CLAIMS

1. A method for purifying a protein comprising an Fc region from a solution comprising the protein and one or more impurities, comprising:

adsorbing the protein to a temperature-responsive protein A material at a temperature at which the protein binds to the material; and

eluting the protein from the material at a temperature below about 35 °C with an elution buffer having a pH of about 6.5 to about 7.5 and comprising a chaotropic agent and a sugar alcohol, wherein the molar concentration ratio of the chaotropic agent to sugar alcohol is about 0.4 to about 4.5.

2. The method of claim 1, wherein the molar concentration ratio of the chaotropic agent to sugar alcohol is about 1.5 to about 2.5.

3. The method of claim 1, wherein the molar concentration ratio of the chaotropic agent to sugar alcohol is about 1.8 to about 2.2.

4. The method of claim 1, wherein the molar concentration ratio of the chaotropic agent to sugar alcohol is about 0.5 to about 1.5.

5. The method of any one of claims 1 to 4, wherein the elution buffer further comprises one or more amino acids.

6. The method of claim 5, wherein the elution buffer comprises a basic amino acid and/or an apolar amino acid.

7. A method for purifying a protein comprising an Fc region, comprising:

contacting a solution comprising the protein and one or more impurities with a temperature-responsive protein A material at a temperature at which the protein binds to the material; and

eluting the protein from the material at a temperature below about 35 °C with an elution buffer having a pH of about 6.5 to about 7.5 and comprising a chaotropic agent, a sugar alcohol, an apolar amino acid, and a basic amino acid, wherein the protein is purified from one or more impurities in the solution.

8. The method of claim 6 or 7, wherein the basic amino acid is histidine, lysine, ornithine, or arginine.

9. The method of claim 8, wherein the basic amino acid is arginine.

10. The method of claim 6 or 7, wherein the apolar amino acid is alanine, cysteine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, or valine.

11. The method of claim 10, wherein the apolar amino acid is proline.

12. The method of any one of claims 6 to 11, wherein the elution buffer comprises arginine and proline.

13. The method of any one of claims 6 to 12, wherein the basic amino acid and/or the apolar amino acid is present at a concentration of about 0.25 M to about 1 M.

14. The method of claim 13, wherein the basic amino acid and/or the apolar amino acid is present at a concentration of about 0.5 M.

15. The method of any one of claims 1 to 14, wherein the chaotropic agent is urea, guanidinium chloride, sodium thiocyanate, potassium thiocyanate, or ammonium thiocyanate.

16. The method of claim 15, wherein the chaotropic agent is urea.

17. The method of claim 16, wherein the urea is present at a concentration of about 2 M to about 4.5 M.

18. The method of claim 17, wherein the urea is present at a concentration of about 4 M.

19. The method of claim 15, wherein the chaotropic agent is guanidinium chloride.

20. The method of any one of claims 1 to 19, wherein the sugar alcohol is sorbitol, mannitol, xylitol, or glycerol.

21. The method of claim 20, wherein the sugar alcohol is sorbitol.

22. The method of any one of claims 1 to 21, wherein the sugar alcohol is present at a concentration of about 1 M to about 4.5 M.

23. The method of claim 22, wherein the sugar alcohol is present at a concentration of about 2 M to about 2.5 M.

24. The method of any one of claims 1 to 23, wherein the elution buffer further comprises a salt.

25. The method of claim 24, wherein the salt is present at a concentration of about 0.25 M to about 0.8 M.

26. The method of claim 24, wherein the salt is sodium chloride.

27. The method of claim 26, wherein the elution buffer comprises about 2 M to about 4.5 M urea, about 1 M to about 4.5 M sorbitol, about 0.25 M to about 1 M arginine, about 0.25 M to about 1 M proline, and 0.25 M to about 0.8 M sodium chloride.

28. The method of claim 27, wherein the elution buffer comprises about 4 M urea, about 2.2 M sorbitol, about 0.5 M arginine, about 0.5 M proline, and about 0.75 M sodium chloride.

29. The method of any one of claims 1 to 28, wherein the elution buffer has a pH of about 7.0 to about 7.4.

30. The method of any one of claims 1 to 29, wherein eluting the protein from the material is performed at a temperature from about 1 °C to about 25 °C.

31. The method of claim 30, wherein eluting the protein from the material is performed at a temperature from about 1 °C to about 6 °C.

32. The method of claim 30, wherein eluting the protein from the material is performed at a temperature from about 20 °C to about 25 °C.

33. The method of any one of claims 1 to 32, wherein the temperature-responsive protein A material is immobilized to a solid phase.

34. The method of claim 33, wherein the solid phase is a bead, a resin, a gel, a film, or a particle.

35. The method of any one of claims 1 to 34, wherein contacting the solution with the material is performed at a temperature of about 10 °C or less.

36. The method of claim 35, wherein contacting the solution with the material is performed at a temperature of about 1°C to about 6 °C.

37. The method of any one of claims 1 to 36, further comprising washing the material having the bound protein with one or more wash solutions prior to eluting the protein from the material.

38. The method of claim 37, wherein the one or more wash solutions comprises sodium chloride at a concentration of about 0.5 M to about 2 M.

39. The method of any one of claims 1 to 38, wherein the protein comprising an Fc region is recombinantly produced in a mammalian cell.

40. The method of claim 39, wherein the mammalian cell is a Chinese Hamster Ovary cell.

41. The method of any one of claims 1 to 40, wherein the protein comprising an Fc region is an antibody.

42. The method of any one of claims 1 to 40, wherein the protein comprising an Fc region is an Fc fusion protein.

43. The method of claim 42, wherein the Fc fusion protein comprises at least one single chain Fv fragment.

44. The method of any one of claims 1 to 43, wherein one or more impurities comprises host cell protein, host cell DNA, cell culture protein, or combinations thereof.

45. The method of any one of claims 1 to 44, wherein the solution comprising the protein and one or more impurities is a harvest from a production bioreactor, a cell culture supernatant, or a cell lysate.

46. The method of any one of claims 1 to 45, wherein at least 70% of the Fc region-containing protein in the eluate from the material is in monomeric form.

47. The method of claim 46, wherein at least 80% of the Fc region-containing protein in the eluate from the material is in monomeric form.

48. The method of claim 47, wherein at least 90% of the Fc region-containing protein in the eluate from the material is in monomeric form.

49. The method of any one of claims 1 to 48, further comprising subjecting the eluted Fc region-containing protein to one or more chromatography steps, preferably selected from ion exchange chromatography, hydrophobic interaction chromatography, mixed mode chromatography, size exclusion chromatography, hydroxyapatite chromatography, metal affinity chromatography, and combinations thereof.

50. The method of any one of claims 1 to 49, further comprising subjecting the eluted Fc region-containing protein to size exclusion chromatography using a mobile phase having a pH of about 6.5 to about 7.5 and comprising a chaotropic agent, a sugar alcohol, an apolar amino acid, and a basic amino acid.