

Claims

1. A method of purifying a bispecific antibody comprising:

A) loading an antibody preparation in a load buffer onto a hydroxyapatite (HA)

5 resin, wherein:

the antibody preparation comprises: I) an intact bispecific antibody of interest; and II) at least one impurity species, wherein the impurity species are selected from the group consisting of: a) a clipped version of the bispecific antibody of interest, wherein the clipped version of the bispecific antibody of interest is a degradation product from the intact bispecific antibody of interest, and has a mass that is less than 1% different than the mass of the intact bispecific antibody of interest; b) a first parent antibody, wherein the first parent antibody is a monospecific antibody having the same antigen specificity as a first arm of the intact bispecific antibody; c) 10 a second parent antibody, wherein the second parent antibody is a monospecific antibody having the same antigen specificity as a second arm of the intact bispecific antibody; and d) high molecular mass species (HMMS); and

B) washing the resin with a wash buffer comprising phosphate ions at

20 concentration between 10 and 50 mM; and

C) eluting the intact bispecific antibody of interest from the HA resin with an elution buffer comprising a phosphate ion at a concentration between 40 and 100 mM, wherein the concentration of the phosphate ions is increased compared to the wash buffer, such that the bispecific antibody of interest is separated from 25 the at least one impurity;

wherein the pH of at least one of the load buffer, wash buffer, and elution buffer is at or between pH 7.0 and 8.0; and

wherein the bispecific antibody is an anti-BCMA / anti-CD3 bispecific antibody comprising an anti-BCMA arm and an anti-CD3 arm, wherein

30 (a) the anti-CD3 arm comprises (i) a VH region comprising an amino acid sequence as shown in SEQ ID NO: 1 and a VL region comprising an amino acid sequence as shown in SEQ ID NO: 3, or (ii) a heavy chain comprising an

amino acid sequence as shown in SEQ ID NO: 2 and a light chain comprising an amino acid sequence as shown in SEQ ID NO: 4; and

5 (b) the anti- BCMA arm comprises (i) a VH region comprising an amino acid sequence as shown in SEQ ID NO: 5 and a VL region comprising an amino acid sequence as shown in SEQ ID NO: 7, or (ii) a heavy chain comprising an amino acid sequence as shown in SEQ ID NO: 6 and a light chain comprising an amino acid sequence as shown in SEQ ID NO: 8.

10 2. The method of claim 1, wherein the antibody is a heterodimeric bispecific antibody.

3. The method of claim 1 or 2, further comprising collecting a purified fraction eluted from the HA resin, wherein the purified fraction comprises the intact antibody of interest, and wherein the purified fraction comprises at least 95%, 96%, 97%, 98%, or 99% by mass intact antibody of interest.

15 4. The method of claim 3, wherein the purified fraction comprises the intact bispecific antibody and the clipped bispecific antibody, further wherein the ratio of clipped bispecific antibody molecules to intact bispecific antibody molecules in the purified fraction is no greater than 1:100.

20 5. The method of any one of claims 1-4, wherein the antibody preparation is loaded onto the HA resin to a density on the resin of between 5 g/L and 20 g/L.

25 6. The method of any one of claims 1-5, wherein at least 1 gram of antibody preparation is loaded onto the HA resin.

7. The method of any one of claims 1-6, wherein the antibody preparation comprises at least 50% but less than 95% by mass intact antibody of interest.

30 8. The method of any one of claims 1-7, wherein the clipped antibody has a mass that is between 5 and 100 Daltons greater than the mass of the intact antibody.

9. The method of any one of claims 1-8, wherein the clipped antibody has a cleaved peptide bond in a polypeptide chain of the antibody, and wherein the cleaved peptide bond is in a heavy chain of the antibody.
- 5 10. The method of any one of claims 1-9, wherein the clipped antibody contains the same number of amino acids and the same amino acid sequences as the intact antibody.
11. The method of any one of claims 1-9, wherein the clipped antibody contains a
10 different number of amino acids as the intact antibody.
12. The method of any one of claims 1-11, wherein the antibody of interest comprises a VH and VL domain which specifically bind to CD3, and wherein the clipped antibody comprises a cleaved peptide bond in the VH domain that specifically binds CD3.
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13. The method of any one of claims 1 to 12, wherein the clipped version of the bispecific antibody has the same amino acid sequences as the intact bispecific antibody, except that the peptide bond between amino acid residue 56 and amino acid residue 57 of the N terminal of the VH region of the CD3 arm of the clipped version of the bispecific
20 antibody is cleaved, and the clipped off 56 amino acids portion of the VH region of the CD3 arm of the clipped version of the bispecific antibody remains bound to the rest of the clipped version of the bispecific antibody through a disulfide bond.
14. The method of any one of claims 1-13, wherein the HA resin is ceramic
25 hydroxyapatite (cHA) resin.
15. The method of any one of claims 1-14, wherein the concentration of the phosphate ion is increased during the elution from 40 mM to 100 mM.
- 30 16. The method of any one of claims 1-15, wherein the antibody preparation contains proteins that were previously loaded onto and eluted from at least one of: i) a protein A resin and ii) an ion exchange resin.

17. The method of any one of claims 1-16, wherein the antibody is isolated and/or purified for use as or in the preparation of pharmaceuticals.

18. An antibody preparation comprising an anti-BCMA / anti-CD3 bispecific antibody
5 comprising an anti-BCMA arm and an anti-CD3 arm, wherein

(a) the anti-CD3 arm comprises

(i) a VH region comprising an amino acid sequence as shown in SEQ ID NO: 1
and a VL region comprising an amino acid sequence as shown in SEQ ID NO: 3, or

(ii) a heavy chain comprising an amino acid sequence as shown in SEQ ID NO: 2
10 and a light chain comprising an amino acid sequence as shown in SEQ ID NO: 4; and

(b) the anti-BCMA arm comprises

(i) a VH region comprising an amino acid sequence as shown in SEQ ID NO: 5
and a VL region comprising an amino acid sequence as shown in SEQ ID NO: 7, or

(ii) a heavy chain comprising an amino acid sequence as shown in SEQ ID NO: 6
15 and a light chain comprising an amino acid sequence as shown in SEQ ID NO: 8; and

wherein the preparation comprises no more than 1% clipped bispecific antibody
as an impurity.

19. The antibody preparation of claim 18, wherein the clipped version of the bispecific
20 antibody has the same amino acid sequences as the intact bispecific antibody, except
that the peptide bond between amino acid residue 56 and amino acid residue 57 of the
N terminal of the VH region of the CD3 arm of the clipped version of the bispecific
antibody is cleaved, and the clipped off 56 amino acids portion of the VH region of the
CD3 arm of the clipped version of the bispecific antibody remains bound to the rest of
25 the clipped version of the bispecific antibody through a disulfide bond.