

ANTI-DRUG ANTIBODY ASSAYS

RELATED APPLICATIONS

[1] This application claims the benefit under 35 U.S.C. § 119(e) to U.S. Provisional Application No: 63/373,426, filed August 24, 2022, which is incorporated herein by reference in its entirety.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[2] Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: 280,159 byte XML Document named "10200-US01-PRI_Sequence_listing"; created on August 24, 2022.

FIELD OF THE INVENTION

[3] The present invention relates methods of detecting anti-drug antibodies and kits for using such assays.

BACKGROUND

[4] Assays for the detection of anti-drug antibodies (ADA) facilitate understanding of potential immune responses to biologic drug candidates. Determining the presence of ADAs and evaluating their clinical implications are a necessary part of any large molecule development program.

[5] All biopharmaceuticals are potentially immunogenic and may induce ADAs. The clinical effects of ADA formation can be highly variable and may cause severe adverse events that put a patient at risk. It is therefore important, both to guide drug development decision-making and as a regulatory requirement, to develop and validate ADA assays with the appropriate sensitivity, specificity, and selectivity for detection. Since ADA responses can affect the pharmacokinetics (PK), pharmacodynamics (PD), safety, and efficacy of a therapeutic candidate, ADA assays should be designed to detect antibodies that could mediate hypersensitivity responses or that have the ability to interfere with interactions between the therapeutic and its target for a neutralizing effect.

[6] In 2019, the FDA issued a Guidance for Industry titled "Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection." The guidance provides recommendations to facilitate industry's development and validation of assays for assessment of the immunogenicity of therapeutic protein products during clinical trials.

[7] There is a need to develop ADA assays that address accuracy, target interference, and at the same time, meet the 2019 FDA guidance.

SUMMARY

[8] Based on the disclosure provided herein, those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following embodiments (E).

- E1. A method of detecting an anti-drug antibody (ADA) in a sample, wherein said ADA binds to denosumab, comprising mixing said sample with:
- (a) a first antibody or antibody conjugate that binds to RANKL, comprising: the heavy chain (HC) CDR-H1, CDR-H2, and CDR-H3 of SEQ ID NO:203, and the light chain (LC) CDR-L1, CDR-L2, and CDR-L3 of SEQ ID NO:208; wherein said first antibody or antibody conjugate can be attached to a solid substrate;
 - (b) (i) a second antibody that binds to RANKL, wherein said second antibody comprises an amino acid sequence that differs from the first antibody by at least one amino acid in one of the six CDR sequences; or (ii) an osteoprotegerin (OPG) protein that binds to RANKL; and
 - (c) a third antibody that binds to said ADA and can be linked to a detectable marker.
- E2. The method of E1, wherein said sample comprises soluble RANKL that interferes with the detection of said ADA.
- E3. A method of reducing false signals of an anti-drug antibody (ADA) assay, wherein said ADA assay is to detect the presence of an anti-denosumab antibody in a sample, comprising mixing said sample with:
- (a) a first antibody or antibody conjugate that binds to RANKL, comprising: the heavy chain (HC) CDR-H1, CDR-H2, and CDR-H3 of SEQ ID NO:203, and the light chain (LC) CDR-L1, CDR-L2, and CDR-L3 of SEQ ID NO:208; wherein said first antibody or antibody conjugate can be attached to a solid substrate;
 - (b) (i) a second antibody that binds to RANKL, wherein said second antibody comprises an amino acid sequence that differs from the first antibody by at least one amino acid in one of the six CDR sequences; or (ii) an osteoprotegerin (OPG) protein that binds to RANKL; and
 - (c) a third antibody that binds to said ADA and can be linked to a detectable marker.
- E4. The method of E3, wherein said false signals are caused by the presence of soluble RANKL that interferes with the detection of said ADA.
- E5. The method of any one of E1-E4, wherein said first antibody conjugate comprises an antibody molecule covalently linked to an immobilizing moiety.

E6. The method of any one of E1-E4, wherein said first antibody conjugate comprises an antibody molecule noncovalently linked to an immobilizing moiety.

E7. The method of E5 or E6, wherein said solid substrate comprises a binding partner, and said immobilizing moiety can be attached to a solid substrate by binding to said binding partner.

E8. The method of E7, wherein said immobilizing moiety is biotin and said binding partner is streptavidin or avidin; or said immobilizing moiety is avidin or streptavidin and said binding partner is biotin.

E9. The method of E7, wherein said immobilizing moiety and said binding partner are one of the following binding pairs: an antigen and an antibody that binds to said antigen; a lectin and a polysaccharide, a steroid and a steroid binding protein, a hormone and a hormone receptor, an enzyme and a substrate of the enzyme, an IgG and Protein A, an IgG and Protein G; or a ligand and a receptor for the ligand.

E10. The method of any one of E1-E4, wherein said first antibody is immobilized to a solid substrate that comprises Protein A or Protein G.

E11. The method of any one E1-E10, wherein said first antibody or antibody conjugate comprise a CDR-H1 comprising SEQ ID NO:200, a CDR-H2 comprising SEQ ID NO:201, and a CDR-H3 comprising SEQ ID NO:202; and a CDR-L1 comprising SEQ ID NO:205, a CDR-L2 comprising SEQ ID NO:206, and a CDR-L3 comprising SEQ ID NO:207.

E12. The method of any one of E1-E11, wherein said first antibody or antibody conjugate comprises a heavy chain variable region (VH) that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:203.

E13. The method of any one of E1-E12, wherein said first antibody or antibody conjugate comprises a light chain variable region (VL) that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:208.

E14. The method of any one of E1-E13, wherein said first antibody or antibody conjugate comprises a human CH1 domain, such as a human IgG1 CH1, a human IgG2 CH1, a human IgG3 CH1, or a human IgG4 CH1.

E15. The method of E14, wherein said human IgG1 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least

88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:182.

E16. The method of E14, wherein:

said human IgG2 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:194; said human IgG3 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:190; or

said human IgG4 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:186.

E17. The method of any one of E1-E16, wherein said first antibody or antibody conjugate comprises a human Fc domain, such as a human IgG1 Fc, a human IgG2 Fc, a human IgG3 Fc, or a human IgG4 Fc.

E18. The method of E17, wherein said human IgG1 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:184.

E19. The method of E17, wherein:

said human IgG2 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:196;

said human IgG3 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:192; or

said human IgG4 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:188.

E20. The method of any one of E1-E13, wherein said first antibody or antibody conjugate comprises a murine CH1 domain, such as a murine IgG1 CH1, a murine IgG2a CH1, a murine IgG2b CH1, or a murine IgG3 CH1.

E21. The method of E20, wherein said murine IgG1 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:172.

E22. The method of E20, wherein said murine IgG2a CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:175.

E23. The method of E20, wherein said murine IgG2b CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:176.

E24. The method of E20, wherein said murine IgG3 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:177.

E25. The method of any one of E1-E13 and E20-E24, wherein said first antibody or antibody conjugate comprises a murine Fc domain, such as a murine IgG1 Fc, a murine IgG2a Fc, a murine IgG2b Fc, or a murine IgG3 Fc.

E26. The method of E25, wherein said murine IgG1 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:174.

E27. The method of E25, wherein said murine IgG2a Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:175.

E28. The method of E25, wherein said murine IgG2b Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:176.

E29. The method of E25, wherein said murine IgG3 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:177.

E30. The method of any one of E1-E29, wherein said first antibody or antibody conjugate comprises a human CL domain, such as a human kappa CL, or a human lambda CL.

E31. The method of E30, wherein said human kappa CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:178 or 179.

E32. The method of E30, wherein said human lambda CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:180 or 181.

E33. The method of any one of E1-E29, wherein said first antibody or antibody conjugate comprises a murine CL domain, such as a murine kappa CL, or a murine lambda CL.

E34. The method of E33, wherein said murine kappa CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:171.

E35. The method of E33, wherein said murine lambda CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:169 or 170.

E36. The method of any one of E1-E35, wherein said third antibody is covalently linked to a detectable marker.

E37. The method of any one of E1-E35, wherein said third antibody is noncovalently linked to a detectable marker.

E38. The method of any one of E1-E37, wherein said detectable marker is an isotope, an enzyme, a fluorescent moiety, a luminescent moiety, a chromatic moiety, a metal, or an electric charge.

E39. The method of any one of E1-E38, wherein said third antibody comprises six CDR sequences that are identical to that of the first antibody.

E40. The method of any one of E1-E39, wherein said third antibody comprise:
(i) the heavy chain (HC) CDR-H1, CDR-H2, and CDR-H3 of SEQ ID NO:203, and the light chain (LC) CDR-L1, CDR-L2, and CDR-L3 of SEQ ID NO:208; or
(ii) a CDR-H1 comprising SEQ ID NO:200, a CDR-H2 comprising SEQ ID NO:201, and a CDR-H3 comprising SEQ ID NO:202; and a CDR-L1 comprising SEQ ID NO:205, a CDR-L2 comprising SEQ ID NO:206, and a CDR-L3 comprising SEQ ID NO:207.

E41. The method of any one of E1-E40, wherein said third antibody comprises a heavy chain variable region (VH) that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:203.

E42. The method of any one of E1-E41, wherein said third antibody comprises a light chain variable region (VL) that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:208.

E43. The method of any one of E1-E42, wherein said third antibody comprises a human CH1 domain, such as a human IgG1 CH1, a human IgG2 CH1, a human IgG3 CH1, or a human IgG4 CH1.

E44. The method of E43, wherein said human IgG1 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:182.

E45. The method of E43, wherein:
said human IgG2 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:194;

said human IgG3 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:190; or
said human IgG4 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:186.

E46. The method of any one of E1-E45, wherein said third antibody comprises a human Fc domain, such as a human IgG1 Fc, a human IgG2 Fc, a human IgG3 Fc, or a human IgG4 Fc.

E47. The method of E46, wherein said human IgG1 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:184.

E48. The method of E46, wherein

said human IgG2 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:196;

said human IgG3 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:192; or

said human IgG4 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:188.

E49. The method of any one of E1-E42, wherein said third antibody comprises a murine CH1 domain, such as a murine IgG1 CH1, a murine IgG2a CH1, a murine IgG2b CH1, or a murine IgG3 CH1.

E50. The method of E49, wherein said murine IgG1 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:172.

E51. The method of E49, wherein said murine IgG2a CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:175.

E52. The method of E49, wherein said murine IgG2b CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:176.

E53. The method of E49, wherein said murine IgG3 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:177.

E54. The method of any one of E1-E42 and E49-E53, wherein said third antibody comprises a murine Fc domain, such as a murine IgG1 Fc, a murine IgG2a Fc, a murine IgG2b Fc, or a murine IgG3 Fc.

E55. The method of E54, wherein said murine IgG1 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:174.

E56. The method of E54, wherein said murine IgG2a Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:175.

E57. The method of E54, wherein said murine IgG2b Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:176.

E58. The method of E54, wherein said murine IgG3 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least

88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:177.

E59. The method of any one of E1-E58, wherein said third antibody comprises a human CL domain, such as a human kappa CL, or a human lambda CL.

E60. The method of E59, wherein said human kappa CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:178 or 179.

E61. The method of E59, wherein said human lambda CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:180 or 181.

E62. The method of any one of E1-E58, wherein said third antibody comprises a murine CL domain, such as a murine kappa CL, or a murine lambda CL.

E63. The method of E62, wherein said murine kappa CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:171.

E64. The method of E62, wherein said murine lambda CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:169 or 170.

E65. The method of any one of E1-E64, wherein said third antibody comprises a VH domain comprising the same sequence as the VH of the first antibody, and a VL domain comprising the same sequence as the VL domain of the first antibody.

E66. The method of any one of E1-E65, wherein said third antibody comprises a heavy chain (HC) comprising the same sequence as the HC of the first antibody, and a light chain (LC) comprising the same sequence as the LC of the first antibody.

E67. The method of any one of E1-E66, wherein said detectable marker comprises a secondary antibody that binds to a constant domain of said third antibody.

- E68. The method of any one of E1-E67, wherein said detectable marker comprises a secondary antibody that binds to the Fc domain of said third antibody.
- E69. The method of any one of E1-E66, wherein said third antibody is covalently linked to a fluorescent moiety, a luminescent moiety, or a chromatic moiety.
- E70. The method of any one of E1-E69, wherein said second antibody binds to the same epitope as denosumab.
- E71. The method of any one of E1-E70, wherein said second antibody competes with denosumab for RANKL binding.
- E72. The method of any one of E1-E71, wherein said second antibody comprises an amino acid sequence that differs from the first antibody by at least one amino acid in each of the six CDR sequences.
- E73. The method of any one of E1-E72, wherein said second antibody comprises an amino acid sequence that differs from the first antibody by at least two, at least three, at least four, at least five, at least six amino acids in CDR sequences.
- E74. The method of any one of E1-E73, wherein said ADA does not bind to said second antibody.
- E75. The method of any one of E1-E74, wherein said second antibody comprises: (i) the heavy chain CDR-H1, CDR-H2, and CDR-H3 of SEQ ID NO: 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, or 167; and (ii) the light chain CDR-L1, CDR-L2, and CDR-L3 of SEQ ID NO: 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, or 168.
- E76. The method of any one of E1-E75, wherein said second antibody comprises:
- (i) a CDR-H1 comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 1, 7, 13, 19, 25, 31, 37, 43, 49, 55, 61, 67, 73, 79, 85, 91, 97, 103, 109, 115, or 121;
 - (ii) a CDR-H2 comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 2, 8, 14, 20, 26, 32, 38, 44, 50, 56, 62, 68, 74, 80, 86, 92, 98, 104, 110, 116, or 122;
 - (iii) a CDR-H3 comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 3, 9, 15, 21, 27, 33, 39, 45, 51, 57, 63, 69, 75, 81, 87, 93, 99, 105, 111, 117, or 123;

- (iv) a CDR-L1 comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:4, 10, 16, 22, 28, 34, 40, 46, 52, 58, 64, 70, 76, 82, 88, 94, 100, 106, 112, 118, or 124;
- (v) a CDR-L2 comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:5, 11, 17, 23, 29, 35, 41, 47, 53, 59, 65, 71, 77, 83, 89, 95, 101, 107, 113, 119, or 125; and
- (vi) a CDR-L3 comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120, or 126.
- E77. The method of any one of E1-E76, wherein said second antibody comprises:
- (1) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 1-6, respectively;
 - (2) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 6-12, respectively;
 - (3) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 13-18, respectively;
 - (4) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 19-24, respectively;
 - (5) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 25-30, respectively;
 - (6) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 31-36, respectively;
 - (7) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 37-42, respectively;
 - (8) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 43-48, respectively;
 - (9) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 49-54, respectively;
 - (10) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 55-60, respectively;
 - (11) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 61-66, respectively;
 - (12) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 67-72, respectively;

(13) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 73-78, respectively;

(14) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 79-84, respectively;

(15) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 85-90, respectively;

(16) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 91-96, respectively;

(17) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 97-102, respectively;

(18) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 103-108, respectively;

(19) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 109-114, respectively;

(20) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 115-120, respectively; or

(21) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 121-126, respectively.

E78. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 1-6, respectively.

E79. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 6-12, respectively.

E80. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 13-18, respectively.

E81. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 19-24, respectively.

E82. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 25-30, respectively.

E83. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 31-36, respectively.

E84. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 37-42, respectively.

E85. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 43-48, respectively.

E86. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 49-54, respectively.

E87. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 55-60, respectively.

E88. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 61-66, respectively.

E89. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 67-72, respectively.

E90. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 73-78, respectively.

E91. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 79-84, respectively.

E92. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 85-90, respectively.

E93. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 91-96, respectively.

E94. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 97-102, respectively.

E95. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 103-108, respectively.

E96. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 109-114, respectively.

E97. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 115-120, respectively.

E98. The method of E77, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 121-126, respectively.

E99. The method of any one of E1-E77, wherein said second antibody comprises a heavy chain variable region (VH) that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, or 167.

E100. The method of any one of E1-E78, wherein said second antibody comprises a light chain variable region (VL) that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, or 168.

E101. The method of any one of E1-E100, wherein said second antibody comprises:

(1) a VH comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 127, and a VL comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 128;

(2) a VH comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 129, and a VL comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 130;

(3) a VH comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 131, and a VL comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 132;

(4) a VH comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 133, and a VL comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 134;

(5) a VH comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 135, and a VL comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 136;

(6) a VH comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical

that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 164.

E121. The method of E101, wherein said second antibody comprises a VH comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 165, and a VL comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 166.

E122. The method of E101, wherein said second antibody comprises a VH comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 167, and a VL comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 168.

E123. The method of any one of E1-E122, wherein said second antibody comprises a human CH1 domain, such as a human IgG1 CH1, a human IgG2 CH1, a human IgG3 CH1, or a human IgG4 CH1.

E124. The method of E123, wherein said human IgG1 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:182.

E125. The method of E123, wherein:

said human IgG2 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:194;

said human IgG3 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:190; or

said human IgG4 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:186.

E126. The method of any one of E1-E125, wherein said second antibody comprises a human Fc domain, such as a human IgG1 Fc, a human IgG2 Fc, a human IgG3 Fc, or a human IgG4 Fc.

E127. The method of E126, wherein said human IgG1 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:184.

E128. The method of E126, wherein

said human IgG2 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:196;

said human IgG3 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:192; or

said human IgG4 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:188.

E129. The method of any one of E1-E122, wherein said second antibody comprises a murine CH1 domain, such as a murine IgG1 CH1, a murine IgG2a CH1, a murine IgG2b CH1, or a murine IgG3 CH1.

E130. The method of E129, wherein said murine IgG1 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:172.

E131. The method of E129, wherein said murine IgG2a CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:175.

E132. The method of E129, wherein said murine IgG2b CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:176.

E133. The method of E129, wherein said murine IgG3 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:177.

E134. The method of any one of E1-E122 and E129-E133, wherein said second antibody comprises a murine Fc domain, such as a murine IgG1 Fc, a murine IgG2a Fc, a murine IgG2b Fc, or a murine IgG3 Fc.

E135. The method of E134, wherein said murine IgG1 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:174.

E136. The method of E134, wherein said murine IgG2a Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:175.

E137. The method of E134, wherein said murine IgG2b Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:176.

E138. The method of E134, wherein said murine IgG3 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:177.

E139. The method of any one of E1-E138, wherein said second antibody comprises a human CL domain, such as a human kappa CL, or a human lambda CL.

E140. The method of E139, wherein said human kappa CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:178 or 179.

E141. The method of E139, wherein said human lambda CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:180 or 181.

E142. The method of any one of E1-E138, wherein said second antibody comprises a murine CL domain, such as a murine kappa CL, or a murine lambda CL.

E143. The method of E142, wherein said murine kappa CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:171.

E144. The method of E142, wherein said murine lambda CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:169 or 170.

E145. The method of any one of E1-E144, wherein said second antibody binds to RANKL with a KD value of 10^{-4} M or less, 10^{-5} M or less, 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, or 10^{-9} M or less.

E146. The method of any one of E1-E145, wherein said sample is pre-treated with said second antibody, when mixed with said first antibody or antibody conjugate and said third antibody.

E147. The method of any one of E1-E145, wherein said sample is pre-treated with said OPG, when mixed with said first antibody or antibody conjugate and said third antibody.

E148. The method of any one of E1-E145, wherein said sample is mixed with said first antibody or antibody conjugate, said second antibody, and said third antibody simultaneously.

E149. The method of any one of E1-E145, wherein said sample is mixed with said first antibody or antibody conjugate, said OPG, and said third antibody complex simultaneously.

E150. The method of any one of E1-E149, wherein said RANKL is human RANKL.

E151. The method of any one of E1-E150, wherein both said first antibody and second antibody bind to a human RANKL epitope comprising SEQ ID NO:214 (DLATE).

E152. The method of any one of E1-E150, wherein both said first antibody and second antibody bind to a human RANKL epitope comprising one or more residues from SEQ ID NO:215.

E153. A diagnostic kit for detecting an anti-drug antibody (ADA) in a sample, wherein said ADA binds to denosumab, comprising:

- (a) a first antibody or antibody conjugate that binds to RANKL, comprising: the heavy chain (HC) CDR-H1, CDR-H2, and CDR-H3 of SEQ ID NO:203, and the light chain (LC) CDR-L1, CDR-L2, and CDR-L3 of SEQ ID NO:208; wherein said first antibody or antibody conjugate can be attached to a solid substrate;
- (b) (i) a second antibody that binds to RANKL, wherein said second antibody comprises an amino acid sequence that differs from the first antibody by at least one amino acid in one of the six CDR sequences; or (ii) an osteoprotegerin (OPG) protein that binds to RANKL;
- (c) a third antibody that binds to said ADA and can be linked to a detectable marker; and
- (d) instructions for use.

E154. The diagnostic kit of E153, wherein said first antibody conjugate comprises an antibody molecule covalently linked to an immobilizing moiety.

E155. The diagnostic kit of E153 or E154, wherein said first antibody conjugate comprises an antibody molecule noncovalently linked to an immobilizing moiety.

E156. The diagnostic kit of E154 or E155, wherein said solid substrate comprises a binding partner, and said immobilizing moiety can be attached to a solid substrate by binding to said binding partner.

E157. The diagnostic kit of E156, wherein said immobilizing moiety is biotin and said binding partner is streptavidin or avidin; or said immobilizing moiety is avidin or streptavidin and said binding partner is biotin.

E158. The diagnostic kit of E156, wherein said immobilizing moiety and said binding partner are one of the following binding pairs: an antigen and an antibody that binds to said antigen; a lectin and a polysaccharide, a steroid and a steroid binding protein, a hormone and a hormone receptor, an enzyme and a substrate of the enzyme, an IgG and Protein A, an IgG and Protein G; or a ligand and a receptor for the ligand.

E159. The diagnostic kit of E153, wherein said first antibody is immobilized to a solid substrate that comprises Protein A or Protein G.

E160. The diagnostic kit of any one E153-E159, wherein said first antibody or antibody conjugate comprise a CDR-H1 comprising SEQ ID NO:200, a CDR-H2 comprising SEQ ID NO:201, and a CDR-H3 comprising SEQ ID NO:202; and a CDR-L1 comprising SEQ ID NO:205, a CDR-L2 comprising SEQ ID NO:206, and a CDR-L3 comprising SEQ ID NO:207.

E161. The diagnostic kit of any one E153-E160, wherein said first antibody or antibody conjugate comprises a heavy chain variable region (VH) that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:203.

E162. The diagnostic kit of any one E153-E161, wherein said first antibody or antibody conjugate comprises a light chain variable region (VL) that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:208.

E163. The diagnostic kit of any one E153-E162, wherein said first antibody or antibody conjugate comprises a human CH1 domain, such as a human IgG1 CH1, a human IgG2 CH1, a human IgG3 CH1, or a human IgG4 CH1.

E164. The diagnostic kit of E163, wherein said human IgG1 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:182.

E165. The diagnostic kit of E163, wherein:

said human IgG2 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:194; said human IgG3 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:190; or

said human IgG4 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:186.

E166. The diagnostic kit of any one of E153-E165, wherein said first antibody or antibody conjugate comprises a human Fc domain, such as a human IgG1 Fc, a human IgG2 Fc, a human IgG3 Fc, or a human IgG4 Fc.

E167. The diagnostic kit of E166, wherein said human IgG1 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:184.

E168. The diagnostic kit of E166, wherein:

said human IgG2 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:196;

said human IgG3 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:192; or

said human IgG4 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:188.

E169. The diagnostic kit of any one of E153-E162, wherein said first antibody or antibody conjugate comprises a murine CH1 domain, such as a murine IgG1 CH1, a murine IgG2a CH1, a murine IgG2b CH1, or a murine IgG3 CH1.

E170. The diagnostic kit of E169, wherein said murine IgG1 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:172.

E171. The diagnostic kit of E169, wherein said murine IgG2a CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:175.

E172. The diagnostic kit of E169, wherein said murine IgG2b CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:176.

E173. The diagnostic kit of E169, wherein said murine IgG3 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:177.

E174. The diagnostic kit of any one of E153-E162 and E169-E173, wherein said first antibody or antibody conjugate comprises a murine Fc domain, such as a murine IgG1 Fc, a murine IgG2a Fc, a murine IgG2b Fc, or a murine IgG3 Fc.

E175. The diagnostic kit of E174, wherein said murine IgG1 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:174.

E176. The diagnostic kit of E174, wherein said murine IgG2a Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:175.

E177. The diagnostic kit of E174, wherein said murine IgG2b Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:176.

E178. The diagnostic kit of E174, wherein said murine IgG3 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:177.

E179. The diagnostic kit of any one of E153-E178, wherein said first antibody or antibody conjugate comprises a human CL domain, such as a human kappa CL, or a human lambda CL.

E180. The diagnostic kit of E179, wherein said human kappa CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:178 or 179.

E181. The diagnostic kit of E179, wherein said human lambda CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:180 or 181.

E182. The diagnostic kit of any one of E153-E178, wherein said first antibody or antibody conjugate comprises a murine CL domain, such as a murine kappa CL, or a murine lambda CL.

E183. The diagnostic kit of E182, wherein said murine kappa CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:171.

E184. The diagnostic kit of E182, wherein said murine lambda CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:169 or 170.

E185. The diagnostic kit of any one of E153-E184, wherein said third antibody is covalently linked to a detectable marker.

E186. The diagnostic kit of any one of E153-E184, wherein said third antibody is noncovalently linked to a detectable marker.

E187. The diagnostic kit of any one of E153-E186, wherein said detectable marker is an isotope, an enzyme, a fluorescent moiety, a luminescent moiety, a chromatic moiety, a metal, or an electric charge.

E188. The diagnostic kit of any one of E153-E187, wherein said third antibody comprises six CDR sequences that are identical to that of the first antibody.

E189. The diagnostic kit of any one E153-E188, wherein said third antibody comprise:

- (i) the heavy chain (HC) CDR-H1, CDR-H2, and CDR-H3 of SEQ ID NO:203, and the light chain (LC) CDR-L1, CDR-L2, and CDR-L3 of SEQ ID NO:208; or
- (ii) a CDR-H1 comprising SEQ ID NO:200, a CDR-H2 comprising SEQ ID NO:201, and a CDR-H3 comprising SEQ ID NO:202; and a CDR-L1 comprising SEQ ID NO:205, a CDR-L2 comprising SEQ ID NO:206, and a CDR-L3 comprising SEQ ID NO:207.

E190. The diagnostic kit of any one of E153-E189, wherein said third antibody comprises a heavy chain variable region (VH) that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:203.

E191. The diagnostic kit of any one of E153-E190, wherein said third antibody comprises a light chain variable region (VL) that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:208.

E192. The diagnostic kit of any one of E153-E191, wherein said third antibody comprises a human CH1 domain, such as a human IgG1 CH1, a human IgG2 CH1, a human IgG3 CH1, or a human IgG4 CH1.

E193. The diagnostic kit of E192, wherein said human IgG1 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:182.

E194. The diagnostic kit of E192, wherein:

said human IgG2 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:194;

said human IgG3 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:190; or

said human IgG4 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:186.

E195. The diagnostic kit of any one of E192-E194, wherein said third antibody comprises a human Fc domain, such as a human IgG1 Fc, a human IgG2 Fc, a human IgG3 Fc, or a human IgG4 Fc.

E196. The diagnostic kit of E195, wherein said human IgG1 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:184.

E197. The diagnostic kit of E195, wherein

said human IgG2 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:196;

said human IgG3 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:192; or

said human IgG4 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:188.

E198. The diagnostic kit of any one of E153-E191, wherein said third antibody comprises a murine CH1 domain, such as a murine IgG1 CH1, a murine IgG2a CH1, a murine IgG2b CH1, or a murine IgG3 CH1.

E199. The diagnostic kit of E198, wherein said murine IgG1 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:172.

E200. The diagnostic kit of E198, wherein said murine IgG2a CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:175.

E201. The diagnostic kit of E198, wherein said murine IgG2b CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:176.

E202. The diagnostic kit of E198, wherein said murine IgG3 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:177.

E203. The diagnostic kit of any one of E153-E191 and E198-E202, wherein said third antibody comprises a murine Fc domain, such as a murine IgG1 Fc, a murine IgG2a Fc, a murine IgG2b Fc, or a murine IgG3 Fc.

E204. The diagnostic kit of E203, wherein said murine IgG1 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:174.

E205. The diagnostic kit of E203, wherein said murine IgG2a Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:175.

E206. The diagnostic kit of E203, wherein said murine IgG2b Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:176.

E207. The diagnostic kit of E203, wherein said murine IgG3 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:177.

E208. The diagnostic kit of any one of E153-E207, wherein said third antibody comprises a human CL domain, such as a human kappa CL, or a human lambda CL.

E209. The diagnostic kit of E208, wherein said human kappa CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:178 or 179.

E210. The diagnostic kit of E208, wherein said human lambda CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:180 or 181.

E211. The diagnostic kit of any one of E153-E207, wherein said third antibody comprises a murine CL domain, such as a murine kappa CL, or a murine lambda CL.

E212. The diagnostic kit of E211, wherein said murine kappa CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:171.

E213. The diagnostic kit of E211, wherein said murine lambda CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:169 or 170.

E214. The diagnostic kit of any one of E153-E213, wherein said third antibody comprises a VH domain comprising the same sequence as the VH of the first antibody, and a VL domain comprising the same sequence as the VL domain of the first antibody.

E215. The diagnostic kit of any one of E153-E214, wherein said third antibody comprises a heavy chain (HC) comprising the same sequence as the HC of the first antibody, and a light chain (LC) comprising the same sequence as the LC of the first antibody.

E216. The diagnostic kit of any one of E153-E215, wherein said detectable marker comprises a secondary antibody that binds to a constant domain of said third antibody.

E217. The diagnostic kit of any one of E153-E216, wherein said detectable marker comprises a secondary antibody that binds to the Fc domain of said third antibody.

E218. The diagnostic kit of any one of E153-E215, wherein said third antibody is covalently linked to a fluorescent moiety, a luminescent moiety, or a chromatic moiety.

E219. The diagnostic kit of any one of E153-E218, wherein said second antibody binds to the same epitope as denosumab.

E220. The diagnostic kit of any one of E153-E219, wherein said second antibody competes with denosumab for RANKL binding.

E221. The diagnostic kit of any one of E153-E220, wherein said second antibody comprises an amino acid sequence that differs from the first antibody by at least one amino acid in each of the six CDR sequences.

E222. The diagnostic kit of any one of E153-E221, wherein said second antibody comprises an amino acid sequence that differs from the first antibody by at least two, at least three, at least four, at least five, at least six amino acids in CDR sequences.

E223. The diagnostic kit of any one of E153-E222, wherein said ADA does not bind to said second antibody.

E224. The diagnostic kit of any one of E153-E223, wherein said second antibody comprises: (i) the heavy chain CDR-H1, CDR-H2, and CDR-H3 of SEQ ID NO: 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, or 167; and (ii) the light chain CDR-L1, CDR-L2, and CDR-L3 of SEQ ID NO: 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, or 168.

E225. The diagnostic kit of any one of E153-E224, wherein said second antibody comprises:

- (i) a CDR-H1 comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 1, 7, 13, 19, 25, 31, 37, 43, 49, 55, 61, 67, 73, 79, 85, 91, 97, 103, 109, 115, or 121;
- (ii) a CDR-H2 comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 2, 8, 14, 20, 26, 32, 38, 44, 50, 56, 62, 68, 74, 80, 86, 92, 98, 104, 110, 116, or 122;
- (iii) a CDR-H3 comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 3, 9, 15, 21, 27, 33, 39, 45, 51, 57, 63, 69, 75, 81, 87, 93, 99, 105, 111, 117, or 123;
- (iv) a CDR-L1 comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 4, 10, 16, 22, 28, 34, 40, 46, 52, 58, 64, 70, 76, 82, 88, 94, 100, 106, 112, 118, or 124;
- (v) a CDR-L2 comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 5, 11, 17, 23, 29, 35, 41, 47, 53, 59, 65, 71, 77, 83, 89, 95, 101, 107, 113, 119, or 125; and
- (vi) a CDR-L3 comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120, or 126.

E226. The diagnostic kit of any one of E153-E225, wherein said second antibody comprises:

- (1) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 1-6, respectively;

- (2) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 6-12, respectively;
- (3) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 13-18, respectively;
- (4) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 19-24, respectively;
- (5) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 25-30, respectively;
- (6) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 31-36, respectively;
- (7) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 37-42, respectively;
- (8) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 43-48, respectively;
- (9) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 49-54, respectively;
- (10) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 55-60, respectively;
- (11) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 61-66, respectively;
- (12) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 67-72, respectively;
- (13) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 73-78, respectively;
- (14) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 79-84, respectively;
- (15) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 85-90, respectively;
- (16) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 91-96, respectively;
- (17) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 97-102, respectively;
- (18) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 103-108, respectively;
- (19) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 109-114, respectively;

(20) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 115-120, respectively; or

(21) a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 121-126, respectively.

E227. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 1-6, respectively.

E228. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 6-12, respectively.

E229. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 13-18, respectively.

E230. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 19-24, respectively.

E231. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 25-30, respectively.

E232. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 31-36, respectively.

E233. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 37-42, respectively.

E234. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 43-48, respectively.

E235. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 49-54, respectively.

E236. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 55-60, respectively.

E237. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 61-66, respectively.

E238. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 67-72, respectively.

E239. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 73-78, respectively.

E240. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 79-84, respectively.

E241. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 85-90, respectively.

E242. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 91-96, respectively.

E243. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 97-102, respectively.

E244. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 103-108, respectively.

E245. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 109-114, respectively.

E246. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 115-120, respectively.

E247. The diagnostic kit of E226, wherein said second antibody comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 comprising SEQ ID NOs. 121-126, respectively.

E248. The diagnostic kit of any one of E153-E247, wherein said second antibody comprises a heavy chain variable region (VH) that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, or 167.

E249. The diagnostic kit of any one of E153-E248, wherein said second antibody comprises a light chain variable region (VL) that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, or 168.

E250. The diagnostic kit of any one of E153-E249, wherein said second antibody comprises:
(1) a VH comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 127, and a VL comprising a sequence that is at least 90%, at least 91%, at least

E272. The diagnostic kit of any one of E153-E271, wherein said second antibody comprises a human CH1 domain, such as a human IgG1 CH1, a human IgG2 CH1, a human IgG3 CH1, or a human IgG4 CH1.

E273. The diagnostic kit of E272, wherein said human IgG1 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:182.

E274. The diagnostic kit of E272, wherein:

said human IgG2 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:194;

said human IgG3 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:190; or

said human IgG4 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:186.

E275. The diagnostic kit of any one of E153-E274, wherein said second antibody comprises a human Fc domain, such as a human IgG1 Fc, a human IgG2 Fc, a human IgG3 Fc, or a human IgG4 Fc.

E276. The diagnostic kit of E275, wherein said human IgG1 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:184.

E277. The diagnostic kit of E275, wherein

said human IgG2 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:196;

said human IgG3 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at

least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:192; or
said human IgG4 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:188.

E278. The diagnostic kit of any one of E153-E271, wherein said second antibody comprises a murine CH1 domain, such as a murine IgG1 CH1, a murine IgG2a CH1, a murine IgG2b CH1, or a murine IgG3 CH1.

E279. The diagnostic kit of E278, wherein said murine IgG1 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:172.

E280. The diagnostic kit of E278, wherein said murine IgG2a CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:175.

E281. The diagnostic kit of E278, wherein said murine IgG2b CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:176.

E282. The diagnostic kit of E278, wherein said murine IgG3 CH1 comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the CH1 domain of SEQ ID NO:177.

E283. The diagnostic kit of any one of E153-E271 and E278-E282, wherein said second antibody comprises a murine Fc domain, such as a murine IgG1 Fc, a murine IgG2a Fc, a murine IgG2b Fc, or a murine IgG3 Fc.

E284. The diagnostic kit of E283, wherein said murine IgG1 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at

least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:174.

E285. The diagnostic kit of E283, wherein said murine IgG2a Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:175.

E286. The diagnostic kit of E283, wherein said murine IgG2b Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:176.

E287. The diagnostic kit of E283, wherein said murine IgG3 Fc comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the Fc domain of SEQ ID NO:177.

E288. The diagnostic kit of any one of E153-E287, wherein said second antibody comprises a human CL domain, such as a human kappa CL, or a human lambda CL.

E289. The diagnostic kit of E288, wherein said human kappa CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:178 or 179.

E290. The diagnostic kit of E288, wherein said human lambda CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:180 or 181.

E291. The diagnostic kit of any one of E153-E287, wherein said second antibody comprises a murine CL domain, such as a murine kappa CL, or a murine lambda CL.

E292. The diagnostic kit of E291, wherein said murine kappa CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at

least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:171.

E293. The diagnostic kit of E291, wherein said murine lambda CL comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:169 or 170.

E294. The diagnostic kit of any one of E153-E293, wherein said second antibody binds to RANKL with a KD value of 10^{-4} M or less, 10^{-5} M or less, 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, or 10^{-9} M or less.

E295. The diagnostic kit of any one of E153-E294, wherein said RANKL is human RANKL.

E296. The diagnostic kit of any one of E153-E295, wherein both said first antibody and second antibody binds to a human RANKL epitope comprising SEQ ID NO:214 (DLATE).

E297. The diagnostic kit of any one of E153-E295, wherein both said first antibody and second antibody binds to a human RANKL epitope comprising one or more residues from SEQ ID NO:215.

E298. A diagnosis kit for use in a method of any one of E1-E152.

BRIEF DESCRIPTION OF THE DRAWINGS

[9] FIG. 1A illustrates an exemplary ADA assay. FIG. 1B illustrates that soluble RANKL interferes with the ADA assay.

[10] FIGs. 2A and 2B illustrate that a second anti-RANKL antibody, or OPG, can reduce signal interference by sequestering soluble RANKL.

DETAILED DESCRIPTION

1. Anti-Drug Antibody (ADA) Assays Overview

[11] In one aspect, the invention provides a method of detecting an anti-drug antibody (ADA) against denosumab in a sample. In general, the sample is obtained from a patient who has been treated with denosumab.

[12] Denosumab (trade names Prolia® and Xgeva®) is a human monoclonal antibody that target RANKL. It comprises a heavy chain comprising SEQ ID NO:198, and a light chain comprising SEQ ID NO:199. Prolia is indicated for the treatment of postmenopausal women with osteoporosis at high risk for fracture, the treatment to increase bone mass in men with osteoporosis at high risk for fracture, the

treatment of glucocorticoid-induced osteoporosis in men and women at high risk of fracture, the treatment to increase bone mass in men at high risk for fracture receiving androgen deprivation therapy for nonmetastatic prostate cancer, and the treatment to increase bone mass in women at high risk for fracture receiving adjuvant aromatase inhibitor therapy for breast cancer. Xgeva is a prescription medicine used to prevent fracture, spinal cord compression, or the need for radiation or surgery to bone in patients with multiple myeloma and in patients with bone metastases from solid tumors.

[13] As taught and exemplified in the Examples, in one study, an unusually high incidence of immunoreactivity in the anti-drug antibody (ADA) assay against denosumab was observed. Based on past experience with denosumab clinical studies, this was believed to be false positive and likely due to nuclear factor- κ B ligand (RANKL) interfering with the ADA assay (FIGs. 1A and 1B).

[14] To address the false positive signals caused by soluble RANKL and to develop a reliable ADA assay, an exemplary anti-RANKL monoclonal antibody that competes with denosumab for binding to RANKL was incorporated into an improved ADA assay. Because the complementarity determining regions (CDRs) of an antibody are the result of random mutations from germline, which then undergo a selection process, the CDRs are the most likely domain to be recognized by the immune system as foreign. Consequently, most anti-drug antibodies to monoclonal antibody therapeutics are CDR-directed. Based on these data, it is believed that to reduce the interference of RANKL ligand in an ADA assay, two strategies may be used: (i) using OPG (which is a decoy receptor for RANKL) to sequester soluble RANKL; or (ii) using an antibody that binds to RANKL to sequester RANKL; this antibody should have different CDR sequences than denosumab, such that the antibody itself does not bind to ADA (FIGs. 2A and 2B).

2. Immunoassays For Detecting ADAs

[15] The most commonly used ADA assays are based on solid-phase immunoassays. Standard solid-phase immunoassays with monoclonal antibodies involve the formation of a complex between an antibody adsorbed on a solid substrate ("capture" molecule), molecule of interest (in this case, ADA against denosumab), and an antibody linked to a detectable marker ("tracer" molecule). Thus, a sandwich is formed: solid substrate-capture molecule-molecule of interest-tracer molecule. In the sandwich, typically the amount of the detectable marker is proportional to the concentration of the ADA in sample. One sandwich assay is the double antigen bridging immunoassay whereby capture and tracer antibodies bind to different epitopes of the antigen. Hoesel, W., et al., in *J. Immunol. Methods* 294 (2004) 101-110, report an anti-EPO double antigen bridging assay whereby a mixture of immobilized rhEPO coupled to amino groups and to carbohydrate groups was used. Immunoassays such as the double antigen bridging ELISA are common assay types in the investigation of an immunogenic answer of a patient to an antibody drug. Mire-Sluis, A.R., et al., in *J. Immunol. Methods* 289 (2004) 1-16, summarize the

recommendations for the design and optimization of immunoassays using detection of host antibodies against biotechnology products.

Capture molecules

[16] A “capture” molecule is typically an antibody that is covalently or non-covalently linked to an immobilizing moiety, and the solid substrate comprises a binding partner that binds to said immobilizing moiety. Through the interaction between the immobilizing moiety and its binding partner, the capture molecule can be immobilized to a solid substrate. The capture molecule is also referred to as the “first antibody or antibody conjugate” herein.

[17] Exemplary binding pairs that can be used as an immobilizing moiety and its binding partner include for example (first component-second component): streptavidin-biotin, avidin-biotin, antibody-antigen (see, for example, Hermanson, G. T., et al., *Bioconjugate Techniques*, Academic Press, 1996), lectin-polysaccharide, steroid-steroid binding protein, hormone-hormone receptor, enzyme-substrate, IgG-Protein A, IgG-Protein G, and ligand-receptor. In an exemplary embodiment, the capture molecule is an antibody conjugated to biotin and immobilization is performed via Avidin or Streptavidin that attached to the surface of a solid substrate.

[18] In certain embodiments, conjugation of the antibody to the immobilizing moiety is by covalent conjugation via N-terminal and/or ϵ -amino groups (lysine), ϵ -amino groups of different lysines, carboxy-, sulfhydryl-, hydroxyl- and/or phenolic functional groups of the amino acid backbone of the antibody and/or sugar alcohol groups of the carbohydrate structure of the antibody.

[19] Since it is believed that ADAs are often caused by CDR sequences, to detect ADAs against denosumab, the capture molecule should comprise an antibody that has the same CDR sequences of denosumab. The amino acid sequences of the heavy chain (HC), light chain (LC), heavy chain variable domain (VH), light chain variable domain (VL), heavy chain complementarity determining regions (CDR-H1, CDR-H2, CDR-H3), and light chain complementarity determining regions (CDR-L1, CDR-L2, CDR-L3) of denosumab is shown in Table F of the Sequence Table.

[20] As used herein, “Complementarity Determining Regions” (CDRs) can be identified according to the definitions of the Kabat, Chothia, the accumulation of both Kabat and Chothia, AbM, contact, North, and/or conformational definitions or any method of CDR determination well known in the art. See, e.g., Kabat et al., 1991, *Sequences of Proteins of Immunological Interest*, 5th ed. (hypervariable regions); Chothia et al., 1989, *Nature* 342:877-883 (structural loop structures). The identity of the amino acid residues in a particular antibody that make up a CDR can be determined using methods well known in the art. AbM definition of CDRs is a compromise between Kabat and Chothia and uses Oxford Molecular's AbM antibody modeling software (Accelrys®). The “contact” definition of CDRs is based on observed

antigen contacts, set forth in MacCallum et al., 1996, J. Mol. Biol., 262:732-745. The “conformational” definition of CDRs is based on residues that make enthalpic contributions to antigen binding (see, e.g., Makabe et al., 2008, J. Biol. Chem., 283:1 156-1 166). North has identified canonical CDR conformations using a different preferred set of CDR definitions (North et al., 2011, J. Mol. Biol. 406: 228-256). In another approach, referred to herein as the “conformational definition” of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding (Makabe et al., 2008, J Biol. Chem. 283:1 156-1 166). Still other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs (or other residue of the antibody) may be defined in accordance with any of Kabat, Chothia, North, extended, AbM, contact, and/or conformational definitions.

[21] For example, the following Table shows several commonly used definitions of CDRs:

Loop	Kabat	AbM	Chothia ¹	Contact ²	IMGT
L1	L24-L34	L24-L34	L26-L32	L30-L36	L27-L32
L2	L50-L56	L50-L56	L50-L52	L46-L55	L50-L51
L3	L89-L97	L89-L97	L91-L96	L89-L96	L89-L97
H1	H31-H35B (Kabat Numbering) ³	H26-H35B	H26-H32..34	H30-H35B	H26-H35B
H1	H31-H35 (Chothia Numbering)	H26-H35	H26-H32	H30-H35	H26-H33
H2	H50-H65	H50-H58	H52-H56	H47-H58	H51-H56
H3	H95-H102	H95-H102	H96-H101	H93-H101	H93-H102

1. some of these definitions (particularly for Chothia loops) vary depending on the individual publication examined. Some papers describe Chothia CDRs as: CDR-L1:L24-34; CDR-L2:L50-56; CDR-L3:L89-97; CDR-H1:H26-32; CDR-H2:H52-56; CDR-H3:H95-102.

2. Any of the numbering schemes can be used for these CDR definitions, except the contact definition uses the Chothia or Martin (Enhanced Chothia) definition.

3. The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop. (This is because the Kabat numbering scheme places the insertions at H35A and H35B.)

[22] The CDR sequences provided in the Sequence Table are based on the Kabat definition. However, other definitions for CDRs may also be used. Accordingly, in some embodiments, the first antibody comprises the CDR-H1, CDR-H2, and CDR-H3 sequences of SEQ ID NO:203, and the CDR-L1, CDR-L2, and CDR-L3 sequences of SEQ ID NO:208. In some embodiments, the first antibody comprises a CDR-H1 comprising SEQ ID NO:200, a CDR-H2 comprising SEQ ID NO:201, and a CDR-H3 comprising SEQ

ID NO:202; and a CDR-L1 comprising SEQ ID NO:205, a CDR-L2 comprising SEQ ID NO:206, and a CDR-L3 comprising SEQ ID NO:207.

[23] Outside the CDR regions, the sequence of the first antibody may differ from that of denosumab; nonetheless, for convenience, denosumab VH and/or VL sequences may be used. Accordingly, in some embodiments, the first antibody or antibody conjugate comprises a heavy chain variable region (VH) that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:203, and/or a light chain variable region (VL) that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:208.

[24] The constant region of the first antibody (or the capture antibody) may be human or murine. Examples of human and murine constant region sequences are provided in the Sequence Table E. For purpose of conducting assays with human samples, in some cases certain murine constant region sequences may be preferred. This could sometimes reduce the cross-reaction or false positives. Endogenous human antibodies within the sample would not interfere with appropriate detection of the ADA molecules. Accordingly, in some embodiments, the first antibody or antibody conjugate comprises a human CH1 domain (such as a human IgG1 CH1, a human IgG2 CH1, a human IgG3 CH1, or a human IgG4 CH1); a human Fc domain (such as a human IgG1 Fc, a human IgG2 Fc, a human IgG3 Fc, or a human IgG4 Fc); a murine IgG1 CH1 (such as a murine IgG2a CH1, a murine IgG2b CH1, or a murine IgG3 CH1); a murine IgG1 Fc (such as a murine IgG2a Fc, a murine IgG2b Fc, or a murine IgG3 Fc), a human CL domain (such as a human kappa CL, or a human lambda CL), or a murine CL domain (such as a murine kappa CL, or a murine lambda CL).

[25] In an exemplary embodiment, the first antibody or antibody conjugate comprises denosumab conjugated to biotin.

[26] The heavy and light chain sequences of denosumab are provided as SEQ ID NOs. 198 and 199, respectively. It should be noted that often, during recombinant production process, terminal lysine and glycine of the heavy chain may be clipped. C-terminal lysine clipping is a common phenomenon occurring during the bioproduction of monoclonal antibodies. Often, the lysine residue is removed via carboxypeptidase D (CpD), which results in generation of a mixture of antibody isoforms bearing zero or one C-terminal lysine residues on each heavy chain. Further, following C-terminal lysine cleavage, peptidylglycine α -amidating monooxygenase (PAM) catalyzes the hydroxylation of glycine and removal of the glyoxylate from the glycine residue, leaving an amidated C-terminal proline. Therefore, during recombinant production of a monoclonal antibody, the product is often a mixture of C-terminal processing

variants, with heavy chain C-terminus ends at (amidated) proline, glycine, or lysine. Therefore, in some embodiments, the terminal lysine of SEQ ID NO:198 may be absent; in some embodiments, the terminal lysine of SEQ ID NO:198 may be present; in some embodiments, the terminal glycine-lysine of SEQ ID NO:198 may be absent; in some embodiments, the terminal glycine-lysine of SEQ ID NO:198 may be present.

[27] "Solid substrate" refers to a non-fluid substance, and includes particles (including microparticles and beads) made from materials such as polymer, metal (paramagnetic, ferromagnetic particles), glass, and ceramic; gel substances such as silica, alumina, and polymer gels; capillaries, which may be made of polymer, metal, glass, and/or ceramic; zeolites and other porous substances; electrodes; microtiter plates; solid strips; cuvettes, tubes, or other spectrometer sample containers. In an ADA assay, solid substrate refers to inert solid surfaces that in general contains a "binding partner" (e.g., avidin or streptavidin) on its surface, which is intended to interact with the "capture" antibody; in this case the first antibody or antibody conjugate comprising an immobilizing moiety (e.g., biotin) that is recognized by the "binding partner." A solid phase may be a stationary component, such as a tube, strip, cuvette, or microtiter plate, or may be a non-stationary component, such as beads and microparticles. Microparticles can also be used as a solid phase for homogeneous assay formats. A variety of microparticles that allow either non-covalent or covalent attachment of proteins and other substances may be used. Such particles include polymer particles such as polystyrene and poly(methylmethacrylate); gold particles such as gold nanoparticles and gold colloids; and ceramic particles such as silica, glass, and metal oxide particles. See for example Martin, C. R., et al., *Analytical Chemistry-News & Features* 70 (1998) 322A-327A.

[28] In general, the first antibody or antibody conjugate is immobilized to the solid substrate by passive adsorption and therefore attached to the solid phase at a site that does not interfere with its binding to ADA. Passive adsorption is, e.g., described by Butler, J. E., *Solid Phases in Immunoassay*, In: *Immunoassays*, Diamandis, E. P. and Christopoulos, T. K. (eds.) Academic Press San Diego (1996), pp. 205-225.

Tracer molecules

[29] In a typically ADA assay, the "tracer" molecule (also referred herein as the "third antibody") needs to be able to bind to the ADA molecule to form a sandwich (e.g., see FIG. 1A). The most convenient way is to have the same CDR sequences as the first antibody, since ADA is typically caused by CDR sequences. The tracer molecule is also linked to a detectable marker to facilitate the detection of ADA.

[30] Accordingly, in certain embodiments, the third antibody comprise the same six CDRs as denosumab. As described in detail above, CDRs can be identified according to the definitions of the Kabat, Chothia, the accumulation of both Kabat and Chothia, AbM, contact, North, and/or conformational definitions or any method of CDR determination well known in the art. The CDR sequences provided in

the Sequence Table are based on the Kabat definition. However, other definitions for CDRs may also be used. Accordingly, in some embodiments, the third antibody comprises an antibody comprising the CDR-H1, CDR-H2, and CDR-H3 sequences of SEQ ID NO:203, and the CDR-L1, CDR-L2, and CDR-L3 sequences of SEQ ID NO:208. In some embodiments, the third antibody comprises an antibody comprising a CDR-H1 comprising SEQ ID NO:200, a CDR-H2 comprising SEQ ID NO:201, and a CDR-H3 comprising SEQ ID NO:202; and a CDR-L1 comprising SEQ ID NO:205, a CDR-L2 comprising SEQ ID NO:206, and a CDR-L3 comprising SEQ ID NO:207.

[31] Outside the CDR regions, the sequence of the third antibody may differ from that of denosumab; nonetheless, for convenience, denosumab VH and/or VL sequences may be used. Accordingly, in some embodiments, the third antibody comprises a heavy chain variable region (VH) that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:203, and/or a light chain variable region (VL) that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:208.

[32] The constant region of the third antibody (or the tracer antibody) may be human or murine. Examples of human and murine constant region sequences are provided in the Sequence Table E. For purpose of conducting assays with human samples, in some cases certain murine constant region sequences may be preferred. This could sometimes reduce the cross-reaction or false positives. Endogenous human antibodies within the sample would not interfere with appropriate detection of the ADA molecules. Accordingly, in some embodiments, the third antibody comprises a human CH1 domain (such as a human IgG1 CH1, a human IgG2 CH1, a human IgG3 CH1, or a human IgG4 CH1); a human Fc domain (such as a human IgG1 Fc, a human IgG2 Fc, a human IgG3 Fc, or a human IgG4 Fc); a murine IgG1 CH1 (such as a murine IgG2a CH1, a murine IgG2b CH1, or a murine IgG3 CH1); a murine IgG1 Fc (such as a murine IgG2a Fc, a murine IgG2b Fc, or a murine IgG3 Fc), a human CL domain (such as a human kappa CL, or a human lambda CL), or a murine CL domain (such as a murine kappa CL, or a murine lambda CL).

[33] In some embodiments, the third antibody comprises a VH domain comprising the same sequence as the VH of the first antibody, and a VL domain comprising the same sequence as the VL domain of the first antibody. In some embodiments, the third antibody comprises a heavy chain (HC) comprising the same sequence as the HC of the first antibody, and a light chain (LC) comprising the same sequence as the LC of the first antibody.

[34] Typically, the “tracer” molecule (or “the third antibody” herein) is covalently or noncovalently linked to a detectable marker. The detectable marker can be an isotope, an enzyme, a fluorescent moiety, a luminescent moiety, a chromatic moiety, a metal, or an electric charge. For example, the detectable marker can be an electrochemiluminescent label, like a ruthenium bispyridyl complex. Chromogens (fluorescent or luminescent groups and dyes), enzymes, NMR-active groups or metal particles, haptens, such as, e.g., digoxigenin, are also examples of detectable markers. The detectable marker can also be a photoactivatable crosslinking group, e.g. an azido or an azirine group. Metal chelates which can be detected by electrochemoluminescence are also preferred signal-emitting groups, with particular preference being given to ruthenium chelates, e.g. a ruthenium (bispyridyl)₃ 2+ chelate. Suitable ruthenium labeling groups are described, for example, in EP 0 580 979, WO 90/05301, WO 90/11511, and WO 92/14138. Further, nearly any antibody can be labeled with biotin, HRP enzyme or one of several fluorophores if needed.

[35] Sometimes, indirect detection method is used. In this case, the “tracer” molecule is considered as a “primary antibody”, which binds to ADA, and the primary antibody is not labeled for direct detection. Instead, a “secondary antibody” that has been labeled with a detectable tag is applied in a second step to probe for the primary antibody-ADA complex. Thus, the ADA is detected indirectly. Another form of indirect detection involves using a primary or secondary antibody that is labeled with an affinity tag such as biotin. Then a secondary (or tertiary) probe, such as streptavidin that is labeled with the detectable enzyme or fluorophore tag, can be used to probe for the biotin tag to yield a detectable signal. Several variants of these probing and detection strategies exist. In general, the strategy depends on a specific probe (e.g., a primary antibody) whose presence is linked directly or indirectly to some sort of detectable marker (e.g., an enzyme whose activity can produce a colored product upon reaction with its substrate).

[36] Most primary antibodies can be produced or manipulated to have mouse, rabbit or one of several other non-human constant domains. Many of these are antibodies of the IgG class, in particular, IgG1 subclass. Therefore, it is relatively easy and economical for manufacturers to produce and supply ready-to-use, labeled secondary antibodies for most applications and detection systems. The choice of secondary antibody depends upon the species of animal in which the primary antibody was raised (the host species). For example, if the primary antibody has a mouse constant domain, then the secondary antibody should be an anti-mouse antibody obtained from a host other than the mouse. Off the shelf secondary antibodies that bind to a constant domain or the Fc domain of primary antibodies are in general readily available.

[37] Biotin-based systems are often used as detectable markers. With biotin-binding proteins as probes, the highly specific affinity interaction between biotin and avidin or streptavidin protein is the basis for many kinds of detection and affinity-purification methods. Biotin is very small (244 Daltons), so its covalent attachment to antibodies or other probes rarely interferes with their functions. Yet its presence

as a label on a probe allows efficient and specific secondary detection with either avidin or streptavidin. Both kinds of biotin-binding proteins are available in purified forms labeled with enzymatic or fluorescent tags that enable detection in many kinds of assays systems.

[38] Enzymatic labels are commonly used as secondary antibody (or streptavidin) tags for detection in blotting and immunoassays. Enzymes provide detectable signal via their activity; reaction with a specific substrate chemical yields a colored, light-emitting, or fluorescent product. While reporter enzymes like beta-galactosidase and luciferase have been successfully used to make probes, alkaline phosphatase (AP) and horseradish peroxidase (HRP) are the two enzymes used most extensively as labels for protein detection. An array of chromogenic, fluorogenic and chemiluminescent substrates is available for use with either enzyme.

[39] Alkaline phosphatase (AP), usually isolated from calf intestine, is a large (140 kDa) protein that catalyzes the hydrolysis of phosphate groups from a substrate molecule resulting in a colored or fluorescent product or the release of light as a byproduct of the reaction. AP has optimal enzymatic activity at a basic pH (pH 8-10) and can be inhibited by cyanides, arsenate, inorganic phosphate and divalent cation chelators, such as EDTA. AP offers certain advantage as a detectable marker. Because its reaction rate remains linear, detection sensitivity can be improved by simply allowing a reaction to proceed for a longer time period.

[40] Horseradish peroxidase (HRP) is a 40 kDa protein that catalyzes the oxidation of substrates by hydrogen peroxide, resulting in a colored or fluorescent product or the release of light as a byproduct of the reaction. HRP functions optimally at a near-neutral pH and can be inhibited by cyanides, sulfides and azides. Antibody-HRP conjugates have shown to be superior to antibody-AP conjugates with respect to the specific activities of both the enzyme and antibody. In addition, its high turnover rate, good stability, low cost and wide availability of substrates makes HRP the enzyme of choice for many applications. Because of the small size of the HRP enzyme, further increases in sensitivity may be achieved by using poly-HRP conjugated secondary antibodies.

[41] Two common fluorophores for labeling probes are fluorescein (fluorescein isothiocyanate, FITC) and rhodamine (tetramethyl rhodamine isothiocyanate, TRITC).

[42] Other detectable markers include fluorescent proteins such as the various forms of green fluorescent protein (GFP) and the phycobiliproteins (allophycocyanin, phycocyanin, phycoerythrin and phycoerythrocyanin).

Reducing Signal Interference

[43] As disclosed herein, in an exemplary ADA assay to detect denosumab ADA, a large number of samples showed false positive signals, and it was believed to be caused by RANKL interfering with the ADA assay (FIGs. 1A and 1B).

[44] The sequence of human RANKL, including membraned-bound and soluble forms of RANKL are provided in the sequence table F. The soluble form of human RANKL comprises residues 69-317 of the membrane-bound form (as numbered in WO 98/46757), shown as SEQ ID NOs:212 and 213. Proteolytic cleavage between residues 139 and 140 of the membrane-bound RANKL also yields a soluble form as shown in SEQ ID NO: 214.

[45] To address the false positive signals caused by soluble RANKL, it is important to include in the ADA assay a molecule that can sequester soluble RANKL. Two strategies are provided herein, one uses a second anti-RANKL antibody, and the other uses decoy receptor OPG.

[46] To effectively sequester RANKL without interfering with the detection of ADA, the second antibody should have the following characteristics: (1) it should have different CDR sequences than denosumab (since ADAs are often CDR-directed, having different CDRs generally ensures that there is no cross-reaction between the second antibody and ADA); and (2) it should bind to RANKL. Preferably, the second antibody binds to the same epitope of RANKL as denosumab; or the second antibody competes (or at least partially competes) with denosumab for RANKL binding. When the second antibody and denosumab cannot bind to RANKL simultaneously, the signal interference caused by RANKL can be reduced most effectively. Nonetheless, competing (or at least partially competing) with denosumab for RANKL binding is not an absolute requirement. For example, in Examples 3-6, the exemplary anti-RANKL mAb was found to not compete with denosumab for RANKL for binding. Epitope binning showing that this anti-RANKL mAb binds to a different epitope bin and does not block denosumab or OPG for RANKL binding. A skilled artisan will be able to readily assess the binding behaviors of the second antibody and design the ADA assays accordingly based on common knowledge. Example 2B also provides an example on how to assess and select suitable second antibodies.

[47] In addition, although it is preferred that the second antibody competes (or at least partially competes) with denosumab for RANKL binding, it is not necessary that the second antibody binds to RANKL with similar affinity (KD value) as denosumab. Even a poor competitor with low binding affinity for RANKL would be adequate to reduce/eliminate the interference. In that case, the competing antibody can be spiked into the serum sample in large quantities and only needs to sequester a small amount of RANKL.

[48] KD is the equilibrium dissociation constant, a ratio of k_{off}/k_{on} , between the antigen binding protein and its target or antigen. KD and KA are inversely related. The KD value relates to the concentration of the antibody (the amount of antibody needed for a particular experiment) and so the lower the KD value

(lower concentration needed) the higher the affinity of the antibody. In exemplary aspects, the K_D value of the second antibody to RANKL is about 10^{-1} M or less, about 10^{-2} M or less, about 10^{-3} M or less, about 10^{-4} M or less, about 10^{-5} M or less, about 10^{-6} M or less, about 10^{-7} M or less, about 10^{-8} M or less, about 10^{-9} M or less, about 10^{-10} M or less, about 10^{-11} M or less, about 10^{-12} M or less, about 10^{-13} M or less, about 10^{-14} M or less, from about 10^{-5} M to about 10^{-15} M, from about 10^{-6} M to about 10^{-15} M, from about 10^{-7} M to about 10^{-15} M, from about 10^{-8} M to about 10^{-15} M, from about 10^{-9} M to about 10^{-15} M, from about 10^{-10} M to about 10^{-15} M, from about 10^{-5} M to about 10^{-14} M, from about 10^{-6} M to about 10^{-14} M, from about 10^{-7} M to about 10^{-14} M, from about 10^{-8} M to about 10^{-14} M, from about 10^{-9} M to about 10^{-14} M, from about 10^{-10} M to about 10^{-14} M, from about 10^{-5} M to about 10^{-13} M, from about 10^{-6} M to about 10^{-13} M, from about 10^{-7} M to about 10^{-13} M, from about 10^{-8} M to about 10^{-13} M, from about 10^{-9} M to about 10^{-13} M, or from about 10^{-10} M to about 10^{-13} M.

[49] In exemplary aspects, the K_D of the second anti-RANKL antibody is micromolar, nanomolar, picomolar or femtomolar. In exemplary aspects, the K_D is within a range of about 10^{-4} to 10^{-6} M, or 10^{-7} to 10^{-9} M, or 10^{-10} to 10^{-12} M, or 10^{-13} to 10^{-15} M. In exemplary aspects, the second antibody binds to the human RANKL with a K_D value of: about 1 μ M or less, about 900 nM or less, about 800 nM or less, about 700 nM or less, about 600 nM or less, about 500 nM or less, about 400 nM or less, about 300 nM or less, about 200 nM or less, about 100 nM or less, about 90 nM or less, about 80 nM or less, about 70 nM or less, about 60 nM or less, about 50 nM or less, about 40 nM or less, about 30 nM or less, about 20 nM or less, about 10 nM or less, about 5 nM or less, about 2 nM or less, about 1 nM or less, about 900 pM or less, about 800 pM or less, about 700 pM or less, about 600 pM or less, about 500 pM or less, about 400 pM or less, about 300 pM or less, about 250 pM or less, about 200 pM or less, about 150 pM or less, about 100 pM or less, about 50 pM or less, about 40 pM or less, about 30 pM or less, about 25 pM or less, about 20 pM or less, about 15 pM or less, about 10 pM or less, about 5 pM or less, or about 1 pM or less.

[50] K_D values can be determined using methods well established in the art. One exemplary method for measuring K_D is surface plasmon resonance (SPR), a method well-known in the art (e.g., Nguyen et al. Sensors (Basel). 2015 May 5; 15(5):10481-510). K_D value may be measured by SPR using a biosensor system such as a BIAcore® system. BIAcore kinetic analysis comprises analyzing the binding and dissociation of an antigen from chips with immobilized molecules (e.g. molecules comprising epitope binding domains), on their surface. Another well-known method in the art for determining the K_D of a protein is by using Bio-Layer Interferometry (e.g., Shah et al. J Vis Exp. 2014; (84): 51383). K_D value may be measured by Bio-Layer Interferometry using OCTET® technology (Octet QKe system, ForteBio). Alternatively or in addition, a KinExA® (Kinetic Exclusion Assay) assay, available from Sapidyne Instruments (Boise, Id.) can also be used. Any method known in the art for assessing the binding affinity between two binding partners is encompassed herein.

[51] In some aspects, the K_D value is measured by surface plasmon resonance (SPR). Antigen (RANKL) may be immobilized, e.g., on a solid surface. The antigen may be immobilized to a chip, for example by covalent coupling (such as amine coupling). The chip may be a CM5 sensor chip. As the analyte binds to the ligand the accumulation of protein on the sensor surface causes an increase in refractive index. This refractive index change is measured in real time (sampling in a kinetic analysis experiment is taken every 0.1 s), and the result plotted as response units (RU) versus time (termed a sensorgram). A response (background response) will also be generated if there is a difference in the refractive indices of the running and sample buffers. This background response must be subtracted from the sensorgram to obtain the actual binding response. The background response is recorded by injecting the analyte through a control or reference flow cell, which has no ligand or an irrelevant ligand immobilized to the sensor surface. The real-time measurement of association and dissociation of a binding interaction allows for the calculation of association and dissociation rate constants and the corresponding affinity constants. One RU represents the binding of 1 pg of protein per square mm. More than 50 pg per square mm of analyte binding is generally needed in practice to generate good reproducible responses.

[52] Dissociation of the antibody from the antigen may be monitored for about 3600 seconds. The SPR analysis may be conducted, and the data collected at between about 15°C and about 37°C. The SPR analysis may be conducted, and the data collected at between about 25°C and 37°C. The SPR analysis may be conducted, and the data collected at about 37°C. The SPR analysis may be conducted, and the data collected at 37°C. The K_D value may be measured by SPR using a BiAcCore T200 instrument. The SPR rates and affinities may be determined by fitting resulting sensorgram data to a 1:1 model in BiAcCore T200 Evaluation software version 1.0. The collection rate may be about 1 Hz.

[53] Another method for determining the K_D of an antibody is by using Bio-Layer Interferometry (BLI), typically using OCTET® technology (Octet QKe system, ForteBio). In some embodiments, biosensor analysis is used. Typically, one interactant is immobilized on the surface of the biosensor ("ligand," such as an antibody) and the other remains in solution ("analyte", such as an antigen). The assay begins with an initial baseline or equilibration step using assay buffer. Next, a ligand (such as an antibody) is immobilized on the surface of the biosensor (loading), either by direct immobilization or capture-based method. After ligand immobilization, biosensors are dipped into buffer solution for a baseline step to assess assay drift and determine loading level of ligand. After the baseline step, biosensors are dipped into a solution containing the ligand's binding partner, the analyte (association). In this step, the binding interaction of the analyte to the immobilized ligand is measured. Following analyte association, the biosensor is dipped into buffer solution without analyte, and the bound analyte is allowed to come off the ligand (dissociation). The series of assay steps is then repeated on new or regenerated biosensors for each analyte being tested. Each binding response is measured and reported in real time on a sensorgram

trace. The instrument may be Octet QKe system, Octet RED96 system, Octet QK384 system, or RED384 system.

[54] In certain aspects, the second antibody binds to the same RANKL epitope as denosumab. Denosumab is known to bind to an epitope comprising a portion of the amino acid sequence of a DE region of RANKL (DE epitope). The DE region of RANKL spans approximately the D and E beta sheet regions and intervening loop sequence (DE loop). The DE region in human RANKL comprises from about amino acid residue 212 to about amino acid residue 250 (GFYYLYANICFRHHETSGDLATEYLQLMVYVTKTSIKIP, SEQ ID NO: 216). However, the amino acid sequence and endpoints of the DE region of human RANKL are merely exemplary, and it is understood that DE regions may have sequences and endpoints which vary. In particular, it has been shown that within DE loop of human RANKL, the amino acid sequence DLATE (SEQ ID NO:215) is important for denosumab binding. See, e.g., WO2001062932.

[55] Accordingly, in some embodiments, the second anti-RANKL antibody binds to an epitope comprising the sequence of "DLATE" (SEQ ID NO:215). In some embodiments, the second anti-RANKL antibody binds to an epitope that is located within DE loop of human RANKL.

[56] An "epitope" refers to the area or region of an antigen to which an antibody specifically binds, e.g., an area or region comprising residues that interacts with the antibody. Epitopes can be linear or conformational. The term "paratope" is derived from the above definition of "epitope" by reversing the perspective, and refers to the area or region of an antibody molecule which is involved in binding of an antigen, e.g., an area or region comprising residues that interacts with the antigen. A paratope may be linear or conformational (such as discontinuous residues in CDRs). The epitope/paratope for a given antibody/antigen binding pair can be defined and characterized at different levels of detail using a variety of experimental and computational epitope mapping methods. The experimental methods include mutagenesis, X-ray crystallography, Nuclear Magnetic Resonance (NMR) spectroscopy, Hydrogen/deuterium exchange Mass Spectrometry (HX-MS) and various competition binding methods. As each method relies on a unique principle, the description of an epitope is intimately linked to the method by which it has been determined. Thus, the epitope/paratope for a given antibody/antigen pair will be defined differently depending on the mapping method employed.

[57] From the fact that descriptions and definitions of epitopes, dependent on the epitope mapping method used, and obtained at different levels of detail, it follows that comparison of epitopes for different antibodies on the same antigen can similarly be conducted at different levels of detail. For example, epitopes described on the amino acid level, e.g., determined from an X-ray structure, are said to be identical if they contain the same set of amino acid residues. Epitopes characterized by competition binding are said to be overlapping if the binding of the corresponding antibodies are mutually exclusive, i.e., binding of one antibody excludes simultaneous or consecutive binding of the other antibody; and

epitopes are said to be separate (unique) if the antigen is able to accommodate binding of both corresponding antibodies simultaneously.

[58] The epitope and paratope for a given antibody/antigen pair may be identified by routine methods. For example, the general location of an epitope may be determined by assessing the ability of an antibody to bind to different fragments RANKL.

[59] In certain aspect, the second antibody competes with denosumab for RANKL binding. The term "compete," as used herein with regard to an antibody, means that binding of a first antibody, or an antigen-binding portion thereof, to an antigen reduces the subsequent binding of the same antigen by a second antibody or an antigen-binding portion thereof. In general, the binding a first antibody creates steric hindrance, conformational change, or binding to a common epitope (or portion thereof), such that the binding of the second antibody to the same antigen is reduced. Standard competition assays may be used to determine whether two antibodies compete with each other. One suitable assay for antibody competition involves the use of the Biacore technology, which can measure the extent of interactions using surface plasmon resonance (SPR) technology, typically using a biosensor system (such as a BIACORE® system). For example, SPR can be used in an in vitro competitive binding inhibition assay to determine the ability of one antibody to inhibit the binding of a second antibody.

[60] Another assay for measuring antibody competition uses an ELISA-based approach. Furthermore, a high throughput process for "binning" antibodies based upon their competition is described in International Patent Application No. WO2003/48731. Competition is present if one antibody (or fragment) reduces the binding of another antibody (or antigen-binding fragment thereof) to RANKL. For example, a sequential binding competition assay may be used, with different antibodies being added sequentially. The first antibody may be added to reach binding that is close to saturation. Then, the second antibody is added. If the binding of second antibody to RANKL is not detected, or is significantly reduced (e.g., at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% reduction) as compared to a parallel assay in the absence of the first antibody (which value can be set as 100%), the two antibodies are considered as competing with each other.

[61] Exemplary anti-RANKL antibodies that can be used in the ADA assays disclosed herein are shown in Sequence Tables. Accordingly, in certain embodiments, the second antibody comprises: (i) the heavy chain CDR-H1, CDR-H2, and CDR3-H3 of SEQ ID NO: 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, or 167; and (ii) the light chain CDR-L1, CDR-L2, and CDR3 of SEQ ID NO: 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, or 168. As described in detail above, CDRs can be identified according to the definitions of the Kabat, Chothia, the accumulation of both Kabat and Chothia, AbM, contact, North, and/or conformational definitions or any method of CDR determination well known in the art. The CDR

sequences provided in the Sequence Table are based on the Kabat definition. However, other definitions for CDRs may also be used.

[62] In some embodiments, the second antibody comprises (i) a CDR-H1 comprising a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 1, 7, 13, 19, 25, 31, 37, 43, 49, 55, 61, 67, 73, 79, 85, 91, 97, 103, 109, 115, or 121; (ii) a CDR-H2 comprising a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 2, 8, 14, 20, 26, 32, 38, 44, 50, 56, 62, 68, 74, 80, 86, 92, 98, 104, 110, 116, or 122; (iii) a CDR-H3 comprising a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 3, 9, 15, 21, 27, 33, 39, 45, 51, 57, 63, 69, 75, 81, 87, 93, 99, 105, 111, 117, or 123; (iv) a CDR-L1 comprising a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 4, 10, 16, 22, 28, 34, 40, 46, 52, 58, 64, 70, 76, 82, 88, 94, 100, 106, 112, 118, or 124; (v) a CDR-L2 comprising a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 5, 11, 17, 23, 29, 35, 41, 47, 53, 59, 65, 71, 77, 83, 89, 95, 101, 107, 113, 119, or 125; and (vi) a CDR-L3 comprising a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120, or 126.

[63] In some embodiments, the second antibody comprises a heavy chain variable region (VH) that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, or 167. In some embodiments, the second antibody comprises a light chain variable region (VL) that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, or 168.

[64] The constant region of the second antibody may be human or murine. Examples of human and murine constant region sequences are provided in the Sequence Table. For purpose of conducting assays with human samples, in some cases certain murine constant region sequences may be preferred. This could sometimes reduce the cross-reaction or false positives. Endogenous antibodies within the sample would not interfere with appropriate detection of the ADA molecules. Accordingly, in some embodiments, the third antibody comprises a human CH1 domain (such as a human IgG1 CH1, a human IgG2 CH1, a human IgG3 CH1, or a human IgG4 CH1); a human Fc domain (such as a human IgG1 Fc, a human IgG2 Fc, a human IgG3 Fc, or a human IgG4 Fc); a murine IgG1 CH1 (such as a murine IgG2a CH1, a murine IgG2b CH1, or a murine IgG3 CH1); a murine IgG1 Fc (such as a murine IgG2a Fc, a murine IgG2b Fc, or a murine IgG3 Fc), a human CL domain (such as a human kappa CL, or a human lambda CL), or a murine CL domain (such as a murine kappa CL, or a murine lambda CL).

[65] Alternatively, Osteoprotegerin (OPG) may be used to sequester soluble RANKL. OPG is also known as osteoclastogenesis inhibitory factor (OCIF) or tumor necrosis factor receptor superfamily member 11B (TNFRSF11B), is a cytokine receptor of the tumor necrosis factor (TNF) receptor superfamily encoded by the TNFRSF11B gene. OPG is a decoy receptor for RANKL. The exemplary sequence of human OPG is shown in Sequence Table F.

[66] In certain embodiments, the OPG is human OPG. In certain embodiment, the OPG comprises a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:210 or 211.

[67] The samples may be pre-treated with the second anti-RANKL antibody or OPG disclosed herein to remove soluble RANKL, then, the first antibody or antibody conjugate and the third antibody may be added to complete the ADA assay. Alternatively, the first antibody or antibody conjugate, the second antibody or OPG, and the third antibody may be added simultaneously. This is particularly feasible when the second antibody competes or partially competes with the first antibody for RANKL binding (i.e., when the first antibody and the second antibody cannot simultaneously bind to RANKL). When the first antibody and the second antibody cannot simultaneously bind to RANKL, the presence of second antibody will prevent RANKL from bridging the immobilized first antibody and the detectable marker.

[68] Excess amount of the second antibody or OPG may be added to ensure the complete sequestration of soluble RANKL in the sample. In certain embodiments, excess amount of the second antibody or OPG is added to a sample, such that the molar ratio of first antibody to second antibody or

OPG is at least 1:2, at least 1:3, at least 1:4, at least 1:5, at least 1:6, at least 1:7, at least 1:8, at least 1:9, at least 1:10, at least 1:15, at least 1:20, at least 1:30, at least 1:40, at least 1:50, at least 1:60, at least 1:70, at least 1:80, at least 1:90, at least 1:100, at least 1:200, at least 1:300, at least 1:400, at least 1:500, at least 1:600, at least 1:700, at least 1:800, at least 1:900, or at least 1:1000.

3. Diagnostic Kits

[69] In certain aspects, this disclosure provides kits that are adapted for determining the presence or absence of an ADA against denosumab in a sample. The kits may comprise instructions and reagents for the ADA assays disclosed herein. For example, the kit may comprise: (a) a first antibody or antibody conjugate that binds to RANKL, comprising: the heavy chain (HC) CDR-H1, CDR-H2, and CDR-H3 of SEQ ID NO:203, and the light chain (LC) CDR-L1, CDR-L2, and CDR-L3 of SEQ ID NO:208; wherein said first antibody or antibody conjugate can be attached to a solid substrate; (b) (i) a second antibody that binds to RANKL, wherein said second antibody comprises an amino acid sequence that differs from the first antibody by at least one amino acid in one of the six CDR sequences; or (ii) an osteoprotegerin (OPG) protein that binds to RANKL; (c) a third antibody that binds to said ADA and can be linked to a detectable marker; and (d) instructions for use.

[70] The kit may also comprise an immobilizing moiety that can be covalently or non-covalently linked to the first antibody. The kit may also comprise a solid substrate, and a binding partner that can bind to the immobilizing moiety. The binding partner may already be fixed to the substrate, or reagents are provided to allow a user to attach the binding partner to the solid substrate.

[71] The kit may also comprise a detectable marker. Reagents that allow a user to covalently or non-covalently link the third antibody to the detectable marker may also be included. Alternatively, the kit may comprise a third antibody that is already attached to a detectable marker covalently or non-covalently.

[72] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purpose

EXAMPLES

Example 1. Background and Rationale

[73] In one denosumab bioequivalence study ("Study A"), an unusually high incidence of immunoreactivity in the anti-drug antibody assay (ADA) was observed. Based on past experience with denosumab clinical studies, this was unexpected, and an investigation was initiated. False positive results due to target interference was suspected because the positive samples gave signal that was uniform and

low magnitude, more in line with treatment-induced shedding of receptor activator of nuclear factor- κ B ligand (RANKL) than an anti-denosumab antibody response.

[74] To investigate this possibility, a subset of antibody positive samples from Study A were treated with osteoprotegerin (OPG), a decoy receptor for RANKL, and retested in the antibody assay. The hypothesis was that if the antibody-positive results were due to soluble RANKL, OPG treatment would reduce the assay signal by competing with denosumab for binding. If the antibody-positive results were caused by anti-denosumab antibodies, the OPG treatment would have no impact on the assay signal, as OPG does not bind to ADA molecules. Specifically, as shown in FIGs. 1A-1B, in an exemplary MSD ADA assay, an anti-drug antibody can bridge the biotin-labeled-denosumab and SULFO-TAG (ruthenium tris bipyridine) labeled denosumab. The complexes are immobilized using streptavidin-coated plates and detected through chemiluminescence (FIG. 1A). It is possible that soluble RANKL trimers can also bridge the labeled denosumab molecules (FIG. 1B) and cause false positive signals. If this is the case, pre-treating the sample with OPG can deplete the soluble RANKL from the samples and eliminate the false positive signals. Indeed, it was confirmed that the OPG treatment led to a clear reduction in signal in most antibody-positive samples tested (data not shown), indicating that many of the positive results were due to target (RANKL) interference.

[75] To address the false positive signals caused by soluble RANKL and to develop a reliable ADA assay, an anti-RANKL monoclonal antibody that binds to human RANKL was incorporated into an improved ADA assay. Because the complementarity determining regions (CDRs) of an antibody are the result of random mutations from germline, which then undergo a selection process, the CDRs may be recognized by the immune system as foreign. Consequently, most anti-drug antibodies to monoclonal antibody therapeutics are CDR-directed. For this reason, screening and selection of an anti-RANKL antibody with a different CDR than denosumab was conducted to ensure that the incorporated anti-RANKL antibody did not inadvertently bind ADA, inhibiting the signal.

Example 2. Materials and Methods for Denosumab ADA Assays

[76] *Meso Scale Discovery (MSD) based bridging immunoassay method.* Serum samples were diluted 1:20 in 300 mM acetic acid to enable antibody complex dissociation prior to analysis. Diluted samples were then incubated with a conjugate/anti-RANKL/neutralization mixture comprising biotinylated denosumab, ruthenylated denosumab, anti-RANKL antibody, 1 M Tris, pH 9.5, and soluble drug (confirmatory assay only). During this incubation, the two antigen binding sites of anti-denosumab antibodies formed a bridge between the conjugated denosumab molecules (FIG. 1A). The sample mixture was then added to a blocked MSD streptavidin standard-bind microtiter plate (MSD, #L15SA-1). The biotinylated-molecule bound to the streptavidin-coated surface of the well, resulting in the immobilization of the bridged complex. The plate was washed to remove any unbound complexes and tripropylamine GOLD read buffer (MSD, #R92TG-2) was added to each well. Using the MSD SECTOR S 600 reader, an

electrical current was placed across the plate-associated electrodes. The result was a series of electrically induced oxidation-reduction reactions involving ruthenium (from the captured complex) and tripropylamine. The subsequent luminescent signal was quantified as electrochemiluminescence (ECL) units. The screening assay was performed on samples to detect the presence of binding antibodies to denosumab. Samples analyzed in the screening assay with responses below the screening assay cut point were considered negative and responses equal to or above the screening assay cut point were then analyzed in the confirmatory assay. Samples tested in the confirmatory assay with % inhibition results equal to or above the confirmatory assay cut point were considered positive for the presence of anti-denosumab antibodies.

[77] *Generation of biotinylated and ruthenylated denosumab molecules.* Denosumab molecules were labeled with biotin (Thermo Scientific, #A39257) or ruthenium (MSD, #R91AO). This process included an initial buffer-exchange, a conjugation reaction, and a post-conjugation buffer-exchange to remove free label from the conjugated AMG 162 molecules.

[78] *Generation of antibodies against human RANKL.* An anti-RANKL monoclonal antibody comprising murine IgG1 Fc was recombinantly expressed from HEK293-6E cells. This antibody is designated as "anti-RANKL mAb" in Examples 3-6 described below. FACS and ELISA assays using soluble RANKL confirmed that this anti-RANKL mAb binds to human RANKL. Binning assays showed that this anti-RANKL mAb binds to a different epitope bin, and does not compete with denosumab or OPG for RANKL binding.

Example 2B. Screening and selection of anti-RANKL molecules and RANKL control

[79] Pooled normal human serum (PNHS) was spiked with a positive control rabbit anti-denosumab polyclonal antibody (control anti-drug antibody or "ADA" for short) at low (10 ng/mL), medium (100 ng/mL), and high (1000 ng/mL) levels. These control ADA samples were then evaluated in 5 different ways (with 5 different reaction buffers): 1) with a conjugate/neutralizing mixture (untreated); 2) with a conjugate/neutralization mixture plus denosumab (confirmatory assay); 3) with a conjugate/neutralization mixture plus a murine anti-RANKL mAb (anti-RANKL1-treated); 4) with a conjugate/neutralizing mixture plus OPG (anti-RANKL2-treated); and 5) with a conjugate/neutralization mixture plus a murine anti-RANKL mAb (anti-RANKL3-treated). Three different RANKL (1250 pg/mL) controls were also prepared (RC-1, RC-2, and RC-3) and included in the experiment for evaluation and selection of the best candidate to serve as the RANKL control. The evaluation served to screen and select the optimal anti-RANKL treatment that did not impact specific ADA signal yet inhibited the specific target (RANKL) signal.

[80] The results from the experiment are shown in Table 9. Increased (untreated) S/N results with increasing ADA concentrations were observed when the untreated conjugate/neutralizing mixture was used. Varying results were observed from the 3 different RANKL Controls (RC) with RC-1 generating

results around the assay cut points, RC-2 generating weak responses that were negative in the assay, and RC-3 generating strong responses that were positive in the assay. Based on the results generated by the 3 RANKL lots, RC-3 was selected for use as the target control. Results from the second evaluation where a conjugate/neutralization mixture plus denosumab (ADA and target inhibition control) was used showed the expected inhibition ($\geq 60\%$) of both the specific ADA signal and the specific target signal, indicating that the control conditions worked as expected. Further, results from the assay where a conjugate/neutralization mixture plus anti-RANKL3 was used showed minimal inhibition of the specific ADA signal, indicating that the use of this anti-RANKL molecule did not impact the specific ADA signal. Because the anti-RANKL3 treatment did not impact the specific ADA signal and inhibited the specific target signal, the anti-RANKL3 murine antibody was considered a preferred agent among the three anti-RANKL treatment agents tested in this experiment.

Table 9. Result summary from anti-RANKL molecule screening

Sample	Reaction Buffer Composition				
	Untreated S/N	denosumab Treated % S/N Inhibition	Anti-RANKL1-Treated % S/N Inhibition	Anti-RANKL2-Treated % S/N Inhibition	Anti-RANKL3-Treated % S/N Inhibition
Negative Control (NC)	1.00	0%	0%	0%	0%
10 ng/mL ADA	2.34	60%	58%	8%	-4%
100 ng/mL ADA	15.39	94%	89%	14%	-3%
1000 ng/mL ADA	156.97	99%	94%	13%	-3%
RC-1	1.21	20%	18%	18%	17%
RC-2	1.05	6%	23%	6%	5%
RC-3	2.22	52%	65%	58%	54%

Example 3. Assessing impact of anti-RANKL antibody on specific ADA signal

[81] Pooled normal human serum (PNHS) was spiked with a positive control rabbit anti-denosumab polyclonal antibody (anti-drug antibody or ADA) at low (10 ng/mL), medium (100 ng/mL), and high (1000 ng/mL) levels. These ADA samples were then evaluated in 3 different ways (with 3 different reaction buffers): 1) with a conjugate/neutralizing mixture (untreated); 2) with a conjugate/neutralization mixture plus anti-RANKL mAb (anti-RANKL mAb-treated); and 3) with a conjugate/neutralizing mixture plus anti-RANKL mAb and denosumab (denosumab + Anti-RANKL mAb-treated, confirmatory assay). A human RANKL (1250 pg/mL) control was also prepared and included in the experiment to confirm efficacy of the added anti-RANKL mAb at inhibiting signal generated by RANKL (target). Results generated by the RANKL control demonstrated that the assay worked as expected (data not shown). The results from the experiment are shown in Table 1A. Increased S/N results with increasing ADA concentrations were observed when the untreated conjugate/neutralizing mixture was used. Results from the assay where a conjugate/neutralization mixture plus anti-RANKL mAb was used showed minimal inhibition ($<20\%$) of the

specific anti-denosumab ADA signal, indicating that the use of the anti-RANKL mAb in the assay did not impact the specific anti-denosumab ADA signal. Results from the assay where a conjugate/neutralization mixture plus anti-RANKL mAb and denosumab were used showed a greater than 20% inhibition of the S/N results.

Table 1A. Impact of anti-RANKL antibody on ADA Signal

Sample	Reaction Buffer Composition				
	Untreated S/N	Anti-RANKL mAb-Treated S/N	Anti-RANKL mAb-Treated % S/N Inhibition	denosumab + Anti-RANKL mAb-Treated S/N	denosumab + Anti-RANKL mAb-Treated % S/N Inhibition
Negative Control (NC)	1.00	1.00	0%	1.00	0%
10 ng/mL ADA	2.06	1.90	7%	1.03	50%
100 ng/mL ADA	13.08	11.65	11%	1.05	92%
1000 ng/mL ADA	129.19	106.69	17%	1.23	99%

$$\% \text{ S/N Inhibition} = (\text{Untreated S/N} - \text{Treated S/N}) / \text{Untreated S/N} * 100$$

[82] An additional experiment was performed where PNHS was spiked with six different positive control anti-denosumab antibodies, including polyclonal (pAb) and additional murine and rat monoclonal antibodies, at low (10 ng/mL), medium (100 ng/mL), and high (1000 ng/mL) levels. These ADA samples were then evaluated in 2 different ways (with 2 different reaction buffers): 1) with a conjugate/neutralizing mixture (untreated); and 2) with a conjugate/neutralization mixture plus anti-RANKL mAb (anti-RANKL mAb-treated). A human RANKL (1250 pg/mL) control was also prepared and included in the experiment to confirm efficacy of the added anti RANKL mAb at inhibiting signal generated by RANKL (target). Results generated by the RANKL control demonstrated that the assay worked as expected (data not shown). The results from the experiment are shown in Table 1B. Increased S/N results with increasing ADA concentrations were observed when the untreated conjugate/neutralizing mixture was used. Results from the assay where a conjugate/neutralization mixture plus anti-RANKL mAb was used showed minimal inhibition (<20%) of the specific anti-denosumab ADA signal, indicating that the use of the anti-RANKL mAb in the assay did not impact the specific anti-denosumab ADA signal.

Table 1B. Impact of anti-RANKL antibody on ADA Signal

Sample	Positive Control Antibody Description	Reaction Buffer Composition		
		Untreated S/N	Anti-RANKL mAb-Treated S/N	Anti-RANKL mAb-Treated % S/N Inhibition
Negative Control (NC)	NA	1.00	1.00	0%
10 ng/mL ADA	rbt anti-denosumab	1.41	1.34	5%
100 ng/mL ADA	pAb Number 1	4.38	4.47	-2%

1000 ng/mL ADA		32.15	33.45	-4%
10 ng/mL ADA	rbt anti-denosumab pAb Number 2	2.29	2.16	6%
100 ng/mL ADA		12.70	12.27	3%
1000 ng/mL ADA		112.58	113.32	-1%
10 ng/mL ADA	rbt anti-denosumab pAb Number 3	2.78	2.69	3%
100 ng/mL ADA		18.58	18.38	1%
1000 ng/mL ADA		163.56	169.21	-3%
10 ng/mL ADA	rbt anti-denosumab pAb Number 4	1.80	1.67	7%
100 ng/mL ADA		8.15	8.56	-5%
1000 ng/mL ADA		67.29	71.30	-6%
10 ng/mL ADA	mu anti-denosumab 2.5.1 mAb	6.21	6.33	-2%
100 ng/mL ADA		50.97	54.34	-7%
1000 ng/mL ADA		447.25	466.03	-4%
10 ng/mL ADA	rat anti-denosumab 3.12 mAb	3.20	3.45	-8%
100 ng/mL ADA		23.37	24.98	-7%
1000 ng/mL ADA		222.09	242.39	-9%

% S/N Inhibition = (Untreated S/N – Treated S/N) / Untreated S/N * 100

[83] These results further demonstrated that the detection of ADA was not impacted when an anti-RANKL mAb was incorporated into the assay.

Example 4. Assessing impact of anti-RANKL antibody on specific RANKL signal

[84] PNHS was spiked with human RANKL at various levels starting with low levels previously shown to generate a low positive (1250 pg/mL), medium (2500 pg/mL), and high (5000 pg/mL) levels. The RANKL-spiked samples were then evaluated in 3 different ways (with 3 different reaction buffers): 1) with a conjugate/neutralizing mixture (untreated); 2) with a conjugate/neutralization mixture plus anti-RANKL mAb; and 3) with a conjugate/neutralizing mixture plus anti-RANKL mAb and denosumab (confirmatory assay).

[85] The results from the experiment are shown in Table 2. Increased S/N results with increasing RANKL concentrations were observed when the untreated conjugate/neutralizing mixture was used. Results from the experiment where a conjugate/neutralization mixture plus anti-RANKL mAb was used showed inhibition (>20%) of the specific RANKL signal at all concentrations tested, indicating that the use of the anti-RANKL mAb in the assay inhibited signal generated by target. Results from the assay where a conjugate/neutralization mixture plus anti-RANKL mAb and denosumab were used also showed a greater than 20% inhibition of the S/N results.

Table 2. Impact of anti-RANKL antibody on RANKL signal

Sample	Reaction Buffer Composition				
	Untreated S/N	Anti-RANKL mAb-Treated S/N	Anti-RANKL mAb-Treated % S/N Inhibition	AMG 162 + Anti-RANKL mAb-Treated S/N	AMG 162 + Anti-RANKL mAb-Treated % S/N Inhibition
Negative Control (NC)	1.00	1.00	0%	1.00	0%
1250 pg/mL huRANKL	1.94	0.99	49%	1.04	46%
2500 pg/mL huRANKL	3.01	1.15	62%	1.00	67%
5000 pg/mL huRANKL	4.86	1.33	73%	0.93	81%

% S/N Inhibition = (Untreated S/N – Treated S/N) / Untreated S/N * 100

[86] These combined results demonstrated that detection of RANKL was inhibited when an anti-RANKL mAb was incorporated into the assay.

Example 5. Assessing impact of anti-RANKL antibody on samples containing both ADA and RANKL

[87] In order to simulate study samples, which may contain both ADA and RANKL, PNHS was spiked with low (10 ng/mL) or medium (100 ng/mL) concentrations of ADA and low (1250 pg/mL), medium (2500 pg/mL), and high (5000 pg/mL) concentrations of RANKL. The combination samples were then evaluated in 3 different ways (with 3 different reaction buffers): 1) with a conjugate/neutralizing mixture (untreated); 2) with a conjugate/neutralization mixture plus anti-RANKL mAb; and 3) with a conjugate/neutralizing mixture plus anti-RANKL mAb and denosumab (confirmatory assay).

[88] The results from the experiment are shown in Table 3A. Increasing S/N values with increasing ADA and/or RANKL concentrations were observed when the untreated conjugate/neutralizing mixture was used. Results from the experiment where a conjugate/neutralization mixture plus anti-RANKL mAb was used showed that a portion of the signal was inhibited, indicating that the specific ADA signal was not inhibited. Results from the assay where a conjugate/neutralization mixture plus anti-RANKL mAb and denosumab were used showed inhibition of the signal (>20%) at all concentrations tested.

Table 3A. Impact of anti-RANKL antibody on samples containing both ADA and RANKL

Sample	Reaction Buffer Composition				
	Untreated S/N	Anti-RANKL mAb-Treated S/N	Anti-RANKL mAb-Treated % S/N Inhibition	AMG 162 + Anti-RANKL mAb-Treated S/N	AMG 162 + Anti-RANKL mAb-Treated % S/N Inhibition
Negative Control (NC)	1.00	1.00	0%	1.00	0%
10 ng/mL ADA / 1250 pg/mL RANKL	2.94	1.92	35%	1.03	65%
10 ng/mL ADA / 2500 pg/mL RANKL	4.01	1.95	51%	1.05	74%
10 ng/mL ADA / 5000 pg/mL RANKL	5.69	2.00	65%	0.97	83%
100 ng/mL ADA / 1250 pg/mL RANKL	13.58	11.49	15%	1.05	92%
100 ng/mL ADA / 2500 pg/mL RANKL	16.23	11.67	28%	1.11	93%
100 ng/mL ADA / 5000 pg/mL RANKL	18.13	11.20	38%	1.11	94%

% S/N Inhibition = (Untreated S/N – Treated S/N) / Untreated S/N * 100

[89] Table 3B shows the S/N results of the combination samples compared to the S/N results of samples containing ADA only (no RANKL) at the same concentrations. At both ADA concentrations (and increasing RANKL concentrations), an increase in S/N was observed. Results from the experiment where the untreated conjugate/neutralization mixture plus anti-RANKL mAb was used showed S/N results consistent with those generated by the ADA-only samples at the same concentrations, indicating that the anti-RANKL mAb was successful at inhibiting the RANKL-specific signal without impacting the specific ADA signal.

Table 3B. Impact of anti-RANKL antibody on samples containing both ADA and RANKL compared to samples containing ADA only

Sample Composition		Reaction Buffer Composition		% Recovery of ADA Signal	%CV of S/N Results of Untreated and Anti-RANKL mAb-Treated Samples containing ADA or ADA + RANKL
ADA Concentration (ng/mL)	RANKL Concentration (pg/mL)	Untreated S/N	Anti-RANKL mAb-Treated S/N		
10	0	2.06	1.90	92%	3%
	1250	2.94	1.92	93%	
	2500	4.01	1.95	95%	

Sample Composition		Reaction Buffer Composition		% Recovery of ADA Signal	%CV of S/N Results of Untreated and Anti-RANKL mAb-Treated Samples containing ADA or ADA + RANKL
ADA Concentration (ng/mL)	RANKL Concentration (pg/mL)	Untreated S/N	Anti-RANKL mAb-Treated S/N		
	5000	5.69	2.00	97%	
100	0	13.08	11.65	89%	6%
	1250	13.58	11.49	88%	
	2500	16.23	11.67	89%	
	5000	18.13	11.20	86%	

% Recovery = (Untreated S/N ÷ Treated S/N) * 100

[90] These results demonstrated that the detection of ADA was not impacted and that the detection of RANKL was inhibited when an anti-RANKL mAb was incorporated into the assay.

[91] As denosumab binds to human RANKL with high affinity, soluble RANKL that may be present in human serum samples may interfere (cause false negative results) or cross-react (cause false positive results in the assay). In order to minimize possible interference or cross-reactivity by RANKL, a human-murine chimeric anti-RANKL monoclonal antibody that competes with denosumab for binding to RANKL was incorporated into the assay.

Example 6. Characterization of anti-RANKL antibodies

[92] *ELISA assays.* Soluble RANKL (sRANKL) at 10ug/ml in 1XPBS/0.05%azide (50ul/well) was used to coat on Costar 3368 medium binding 96 well plate; samples were incubated overnight 4°C. Plates were washed using Titertek with RO (reverse osmosis) water with 3-cycle wash; then blocked with 250ul of 1XPBS/1% milk, incubated at least 30 min RT; then washed using Titertek with RO water with 3-cycle wash again. Primary antibodies were purified mAbs clones, titrated 1:3 from 1ug/ml 1XPBS/1% milk (50ul/well), and incubated 1 hour at RT. Positive control 1 (dmab-IgG1) was a modified denosumab where the original IgG2 Fc region of denosumab was replaced with IgG1 Fc region. IgG1 Fc was more convenient for detection. Positive control 2 was OPG-Fc titrated 1:3 from 1 ug/ml. After incubating with primary antibodies, the plates were washed using Titertek with RO water 3-cycle wash, then incubated with secondary antibody. Secondary antibody was goat anti-human IgG Fc (cross-adsorbed)-HRP (Thermo Fisher, 31413) at 0.4ug/ml, in 1XPBS/1% milk (50ul/well), incubated 1 hour at RT. Samples were then washed using Titertek with RO water 3-cycle wash. Substrate was treated with 1 step TMB (Neogen, 308177) (50ul/well) quenched with 1N Hydrochloric acid (50ul/well) after 30 minutes at RT. Plates were read at 450nm, and the results were shown in Tables 4.

Table 4. Soluble human RANKL Binding Data

Purified Mabs\Conc.	sHuman RANK-L @ 10ug/mL							
	1ug/ml	0.333	0.111	0.037	0.012	0.004	0.001	0
anti-RANKL mAb	5.1	4.4	4.0	2.6	1.5	0.7	0.4	0.2
Positive control 1	6.0	6.0	6.0	6.0	3.0	1.5	0.7	0.2
OPG-Fc	6.0	5.5	5.0	4.5	3.2	1.6	0.6	0.2
Neg control 1	0.3	0.3	0.3	0.3	1.5	0.3	0.23	0.3
Neg control 2	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2

[93] *FACS assays.* Standard FACS protocols are as follows: Ren 218.9 stable cells (CHO/RANKL) were counted and seeded in FACS buffer (1X PBS + 2% Fetal Bovine Serum) such that there were 50,000 cells/well in 50uls/well final volume. Cells and primary antibody at 5ug/mL and 0.25ug/mL were incubated 1 hour at 4°C, then washed 2x with 200uls/well FACS buffer. 100uls/well of secondary antibody mixed with dead cell stain 7-Aminoactinomycin D (7AAD) (Sigma, A9400-5MG) were then added. Goat anti human IgG Cy5 secondary antibody (Jackson, 109-175-098) and 7AAD were both used at 5.0 ug/mL and were incubated with the samples for 15 minutes at 4°C and then washed 1x with 200uls/well FACS buffer. Cells were then resuspended in 100ul FACS buffer and read by flow cytometry with high throughput screening (HTS) head.

Table 5 FACS binding Data

Purified Mabs\Conc.	mAb @ 5 ug/mL X Geo Mean	mAb @ 0.25 ug/mL X Geo Mean
anti-RANKL mAb	5353.4	1981.38
Positive control 1	5567.8	3393.54
Neg control 1	4.98	4.92
Neg control 2	4.49	4.74

[94] *Additional ELISA assays with human mouse and rat soluble RANKL.* An ELISA protocol was used that was nearly identical to ELISA assays described above, except the human, mouse and rat were photobiotinylated (pb) and captured onto neutravidin (NA). Tables 6-7 show results of ELISA assays using human, mouse, and rat RANKL.

Table 6. Soluble human and mouse RANKL Binding Data

Purified Mabs\Conc.	Human RANK-L @ 250 ng/mL				Mouse RANKL			
	1ug/ml	0.1	0.01	0.001	1ug/ml	0.1	0.01	0.001
anti-RANKL mAb	4.710	3.525	1.946	0.264	0.064	0.061	0.055	0.057

Table 7. Soluble rat RANKL Binding Data

Purified Mabs\Conc.	Rat RANK-L @ 250 ng/mL			
	1ug/ml	0.1	0.01	0.001
anti-RANKL mAb	0.079	0.067	0.068	0.070

[95] Table 8 summarizes the characteristics of certain anti-RANKL antibodies disclosed herein.

Table 8 Characteristics of antibodies to human RANKL

Antibody	Immunogen		Neutralizing	Epitope
H1	human RANKL 143-317	monoclonal	Yes	DE loop
Denosumab	human RANKL on CHO	monoclonal	Yes	DE loop
C1	rec. human RANKL	monoclonal	Yes	DE loop
A1	rec. human RANKL or human RANKL on CHO	monoclonal	Yes	DE loop
B1	rec. human RANKL	monoclonal	Yes	DE loop
F1	rec. human RANKL	monoclonal	Yes	ND
E1	human RANKL on CHO	monoclonal	Yes	ND
D1	rec. human RANKL	monoclonal	Yes	DE loop

[96] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[97] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the disclosure (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted.

[98] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range and each endpoint, unless otherwise indicated herein, and each separate value and endpoint is incorporated into the specification as if it were individually recited herein.

[99] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the disclosure and does not pose a limitation on the scope of the disclosure unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the disclosure.

[100] Preferred embodiments of this disclosure are described herein, including the best mode known to the inventors for carrying out the disclosure. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the disclosure to be practiced otherwise than as specifically described herein. Accordingly, this disclosure includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the disclosure unless otherwise indicated herein or otherwise clearly contradicted by context.

SEQUENCE TABLES

TABLE A: Sequence ID numbers for CDRs (AA)

Antibody Name	Heavy Chain (HC)			Light Chain (LC)		
	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
A1	1	2	3	4	5	6
B1	7	8	9	10	11	12
C1	13	14	15	16	17	18
D1	19	20	21	22	23	24
E1	25	26	27	28	29	30
F1	31	32	33	34	35	36
G1	37	38	39	40	41	42
H1	43	44	45	46	47	48
I1	49	50	51	52	53	54
J1	55	56	57	58	59	60
K1	61	62	63	64	65	66
L1	67	68	69	70	71	72
M1	73	74	75	76	77	78
N1	79	80	81	82	83	84
O1	85	86	87	88	89	90
P1	91	92	93	94	95	96
Q1	97	98	99	100	101	102
R1	103	104	105	106	107	108
S1	109	110	111	112	113	114
T1	115	116	117	118	119	120
U1	121	122	123	124	125	126

TABLE B: Sequence ID numbers for Variable Regions (AA)

Antibody Name	HC variable (VH)	LC variable (VL)
A1	127	128
B1	129	130
C1	131	132
D1	133	134
E1	135	136
F1	137	138
G1	139	140
H1	141	142
I1	143	144
J1	145	146
K1	147	148
L1	149	150
M1	151	152
N1	153	154
O1	155	156
P1	157	158
Q1	159	160
R1	161	162
S1	163	164
T1	165	166
U1	167	168

Table C: CDR sequences

SEQ ID NO.	Name	Sequence
1	A1_VH CDR1	SNGMH
2	A1_VH CDR2	GIGTAGGTYADSVKG
3	A1_VH CDR3	KNWGWFDP
4	A1_VL CDR1	RASQSVNSYLA
5	A1_VL CDR2	DASNRAT
6	A1_VL CDR3	QQRSNWPPFT
7	B1_VH CDR1	SNGMH
8	B1_VH CDR2	GIGTAGGTYADSVKG
9	B1_VH CDR3	KDWGWFDP
10	B1_VL CDR1	RASQSISSYLA
11	B1_VL CDR2	DASNRAT
12	B1_VL CDR3	QQRSKWPPYT
13	C1_VH CDR1	SYGMH
14	C1_VH CDR2	GIGTGGGTYADSVKG
15	C1_VH CDR3	KNWGWFDY
16	C1_VL CDR1	RASQSISSYLA
17	C1_VL CDR2	DASNRAT

SEQ ID NO.	Name	Sequence
18	C1_VL CDR3	QQRSKWPPYT
19	D1_VH CDR1	GHALH
20	D1_VH CDR2	GIGTHGGTTYADSVKG
21	D1_VH CDR3	RNWGQFDY
22	D1_VL CDR1	RASQSVSSYLA
23	D1_VL CDR2	DASNRAT
24	D1_VL CDR3	QQRSNWPPYT
25	E1_VH CDR1	AYPMH
26	E1_VH CDR2	GIGSGGGTNYADSVKG
27	E1_VH CDR3	GRNSFDY
28	E1_VL CDR1	RASQGISTWLA
29	E1_VL CDR2	AASSLQS
30	E1_VL CDR3	QQYNSYPPT
31	F1_VH CDR1	HYPLH
32	F1_VH CDR2	GIHTGGGTYYTDSVKG
33	F1_VH CDR3	GRNSFDY
34	F1_VL CDR1	RASQGISSWLA
35	F1_VL CDR2	AASSLQS
36	F1_VL CDR3	QQYNSYPPT
37	G1_VH CDR1	RYAMN
38	G1_VH CDR2	GISTSGGSTYYADSVKG
39	G1_VH CDR3	DGDRGVIMTVFDY
40	G1_VL CDR1	RASQSVRSSYLA
41	G1_VL CDR2	GASSRAT
42	G1_VL CDR3	QQYGSSPRT
43	H1_VH CDR1	NYAIH
44	H1_VH CDR2	WINAGNGNTKFSQKFQG
45	H1_VH CDR3	DSSNMVRGIIIAYYFDY
46	H1_VL CDR1	RASQISRYLN
47	H1_VL CDR2	GASSLQS
48	H1_VL CDR3	QHTRA
49	I1_VH CDR1	NYAIH
50	I1_VH CDR2	WINAGNGNTKFSQKFQG
51	I1_VH CDR3	DSSNMVRGIIIAYYFDY

SEQ ID NO.	Name	Sequence
52	I1_VL CDR1	RASQSVGSYLA
53	I1_VL CDR2	DATNRAT
54	I1_VL CDR3	QHRRT
55	J1_VH CDR1	DYAMH
56	J1_VH CDR2	GISWNSGRIGYADSVKG
57	J1_VH CDR3	GGSTSARYSSGWYY
58	J1_VL CDR1	SGDALPKQYVY
59	J1_VL CDR2	EDSERPS
60	J1_VL CDR3	QSTDSSGTYVV
61	K1_VH CDR1	NYPMH
62	K1_VH CDR2	VISYDGNKYYADSVKG
63	K1_VH CDR3	GGGGFDY
64	K1_VL CDR1	RASQSVSSSLA
65	K1_VL CDR2	GASSRAT
66	K1_VL CDR3	QQYGA
67	L1_VH CDR1	NYWMS
68	L1_VH CDR2	SISSSSSYIYYADSVKG
69	L1_VH CDR3	QSESSVYGMDV
70	L1_VL CDR1	SGSHSNIASSSAN
71	L1_VL CDR2	GNDQRPS
72	L1_VL CDR3	AAWDDSLSGVV
73	M1_VH CDR1	NAWMS
74	M1_VH CDR2	SISSSSSYIYYADSVKG
75	M1_VH CDR3	DGSIFGVVTPFDY
76	M1_VL CDR1	TGSSSNIGAGYDVH
77	M1_VL CDR2	SNNQRPS
78	M1_VL CDR3	AAWDDSLRGPHWV
79	N1_VH CDR1	NTWMN
80	N1_VH CDR2	SISVNSNYIYYADSVKG
81	N1_VH CDR3	DSEYAFQN
82	N1_VL CDR1	TGSSSNIGAGYDVH
83	N1_VL CDR2	DNEWRP
84	N1_VL CDR3	ATWDDSLHWV
85	O1_VH CDR1	DHWMS

SEQ ID NO.	Name	Sequence
86	O1_VH CDR2	SISSSSSYIYYADSVKG
87	O1_VH CDR3	HEDTYYYYGMDV
88	O1_VL CDR1	SGSFSNIGGNTVN
89	O1_VL CDR2	DNDQRPS
90	O1_VL CDR3	ASWDDSLSGVV
91	P1_VH CDR1	KDWMS
92	P1_VH CDR2	VISYDGSNKYYADSVKG
93	P1_VH CDR3	AAPHYYYGLDV
94	P1_VL CDR1	SGSSSNIGSNTVN
95	P1_VL CDR2	YDDLPS
96	P1_VL CDR3	GSWDSSL SAVV
97	Q1_VH CDR1	GFGMH
98	Q1_VH CDR2	VISYDGRNKYYADSVKG
99	Q1_VH CDR3	SLSGTYWGYGMDV
100	Q1_VL CDR1	RASQSIRSWLA
101	Q1_VL CDR2	KASSLES
102	Q1_VL CDR3	QQYNSYLWT
103	R1_VH CDR1	GYGIH
104	R1_VH CDR2	VISYDGTNKYYGDSVKG
105	R1_VH CDR3	SLSGTYWGYGMDV
106	R1_VL CDR1	RASQNIGNWLA
107	R1_VL CDR2	KASSLQN
108	R1_VL CDR3	QRYNSYSWT
109	S1_VH CDR1	GYGMH
110	S1_VH CDR2	VISYDGSNKHYADSVKG
111	S1_VH CDR3	SLSGTYWGYGMDV
112	S1_VL CDR1	RASQNISSWLA
113	S1_VL CDR2	KASSLES
114	S1_VL CDR3	QQYNRYSWT
115	T1_VH CDR1	NYYIE
116	T1_VH CDR2	VINPGWGD TNYNEKFKG
117	T1_VH CDR3	RDTAHGYYALDP
118	T1_VL CDR1	KASONVGTNVA
119	T1_VL CDR2	SASYRYS

SEQ ID NO.	Name	Sequence
120	T1_VL CDR3	QQYWDYPLT
121	U1_VH CDR1	NYYIE
122	U1_VH CDR2	VINPGWGDNTYNEKFKG
123	U1_VH CDR3	RDTRHGYALDY
124	U1_VL CDR1	KASONVGTNVA
125	U1_VL CDR2	SASYRYS
126	U1_VL CDR3	QQYNDYPLT

Table D: VH VL sequences

SEQ ID NO	Name	Sequence
127	A1 VH	EVQLVQSGGGLVHPGGSRLRLSCEGSGFTFSSNGMHWVRQTPGKGLEWVSGIGTAGGTYADSVKG RFTISRDNVKKSLYLQMNSLRAEDMAIYYCVRNWGWFDPPWGQALVTVSS
128	A1 VL	EIVLTQSPATLSLSPGERATLSCRASQSVNSYLAWFQQKPGQAPRLLIYDASNRATGIPARFSGS GSGTDFTLTISSLEPEDFAIYYCQQRSNWPPPTFGQGTKLEIKR
129	B1 VH	EVQLVQSGGGLVHPGGSRLRLSCEGSGFTFSSNGMHWVRQTPGKGLEWVSGIGTAGGTYADSVKG RFTISRDNVKKSLYLQMNSLRAEDMAIYYCVRKDWGWFDPPWGQALVTVSS
130	B1 VL	EIVLTQSPATLSLSPGERATLSCRASQSISSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGS GSGTDFTLTISSLEPEDFAVYYCQQRSKWPPPTFGQGTKLEIKR
131	C1 VH	EVQLVQSGGGLVHPGGSRLRLSCAGSGFTFSSYGMHWVRQAPGKGLEWVSGIGTGGGTYADSVKG RFTISRDNVKNLSLYLQMNSLRAEDMAVYYCARKNWGWFDYWGQGTLTVTSS
132	C1 VL	EIVLTQSPATLSLSPGERATLSCRASQSISSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGS GSGTDFTLTISSLEPEDFAVYYCQQRSKWPPPTFGQGTKLEIKR
133	D1 VH	EVQLVQSGGGLVHPGGSRLRLSCAGSGFTFSGHALHWVRQAPGKGLEWVSGIGTHGGTYADSVKG RFTISRDNVKNLSLYLQMNSLRAEDMAVYYCTRNRWGWFDYWGQGTLTVTSS
134	D1 VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGS GSGTDFTLTISSLEPEDFAVYYCQQRSNWPPPTFGQGTKLEIKR
135	E1 VH	EVQLVQSGGGLVHPGGSRLRLSCVGSRTFSAYPEMHWVRQAPGKGLEWVSGIGSGGGTNYADSVKG RFTISRDTAKNSLYLQMNSLRAEDMAVYYCARGRNSFDYWGQGTLTVTSS
136	E1 VL	DIQMTQSPSSLSASVGDRTITCRASQGISTWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGS GSGTDFTLTISLQPEDFATYYCQQYNSYPPTFGQGTKVEIKR
137	F1 VH	EVQLVQSGGDLVHPGGSRLRLSCVGSRTFSSHYPHWRQAPGKGLEWISGIHTGGGTYTDSVKG RFTISSDNVKNLSLYLQMNTLRAEDMAVYYCARGRNSFDYWGQGTLTIVSS
138	F1 VL	DIQMTQSPSSLSASVGDRTITCRASQGISWYLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGS GSGTDFTLTISLQSEDFATYYCQQYNSYPPTFGQGTREIKR
139	G1 VH	EVHLLSGGGLVQPGGSRLRLSCAASGFTFSRYAMNWVRQAPGKGLEWVSGISTSGGSTYYADSVK GRFTISRDISRNTLNLMNSLRAEDTAVYYCAKDGDRGVIMTVFDYWGQGTLTVTSS
140	G1 VL	EIVLTQSPGTLSPGERATLSCRASQSVRSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG SGSGTDFTLTISRLEPEDFAVYYCQQYGSPPPTFGQGTKVEIKR
141	H1 VH	QVQLVQSGAEVRKPGASVKVSKASGYDFSNIYAIHWVRQAPGQRLEWMGWINAGNNTKFSQKFQ GRITVTRDTAASTAYMELRSLRSEDYAVYYCARDSSNMVRGII IAYYFDYWGQGTLTVTSS
142	H1 VL	EIVMTQSPSSLSASVGDRTITCRASQISRYLNWYQLKPGKAPRLLIYGASSLQSGVPSRFSGS GSGAEFTLTISLQPEDATYYCQHTRAFGQTKVEIKR
143	I1 VH	EVQLVQSGAEVRKPGASVKVSKASGYDFSNIYAIHWVRQAPGQRLEWMGWINAGNNTKFSQKFQ GRITVTRDTAASTAYMELRSLRSEDYAVYYCARDSSNMVRGII IAYYFDYWGQGTLTVTSS
144	I1 VL	EIVLTQSPATLSLSPGERATLSCRASQSVGSYLAWYQQKPGQAPRLLIYDATNRATGIPTRFSGS GSGTDFTLTISSLEPEDFATYYCQHRRTFGRGTKLEIKR
145	J1 VH	EVQLLESGGGLVQPGRSRLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGRIGYADSVK GRFTISRDNVKNLSLYLQMNSLRPEDTAVYYCAKGGSTASRYSSGWYYWGQGTLTVTSS
146	J1 VL	QSVLTQPPSVSVSPGQTATITCSGDALPKQYVYWRQKPGQAPLLVIYEDSERPSGIPERFSGSS SGTEVTLTSLSGVQAEDADYYCQSTDSSTGYVVFGGGKLTVLTS

SEQ ID NO	Name	Sequence
147	K1 VH	EVQLVQSGGGLVQPGGSLRSLCSLVSGFTFNNYPMHWVRQAPGKGLEWVAVISYDGNKKYYADSVK GRFTISRDN SKNTLYLQMN SLRSED TAVYYCARGGGGFDYWGQGT LVTVSS
148	K1 VL	EIVMTQSPGTL SLSPGERATL SCRASQSVSSSLAWYQQKPGQAPRLLIY GASSRATGIPDRFSG SGSGTDFTLTISRLEPEDFAVYYCQQYGAFGGG TKVEIKR
149	L1 VH	EVQLLES GGGGLVQPGGSLRSLCAASGFTF SNYWMSWVRQAPGKGLEWVSSISSSSSYIYYADSVK GRFTISRDN SKNTLYLQMN SLRAED TAVYYCARQSESSVYGM DVWGQGT LVTVSS
150	L1 VL	QSVLTQPPSASGTPGQ RVTISCSGSHSN IASSANWYQQLPGTAPKLLIY GNDQRPSGVPDRFSG SKSGTSASLAISGLRSEDEADYYCAAWDDSLSGVVF GGGTKLTVLGQP
151	M1 VH	EVQLLES GGGGLVQPGGSLRSLCAASGFTF SNAWMSWVRQAPGKGLEWVSSISSSSSYIYYADSVK GRFTISRDN SKNTLYLQMN SLRAED TAVYYCARDGSI FG VVTPFDYWGQGT LVTVSS
152	M1 VL	QSVLTQPPSASGTPGQ RVTISCTGSSSN IAGYDVHWYQQLPGTAPKLLIY SNNQRPSGVPDRFS GSKSGTSASLAISGLRSEDEADYYCAAWDDSLRGPHWVF GGGTKLTVLGQP
153	N1 VH	EVQLLES GGGGLVQPGGSLRSLCAASGFTF SNTWMNWVRQAPGKGLEWVSSISVNSNYIYYADSVK GRFTISRDN SKNTLYLQMN SLRAED TAVYYCARDSEYAFQNWGQGT LVTVSS
154	N1 VL	QSVLTQPPSASGTPGQ RVTISCTGSSSN IAGYDVHWYQQLPGTAPKLLIY DNEWRPSGVPDRFS GSKSGTSASLAISGLRSEDEADYYCATWDDSLHWVF GGGTKLTVLGQP
155	O1 VH	EVQLLES GGGGLVQPGGSLRSLCAASGFTF SDHWSWVRQAPGKGLEWVSSISSSSSYIYYADSVK GRFTISRDN SKNTLYLQMN SLRAED TAVYYCARHEDTYYYYGMDVWGQGT LVTVSS
156	O1 VL	QSVLTQPPSASGTPGQ RVTISCSGFSN IGNTVNWYQQLPGTAPKLLIY DNDQRPSGVPDRFSG SKSGTSASLAISGLRSEDEADYYCASWDDSLSGVVF GGGTKLTVLGQP
157	P1 VH	EVQLLES GGGGLVQPGGSLRSLCAASGFTF RKDWSWVRQAPGKGLEWVAVISYDGSNKYYADSVK GRFTISRDN SKNTLYLQMN SLRAED TAVYYCTRAAPHY YYGLDVWGQGT LVTVSS
158	P1 VL	QSVLTQPPSASGTPGQ RVTISCSGSSSN IGSTVNWYQQLPGTAPKLLIY YD DLLPSGVPDRFSG SKSGTSASLAISGPRSEDEADYYCGSWDSSL SAVVF GGGTKLTVLGQP
159	Q1 VH	QVQLVESGGGVVQPGRSLRSLCAASGFTF SFGFMHWVRQAPGKGLEWVAVISYDGRNKYYADSVK GRFTISRDN SKNTLYLQMN SLRAED TAVYYCAKSLSGTYWGYGMDVWGQGT TVTVS
160	Q1 VL	DIQMTQSPSTLSASV GDRVTITCRASQSI RSWLAWYQQKPGKAPKLLIY KASSLES GVP SRFSGS GSGTEFTLTISLQPD DFATYYCQYNSYLWTFGQGT KVEIK
161	R1 VH	QVQLVESGGGVVQPGRSLRSLCAASGFTF SFGYGIHWVRQAPDKGLEWVAVISYDGTNKYYGDSVK GRFTISRDN SKNTLYLQMN SLRTE D TAVYYCAKSLSGTYWGYGMDVWGQGT TVTVS
162	R1 VL	DIQMTQSPSTLSASV GDRVTITCRASQNI GNWLAWYQQKPGKAPKLLIY KASSLQNGVPSRFSGS GSGTEFTLTISLQPD DFATYYCQRYNSYSWTFGQGT KVEIK
163	S1 VH	QVQLVESGGGVVQPGRSLRSLCAASGFTF SFGYGMHWVRQAPGKGLDWVTVISYDGSNKHYYADSVK GRFTISRDN SKNTLYLQMN SSLGPED TAVYYCAKSLSGTYWGYGMDVWGQGT TVTVS
164	S1 VL	DIQMTQSPSTLSASV GDRVTITCRASQNI SSWLAWYQQKPGKAPKLLIY KASSLES GVP SRFSGS GSGTEFTLTVSS LQPD DFATYYCQYNNRYSWTFGQGT KVEIK
165	T1 VH	QVQLVOSGA EVKPKGSSVKVSCASGYAFTNYI IEWVRQAPGQGLEWMGVINPGWGD TNYNEKFK GRVTITADKSTSTAYMELSSL RSED TAVYYCARRDTAHGYALDPWGQGT TVTVSS
166	T1 VL	DIQMTQSPSSLSASV GDRVTITKASON VGTNVAWYQQKPGKAPKLLIY SASYRYSGVPSRFSGS GSGTDFTLTISLQPD DFATYYCQQYWDYPLTFGGG TKVEIK
167	U1 VH	QVQLQQSGAELVRPGTSVKVSCASGYAFTNYI IEWLKORPGQGLEWIGVINPGWGD TNYNEKFK GKATLTADKSSSTAYMQLSSLTSD SAVFFCARRDTRHGYALDYWGQGT SVTVSS
168	U1 VL	DIVMTQSQKFMSTSV GDRVSVTC KASON VGTNVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGS GSGTDFTLTISNVOSED LAEYFCQQYNDYPLTFGAGTRLEIK

Table E: Constant Region sequences

SEQ ID	Name	Sequence
169	Murine LC 1 (Lambda)	QPKSSPSVTLFPSPSSEELTNKATLVCTITDFYPGVVTVDWKVDGTPVTQGMETTQPS KQSNKYM ASSYLTLTARAWERHSSYSCQVTHEGHTVEKSLSRADCS
170	Murine LC 2 (Lambda)	QPKSTPTLT VFPSPSSEELKENKATLVCLISNFPSPSGVTVAWKANGTPITQGVDTSNPT KEGNKFMASSFLHLTSDQWRSHNSFTCQVTHEGDTVEKSLSPAECLE

SEQ ID	Name	Sequence
171	Murine LC (Kappa)	(R)ADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSEKQNGVLNSW TDQDSKDSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC
172	Murine IgG1 CH1	AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQ SDLYTLSSSVTVPESSPRPSETVTCNVAHPASSTKVDDKKI
173	Murine IgG1 hinge	VPRDCGCKPCICT
174	Murine IgG1 HC Fc (CH2-CH3)	VPEVSSVFIFPPKPKDVLITITLTPKVTCTVVDISKDDPEVQFSWFVDDDEVHTAQTP REEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVY TIPPKEQMAKDKVSLTCLMITDFFPEDITVEWQWNGQPAENYKNTQPIMNNTNGSYFVY SKLNVQKSNWEAGNTFTCSVLHEGLHNHHTTEKSLSHSPGK
175	Murine HC IgG2a	AKTTAPSVYP LVPVCGGTG SSVTLGCLVK GYFPEPVTLT WNSGSLSSGV HTFPALLQSG LYTLSSSVTV TSNTWPSQTI TCNVAHPASS TKVDKKIEPR VPITQNPCCP HQRVPPCAAP DLLGGPSVFI FPPKIKDVLMI ISLSPMVTCTV VVDVSEDDPD VQISWFVNNV EVHTAQQTQTH REDYNSTLRV VSALPIQHQD WMSGKEFKCK VNNRALPSPI EKTISKPRGP VRAPQVYVLP PPAEEMTKKE FSLTCLMITGF LPAEIAVDWT SNGRTEQNYK NTATVLDSDG SYFMYSKLRV QKSTWERGSL FACSIVHEVL HNHLTKTKTIS RSLGK
176	Murine HC IgG2b	KTTTPPSVYPLAPGCGDTTGSSVTLGCLVKGYFPESVTVTWNSGSLSSSVHTFPALLQS GLYTMSSSVTVPSSTWPSQTVTCSVAHPASSTVDDKKLEPSGPISTINPCPPCKECKH CPAPNLEGGPSVFI FPPNPKDVLMI SLTPKVTCTVVDVSEDDPDVQISWFVNNVEVHT AQQTQTHREDYNSTIRVSTLPIQHQDWMSGKEFKCKVNNKDLPSPIERTISKIKGLVR APQVYILPPPAEQLSRKDVSLTCLVVGFNPGDISVEWTSNGHTEENYKDTAPVLDSDG SYFIYSKLNMTSKWEKTDSCNVRHEGLKNYYLKKTISRSPGK
177	Murine HC IgG3	TTTAPSVYPLVPGCSDTSGSSVTLGCLVKGYFPEPVTVKWNYGALSSGVRTVSSVLQS GFYSLSSLVTVPSSTWPSQTVICNVAHPASKTELIKRIEPRIPKPPSTPPGSSCPPGNI LGGPSVFI FPPKPKDALMI SLTPKVTCTVVDVSEDDPDVHVSWFVDNKEVHTAWTQPR EAQYNSTFRVVSALPIQHQDWMRGKEFKCKVNNKALPAPIERTISKPKGRAQTPQVYT IPPPREQMSKKKVSILTCLVTNFFSEATSVIEWERNGELEQDYKNTPPILDSGDGTFLYS KLTVDTDSDLQGEIFTCSVVHEALHNHHTQKNLSRSPGK
178	Human LC (kappa)	TVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD SKDSTYSLSS TLTLKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC
179	Human LC (kappa) with optional junction residue	RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD SKDSTYSLSS TLTLKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC
180	Human LC (lambda)	QPKANPTVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADGSPVK AGVETTKPSK QSNNKYAASS YLSLTPEQWK SHRSYSCQVT HEGSTVEKTV APTECS
181	Human LC (lambda) with optional junction residue	GQPKANPTVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADGSPVK AGVETTKPSK QSNNKYAASS YLSLTPEQWK SHRSYSCQVT HEGSTVEKTV APTECS
182	Human IgG1 HC CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDDKKV

SEQ ID	Name	Sequence
183	Human IgG1 HC hinge	EPKSCDKTHTCP
184	Human IgG1 HC Fc (CH2-CH3)	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (K)
185	Human IgG1 HC C-domain	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (K)
186	Human IgG4 CH1	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKRV
187	Human IgG4 hinge	ESKYGPPCPSCP
188	Human IgG4 Fc (CH2-CH3)	APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
189	Human IgG4 HC C-domain	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPAEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
190	Human IgG3 CH1	ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRV
191	Human IgG3 hinge	ELKTFPLGDTTHTCPRCPEPKSCDTFPPCPRCPEPKSCDTFPPCPRCPEPKSCDTFPPCPRC
192	Human IgG3 Fc (CH2-CH3)	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVSVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTTPMLDSGDSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRTQKSLSLSPG
193	Human IgG3 HC C-domain	ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTFPLGDTTHTCPRCPEPKSCDTFPPCPRCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVSVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTTPMLDSGDSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRTQKSLSLSPG
194	Human IgG2 CH1	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTV
195	Human IgG2 hinge	ERKCCVECPPCP
196	Human IgG2 Fc (CH2-CH3)	APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVSVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID	Name	Sequence
197	Human IgG2 HC C-domain	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPSSNFGTQT YTCNVDPKPSNTKVDKTVERKCCVECPPECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQFNSTFRVSVLTQVHQLDWSLGKEYKCKVSNKGLPAPAEKTIKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFNFSVMDHMEALHNHYTQKSLSLSPGK

Table F: other sequences

SEQ ID	Name	Sequence
198	Denosumab heavy chain	EVQLLES GGG LVQPGGSLRL SCAASGFTFS SYAMSWVRQA PGKGLEWVSG ITGSGGSTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDP GT TVIMSWFD PWGQ GLVTV SS ASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPVPSNFGT QTYTCNVDPKPSNTKVDKTVERKCCVECPPECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQFNSTFRVSVLTQVHQLDWSLGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFNFSVMDHMEALHNHYTQKSLSLSPGK (Kabat CDRs underlined, VH in bold)
199	Denosumab light chain	EIVLTQSPGT LSLSPGERAT LSCRASQSVR GRYLAWYQQK PGQAPRLLIY GASSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVFCYQ QYGSSPRTFG QGT KVEIKRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (Kabat CDRs underlined, VL in bold)
200	Denosumab CDR-H1	SYAMS
201	Denosumab CDR-H2	GITGSGGSTYYADSVKG
202	Denosumab CDR-H3	DPGTTVIMSWFDP
203	Denosumab VH	EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYAMSWVRQA PGKGLEWVSG ITGSGGSTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDP GTTVIMSWFD PWGQGLVTV SS
204	Denosumab CH	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPVPSNFGTQTYTCNVDPKPSNTKVDKTVERKCCVECPPECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQFNSTFRVSVLTQVHQLDWSLGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFNFSVMDHMEALHNHYTQKSLSLSPGK
205	Denosumab CDR-L1	RASQSVRGRYLA
206	Denosumab CDR-L2	GASSRAT
207	Denosumab CDR-L3	QYGSPPRT
208	Denosumab VL	EIVLTQSPGT LSLSPGERAT LSCRASQSVR GRYLAWYQQK PGQAPRLLIY GASSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVFCYQ QYGSPPRTFG QGTKEIKRT V
209	Denosumab CL	AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID	Name	Sequence
210	Human OPG	ETFPKYLHYDEETSHQLLCDKCPGTYLKQHCTAKWKTVCAPCPDHYTDSWHTSDE CLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLKHRSCPPGFGVQAGTPE RNTVCKRCPDGGFSNETSSKAPCRKHTNCSVFGLLLTQKGNATHDNICSGNSESTQKC GIDVTLCEEAFFRFAVPTKFTPNWLSVLVDNLPGTKVNAESVERIKRQHSSQEQTQQL LKLWKHQNKDQDIVKKIIQDIDLCENSQVRHIGHANLTFEQLRSLMESLPGKKVGAED IEKTIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHALKHSKTYHFPKTVTQSLKTTI RFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL
211	Human OPG with signal peptide	MNNLLCCALVFLDISIKWTTQETFFPKYLHYDEETSHQLLCDKCPGTYLKQHCTAKW KTVCAPCPDHYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIE FCLKHRSCPPGFGVQAGTPERNTVCKRCPDGGFSNETSSKAPCRKHTNCSVFGLLLT QKGNATHDNICSGNSESTQKCGIDVTLCEEAFFRFAVPTKFTPNWLSVLVDNLPGTKV NAESVERIKRQHSSQEQTQQLLKLWKHQNKDQDIVKKIIQDIDLCENSQVRHIGHANL TFEQLRSLMESLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHA LKHSKTYHFPKTVTQSLKTTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL
212	Human RANKL (membrane bound)	MRRASRDYTKYLRGSEEMGGGPGAPHEGPLHAPPPAPHQPPAASRSMFVALLGLGLG QVVCVALFFYFRAQMDPNRISEDTGTHCIYRILRLHENADFQDTTLESQDTKLIPDSC RRIKQAFQGAVQKELQHIVGSQHIRAEKAMVDGSLDLAKRSKLEAQPFAHLTINATD IPSGSHKVSLSWYHDRGWAKISNMTFSNGKLIVNQDGFYYLYANICFRHHETSGDLA TEYLQLMVYVTKTSIKIPSSHTLMKGGSTKYWSGNSEFHFYSINVGGFFKLRSGEESIS IEVSNPSLLDPDQDATYFGAFKVRDID
213	RANKL (soluble form)	FYFRAQMDPNRISEDTGTHCIYRILRLHENADFQDTTLESQDTKLIPDSCRRIKQAFQG AVQKELQHIVGSQHIRAEKAMVDGSLDLAKRSKLEAQPFAHLTINATDIPSGSHKVS LSSWYHDRGWAKISNMTFSNGKLIVNQDGFYYLYANICFRHHETSGDLATEYLQLMVY VTKTSIKIPSSHTLMKGGSTKYWSGNSEFHFYSINVGGFFKLRSGEESISIEVSNPSLL DPDQDATYFGAFKVRDID
214	RANKL (soluble, via proteolytic cleavage)	IRAEKAMVDGSLDLAKRSKLEAQPFAHLTINATDIPSGSHKVSLSWYHDRGWAKIS NMTFSNGKLIVNQDGFYYLYANICFRHHETSGDLATEYLQLMVYVTKTSIKIPSSHTL MKGGSTKYWSGNSEFHFYSINVGGFFKLRSGEESISIEVSNPSLLDPDQDATYFGAFKV RDID
215	RANKL epitope	DLATE
216	RANKL DE loop	GFYYLYANICFRHHETSGDLATEYLQLMVYVTKTSIKIP

CLAIMS

1. A method of detecting an anti-drug antibody (ADA) in a sample, wherein said ADA binds to denosumab, comprising mixing said sample with:
 - (a) a first antibody or antibody conjugate that binds to RANKL, comprising: the heavy chain (HC) CDR-H1, CDR-H2, and CDR-H3 of SEQ ID NO:203, and the light chain (LC) CDR-L1, CDR-L2, and CDR-L3 of SEQ ID NO:208; wherein said first antibody or antibody conjugate can be attached to a solid substrate;
 - (b) (i) a second antibody that binds to RANKL, wherein said second antibody comprises an amino acid sequence that differs from the first antibody by at least one amino acid in one of the six CDR sequences; or (ii) an osteoprotegerin (OPG) protein that binds to RANKL; and
 - (c) a third antibody that binds to said ADA and can be linked to a detectable marker.
2. The method of claim 1, wherein said first antibody conjugate comprises an antibody molecule noncovalently or covalently linked to an immobilizing moiety, wherein said solid substrate comprises a binding partner, and said immobilizing moiety can be attached to a solid substrate by binding to said binding partner.
3. The method of claim 1 or 2, wherein said first antibody or antibody conjugate comprise a CDR-H1 comprising SEQ ID NO:200, a CDR-H2 comprising SEQ ID NO:201, and a CDR-H3 comprising SEQ ID NO:202; and a CDR-L1 comprising SEQ ID NO:205, a CDR-L2 comprising SEQ ID NO:206, and a CDR-L3 comprising SEQ ID NO:207.
4. The method of any one of claims 1-3, wherein said first antibody or antibody conjugate comprises a heavy chain variable region (VH) that is at least 90% identical to SEQ ID NO:203, and a light chain variable region (VL) that is at least 90% identical to SEQ ID NO:208.
5. The method of any one of claims 1-4, wherein said first antibody or antibody conjugate comprises a human heavy chain constant domain (CH) or a murine heavy chain constant domain (CH).
6. The method of any one of claims 1-5, wherein said first antibody or antibody conjugate comprises a human Fc domain or a murine Fc domain.
7. The method of any one of claims 1-6, wherein said first antibody or antibody conjugate comprises a human light chain constant (CL) domain or a murine light chain constant (CL) domain.
8. The method of any one of claims 1-7, wherein said third antibody is covalently or noncovalently linked to a detectable marker.

9. The method of any one of claims 1-8, said detectable marker is an isotope, an enzyme, a fluorescent moiety, a luminescent moiety, a chromatic moiety, a metal, or an electric charge.
10. The method of any one of claims 1-9, wherein said third antibody comprises six CDR sequences that are identical to that of the first antibody.
11. The method of any one of claims 1-10, wherein said third antibody comprises a VH domain comprising the same sequence as the VH of the first antibody, and a VL domain comprising the same sequence as the VL domain of the first antibody.
12. The method of any one of claims 1-11, wherein said third antibody comprises a heavy chain (HC) comprising the same sequence as the HC of the first antibody, and a light chain (LC) comprising the same sequence as the LC of the first antibody.
13. The method of any one of claims 1-12, wherein said second antibody binds to the same epitope as denosumab.
14. The method of any one of claims 1-13, wherein said second antibody competes with denosumab for RANKL binding.
15. The method of any one of claims 1-14, wherein said second antibody comprises an amino acid sequence that differs from the first antibody by at least one amino acid in each of the six CDR sequences.
16. The method of any one of claims 1-15, wherein said second antibody comprises: (i) the heavy chain CDR-H1, CDR-H2, and CDR3-H3 of SEQ ID NO: 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, or 167; and (ii) the light chain CDR-L1, CDR-L2, and CDR3 of SEQ ID NO: 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, or 168.
17. The method of any one of claims 1-16, wherein said second antibody comprises a human heavy chain constant domain (CH) or a murine heavy chain constant domain (CH).
18. The method of any one of claims 1-17, wherein said second antibody comprises a human Fc domain or a murine Fc domain.
19. The method of any one of claims 1-18, wherein said second antibody comprises a human CL domain or a murine CL domain.
20. A diagnostic kit for detecting an anti-drug antibody (ADA) in a sample, wherein said ADA binds to denosumab, comprising:

- (a) a first antibody or antibody conjugate that binds to RANKL, comprising: the heavy chain (HC) CDR-H1, CDR-H2, and CDR-H3 of SEQ ID NO:203, and the light chain (LC) CDR-L1, CDR-L2, and CDR-L3 of SEQ ID NO:208; wherein said first antibody or antibody conjugate can be attached to a solid substrate;
- (b) (i) a second antibody that binds to RANKL, wherein said second antibody comprises an amino acid sequence that differs from the first antibody by at least one amino acid in one of the six CDR sequences; or (ii) an osteoprotegerin (OPG) protein that binds to RANKL; and
- (c) a third antibody that binds to said ADA and can be linked to a detectable marker; and
- (d) instructions for use.