain variable region and a light chain variable region, the light chain variable region having a cysteine at position 80 ("Cys80") and an amino acid other than Phe, Lys, or Cys at position 83.
- Embodiment 48. The immunoglobulin of embodiment 47, wherein the light chain variable region is a kappa light chain variable region.
- Embodiment 49. The immunoglobulin of embodiment 47 or 48, wherein the light chain variable region is derived from *Oryctolagus cuniculus*.
- Embodiment 50. The immunoglobulin of any one of embodiments 47-49, wherein the amino acid other than Phe, Lys, or Cys is a polar or hydrophobic amino acid.
- Embodiment 51. The immunoglobulin of any one of embodiments 47-50, wherein the Cys80 is unpaired.
- Embodiment 52. The immunoglobulin of any one of embodiments 47-51, wherein the Cys80 is decapped.
- Embodiment 53. The immunoglobulin of any one of embodiments 47-52, wherein the immunoglobulin is chimerized.
- Embodiment 54. The immunoglobulin of any one of embodiments 47-53, wherein the immunoglobulin is humanized.
- Embodiment 55. The immunoglobulin of any one of embodiments 47-54, wherein the immunoglobulin immunospecifically binds to human CA9.
- Embodiment 56. The immunoglobulin of embodiment 55, comprising:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-141 of xi155D5HC (SEQ ID NO:52) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi155D5LC (SEQ ID NO:78);
- b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-144 of zu155D5HC (SEQ ID NO:54) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu155D5LC-3 (SEQ ID NO:84), zu155D5LC-4 (SEQ ID NO:86), zu155D5LC-5 (SEQ ID NO:88), zu155D5LC-6 (SEQ ID NO:90), zu155D5LC-7 (SEQ ID NO:92), zu155D5LC-huVK2-40 (SEQ ID NO:96), zu155D5LC-huVK4-1 (SEQ ID NO:100), zu155D5LC-huVK6-21 (SEQ ID NO:102), zu155D5LC-huVK6D-41 (SEQ ID NO:104); or zu155D5LC-huVK7-3-Glu81 (SEQ ID NO:106);
- c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-138 of xi1E4HC (SEQ ID NO:58) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi1E4LC (SEQ ID NO:110);
- d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-140 of zu1E4HC (SEQ ID NO:60) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu1E4LC-CXXA (SEQ ID NO:114);
- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi166B3HC (SEQ ID NO:74) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi166B3LC (SEQ ID NO:132); or
- f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu166B3HC (SEQ ID NO:76) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu166B3LC-CXXA (SEQ ID NO:136).

Embodiment 57. The immunoglobulin of embodiment 56, comprising:

- a. a heavy chain CDR1, CDR2, and CDR3 of xi155D5HC as set forth as SEQ ID NO:146, 148, and 150, respectively, and a light chain CDR1, CDR2, and CDR3 of xi155D5LC as set forth as SEQ ID NO:224, 226, and 228, respectively;

- b. a heavy chain CDR1, CDR2, and CDR3 of zu155D5HC as set forth as SEQ ID NO:152, 154, and 156, respectively, and a light chain CDR1, CDR2, and CDR3 of zu155D5LC-3 as set forth as SEQ ID NO:242, 244, and 246, respectively, zu155D5LC-4 as set forth as SEQ ID NO:248, 250, and 252, respectively, zu155D5LC-5 as set forth as SEQ ID NO:254, 256, and 258, respectively, zu155D5LC-6 as set forth as SEQ ID NO:260, 262, and 264, respectively, zu155D5LC-7 as set forth as SEQ ID NO:266, 268, and 270, respectively, zu155D5LC-huVK2-40 as set forth as SEQ ID NO 278, 280, and 282, respectively, zu155D5LC-huVK4-1 as set forth as SEQ ID NO 290, 292, and 294, respectively, zu155D5LC-huVK6-21 as set forth as SEQ ID NO 296, 298, and 300, respectively, zu155D5LC-huVK6D-41 as set forth as SEQ ID NO 302, 304, and 306, respectively; or zu155D5LC-huVK7-3-Glu81 as set forth as SEQ ID NO 308, 310, and 312, respectively;
- c. a heavy chain CDR1, CDR2, and CDR3 of xi1E4HC as set forth as SEQ ID NO:164, 166, and 168, respectively, and a light chain CDR1, CDR2, and CDR3 of xi1E4LC as set forth as SEQ ID NO:320, 322, and 324, respectively;
- d. a heavy chain CDR1, CDR2, and CDR3 of zu1E4HC as set forth as SEQ ID NO:170, 172, and 174, respectively, and a light chain CDR1, CDR2, and CDR3 of zu1E4LC-CXXA as set forth as SEQ ID NO:332, 334, and 336, respectively;
- e. a heavy chain CDR1, CDR2, and CDR3 of xi166B3HC as set forth as SEQ ID NO:212, 214, and 216, respectively, and a light chain CDR1, CDR2, and CDR3 of xi166B3LC as set forth as SEQ ID NO:386, 388, and 390, respectively; or
- f. a heavy chain CDR1, CDR2, and CDR3 of zu166B3HC as set forth as SEQ ID NO:218, 220, and 222, respectively, and a light chain CDR1, CDR2, and CDR3 of zu166B3LC-CXXA as set forth as SEQ ID NO:398, 400, and 402, respectively.

Embodiment 58. The immunoglobulin of any one of embodiments 46-54, wherein the immunoglobulin immunospecifically binds to human TEM1.

Embodiment 59. The immunoglobulin of embodiment 58, comprising:
a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-139 of xi1-55-2HC (SEQ ID NO:56) and a light chain

variable region having an amino acid sequence at least 90% identical to amino acids 20-129 of xi1-55-2LC (SEQ ID NO:108).

Embodiment 60. The immunoglobulin of embodiment 59, comprising:

a heavy chain CDR1, CDR2, and CDR3 of xi1-55-2HC as set forth as SEQ ID NO:158, 160, and 162, respectively, and a light chain CDR1, CDR2, and CDR3 of xi1-55-2LC as set forth as SEQ ID NO:314, 316, and 318, respectively.

Embodiment 61. The immunoglobulin of any one of embodiments 46-54, wherein the immunoglobulin immunospecifically binds to human mesothelin.

Embodiment 62. The immunoglobulin of embodiment 61, comprising:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi33O11HC (SEQ ID NO:62) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of xi33O11LC (SEQ ID NO:116);
- b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu33O11HC (SEQ ID NO:64) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of zu33O11LC-CXXA (SEQ ID NO:120) or zu33O11LC-CXXI (SEQ ID NO:122);
- c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi324O5HC (SEQ ID NO:66) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi324O5LC (SEQ ID NO:124);
- d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi178F16HC (SEQ ID NO:68) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi178F16LC (SEQ ID NO:126);
- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-132 of xi237N18HC (SEQ ID NO:70) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi237N18LC (SEQ ID NO:128); or

- f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi383I18HC (SEQ ID NO:72) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi383I18LC (SEQ ID NO:130).

Embodiment 63. The immunoglobulin of embodiment 62, comprising:

- a. a heavy chain CDR1, CDR2, and CDR3 of xi33O11HC as set forth as SEQ ID NO: 176, 178, and 180, respectively, and a light chain CDR1, CDR2, and CDR3 of xi33O11LC as set forth in SEQ ID NO:338, 340, and 342, respectively;
- b. a heavy chain CDR1, CDR2, and CDR3 of zu33O11HC as set forth as SEQ ID NO:182, 184, and 186, respectively, and a light chain CDR1, CDR2, and CDR3 of zu33O11LC-CXXA as set forth as SEQ ID NO:350, 352, and 354, respectively, or zu33O11LC-CXXI as set forth as SEQ ID NO:356, 358, and 360, respectively;
- c. a heavy chain CDR1, CDR2, and CDR3 of xi324O5HC as set forth as SEQ ID NO:188, 190, and 192, respectively, and a light chain CDR1, CDR2, and CDR3 of xi324O5LC as set forth as SEQ ID NO:362, 364, and 366, respectively;
- d. a heavy chain CDR1, CDR2, and CDR3 of xi178F16HC as set forth as SEQ ID NO:194, 196, and 198, respectively, and a light chain CDR1, CDR2, and CDR3 of xi178F16LC as set forth as SEQ ID NO:368, 370, and 372, respectively;
- e. a heavy chain CDR1, CDR2, and CDR3 of xi237N18HC as set forth as SEQ ID NO:200, 202, and 204, respectively, and a light chain CDR1, CDR2, and CDR3 of xi237N18LC as set forth as SEQ ID NO:374, 376, and 378, respectively; or
- f. a heavy chain CDR1, CDR2, and CDR3 of xi383I18HC as set forth as SEQ ID NO:206, 208, and 210, respectively, and a light chain CDR1, CDR2, and CDR3 of xi383I18LC as set forth as SEQ ID NO:380, 382, and 384, respectively.

Embodiment 64. A conjugated immunoglobulin comprising:

the immunoglobulin of any one of embodiments 46-63, wherein the cysteine at position 80 is conjugated to a thiol-reactive compound, the thiol-reactive compound comprising a thiol-reactive group.

Embodiment 65. The conjugated immunoglobulin of embodiment 64, wherein the thiol-reactive group is maleimide or haloacetyl.

- Embodiment 66. The conjugated immunoglobulin of embodiment 64 or 65, wherein the thiol-reactive group is appended to a linker.
- Embodiment 67. The conjugated immunoglobulin of embodiment 66, wherein the linker is a non-cleavable linker or a cleavable linker.
- Embodiment 68. The conjugated immunoglobulin of embodiment 67, wherein the linker is a disulfide containing linker, an acetal-based linker or a ketal-based linker.
- Embodiment 69. The conjugated immunoglobulin of any one of embodiments 64-68, wherein the thiol-reactive compound further comprises a functional agent.
- Embodiment 70. The conjugated immunoglobulin of embodiment 69, wherein the functional agent comprises a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug.
- Embodiment 71. The conjugated immunoglobulin of embodiment 70, wherein the functional agent is auristatin F.
- Embodiment 72. The conjugated immunoglobulin of any one of embodiments 69-71, wherein the conjugated immunoglobulin has an immunoglobulin:functional agent ratio of 2:1.
- Embodiment 73. A method of treating cancer in a subject comprising administering to the subject a pharmaceutically effective amount of a conjugated mesothelin immunoglobulin, wherein the conjugated mesothelin immunoglobulin comprises:
the immunoglobulin of any one of embodiments 61-63, and
a thiol-reactive compound comprising a thiol-reactive group, a linker, and a functional agent.
- Embodiment 74. The method of embodiment 73, wherein the functional agent is auristatin F.

Embodiment 75. The method of embodiment 73 or 74, wherein the cancer is a mesothelin-expressing cancer.

Embodiment 76. A antigen-binding molecule comprising:
a first conjugated immunoglobulin comprising a first heavy chain variable region and a first light chain variable region, the first light chain variable region having a cysteine at position 80 ("Cys80¹"), wherein the Cys80¹ is conjugated to a first thiol-reactive compound comprising a first thiol-reactive group, and
a second conjugated immunoglobulin comprising a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at position 80 ("Cys80²") wherein the Cys80² is conjugated to a second thiol-reactive compound comprising a second thiol-reactive group.

Embodiment 77. The antigen-binding molecule of embodiment 76, wherein the first light chain variable region, the second light chain variable region, or both, is derived from *Oryctolagus cuniculus*.

Embodiment 78. The antigen-binding molecule of embodiment 76 or 77, wherein Cys80¹, Cys80², or both, is unpaired.

Embodiment 79. The antigen-binding molecule of any one of embodiments 76-78, wherein the first immunoglobulin, the second immunoglobulin, or both, is chimerized.

Embodiment 80. The antigen-binding molecule of any one of embodiments 76-78, wherein the first immunoglobulin, the second immunoglobulin, or both, is humanized.

Embodiment 81. The antigen-binding molecule of any one of embodiments 76-80, wherein the amino acid at position 83 of the first immunoglobulin, the amino acid at position 83 of the second immunoglobulin, or both, is an amino acid residue other than Phe, Lys, or Cys.

Embodiment 82. The antigen-binding molecule of embodiment 81, wherein the amino acid residue other than Phe, Lys, or Cys is a polar or hydrophobic residue.

- Embodiment 83. The antigen-binding molecule of any one of embodiments 76-82, wherein the first immunoglobulin and the second immunoglobulin bind to different antigens.
- Embodiment 84. The antigen-binding molecule of any one of embodiments 76-83, wherein the first thiol-reactive group, the second thiol-reactive group, or both, is maleimide or haloacetyl.
- Embodiment 85. The antigen-binding molecule of any one of embodiments 76-84, wherein the first thiol-reactive compound further comprises a first linker, the second thiol-reactive compound further comprises a second linker, or both.
- Embodiment 86. The antigen-binding molecule of embodiment 85, wherein the first linker, the second linker, or both, is a cleavable linker or a non-cleavable linker.
- Embodiment 87. The antigen-binding molecule of embodiment 86, wherein the first linker, the second linker, or both, is a disulfide containing linker, an acetal-based linker or a ketal-based linker.
- Embodiment 88. The antigen-binding molecule of any one of embodiments 76-87, wherein the first thiol-reactive compound further comprises a first functional agent, the second thiol-reactive compound further comprises a second functional agent, or both.
- Embodiment 89. The antigen-binding molecule of embodiment 88, wherein the first functional agent, the second functional agent, or both, is a chemical linker.
- Embodiment 90. The antigen-binding molecule of any one of embodiments 76-89, wherein the first thiol-reactive compound, the second thiol-reactive compound, or both, is maleimido-PEG4-azide or maleimido-PEG4-dibenzocyclooctyne.
- Embodiment 91. The antigen-binding molecule of any one of embodiments 76-90, wherein the first thiol-reactive compound differs from the second thiol-reactive compound.
- Embodiment 92. The antigen-binding molecule of any one of embodiments 76-91, wherein the first immunoglobulin, second immunoglobulin, or both is a Fab.

- Embodiment 93. A light chain variable region for use in a conjugated immunoglobulin, the light chain variable region having a cysteine at amino acid position 80 ("Cys80") and an amino acid residue other than Phe, Lys, or Cys at amino acid position 83, wherein the Cys80 is unpaired.
- Embodiment 94. The light chain variable region of embodiment 93, wherein the light chain variable region has a Cys80-Xaa₁-Xaa₂-Xaa₃ motif, wherein Xaa₃ is any amino acid other than Phe, Lys, or Cys.
- Embodiment 95. The light chain variable region of embodiment 93 or 94, wherein the light chain variable region is derived from *Oryctolagus cuniculus*.
- Embodiment 96. The light chain variable region of any one of embodiments 93-95, wherein the light chain variable region is chimerized.
- Embodiment 97. The light chain variable region of any one of embodiments 93-95, wherein the light chain variable region is humanized.
- Embodiment 98. A nucleic acid molecule encoding the immunoglobulin of any one of embodiments 47-63.
- Embodiment 99. A host cell comprising the nucleic acid molecule of embodiment 98.

What is Claimed:

1. A method for generating a conjugated immunoglobulin, the method comprising:
decapping a cysteine at amino acid position 80 ("Cys80") in a light chain variable region of an immunoglobulin derived from rabbit, wherein the immunoglobulin comprises a heavy chain variable region and the light chain variable region; and
conjugating a thiol-reactive compound to the Cys80, wherein the thiol-reactive compound comprises a thiol-reactive group.
2. The method of claim 1, wherein the decapping comprises incubating the immunoglobulin with a reducing buffer followed by incubating the immunoglobulin with an oxidizing buffer.
3. The method of claim 2, further comprising immobilizing the immunoglobulin on a matrix prior to the incubating with the reducing buffer and eluting the immunoglobulin from the matrix following the incubating with the oxidizing buffer.
4. The method of any one of the previous claims, wherein the thiol-reactive compound is attached to a functional agent.
5. The method of claim 4, wherein the functional agent comprises a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug.
6. The method of any one of the previous claims, wherein the thiol-reactive compound is bound to a second thiol-reactive compound, the second thiol-reactive compound being bound to a second immunoglobulin having a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at amino acid position 80 ("Cys80²"), wherein the second thiol-reactive compound comprises a second thiol-reactive group bound to the Cys80².
7. The method of any one of the previous claims, wherein the Cys80 is unpaired.
8. The method of any one of the previous claims, further comprising substituting an amino acid at position 83 with an amino acid residue other than Phe, Lys, or Cys.

9. A method for generating an antigen-binding molecule, the method comprising incubating a first conjugated immunoglobulin with a second conjugated immunoglobulin to generate the antigen-binding molecule, wherein:
- the first conjugated immunoglobulin comprises a first heavy chain variable region and a first light chain variable region, the first light chain variable region having a cysteine at position 80 ("Cys80¹") wherein the Cys80¹ is conjugated to a first thiol-reactive compound comprising a first thiol-reactive group; and
- the second conjugated immunoglobulin comprises a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at position 80 ("Cys80²") wherein the Cys80² is conjugated to a second thiol-reactive compound comprising a second thiol-reactive group.
10. The method of claim 9, wherein the Cys80¹, the Cys80², or both, is unpaired.
11. The method of claim 9 or 10, further comprising, prior to the incubating step,
- decapping the Cys80¹, Cys80², or both; and
- conjugating a first thiol-reactive compound to the Cys80¹, a second thiol-reactive compound to the Cys80², or both, wherein the first thiol-reactive compound comprises a first thiol-reactive group and the second thiol-reactive compound comprises a second thiol-reactive group.
12. The method of any one of claims 9-11, wherein the first thiol-reactive compound further comprises a first functional agent, the second thiol-reactive compound further comprises a second functional agent, or both.
13. The method of any one of claims 9-12, wherein the first immunoglobulin is a first Fab, the second immunoglobulin is a second Fab, or both.
14. The method of any one of claims 9-13, further comprising substituting an amino acid at position 83 of the first light chain variable region with an amino acid residue other than Phe, Lys, or Cys, substituting an amino acid at position 83 of the second light chain variable region with an amino acid residue other than Phe, Lys, or Cys, or both.

15. The antigen-binding molecule produced according to the method of any one of claims 9-14.
16. An immunoglobulin comprising a heavy chain variable region and a light chain variable region, the light chain variable region having a cysteine at position 80 ("Cys80") and an amino acid other than Phe, Lys, or Cys at position 83.
17. The immunoglobulin of claim 16, wherein the Cys80 is unpaired.
18. The immunoglobulin of claim 16 or 17, wherein the Cys80 is decapped.
19. The immunoglobulin of any one of claims 16-18, comprising:
 - a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-141 of xi155D5HC (SEQ ID NO:52) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi155D5LC (SEQ ID NO:78);
 - b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-144 of zu155D5HC (SEQ ID NO:54) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu155D5LC-3 (SEQ ID NO:84), zu155D5LC-4 (SEQ ID NO:86), zu155D5LC-5 (SEQ ID NO:88), zu155D5LC-6 (SEQ ID NO:90), zu155D5LC-7 (SEQ ID NO:92), zu155D5LC-huVK2-40 (SEQ ID NO:96), zu155D5LC-huVK4-1 (SEQ ID NO:100), zu155D5LC-huVK6-21 (SEQ ID NO:102), zu155D5LC-huVK6D-41 (SEQ ID NO:104); or zu155D5LC-huVK7-3-Glu81 (SEQ ID NO:106);
 - c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-138 of xi1E4HC (SEQ ID NO:58) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi1E4LC (SEQ ID NO:110);
 - d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-140 of zu1E4HC (SEQ ID NO:60) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu1E4LC-CXXA (SEQ ID NO:114);

- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi166B3HC (SEQ ID NO:74) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of xi166B3LC (SEQ ID NO:132); or
- f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu166B3HC (SEQ ID NO:76) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-130 of zu166B3LC-CXXA (SEQ ID NO:136).

20. The immunoglobulin of claim 19, comprising:

- a. a heavy chain CDR1, CDR2, and CDR3 of xi155D5HC as set forth as SEQ ID NO:146, 148, and 150, respectively, and a light chain CDR1, CDR2, and CDR3 of xi155D5LC as set forth as SEQ ID NO:224, 226, and 228, respectively;
- b. a heavy chain CDR1, CDR2, and CDR3 of zu155D5HC as set forth as SEQ ID NO:152, 154, and 156, respectively, and a light chain CDR1, CDR2, and CDR3 of zu155D5LC-3 as set forth as SEQ ID NO:242, 244, and 246, respectively, zu155D5LC-4 as set forth as SEQ ID NO:248, 250, and 252, respectively, zu155D5LC-5 as set forth as SEQ ID NO:254, 256, and 258, respectively, zu155D5LC-6 as set forth as SEQ ID NO:260, 262, and 264, respectively, zu155D5LC-7 as set forth as SEQ ID NO:266, 268, and 270, respectively, zu155D5LC-huVK2-40 as set forth as SEQ ID NO 278, 280, and 282, respectively, zu155D5LC-huVK4-1 as set forth as SEQ ID NO 290, 292, and 294, respectively, zu155D5LC-huVK6-21 as set forth as SEQ ID NO 296, 298, and 300, respectively, zu155D5LC-huVK6D-41 as set forth as SEQ ID NO 302, 304, and 306, respectively; or zu155D5LC-huVK7-3-Glu81 as set forth as SEQ ID NO 308, 310, and 312, respectively;
- c. a heavy chain CDR1, CDR2, and CDR3 of xi1E4HC as set forth as SEQ ID NO:164, 166, and 168, respectively, and a light chain CDR1, CDR2, and CDR3 of xi1E4LC as set forth as SEQ ID NO:320, 322, and 324, respectively;
- d. a heavy chain CDR1, CDR2, and CDR3 of zu1E4HC as set forth as SEQ ID NO:170, 172, and 174, respectively, and a light chain CDR1, CDR2, and CDR3 of zu1E4LC-CXXA as set forth as SEQ ID NO:332, 334, and 336, respectively;

- e. a heavy chain CDR1, CDR2, and CDR3 of xi166B3HC as set forth as SEQ ID NO:212, 214, and 216, respectively, and a light chain CDR1, CDR2, and CDR3 of xi166B3LC as set forth as SEQ ID NO:386, 388, and 390, respectively; or
- f. a heavy chain CDR1, CDR2, and CDR3 of zu166B3HC as set forth as SEQ ID NO:218, 220, and 222, respectively, and a light chain CDR1, CDR2, and CDR3 of zu166B3LC-CXXA as set forth as SEQ ID NO:398, 400, and 402, respectively.

21. The immunoglobulin of any one of claims 16-18, comprising:

a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-139 of xi1-55-2HC (SEQ ID NO:56) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-129 of xi1-55-2LC (SEQ ID NO:108).

22. The immunoglobulin of claim 21, comprising:

a heavy chain CDR1, CDR2, and CDR3 of xi1-55-2HC as set forth as SEQ ID NO:158, 160, and 162, respectively, and a light chain CDR1, CDR2, and CDR3 of xi1-55-2LC as set forth as SEQ ID NO:314, 316, and 318, respectively.

23. The immunoglobulin of any one of claims 16-18, comprising:

- a. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-142 of xi33O11HC (SEQ ID NO:62) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of xi33O11LC (SEQ ID NO:116);
- b. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-145 of zu33O11HC (SEQ ID NO:64) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-131 of zu33O11LC-CXXA (SEQ ID NO:120) or zu33O11LC-CXXI (SEQ ID NO:122);
- c. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi324O5HC (SEQ ID NO:66) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi324O5LC (SEQ ID NO:124);
- d. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi178F16HC (SEQ ID NO:68) and a light chain variable

- region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi178F16LC (SEQ ID NO:126);
- e. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-132 of xi237N18HC (SEQ ID NO:70) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi237N18LC (SEQ ID NO:128); or
 - f. a heavy chain variable region having an amino acid sequence at least 90% identical to amino acids 20-137 of xi383I18HC (SEQ ID NO:72) and a light chain variable region having an amino acid sequence at least 90% identical to amino acids 20-127 of xi383I18LC (SEQ ID NO:130).
24. The immunoglobulin of claim 23, comprising:
- a. a heavy chain CDR1, CDR2, and CDR3 of xi33O11HC as set forth as SEQ ID NO: 176, 178, and 180, respectively, and a light chain CDR1, CDR2, and CDR3 of xi33O11LC as set forth in SEQ ID NO:338, 340, and 342, respectively;
 - b. a heavy chain CDR1, CDR2, and CDR3 of zu33O11HC as set forth as SEQ ID NO:182, 184, and 186, respectively, and a light chain CDR1, CDR2, and CDR3 of zu33O11LC-CXXA as set forth as SEQ ID NO:350, 352, and 354, respectively, or zu33O11LC-CXXI as set forth as SEQ ID NO:356, 358, and 360, respectively;
 - c. a heavy chain CDR1, CDR2, and CDR3 of xi324O5HC as set forth as SEQ ID NO:188, 190, and 192, respectively, and a light chain CDR1, CDR2, and CDR3 of xi324O5LC as set forth as SEQ ID NO:362, 364, and 366, respectively;
 - d. a heavy chain CDR1, CDR2, and CDR3 of xi178F16HC as set forth as SEQ ID NO:194, 196, and 198, respectively, and a light chain CDR1, CDR2, and CDR3 of xi178F16LC as set forth as SEQ ID NO:368, 370, and 372, respectively;
 - e. a heavy chain CDR1, CDR2, and CDR3 of xi237N18HC as set forth as SEQ ID NO:200, 202, and 204, respectively, and a light chain CDR1, CDR2, and CDR3 of xi237N18LC as set forth as SEQ ID NO:374, 376, and 378, respectively; or
 - f. a heavy chain CDR1, CDR2, and CDR3 of xi383I18HC as set forth as SEQ ID NO:206, 208, and 210, respectively, and a light chain CDR1, CDR2, and CDR3 of xi383I18LC as set forth as SEQ ID NO:380, 382, and 384, respectively.
25. A conjugated immunoglobulin comprising:

the immunoglobulin of any one of claims 16-24, wherein the cysteine at position 80 is conjugated to a thiol-reactive compound, the thiol-reactive compound comprising a thiol-reactive group.

26. The conjugated immunoglobulin of claim 25, wherein the thiol-reactive compound further comprises a functional agent.
27. The conjugated immunoglobulin of claim 26, wherein the functional agent comprises a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug.
28. A method of treating cancer in a subject comprising administering to the subject a pharmaceutically effective amount of a conjugated mesothelin immunoglobulin, wherein the conjugated mesothelin immunoglobulin comprises:
the immunoglobulin of any one of claims 23-24, and
a thiol-reactive compound comprising a thiol-reactive group, a linker, and a functional agent.
29. The method of claim 28, wherein the functional agent is auristatin F.
30. A antigen-binding molecule comprising:
a first conjugated immunoglobulin comprising a first heavy chain variable region and a first light chain variable region, the first light chain variable region having a cysteine at position 80 ("Cys80¹"), wherein the Cys80¹ is conjugated to a first thiol-reactive compound comprising a first thiol-reactive group, and
a second conjugated immunoglobulin comprising a second heavy chain variable region and a second light chain variable region, the second light chain variable region having a cysteine at position 80 ("Cys80²") wherein the Cys80² is conjugated to a second thiol-reactive compound comprising a second thiol-reactive group.
31. The antigen-binding molecule of claim 30, wherein Cys80¹, Cys80², or both, is unpaired.

32. The antigen-binding molecule of claim 30 or 31, wherein the amino acid at position 83 of the first immunoglobulin, the amino acid at position 83 of the second immunoglobulin, or both, is an amino acid residue other than Phe, Lys, or Cys.
33. The antigen-binding molecule of any one of claims 30-32, wherein the first thiol-reactive compound further comprises a first functional agent, the second thiol-reactive compound further comprises a second functional agent, or both.
34. The antigen-binding molecule of any one of claims 30-33, wherein the first immunoglobulin, second immunoglobulin, or both is a Fab.
35. A light chain variable region for use in a conjugated immunoglobulin, the light chain variable region having a cysteine at amino acid position 80 ("Cys80") and an amino acid residue other than Phe, Lys, or Cys at amino acid position 83, wherein the Cys80 is unpaired.
36. The light chain variable region of claim 35, wherein the light chain variable region has a Cys80-Xaa₁-Xaa₂-Xaa₃ motif, wherein Xaa₃ is any amino acid other than Phe, Lys, or Cys.
37. A nucleic acid molecule encoding the immunoglobulin of any one of claims 16-24.
38. A host cell comprising the nucleic acid molecule of claim 37.

rb-IGKV1S2*01

hu-IGKV1-5*01

Consensus

AOVITOTESPVSA

DIQMTQSPSTLSASV

ITQS S LSA Vg

PGGTVTINCOASQSVYDNNWLSWYQKPGQPKLLIYDASKLASGVPSRFSGSGSGTQFTLTITSGVOCDDAATYYCGSYYSSGWY

GVDRVTITCRASQSISS--WLAWYQKPGKAPKLLIYDASSLFSGVPSRFSGSGSGTETLTITSSLSLQ

VTI C ASQSI WLAWYQKPG KLLIYDAS L SGVPSRFSGSGGT FTLTIS LQ DD ATYYCQ S

rb-IGKC1*01

hu-IGKC*01

Consensus

-DPVAPTVLIRPPAADQVATGTVTI

RTVAAPSVFIPEPSPDEQLKSGTASV

APSV IPEPA DQL SGT SI

VCVANKYFP--DVTVTWEVDGTTOTTGIENSKTPONSADCTYNLSSTLTITSTQYN

VVCLLNFFYPREAKVQWVDNALQSGNSQESVTEQDSKDS

VTI SIVCL N FFP D V W VD QS S T Q S D TY LSSTLTIS Y HK Y C VT SS V SFNRGDC

FIG. 1

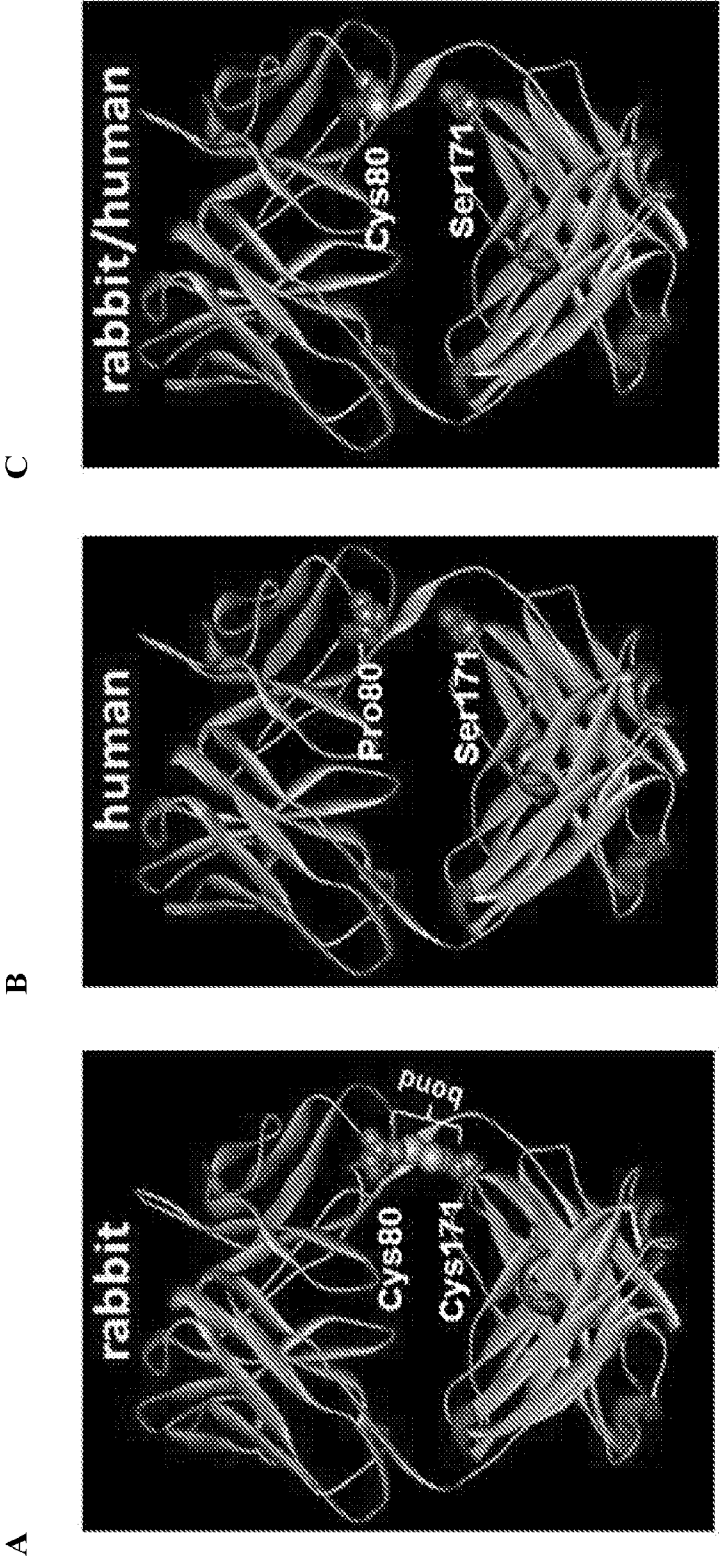


FIG. 2

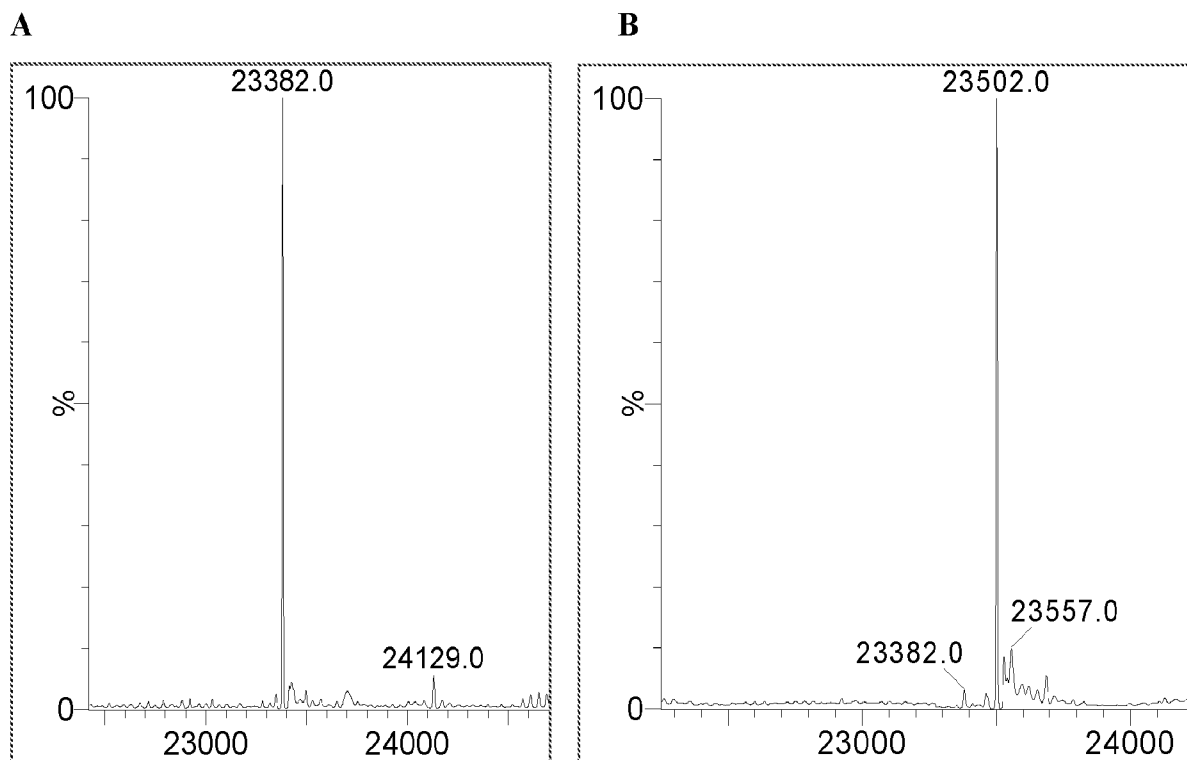
FIG. 3

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IGKV1S2*01 AQVLTQTTPSPVSAVGGTVTINC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:408)
IGKV1S2*02 AQVLTQTTPSPVSAVGGTVTINC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:409)
IGKV1S3*01 AQVLTQTTPSPVSAVGGTVTINC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:410)
IGKV1S3*02 AQVMTQTTPSPVSAVGGTVTIC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:411)
IGKV1S4*01 AIDMTQTTPSPVSAVGGTVTINC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:412)
IGKV1S5*01 DPVMTQTTPSSSTAAGGTVTINC WFQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:413)
IGKV1S6*01 DGVMTQTTPSPVSAVGGTVTINC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:414)
IGKV1S7*01 DVVMTQTTPSPVSAVGGTVTINC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:415)
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IGKV1S17*01 ALVMTQTTPSPVSAVGGTVTIC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:426)
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IGKV1S27*01 YVMMTQTTPSSVSEAVGGTVIYC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:436)
IGKV1S28*01 DPMLTQTASPVSAAVGGTVTINC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:437)
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IGKV1S55*01 DPMLTQTASPVSAAVGGTVTISC WFQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:462)
IGKV1S56*01 DPVLTQTASPVSAAVGGTVTISC WFQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:463)
IGKV1S57*01 DPMLTQTASPVSAAVGGTVTISC WFQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:464)
IGKV1S58*01 DVVMTQTTPSPVSAVGGTVTINC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:465)
IGKV1S59*01 AAVLTQTTPSPVSAVGGTVTISC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:466)
IGKV1S60*01 DPVLTQTTPSPVSAVGGTVTINC WFQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:467)
IGKV1S61*01 ALVMTQTTPSSSTSEVGGTVTINC WFQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:468)
IGKV1S62*01 DVVMTQTASPVSAAVGGTVTISC WFQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:469)
IGKV1S63*01 AAVLTQTTPSPVSAVGGTVTINC WFQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:470)
IGKV1S64*01 DPVLTQTASPVSAAVGGTVTISC WFQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:471)
IGKV1S65*01 YVMMTQTTPSSVSEAVGGTVIYC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:472)
IGKV1S66*01 DPMLTQTASPVSAAVGGTVTISC WFQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:473)
IGKV1S67*01 DVVMTQTTPSSKSAAGGTVTIC WYQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:474)
IGKV1S68*01 AAVLTQTTPSPVSAVGGTVTINC WFQQKPGQPPKLLIY GVPSPFKSGSGGTQFTLTI SGVQCDDAATYYC (SEQ ID NO:475)

```

FIG. 4



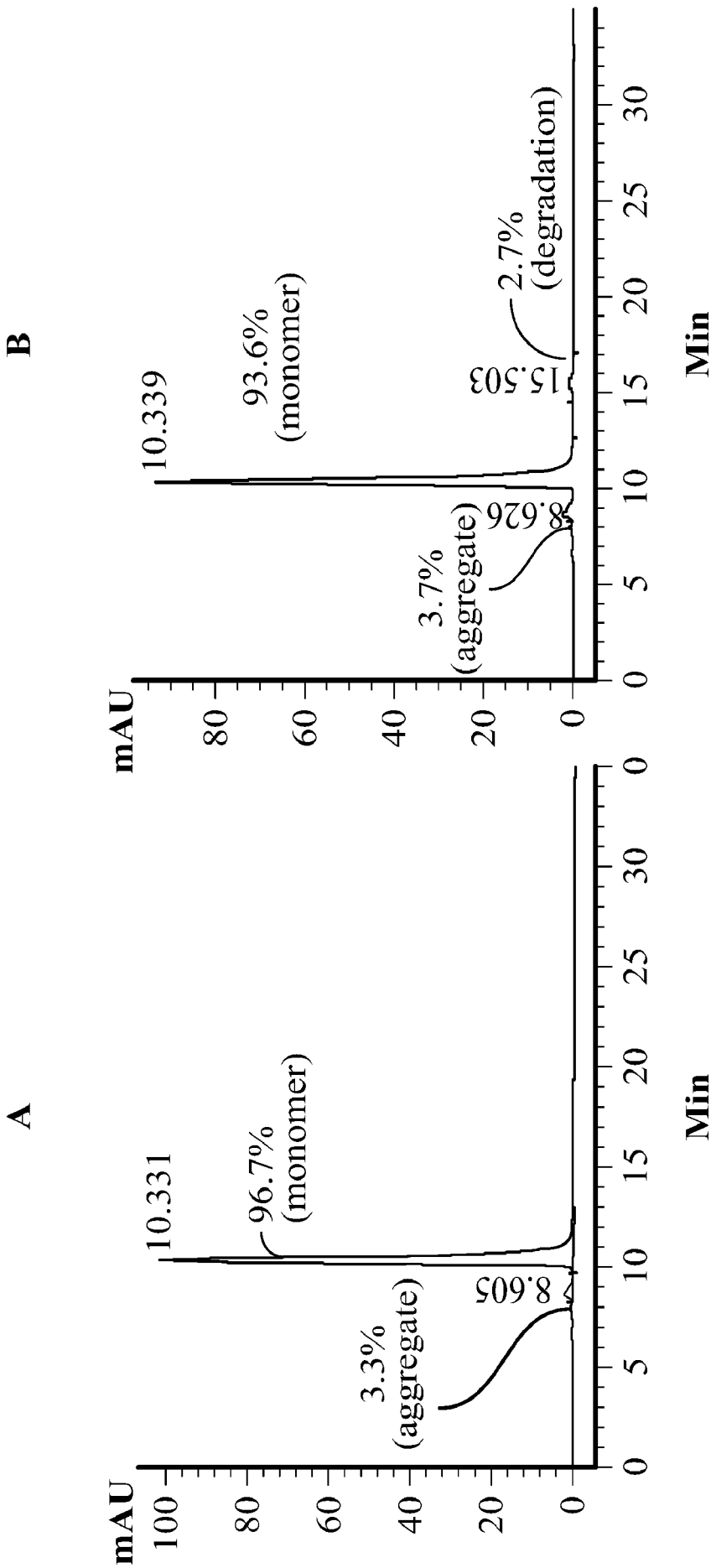


FIG. 5

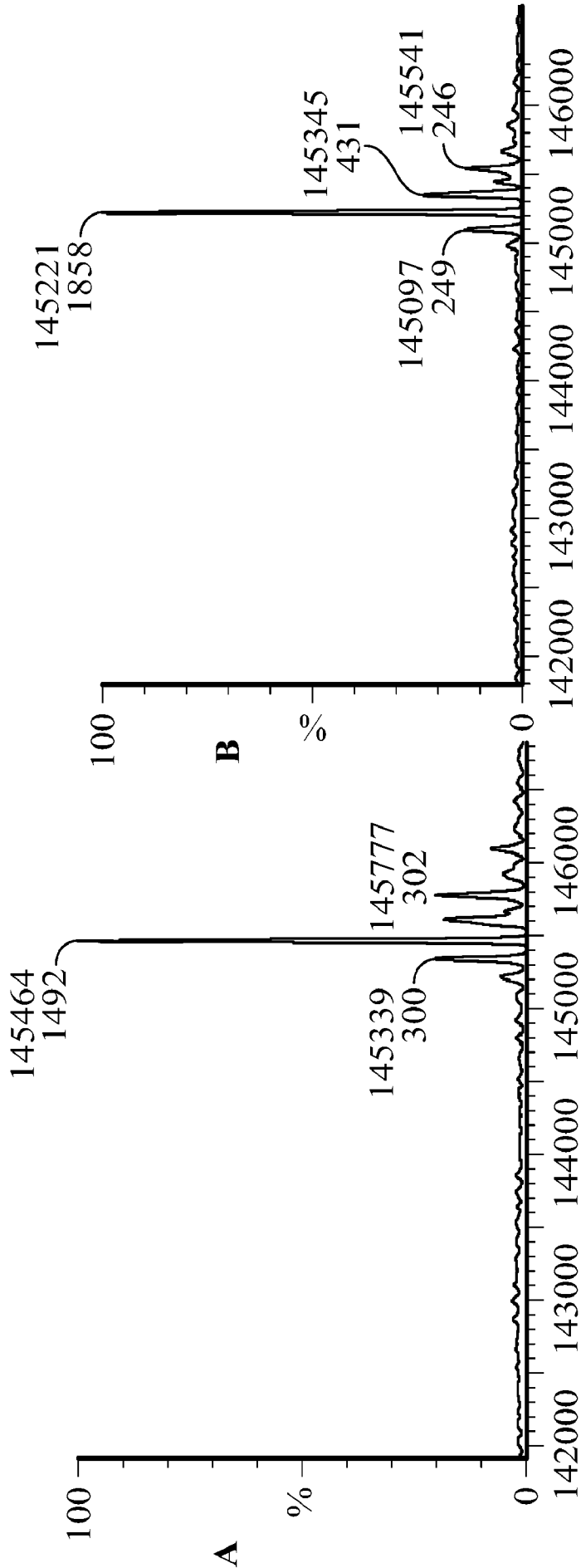


FIG. 6

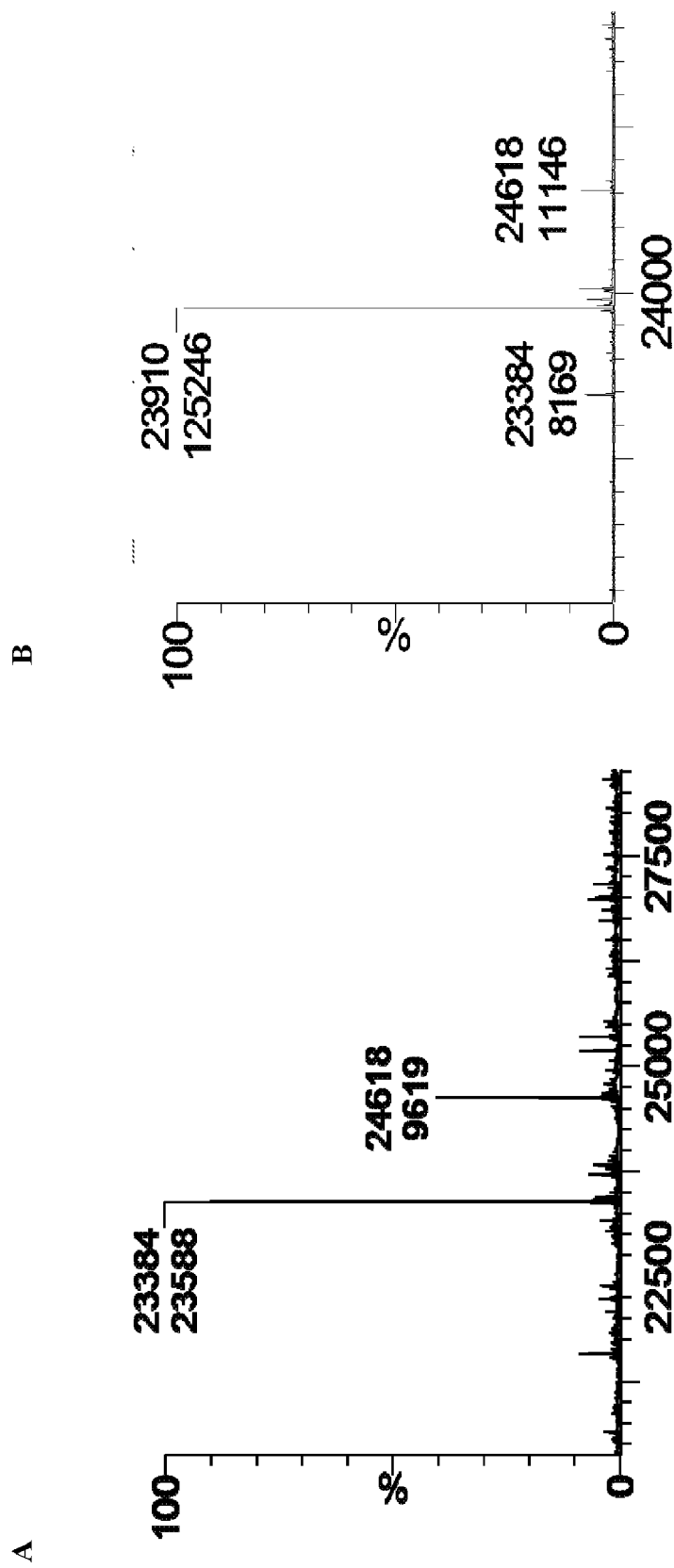


FIG. 7

155D5-VH	<-----FWR1----->	<---CDR1--->	<-----FWR2----->	<-----CDR2----->	<-----FWR3----->	(SEQ ID NO: 476)
	-QSVKESGRLVTPGTPLTITCTVSGFSIN	SYAMI	WVRQAPGEGLEYIG	FITT---GGTTYASMAKG	RFTISR--TSTVYDLKLTPTPTEDTATYFCAR	(SEQ ID NO: 477)
IGHV3-64*04	QVQLV...G..Q..GS.R.S.SA...	...H	...K...VS	A.SSN--...S....DSV..	DNSKN.IY.QMNSLRA....V.Y..	(SEQ ID NO: 478)
IGHV3-49*04	-----G..Q..RS.R.S.A...TFG	D...S	...K...WV.	..PSKAY...E...ASV..	DDSKSIAY.QMNSLK.....V.Y.T.	(SEQ ID NO: 479)
IGHV3-64*01	-----G..Q..GS.R.S.SA...TFS	...H	...K...VS	A.SSN--...S....NSV..	DNSKN.IY.QMGSIARA..N.V.Y...	
155D5-VL	<-----FWR1----->	<---CDR1--->	<-----FWR2----->	<-----CDR2>	<-----FWR3----->	(SEQ ID NO: 480)
	ELVMTQTPSSVSAAVGGTVTINC	QASQISSYLA	WYQOKPGQPPKLLIY	YASTIAS	GVPSRFKSGSGTEFTLTITGVQCDDAATYYC	(SEQ ID NO: 481)
IGKV1-5*03	DIQ...S..TL..S..DR...T.	R.....W..	...KA.....	K..S.E.	SSL.P..F.....	(SEQ ID NO: 482)
IGKV1-5*01	DIQ...S..TL..S..DR...T.	R.....W..	...KA.....	D..S.E.	SSL.P..F.....	(SEQ ID NO: 483)
IGKV1-8*01	---S...S...F..ST.DR...T.	R...G.....	...KA.....	A....Q.	SCL.SE.F.....	

FIG. 8

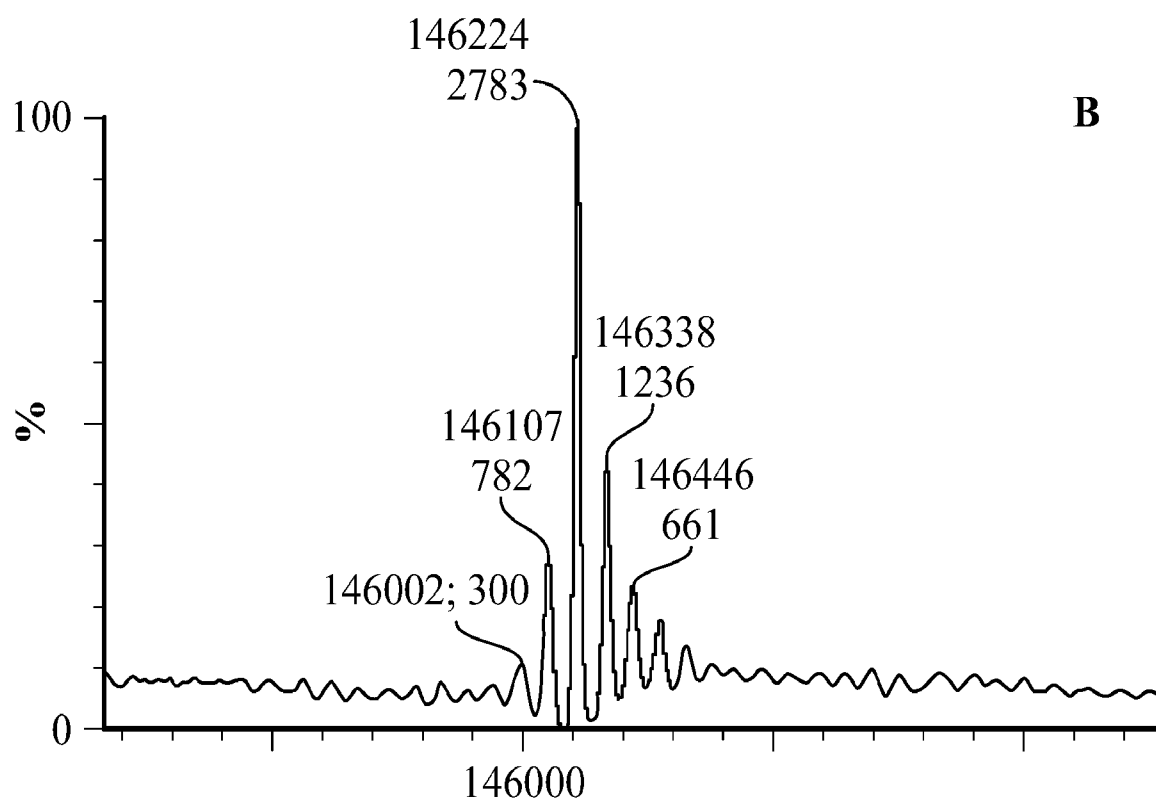
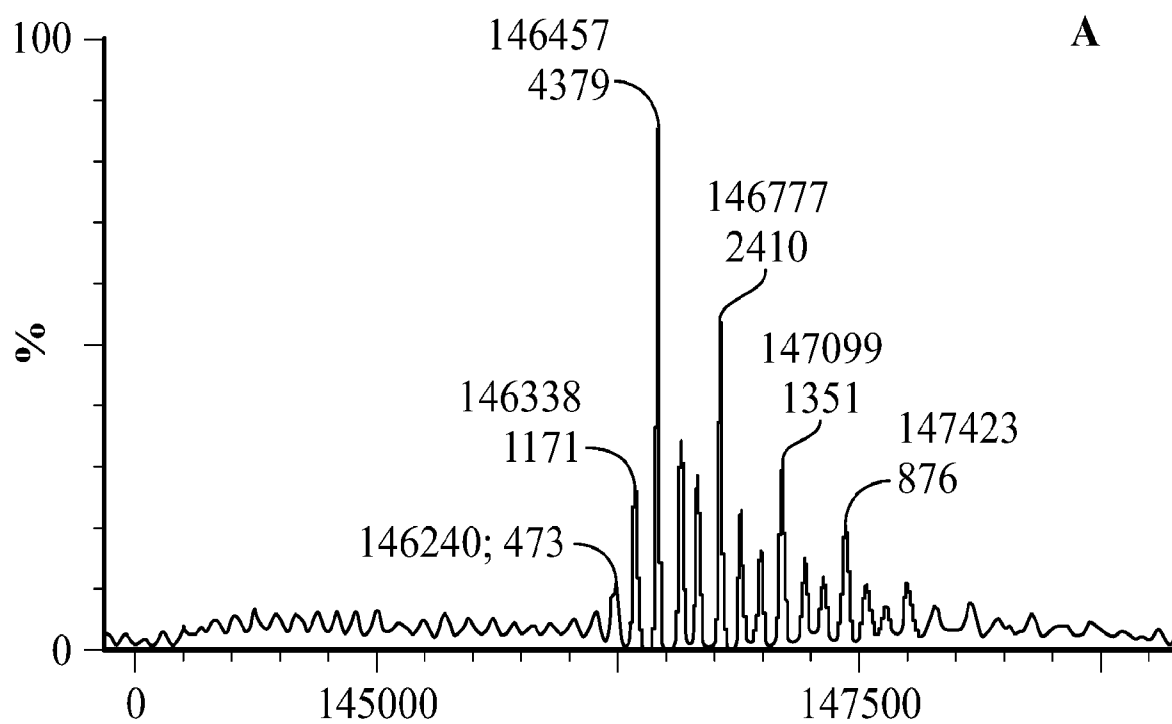


FIG. 9

FIG. 10

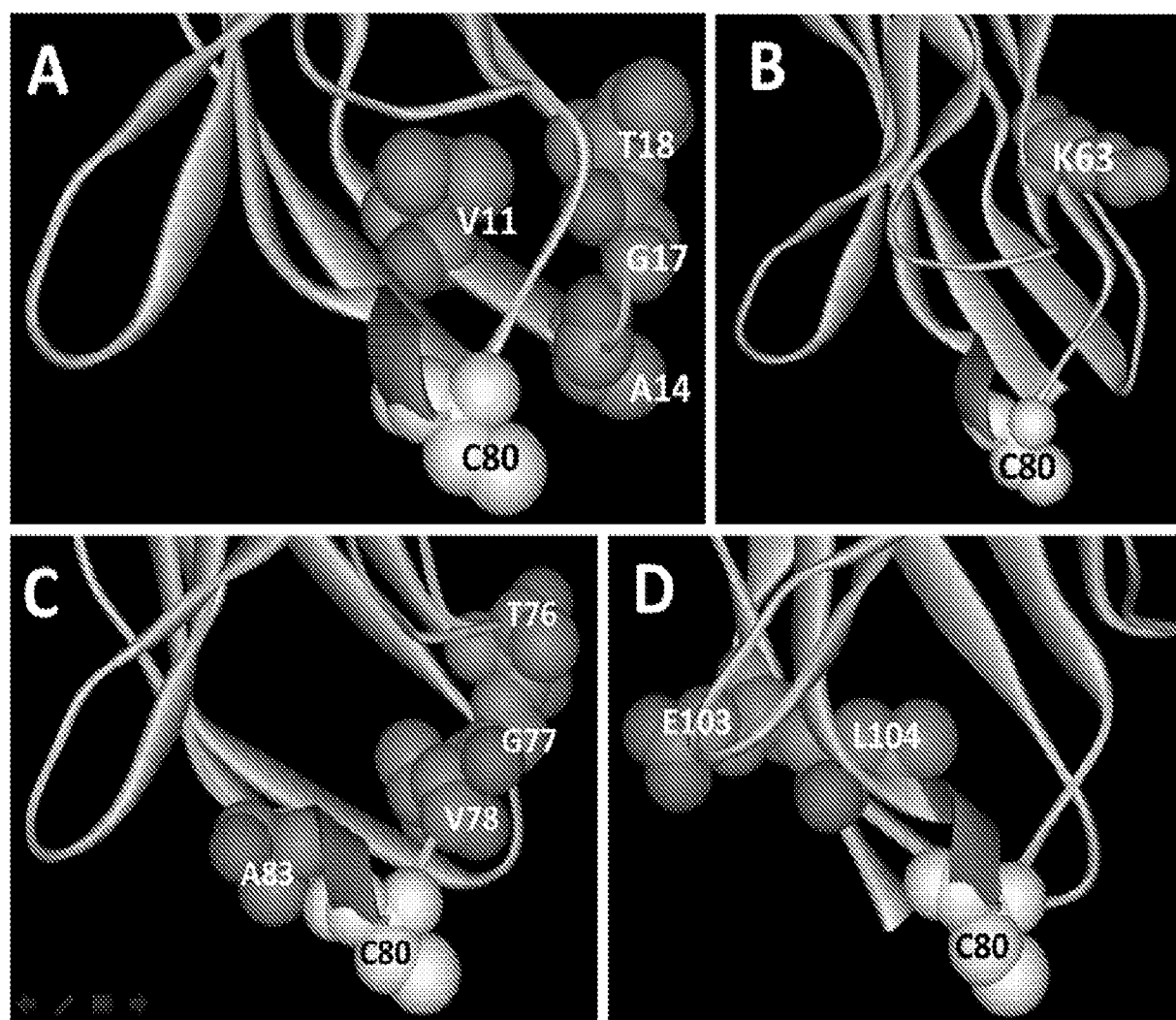
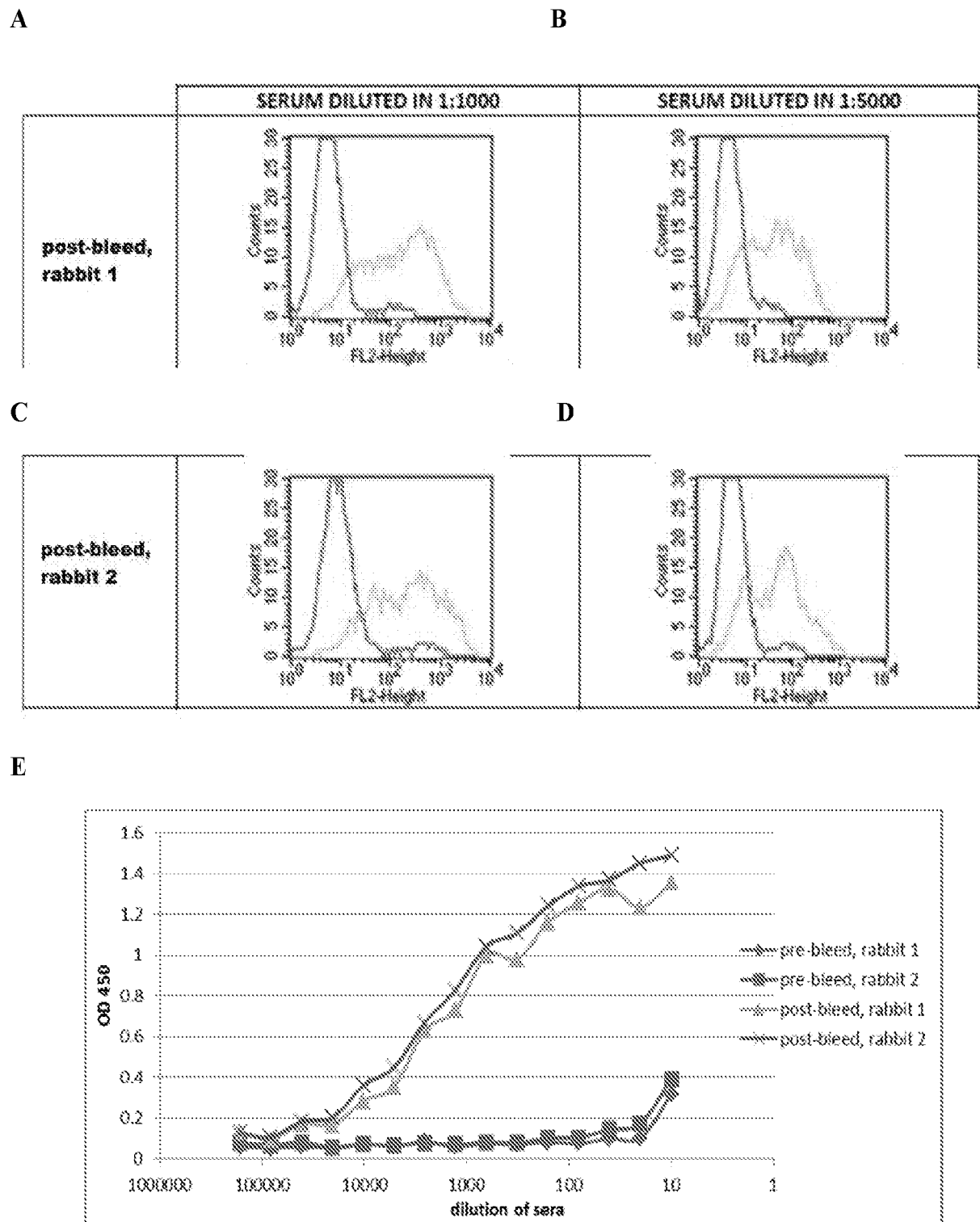


FIG. 11

FIG. 12



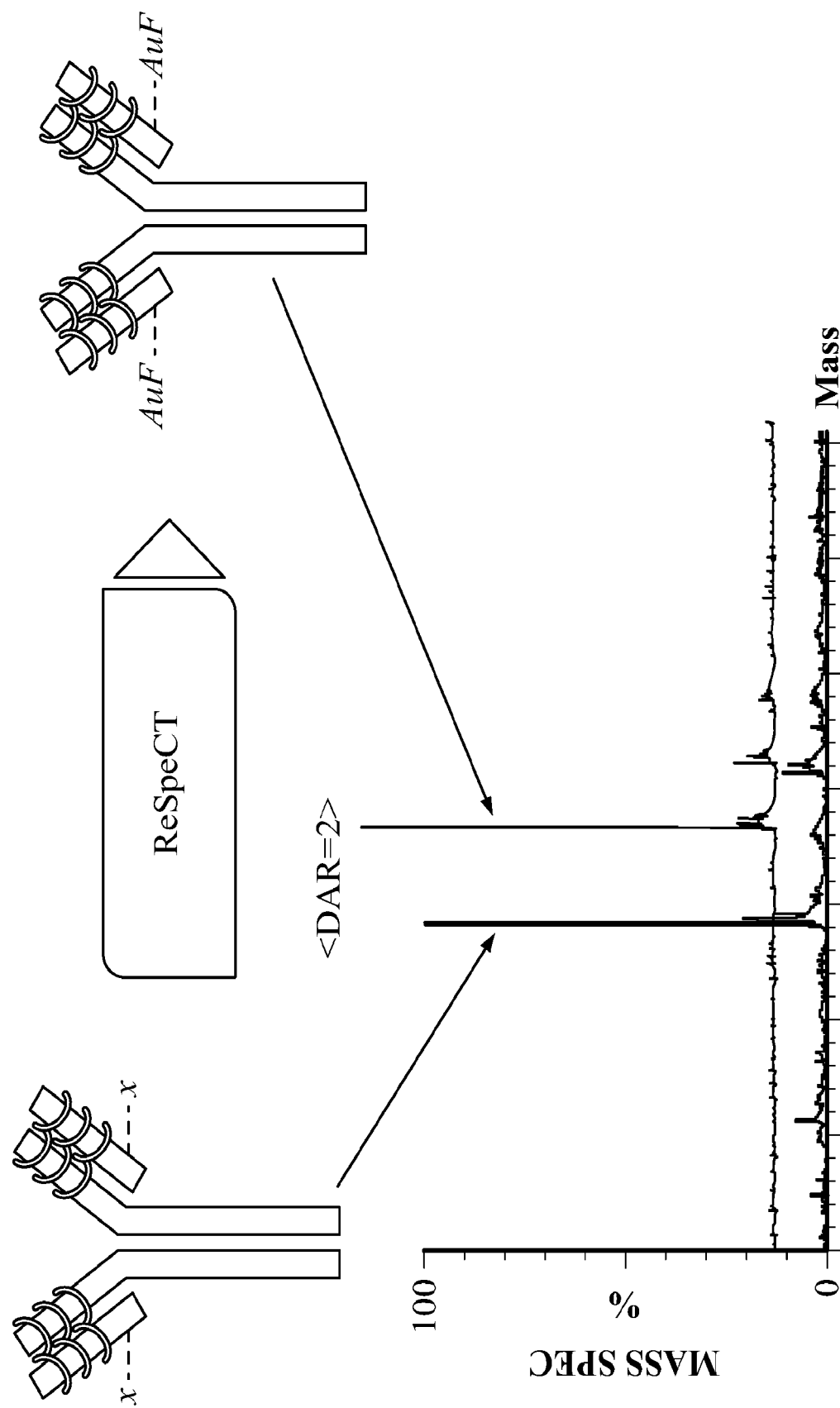
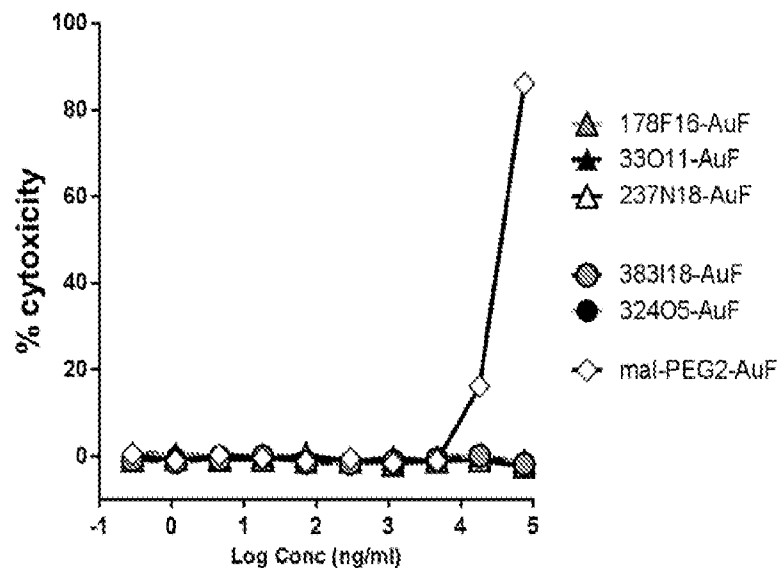


FIG. 13

FIG. 14

A



B

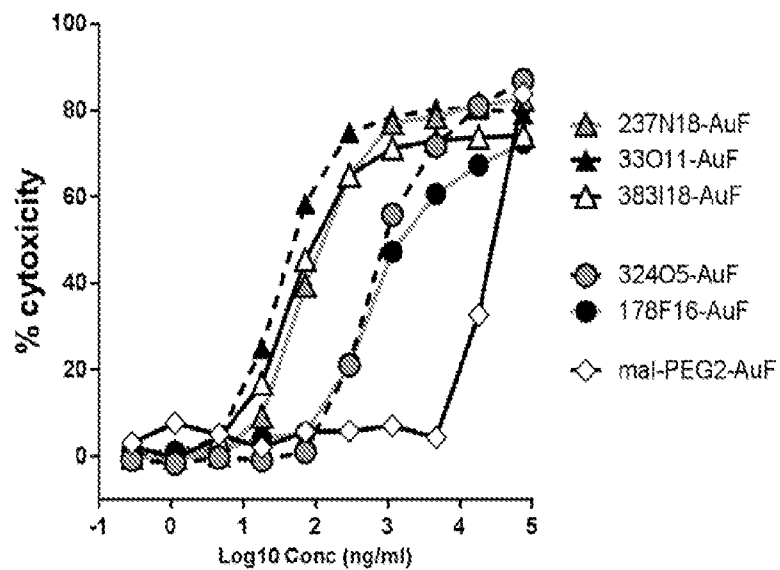


FIG. 15

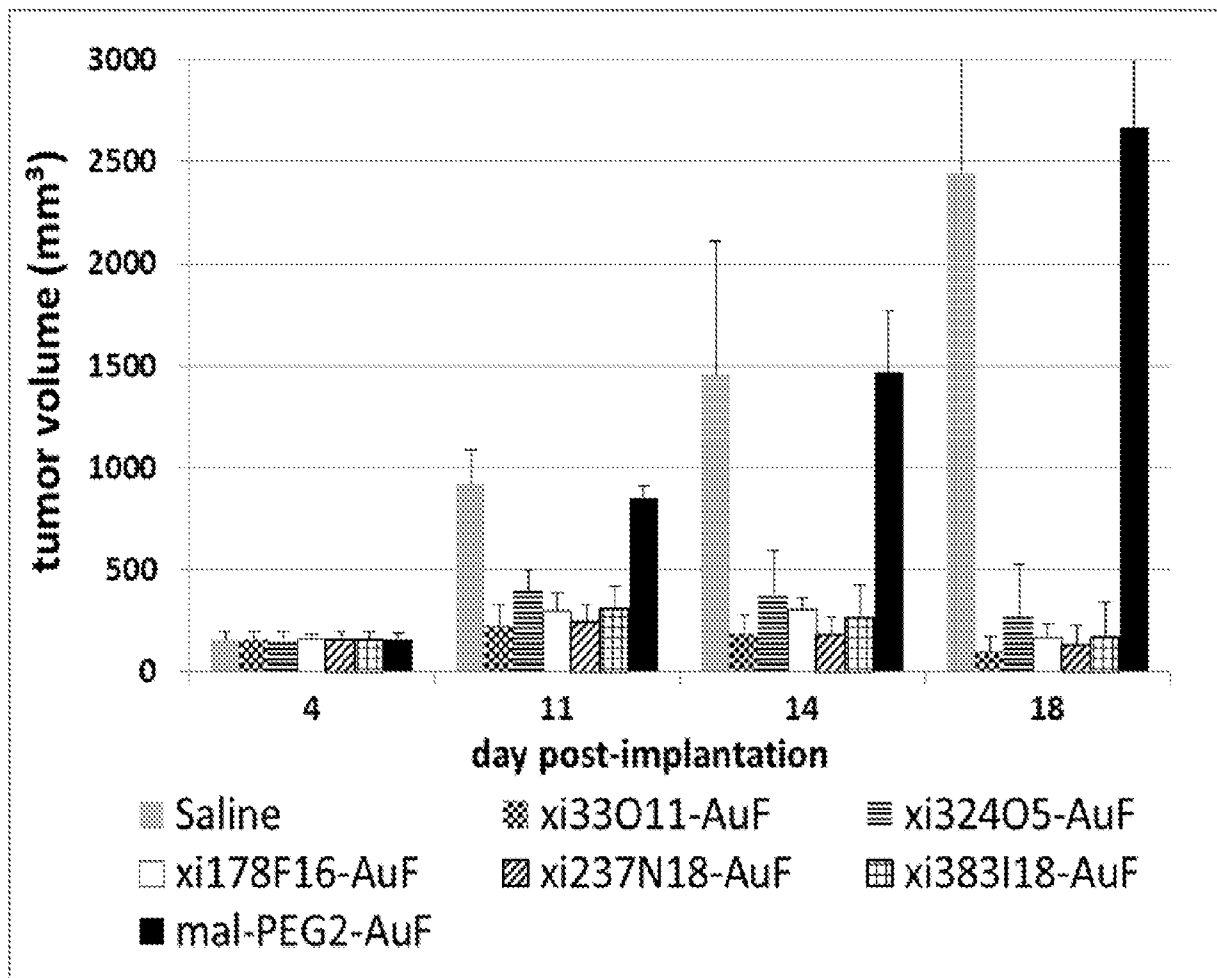
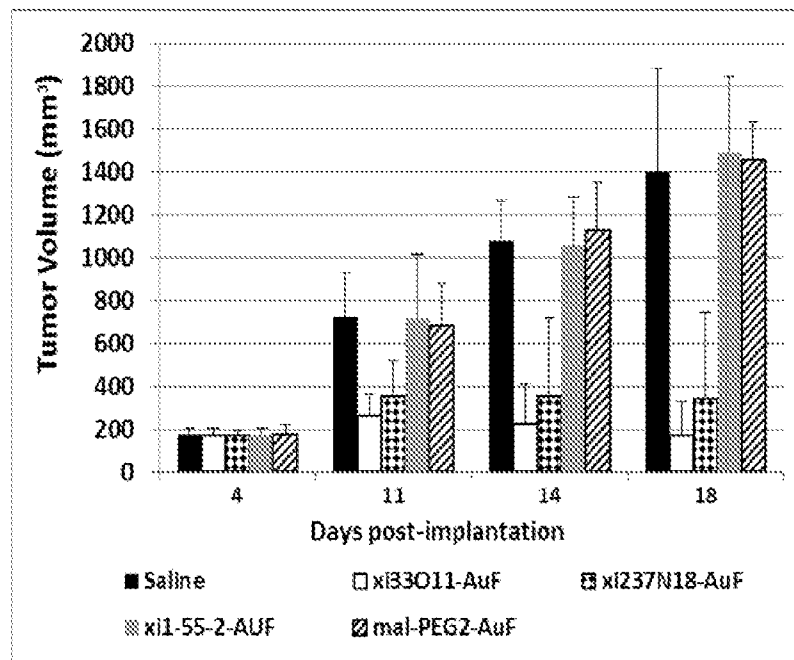
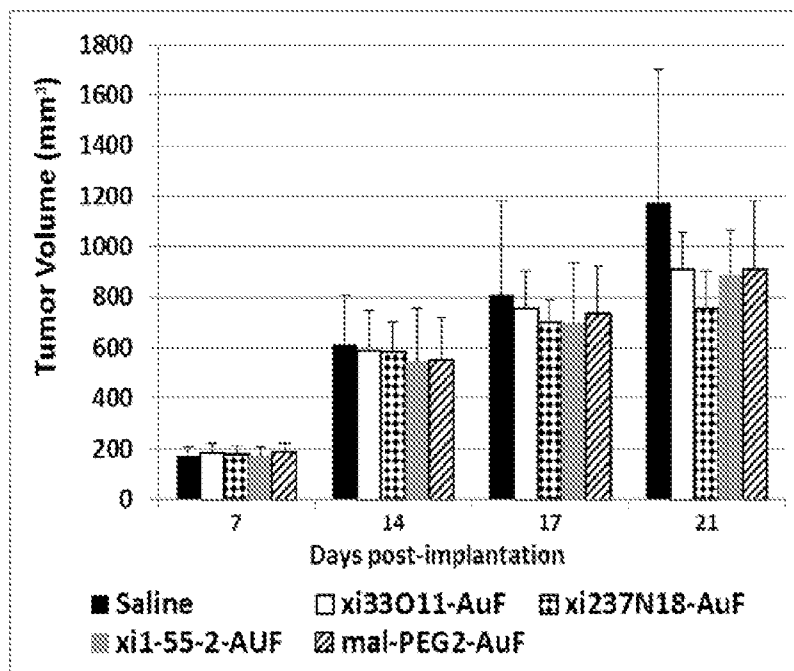


FIG. 16

A



B



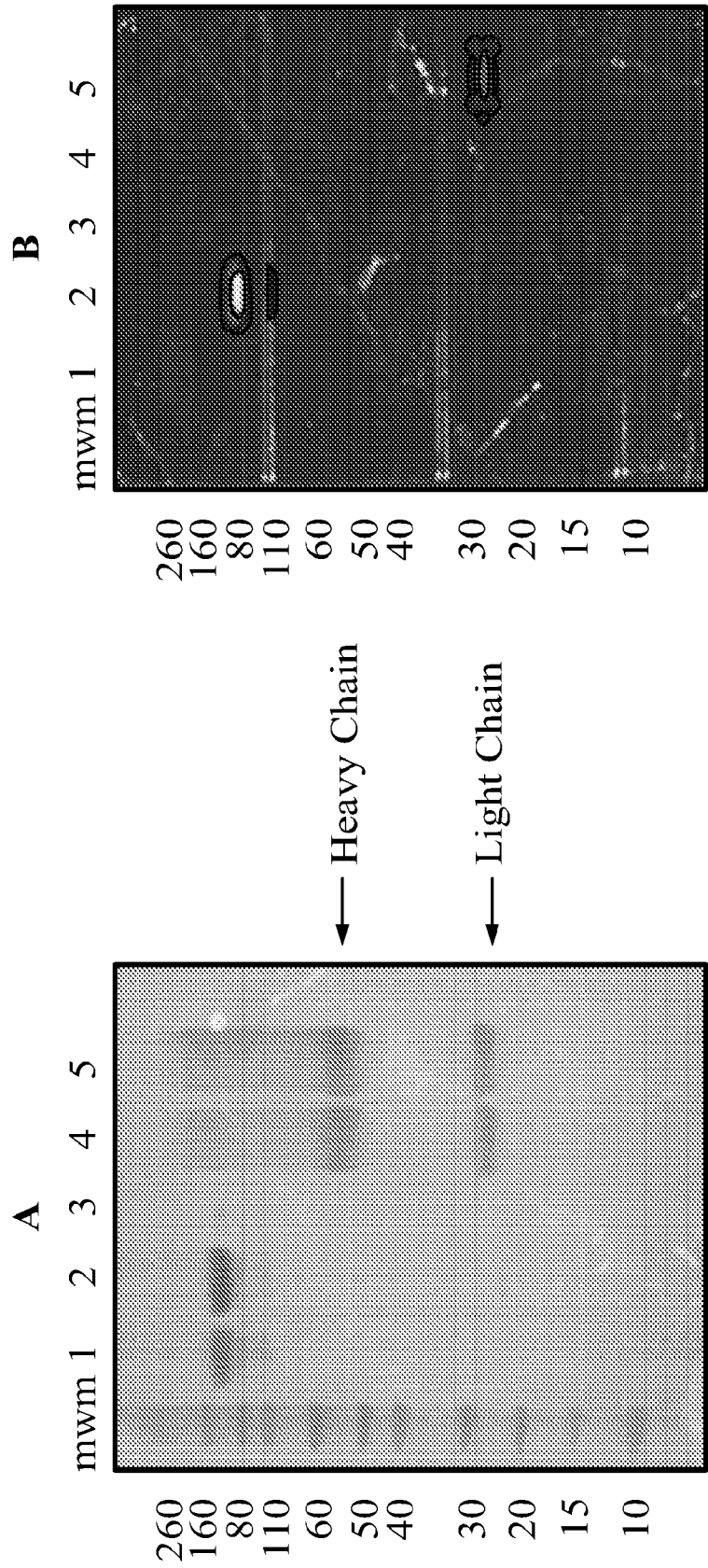
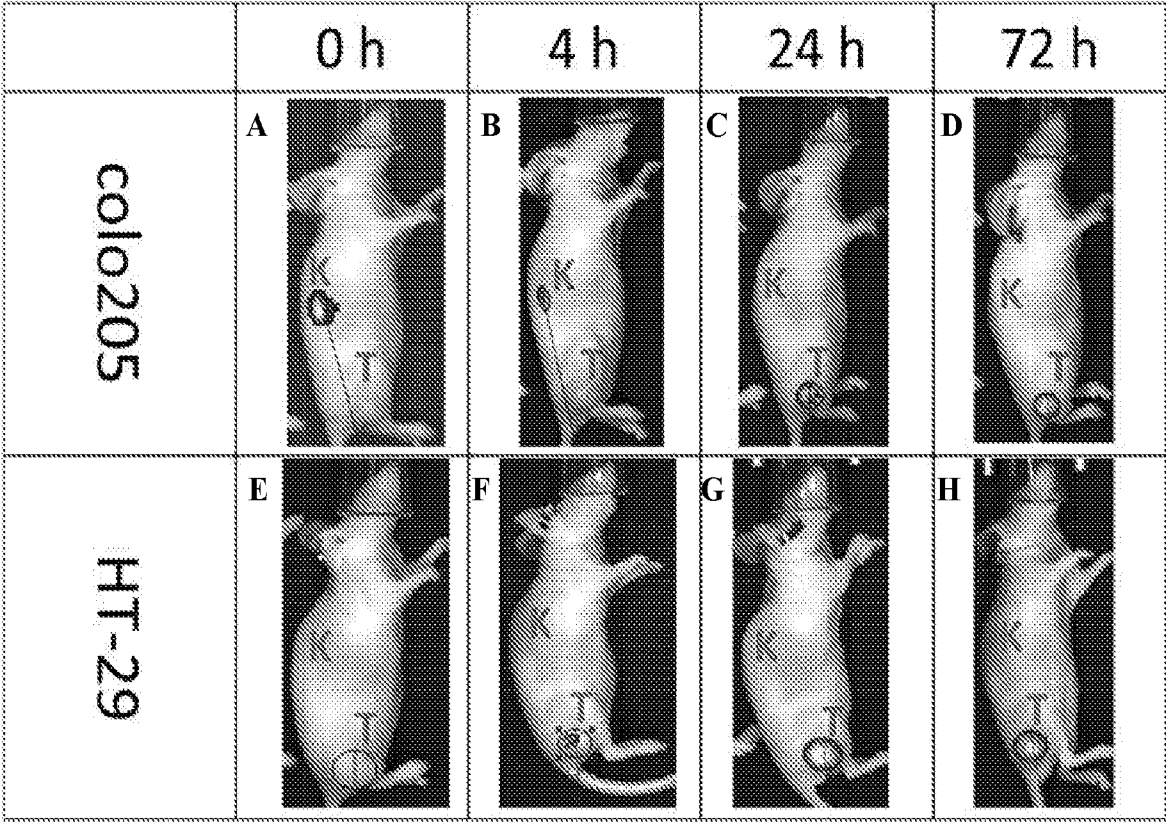


FIG. 17

FIG. 18



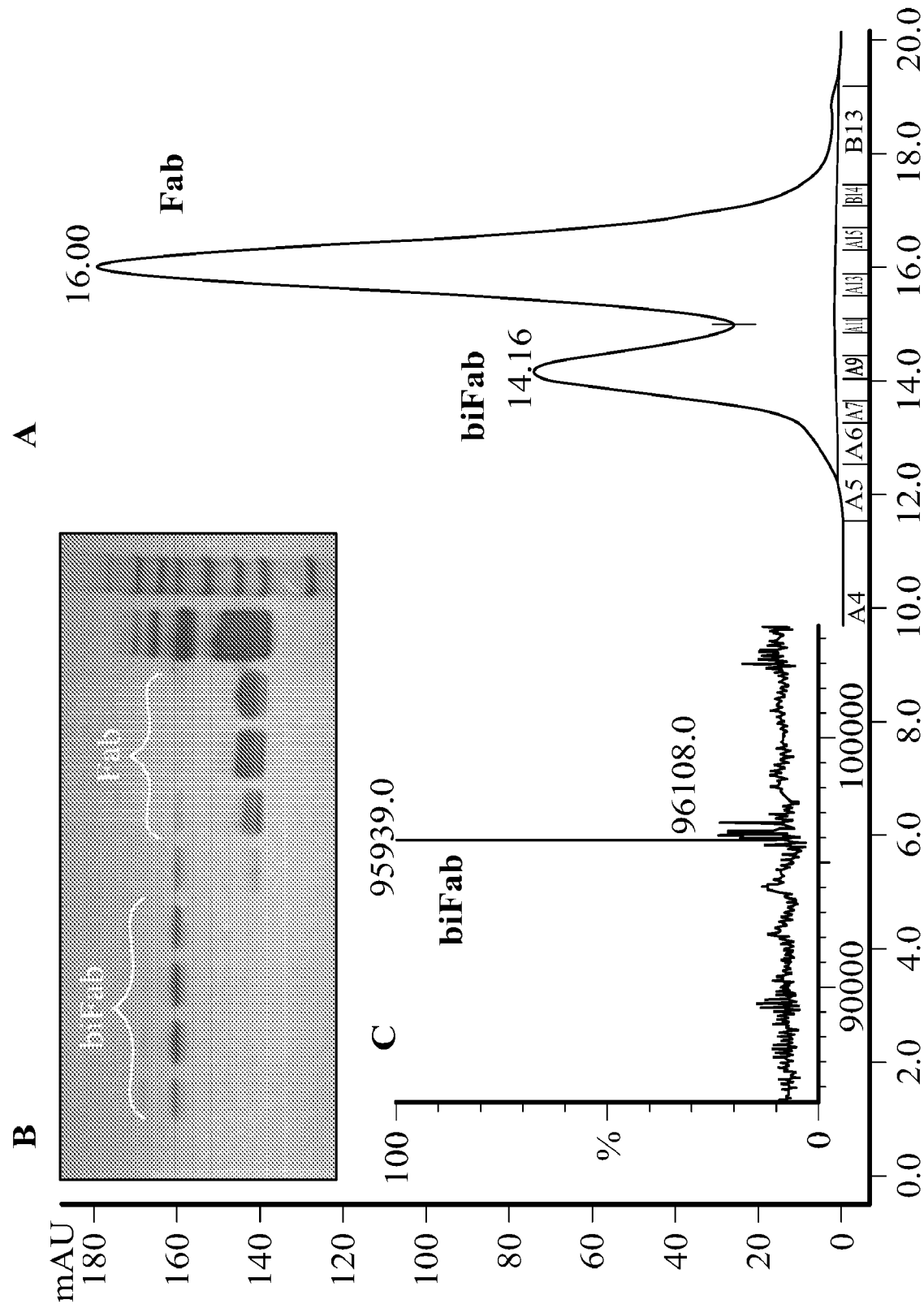


FIG. 19

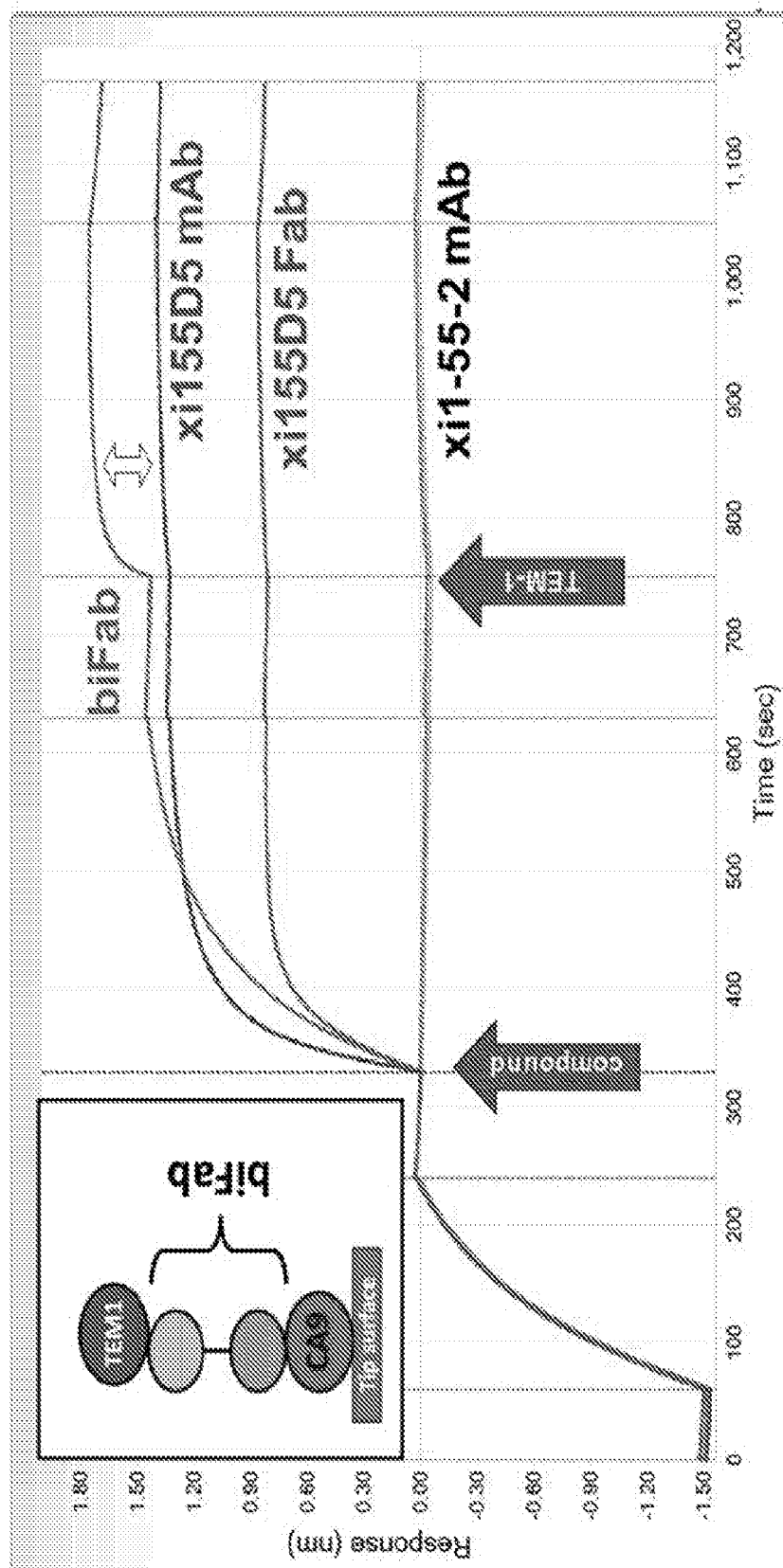
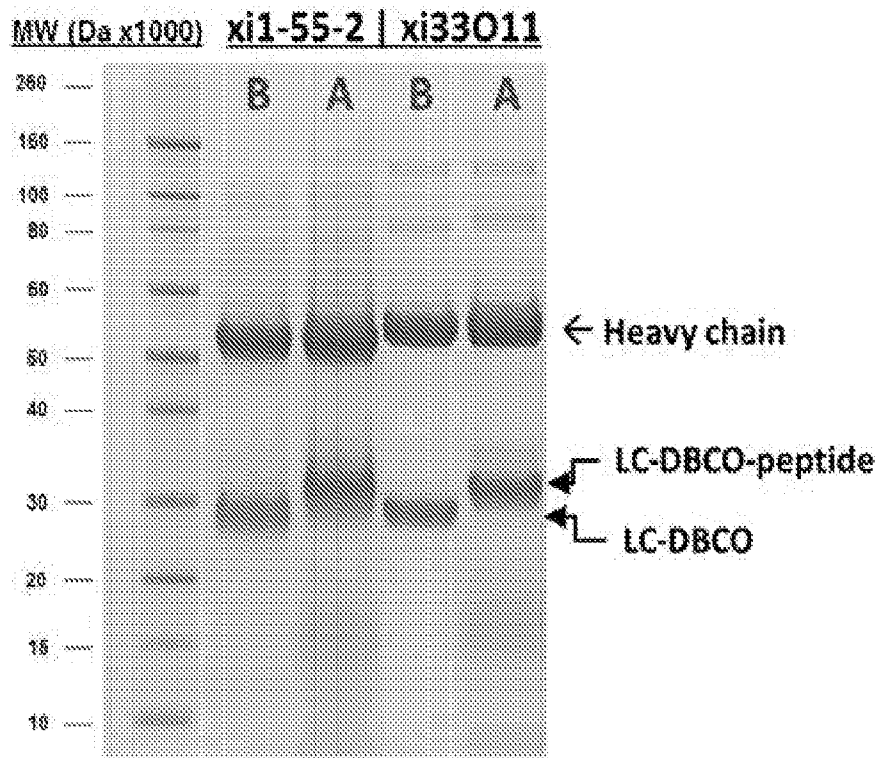


FIG. 20

FIG. 21



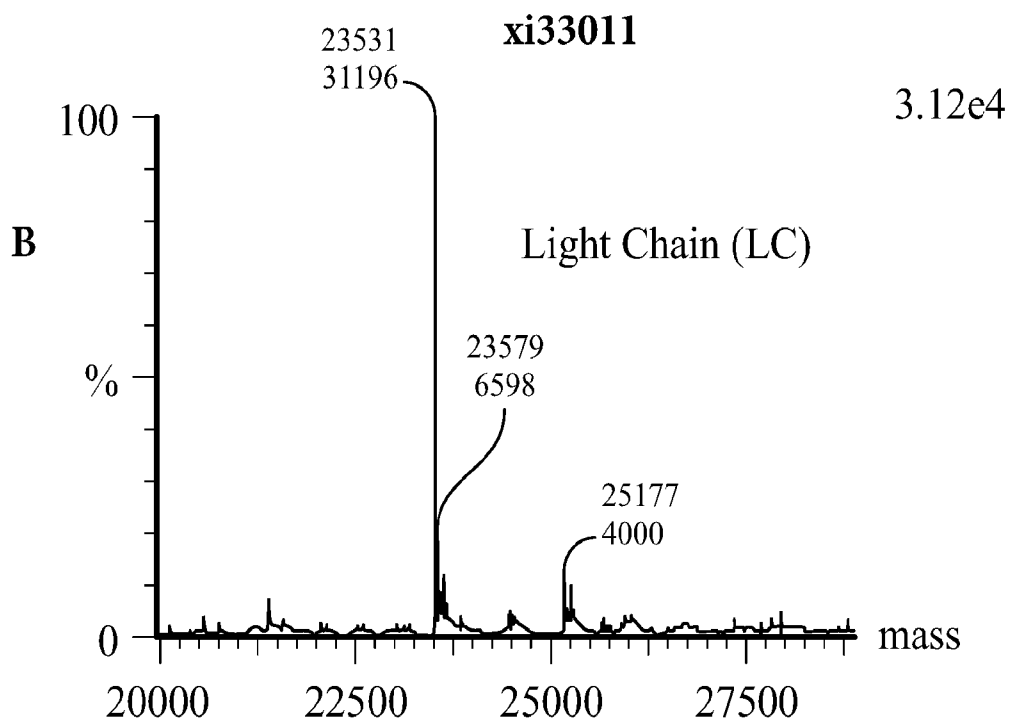
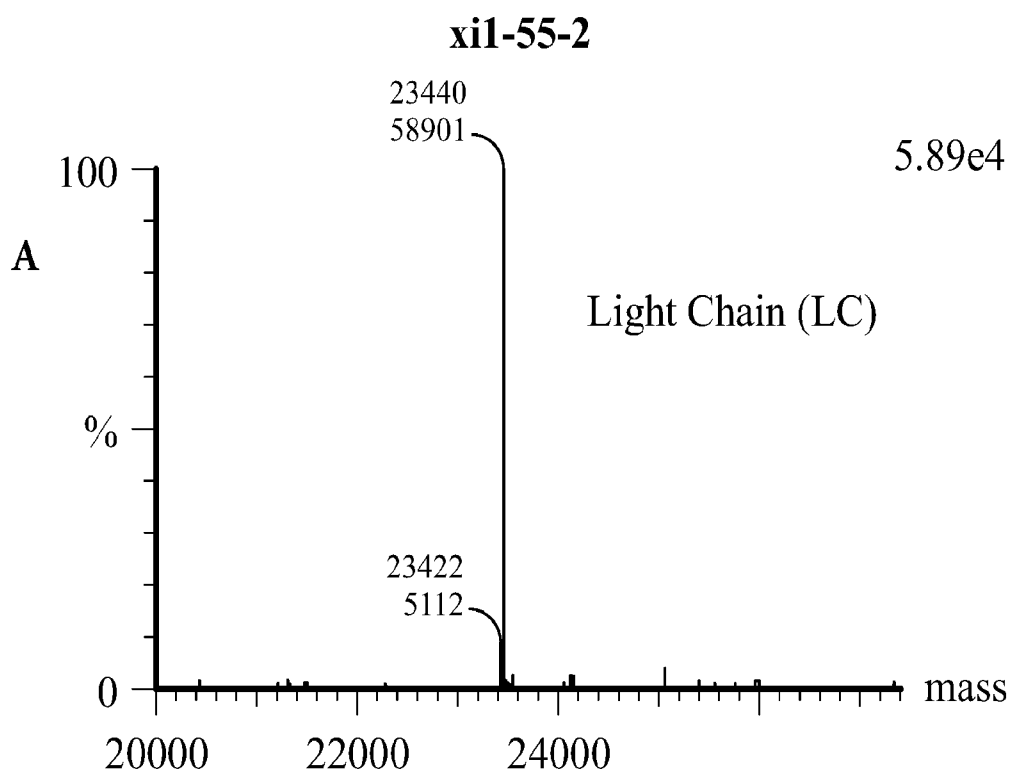


FIG. 22

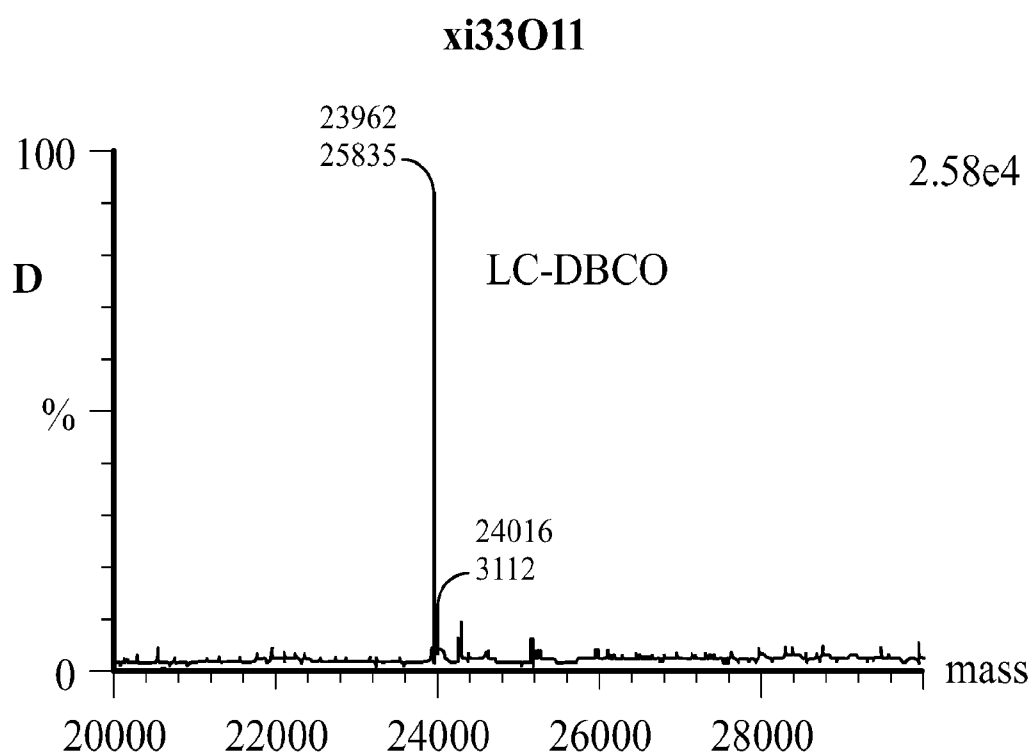
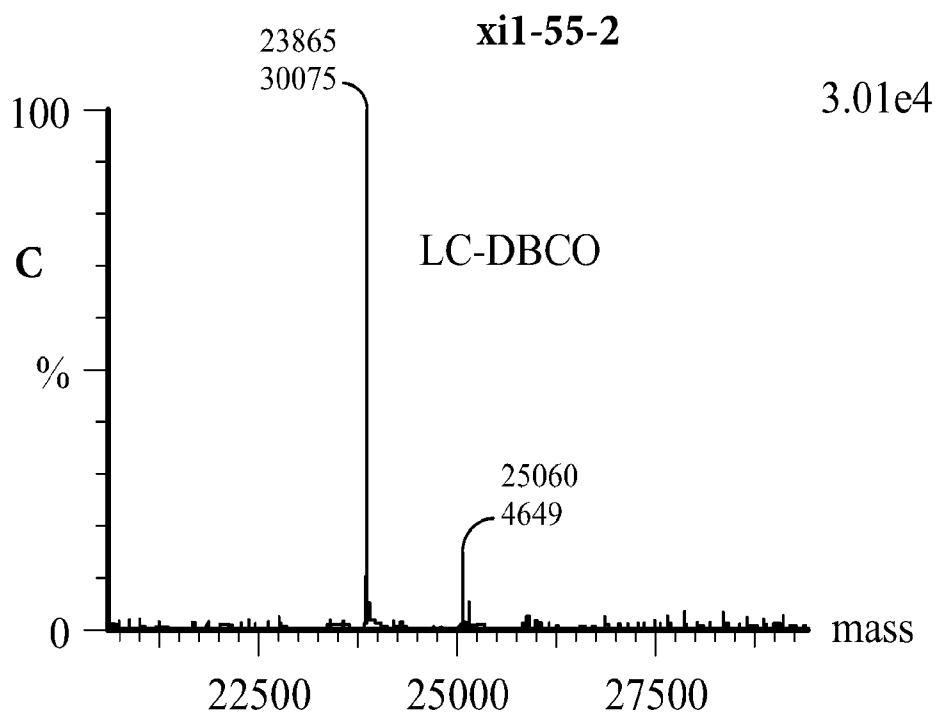


FIG. 22 (Continued)

SUBSTITUTE SHEET (RULE 26)

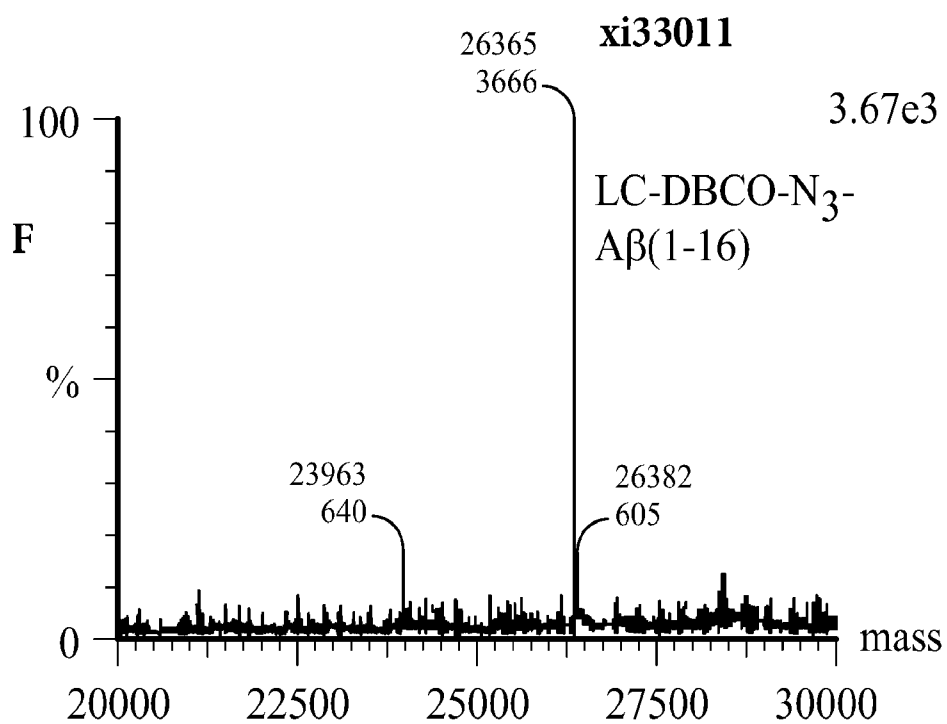
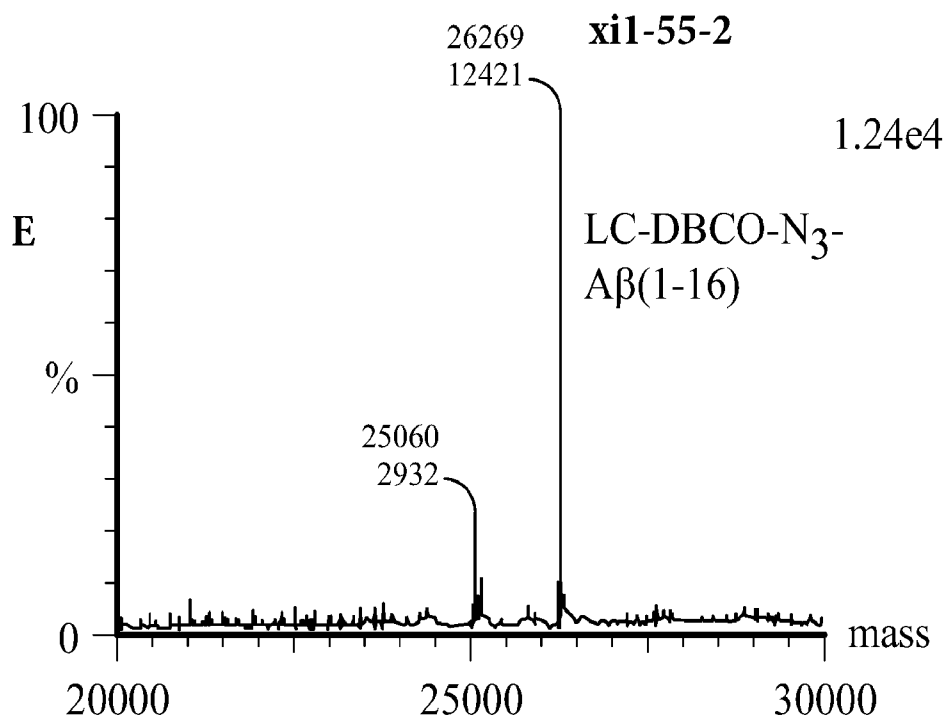


FIG. 22 (Continued)

SUBSTITUTE SHEET (RULE 26)

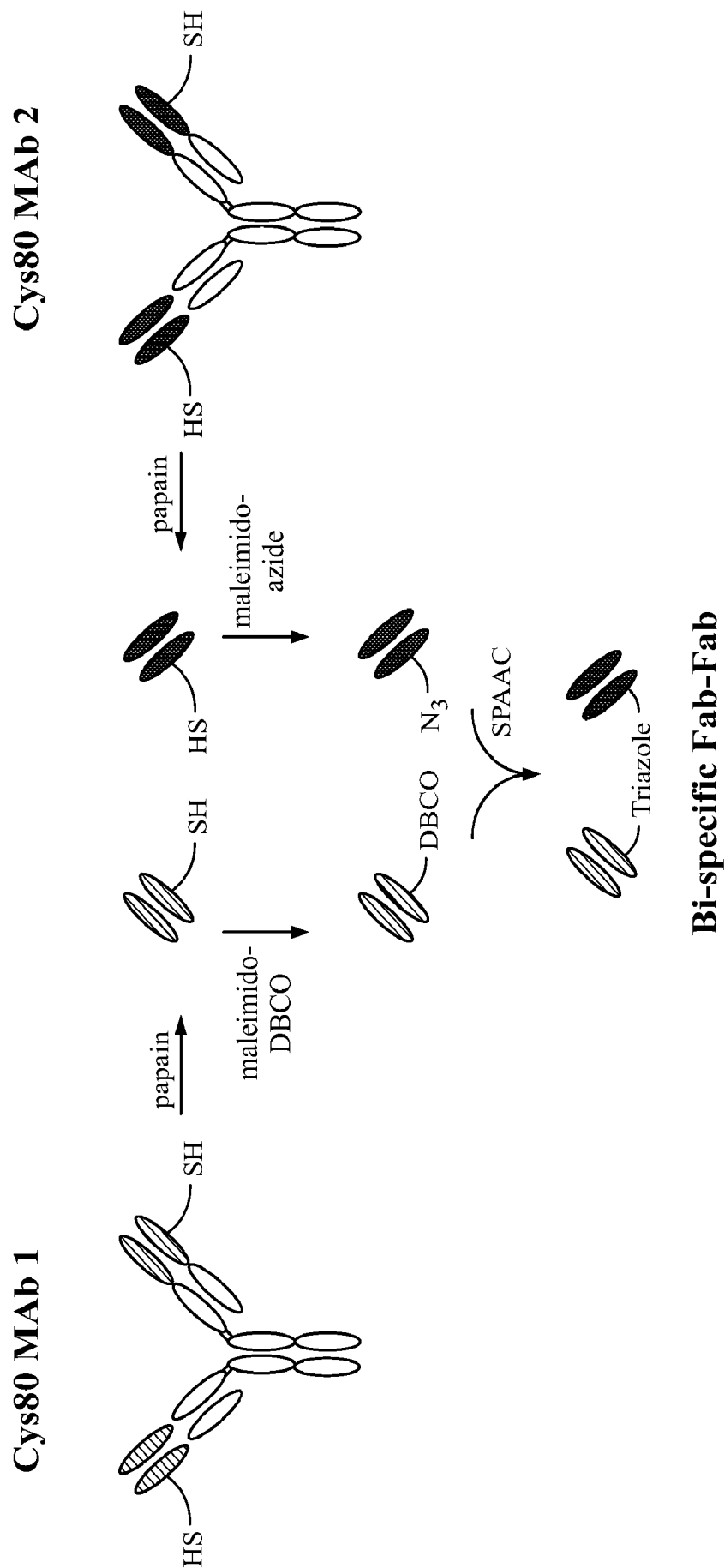


FIG. 23

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/038041

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/28 A61K47/48 A61K49/00 C07K16/30
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/136694 A1 (TECHNOPHAGE INVESTIGACAO E DES [PT]; BRAZ GONCALVES JOAO MANUEL [PT];) 13 November 2008 (2008-11-13)	1-38
Y	claims 50-51; table 9	1-38
X	WO 2011/156328 A1 (GENENTECH INC [US]; BHAKTA SUNIL [US]; JUNUTULA JAGATH R [US]) 15 December 2011 (2011-12-15) page 56; claim 1; table 4; sequence 109	1-38
X	WO 2008/144757 A1 (ALDER BIOPHARMACEUTICALS INC [US]; LATHAM JOHN [US]; KOVACEVICH BRIAN) 27 November 2008 (2008-11-27) figure 2	16,35-38
	----- -/-	



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

9 September 2016

Date of mailing of the international search report

29/09/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/038041

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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X	WO 2014/031476 A1 (US HEALTH [US]; GAO WEI [US]; HASSAN RAFFIT [US]) 27 February 2014 (2014-02-27) sequences 15,19,23 -----	1-38
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Y	LYON ROBERT P ET AL: "Chapter 6: CONJUGATION OF ANTICANCER DRUGS THROUGH ENDOGENOUS MONOCLONAL ANTIBODY CYSTEINE RESIDUES", METHODS IN ENZYMOLOGY, ACADEMIC PRESS, US, vol. 502, 1 January 2012 (2012-01-01), pages 123-138, XP008157545, ISSN: 0076-6879, DOI: 10.1016/B978-0-12-416039-2.00006-9 page 126, paragraph 1 page 124, paragraph 3 -----	1-38
X	WO 2014/033074 A1 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 6 March 2014 (2014-03-06) the whole document -----	19-24

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/038041

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
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- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
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2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/038041

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/038041

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