

Claims

It is claimed:

1. A method of purifying an antibody comprising:

5 A) loading an antibody preparation in a load buffer onto a hydroxyapatite (HA) resin, wherein:

 the antibody preparation comprises: I) an intact antibody of interest and II) a clipped version of the antibody of interest, wherein the clipped version of the antibody of interest is a degradation product from the intact antibody of interest, and has a mass that is less than 10% different than the mass of the intact antibody of interest; and

 B) eluting the intact antibody of interest from the HA resin with an elution buffer comprising an ion, wherein the concentration of the ion in the elution buffer is increased during the elution.

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2. A method of purifying a bispecific antibody comprising:

 A) loading an antibody preparation in a load buffer onto a hydroxyapatite (HA) 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 10% 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) 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

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B) eluting the intact bispecific antibody of interest from the HA resin with an elution buffer comprising an ion, wherein the concentration of the ion in the elution buffer is increased during the elution.

5 3. A method of purifying a bispecific antibody comprising:

A) loading an antibody preparation in a load buffer onto a hydroxyapatite (HA) resin, wherein:

10 I) the antibody preparation comprises: a) an intact bispecific antibody of interest and b) a clipped version of the bispecific antibody of interest, wherein the clipped version of the antibody of interest is a degradation product from the intact bispecific antibody of interest, and has a mass that is less than 10% different than the mass of the intact bispecific antibody of interest; and

15 II) the ratio of molecules of the clipped bispecific antibody to molecules of the intact bispecific antibody in the antibody preparation is between is between at least 1:50 and no greater than 1:5;

B) eluting the intact bispecific antibody from the HA resin with an elution buffer comprising an ion, wherein the concentration of the ion in the elution buffer is increased during the elution, and optionally,

20 C) collecting a purified fraction eluted from the HA resin, wherein the purified fraction comprises the intact bispecific antibody.

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

25 5. 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.

30 6. 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.

7. The method of claim 1, wherein the antibody of interest is an anti-CD3 antibody, and wherein the antibody comprises at least one of the following: i) a VH region comprising an amino acid sequence as shown in SEQ ID NO: 1; ii) a heavy chain comprising an amino acid sequence as shown in SEQ ID NO: 2; iii) 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 iv) 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.
8. The method of any one of claims 2-6, wherein the bispecific antibody is: i) an anti-BCMA / anti-CD3 bispecific antibody comprising an anti-BCMA arm and an anti-CD3 arm, or ii) an anti-FLT3 / anti-CD3 bispecific antibody comprising an anti-FLT3 arm and an anti-CD3 arm.
9. The method of claim 8, wherein the anti-CD3 arm comprises at least one of the following: i) a VH region comprising an amino acid sequence as shown in SEQ ID NO: 1; ii) a heavy chain comprising an amino acid sequence as shown in SEQ ID NO: 2; iii) 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 iv) 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.
10. The method of claim 8 or 9, wherein the anti-BCMA arm comprises at least one of the following: i) a VH region comprising an amino acid sequence as shown in SEQ ID NO: 5; ii) a heavy chain comprising an amino acid sequence as shown in SEQ ID NO: 6; iii) 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 iv) 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.
11. The method of claim 8 or 9, wherein the anti-FLT3 arm comprises at least one of the following: i) a VH region comprising an amino acid sequence as shown in SEQ ID

- NO: 9; ii) a heavy chain comprising an amino acid sequence as shown in SEQ ID NO: 10; iii) a VH region comprising an amino acid sequence as shown in SEQ ID NO: 9 and a VL region comprising an amino acid sequence as shown in SEQ ID NO: 11; or iv) a heavy chain comprising an amino acid sequence as shown in SEQ ID NO: 10 and a light chain comprising an amino acid sequence as shown in SEQ ID NO: 12.
12. The method of any one of claims 1-11, 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.
13. The method of any one of claims 1-12, wherein at least 1 gram of antibody preparation is loaded onto the HA resin.
14. The method of any one of claims 1-13, wherein the antibody preparation comprises at least 50% but less than 95% by mass intact antibody of interest.
15. The method of any one of claims 1-14, wherein the clipped antibody has a mass that less than 1% different than the mass of the intact antibody.
16. The method of any one of claims 1-15, wherein the clipped antibody has a mass that is between about 5 and 100 Daltons greater than the mass of the intact antibody.
17. The method of any one of claims 1-16, 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.
18. The method of any one of claims 1-17, wherein the clipped antibody contains the same number of amino acids and the same amino acid sequences as the intact antibody.
19. The method of any one of claims 1-17, wherein the clipped antibody contains a different number of amino acids as the intact antibody.

20. The method of any one of claims 1-19, 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.
- 5 21. The method of any one of claims 1-20, wherein the HA resin is ceramic hydroxyapatite (cHA) resin.
22. The method of any one of claims 1-21, wherein after loading the antibody preparation onto the HA resin but prior to eluting the intact bispecific antibody the resin
10 is washed with a wash buffer comprising phosphate ions at concentration between 10 and 50 mM.
23. The method of any one of claims 1-22, wherein the ion in the elution buffer is phosphate.
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24. The method of any one of claims 1-23, wherein the concentration of the phosphate ion is increased during the elution from about 40 mM to 100 mM.
25. The method of any one of claims 1-24, wherein the pH of at least one of the load
20 buffer, wash buffer, and elution buffer is at or between about pH 7.0 and 8.0.
26. The method of any one of claims 1-25, 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.
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27. The method of any one of claims 1-26, wherein the antibody is isolated and/or purified for use as or in the preparation of pharmaceuticals.