CLAIMS

1. A method of purifying a polypeptide comprising an Fc region, the method comprising the steps of:

(a) contacting a Protein A chromatography matrix with a sample comprising (i) the polypeptide comprising the Fc region, and (ii) one or more impurities, under a condition that the polypeptide comprising the Fc region binds to Protein A; and

(b) washing the matrix with a wash solution, wherein the wash solution comprises one or both of (i) a benzoate salt at a concentration of 0.1 M to 1.0 M and (ii) benzyl alcohol at a concentration of 0.5% to 4% volume/volume (v/v), and wherein the wash solution has a pH of 4.0 to 10.0.

2. The method of claim 1, wherein the wash solution comprises: 1) benzoate salt; 2) benzyl alcohol; or 3) benzoate salt and benzyl alcohol.

3. The method of claim 1 or claim 2, wherein the benzoate salt is at a concentration from 0.1 M to 0.5 M.

4. The method of any one of claims 1-3, wherein the benzoate salt is a benzoate alkali salt.

5. The method of any one of claims 1-4, wherein the benzoate salt is sodium benzoate.

6. The method of claim 5, wherein the sodium benzoate is at a concentration from 0.1 M to 0.3 M.

7. The method of claim 5, wherein the sodium benzoate is at a concentration of 0.3 M.

8. The method of claim 5, wherein the sodium benzoate is at a concentration of 0.5 M.

9. The method of any one of claims 1-8, wherein the benzyl alcohol is at a concentration from 1% to 4% (v/v).

10. The method of any one of claims 1-8, wherein the benzyl alcohol is at a concentration from % to 2% (v/v).

11. The method of any one of claims 1-8, wherein the benzyl alcohol is at a concentration of 2% (v/v) to 4% (v/v).

12. The method of any one of claims 1-11, wherein the wash solution further comprises a buffering agent.

13. The method of claim 11, wherein the buffering agent is selected from the group consisting of phosphate, tris, arginine, acetate, and citrate.

14. The method of claim 11 or claim 13, wherein the buffering agent is at a concentration of 10 mM to 500 mM.

15. The method of claim 11 or claim 13, wherein the buffering agent is at a concentration of 50 mM or 500mM.

16. The method of any one of claims 1-15 wherein the wash solution has a pH of 5.0 to 10.0.

17. The method of any one of claims 1-16, wherein the wash solution has a pH of 5.0 to 9.0.

18. The method of any one of claims 1-16, wherein the wash solution has a pH of 5.0, 6.0, 7.0, 9.0, or 10.0.

19. The method of any one of claims 1-18, wherein the wash solution further comprises sodium benzenesulfonate.

20. The method of claim 19, wherein the sodium benzenesulfonate is at a concentration of 0.1 M to 0.5 M.

21. The method of any one of claims 1-20, wherein the wash solution further comprises caprylic acid.

22. The method of claim 21, wherein the caprylic acid is at a concentration of 10 mM to 50 mM.

23. The method of any one of claims 1-22, wherein the wash solution further comprises hexylene glycol.

24. The method of claim 23, wherein the hexylene glycol is at a concentration of 1% to 10% (v/v).

25. The method of any one of claims 1-24, wherein the wash solution further comprises creatine.

26. The method of claim 25, wherein the creatine is at a concentration of 10 mM to 100 mM.

27. The method of any one of claims 1-26, wherein the wash solution further comprises arginine.

28. The method of claim 27, wherein the arginine is at a concentration of 0.1 M to 1.0 M.

29. The method of claim 27, wherein the arginine is at a concentration of 0.5 M.

30. The method of any one of claims 27-29, wherein the arginine is arginine-HCl.

31. The method of any one of claims 27-30, wherein the wash solution comprising arginine has a pH of 4.0 to 6.0.

32. The method of any one of claims 27-30, wherein the wash solution comprising arginine has a pH of 8.0 to 10.0.

33. The method of any one of claims 1-32, wherein the wash solution further comprises one or more non-buffering salts.

34. The method of claim 33, wherein the one or more non-buffering salts are selected from the group consisting of sodium chloride, sodium bromide, potassium chloride, potassium

bromide, magnesium chloride, magnesium bromide, calcium chloride, calcium bromide, and any combinations thereof.

35. The method of claim 33, wherein the one or more non-buffering salts are sodium chloride and/or potassium chloride.

36. The method of any one of claims 33-35, wherein the one or more non-buffering salts are at a concentration of 0.1 M to 1.0 M.

37. The method of any one of claims 1-36, wherein the wash solution is a solution selected from the group consisting of:

(i) a solution comprising sodium benzoate at a concentration of 0.5 M, and sodium bicarbonate at a concentration of 50 mM, having a pH of 10.0;

(ii) a solution comprising sodium benzoate at a concentration of 0.5 M, benzyl alcohol at a concentration of 2%, arginine at a concentration of 0.5 M, and sodium phosphate at a concentration of 50mM, having a pH of 9.0;

(iii) a solution comprising sodium benzoate at a concentration of 0.5 M and benzyl alcohol at a concentration of 2% (v/v), having a pH of 7.0;

(iv) a solution comprising sodium benzoate at a concentration of 0.5 M, benzyl alcohol at a concentration of 2% (v/v), and sodium chloride at a concentration of 0.5 M, having a pH of 7.0;

(v) a solution comprising hexylene glycol at a concentration of 10% (v/v), sodium benzoate at a concentration of 0.5 M, and benzyl alcohol at a concentration of 2% (v/v), having a pH of 7.0;

(vi) a solution comprising benzenesulfonate at a concentration of 0.5 M, sodium benzoate at a concentration of 0.5 M, and benzyl alcohol at a concentration of 2% (v/v), having a pH of 7.0;

(vii) a solution comprising caprylic acid at a concentration of 50 mM, sodium benzoate at a concentration of 0.5 M, arginine at a concentration of 0.5 M, and sodium chloride at a concentration of 0.5 M, having a pH of 7.0;

(viii) a solution comprising sodium benzoate at a concentration of 0.5 M, benzyl alcohol at a concentration of 2% (v/v), and arginine at a concentration of 0.5 M, having a pH of 6.0;

(ix) a solution comprising sodium benzoate at a concentration of 0.5 M, benzyl alcohol at a concentration of 2% (v/v), and arginine at a concentration of 0.5 M, having a pH of 5.0; and

(x) a solution comprising benzyl alcohol at a concentration of 2% (v/v) and arginine at a concentration of 0.5 M, having a pH of 5.0.

38. The method of any one of claims 1-37, further comprising a step of washing the matrix with a first solution prior to washing the matrix with the wash solution of step (b) in claim 1.

39. The method of claim 38, wherein the first solution comprises a buffer selected from the group consisting of a phosphate buffer, a tris buffer, an acetate buffer, a carbonate buffer, a citrate buffer, and any combinations thereof.

40. The method of claim 39, wherein the first solution comprises the buffer at a concentration of 10 mM to 100 mM.

41. The method of any one of claims 38-40, wherein the first solution is a phosphate buffer.

42. The method of any one of claims 1-41, further comprising a step of washing the matrix with a second solution after washing the matrix with the wash solution of step (b) in claim 1.

43. The method of claim 42, wherein the second solution comprises a buffer selected from the group consisting of a phosphate buffer, a tris buffer, an acetate buffer, a carbonate buffer, a citrate buffer, and any combinations thereof.

44. The method of claim 43, wherein the second solution comprises the buffer at a concentration of 10 mM to 100 mM.

45. The method of any one of claims 42-44, wherein the second solution has a pH of 5.0 to 7.0.

46. The method of any one of claims 42-45, wherein the second solution comprises substantially low salt or no salt.

47. The method of any one of claims 1-46, further comprising a step of contacting the Protein A chromatography matrix with an elution solution after one or more washings steps.

48. The method of claim 47, further comprising the step of collecting an eluate comprising the polypeptide comprising the Fc region.

49. The method of acclaim 48, further comprising a step of filtering the eluate via depth filtration.

50. The method of claim 48 or claim 49, wherein the eluate comprises less than 500 parts per million (ppm) of the one or more impurities.

51. The method of any one of claims 1-50, wherein the method results in the polypeptide comprising the Fc region being purified away from the one or more impurities to a higher degree than a corresponding method lacking the step of washing the matrix with the wash solution.

52. The method of any one of claims 1-51, wherein the one or more impurities are host cell proteins (HCPs).

53. The method of claim 52, wherein the one or more HCPs are selected from the group consisting of phospholipases, clusterin, serine proteases, elongation factors, and any combinations thereof.

54. The method of claim 52 or claim 53, wherein the HCP is Putative Phospholipase B-like 2 (PLBL2).

55. The method of claim 52 or claim 53, wherein the host cell is a mammalian host cell.

56. The method of any one of claims 52-55, wherein the host cell is a Chinese hamster ovary (CHO) cell.

57. The method of any one of claims 1-56, wherein the Fc region is a human Fc region.

58. The method of claim 57, wherein the human Fc region comprises a human IgG1, IgG2, or IgG4 Fc region.

59. The method of any one of claims 1-56, wherein the Fc region is a mouse Fc region.

60. The method of claim 59, wherein the mouse Fc region comprises a mouse IgG1, IgG2, or IgG3 Fc region.

61. The method of any one of claims 1-60, wherein the polypeptide comprising the Fc region is an antibody.

62. The method of claim 61, wherein the antibody is a human antibody, a humanized antibody, or a chimeric antibody.

63. The method of claim 61 or claim 62, wherein the antibody is a monoclonal antibody.

64. The method of claim 61 or claim 62, wherein the antibody is a bispecific antibody or a trispecific antibody.

65. The method of any one of claims 1-64, further comprising, before step (a), adjusting a harvest comprising the polypeptide comprising the Fc region to achieve a final concentration of a benzoate salt of between 0.1 M and 0.5 M and a pH between 7.0 and 9.0 to produce the sample comprising (i) the polypeptide comprising the Fc region, and (ii) one or more impurities.

66. A method of purifying a polypeptide comprising an Fc region, the method comprising the steps of:

(A) adjusting a harvest comprising the polypeptide comprising the Fc region to achieve a final concentration of a benzoate salt of 0.1M and 0.5M and a pH between 7.0 and 9.0 to produce a sample comprising (i) the polypeptide comprising the Fc region, and (ii) one or more impurities; and

(B) contacting the sample with at least one chromatography matrix.

67. The method of claim 65 or 66, wherein the benzoate salt is a benzoate alkali salt.

68. The method of claim 67, wherein the benzoate salt is sodium benzoate.

69. The method of any one of claims 65-68, wherein the final concentration of the benzoate salt in the harvest is between 0.4M and 0.5M.

70. The method of any one of claims 65-69, wherein the pH of the harvest is between 7.0 and 8.0.

71. The method of any one of claims 65-69, wherein the pH of the harvest is between 8.0 and 9.0.

72. The method of any one of claims 65-67, wherein the harvest is generated from a culture comprising a host cell engineered to express the polypeptide.

73. The method of claim 72, wherein the host cell is a eukaryotic host cell.

74. The method of claim 73, wherein the eukaryotic host cell is a Chinese Hamster Ovary (CHO) cell.

75. The method of any one of claims 65-74, wherein the harvest is clarified prior to the adjusting.

76. The method of any one of claims 65-74, wherein the harvest is clarified following the adjusting.

77. The method of any one of claims 66-76, wherein the at least one chromatography matrix comprises an affinity chromatography matrix.

78. The method of claim 77, wherein the affinity chromatography matrix is a Protein A chromatography matrix or a Protein G chromatography matrix.

79. The method of any one of claims 65-78, further comprising a step of contacting the at least one chromatography matrix with at least one wash solution.

80. The method of anyone of claims 65-79, further comprising a step of contacting the at least one chromatography matrix with an elution solution.

81. The method of any one of claims 80, further comprising the step of collecting an eluate comprising the polypeptide comprising the Fc region.

82. The method of claim 81, further comprising a step of filtering the eluate via depth filtration.

83. The method of claim 81 or claim 82, wherein the eluate comprises less than 500 parts per million (ppm) of the one or more impurities.

84. The method of any one of claims 66-83, wherein the method results in the polypeptide comprising the Fc region being purified away from the one or more impurities to a higher degree than a corresponding method lacking the step of adjusting the harvest comprising the polypeptide comprising the Fc region to produce the sample.

85. The method of any one of claims 66-84, wherein the one or more impurities are host cell proteins (HCPs).

86. The method of claim 85, wherein the one or more HCPs are selected from the group consisting of phospholipases, clusterin, serine proteases, elongation factors, and any combinations thereof.

87. The method of claim 85 or claim 86, wherein the HCP is Putative Phospholipase B-like 2 (PLBL2).

88. The method of any one of claims 66-87, wherein the Fc region is a human Fc region.

89. The method of claim 82, wherein the human Fc region comprises a human IgG1, IgG2, or IgG4 Fc region.

90. The method of any one of claims 66-89, wherein the Fc region is a mouse Fc region.

91. The method of claim 90, wherein the mouse Fc region comprises a mouse IgG1, IgG2, or IgG3 Fc region.

92. The method of any one of claims 66-91, wherein the polypeptide comprising the Fc region is an antibody.

93. The method of claim 92, wherein the antibody is a human antibody, a humanized antibody, or a chimeric antibody.

94. The method of claim 92 or claim 93, wherein the antibody is a monoclonal antibody.

95. The method of claim 92 or claim 93, wherein the antibody is a bispecific antibody or a trispecific antibody.