

CLAIMS

1. A method for quantitating an amount of a polypeptide comprising a portion of an antibody heavy chain constant region in a sample comprising:
 - (a) digesting the sample comprising the polypeptide comprising the portion of the antibody heavy chain constant region, wherein the portion of the antibody heavy chain constant region comprises an engineered mutation that is unique in non-human and human proteome backgrounds, and wherein digestion produces a peptide fragment derived from the antibody heavy chain constant region that is between 5 and 26 amino acids long and comprises the engineered mutation,
 - (b) analyzing the digested sample by mass spectrometry to determine quantity of the peptide fragment, thereby determining the quantity of the polypeptide comprising the portion of the antibody heavy chain constant region in the sample.
2. The method of claim 1, wherein the peptide fragment does not comprise a methionine (M) or cysteine (C).
3. The method of claim 1 or claim 2, wherein the peptide fragment does not comprise an asparagine (N) followed by a glycine (G) or serine (S).
4. The method of any one of claims 1-3, further comprising purifying and concentrating the digested sample prior to the mass spectrometry analysis.
5. The method of claim 4, wherein the digested sample is purified and concentrated via solid phase extraction (SPE).
6. The method of claim 4, wherein the digested sample is purified and concentrated via SISCAPA (Stable Isotope Standards and Capture by Anti-Peptide Antibodies).
7. The method of any one of claims 1-6, wherein the sample is a whole blood sample, a serum sample, a plasma sample, or a tissue sample.
8. The method of any one of claims 1-7, wherein the sample is from a mouse, a non-human primate, or a human.
9. The method of claim 8, wherein the non-human primate is a cynomolgus monkey or a rhesus monkey.
10. The method of any one of claims 1-9, wherein the sample is digested with at least one enzyme.
11. The method of claim 9, wherein the at least one enzyme is trypsin, chymotrypsin, glutamyl endopeptidase, lysyl endopeptidase, Asp-N, Arg-C, Glu-C, cyanogen bromide (CnBr), or combinations thereof.
12. The method of any one of claims 1-11, wherein the mass spectrometry is liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS).
13. The method of any one of claims 1-12, wherein the polypeptide comprises a CH1 domain, and wherein the CH1 domain comprises the engineered mutation.
14. The method of any one of claims 1-12, wherein the polypeptide comprises a CH2 domain, and wherein the CH2 domain comprises the engineered mutation.

15. The method of any one of claims 1-12, wherein the polypeptide comprises a CH3 domain, and wherein the CH3 domain comprises the engineered mutation.
16. The method of claim 15, wherein the engineered mutation in the CH3 domain of the antibody heavy chain constant region is T366Y, T366W, T366S, L368A, T394W, T394S, F405A, F405W, Y407T, Y407V, or Y407A.
17. The method of claim 16, wherein the engineered mutation in the CH3 domain of the antibody heavy chain constant region is Y407V, and wherein the CH3 domain comprises an amino acid sequence set forth in SEQ ID NO: 6 (DGSFFLVVS).
18. The method of claim 17, wherein the digestion produces a peptide fragment comprising the amino acid sequence TTPPVLDSDGSFFLVSK (SEQ ID NO: 7), DGSFFLVSKLTV (SEQ ID NO: 8), or GSFFLVSKLTVD (SEQ ID NO: 9).
19. The method of claim 18, wherein the sample is digested with trypsin, and wherein the digestion produces a peptide fragment consisting of the amino acid sequence TTPPVLDSDGSFFLVSK (SEQ ID NO: 7).
20. The method of claim 18, wherein the sample is digested with Asp-N, and wherein the digestion produces a peptide fragment consisting of the amino acid sequence DGSFFLVSKLTV (SEQ ID NO: 8).
21. The method of claim 18, wherein the sample is digested with Glu-C, and wherein the digestion produces a peptide fragment consisting of the amino acid sequence GSFFLVSKLTVD (SEQ ID NO: 9).
22. The method of any one of claims 16-21, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 4.
23. The method of claim 15, wherein the engineered mutation in the CH3 domain of the antibody heavy chain constant region is N434S.
24. The method of claim 23, wherein the sample is digested with Glu-C and trypsin, and wherein the digestion produces a peptide fragment consisting of the amino acid sequence ALHSHYTQK (SEQ ID NO: 11).
25. The method of claim 23 or 24, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 3.
26. The method of any one of claims 1-25, wherein the polypeptide comprising the portion of the antibody heavy chain constant region is an antibody, an Fc-fusion protein, or an immunoadhesin.
27. The method of claim 26, wherein the polypeptide comprising the portion of the antibody heavy chain constant region is an antibody.
28. The method of any one of claims 27, wherein the antibody is a therapeutic antibody.
29. The method of claim 27 or 28, wherein the antibody is a chimeric antibody, a humanized antibody, or a human antibody.
30. The method of any one of claims 27-29, wherein the antibody is a monospecific antibody, a bispecific antibody, a trispecific antibody, or a multispecific antibody.

31. The method of claim 30, wherein the antibody is a trispecific antibody.
32. The method of claim 31, wherein the trispecific antibody comprises four polypeptide chains that form three antigen binding sites that specifically bind one or more antigen targets or target proteins, wherein a first polypeptide comprises a structure represented by the formula: V_{L2} - L_1 - V_{L1} - L_2 -CL; the second polypeptide chain comprises a structure represented by the formula: V_{H1} - L_3 - V_{H2} - L_4 - C_{H1} -hinge- C_{H2} - C_{H3} ; the third polypeptide chain comprises a structure represented by the formula: V_{H3} - C_{H1} -hinge- C_{H2} - C_{H3} ; the fourth polypeptide chain comprises a structure represented by the formula: V_{L3} -CL, wherein V_{L1} is a first immunoglobulin light chain variable domain; V_{L2} is a second immunoglobulin light chain variable domain; V_{L3} is a third immunoglobulin light chain variable domain; V_{H1} is a first immunoglobulin heavy chain variable domain; V_{H2} is a second immunoglobulin heavy chain variable domain; V_{H3} is a third immunoglobulin heavy chain variable domain; C_L is an immunoglobulin light chain constant domain; C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and L_1 , L_2 , L_3 and L_4 are amino acid linkers; wherein the first and second polypeptides form a cross-over light chain-heavy chain pair; and wherein the second polypeptide chain or the third polypeptide chain comprises the amino acid sequence TTPVLDSDGSFFLVSK (SEQ ID NO: 7), DGSFFLVSKLTV (SEQ ID NO: 8), or GSFFLVSKLTV (SEQ ID NO: 9).
33. The method of 32, wherein the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 12; the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 13; the third polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 14, and the fourth polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 15.
34. The method of any one of claims 27-33, wherein the antibody is conjugated to a drug or a label.
35. The method of claim 34, wherein the drug is selected from a chemotherapeutic agent, a cytotoxic agent, or a growth-inhibitory agent.
36. The method of claim 35, wherein the label is a radioisotope, a fluorescent dye, or an enzyme.
37. The method of any one of claims 27-36, wherein the antibody is a human IgG antibody.
38. The method of claim 37, wherein the IgG antibody is a human IgG1 antibody or a human IgG4 antibody.
39. The method of any one of claims 27-32 and 34-38, wherein the antibody binds A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4/VTN1, B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2/MCP-1, CCL3/MIP-1a, CCL4/MIP-1b, CCL5/RANTES, CCL7/MCP-3, CCL8/mcp-2, CCL11/eotaxin, CCL15/MIP-1d, CCL17/TARC, CCL19/MIP-3b, CCL20/MIP-3a, CCL21/MIP-2, CCL24/MIP-2/eotaxin-2, CCL25/TECK, CCL26/eotaxin-3, CCR3, CCR4, CD3, CD19, CD20, CD23/FCER2, CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80/ B7-1, CD86/B7-2, CD122, CD137/41BB, CD137L, CD152/CTLA4, CD154/CD40L, CD160, CD272, CD273/ PDL2, CD274/PDL1, CD275/B7H2, CD276/B7H3, CD278/ ICOS, CD279/PD-1, CDH1/E-cadherin, chitinase, CLEC9, CLEC91, CRTH2, CSF-1/M-CSF, CSF-2/GM-CSF, CSF-3/GCSF, CX3CL1/SCYD1, CXCL12/SDF1, CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFN α , IgE, IGF1R, IL2R β , IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17R β /IL25, IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4/b4 integrin, ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2/receptor for IL33,

STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP/ IL7Ra, TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, or XCR/GPR5/CCXCR1.

40. The method of any one of claims 1-39, wherein the method is for use in pharmacokinetic study of the polypeptide comprising an antibody heavy chain constant region in a mouse, a non-human primate, and a human.