

CLAIMS

1. An Immunoglobulin (Ig) binding protein, comprising one or more Ig binding domains, wherein at least one Ig binding domain comprises a variant of a parental amino acid sequence of SEQ ID NO: 1, or SEQ ID NO: 2, having at least 3 substitutions as compared to SEQ ID NO: 1 selected from the group consisting of an amino acid substitution to Isoleucine at position 1, an amino acid substitution to alanine, glutamic acid, or isoleucine at position 11, an amino acid substitution to arginine or isoleucine at position 35, and an amino acid substitution to leucine at position 42.
2. The Ig binding protein of claim 1, wherein said Ig binding domain comprises a variant of parental amino acid sequences selected from the group consisting of SEQ ID NO: 3, 4, 10, 14, 21, 25, 47, 48, 49, and 50 having at least 3 amino acid substitutions selected from the group consisting of an amino acid substitution of alanine or proline to isoleucine at position 1, an amino acid substitution of serine to alanine, glutamic acid, or isoleucine at position 11, an amino acid substitution of lysine at position 35 to arginine or isoleucine, and an amino acid substitution of lysine to leucine at position 42.
3. The Ig binding protein of claim 1, wherein said Ig binding domain comprises a variant of parental amino acid sequence of any one of SEQ ID NOs: 5-8, wherein the variant has at least 3 amino acid substitutions selected from the group consisting of an amino acid substitution to isoleucine at position 1, an amino acid substitution to alanine, glutamic acid, or isoleucine at position 11, an amino acid substitution at position 35 to arginine or isoleucine, and an amino acid substitution to leucine at position 42.
4. The Ig binding domain protein of any one of claims 1-3, wherein said variant of the parental amino acid sequence has 4 amino acid substitutions.
5. The Ig binding protein of any one of the preceding claims, wherein the variant comprises a substitution or a plurality of substitutions selected from the group consisting of:
1I/11A/35R; 1I/11E/35R; 1I/11I/35R; 1I/11A/42L; 1I/11E/42L; 1I/11I/42L; 1I/11A/35I;
1I/11E/35I; 1I/11I/35I; 1I/35R/42L; 1I/35I/42L; 11I/35R/42L; 11I/35I/42L; 11A/35R/42L;
11A/35I/42L; 11E/35R/42L; 11E/35I/42L; 1I/11A/35R/42L; 1I/11E/35R/42L;
1I/11I/35R/42L; 1I/11A/35I/42L; 1I/11E/35I/42L; 1I/11I/35I/42L;
1I/11A/28N/35R/42L; 1I/11E/28N/35R/42L; 1I/11I/28N/35R/42L; 1I/11A/28N/35I/42L;
1I/11I/28N/35I/42L; and 1I/11E/28N/35I/42L.

6. The Ig binding protein of claim 5, wherein 3 or 4 of the amino acid positions are selected from the group consisting of 1I, 11A, 35R, and 42L.
- 5 7. The Ig binding protein of any one of claims 1 to 6, wherein said Ig binding domain further comprises 1, 2, 3, 4, 5, or 6 modifications, wherein each individual modification is selected from the group consisting of a single amino acid substitution, a single amino acid deletion, and a single amino acid insertion.
- 10 8. An Ig binding protein, comprising one or more Ig binding domains, wherein at least one Ig binding domain comprises an amino acid sequence of SEQ ID NO: 52, wherein the amino acid at position 5 is selected from H or F, the amino acid at position 7 is selected from K or E, the amino acid at position 8 is selected from D, A, or E, the amino acid at position 25 is selected from D or E, the amino acid at position 28 is selected from S or N,

15 the amino acid at position 42 is selected from L or K, the amino acid at position 44 is selected from I or V, the amino acid at position 46 is selected from G or A, the amino acid at position 49 is selected from K or Q, the amino acid at position 54 is selected from A or S, and the amino acid at position 58 is selected from P or K.
- 20 9. The Ig binding protein of claim 8, wherein acid at position 42 is L.
10. The Ig binding protein of claim 8 or 9, wherein said Ig binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 26, 30, and 42-45, or is a sequence with at least 89.5 % sequence identity to an amino acid

25 sequence selected from the group consisting of SEQ ID Nos: 20, 26, 30, and 42-45.
11. The Ig binding protein of any one of the preceding claims, wherein the protein comprises 2, 3, 4, 5, 6, 7, or 8 Ig binding domains linked to each other.
- 30 12. The Ig binding protein of any one of the preceding claims, wherein the protein is conjugated to a solid support.
13. The Ig binding protein of claim 13, wherein said Ig binding protein further comprises an

35 attachment site for site-specific covalent coupling of said Ig binding protein to a solid support.

14. The Ig binding protein of any one of claims 1 to 14, wherein said Ig binding protein binds to IgG1, IgG2, IgG4, IgM, IgA, Ig fragments comprising the Fc region, fusion proteins comprising an Fc region of an Ig, and conjugates comprising an Fc region of an Ig.

5 15. An affinity separation matrix comprising the Ig binding protein as defined in any one of claims 1 to 14.

16. Use of the Ig binding protein of any one of claims 1 to 14 or of the affinity separation matrix of claim 15 for affinity purification of immunoglobulins.

10 17. A method of affinity purification of immunoglobulins, the method comprising: (a) providing a liquid containing immunoglobulins; (b) providing an affinity separation matrix comprising at least one Ig binding protein of any one of claims 1 to 14 coupled to said affinity separation matrix; (c) contacting said liquid and said affinity separation matrix,
15 wherein said immunoglobulin binds to said Ig binding protein; and (d) eluting said immunoglobulin from said matrix, thereby obtaining an eluate containing said immunoglobulin.

20 18. The method of claim 17, further comprising washing the affinity matrix between steps (c) and (d) under conditions sufficient to remove from the affinity separation matrix some or all molecules that are non-specifically bound thereto.