

What is claimed is

1. A method of purifying a multispecific protein from a composition comprising the multispecific protein and at least one product-related impurity, the method comprising
equilibrating a cation exchange chromatography medium with an equilibration buffer comprising 94-105 mM sodium chloride;
loading the composition on to the cation exchange medium in a load buffer comprises 94-105 mM sodium chloride;
washing the column with at least one wash buffer comprising 94-105 mM sodium chloride; and
eluting the multispecific protein from the cation exchange chromatography medium.
2. The method according to claim 1, wherein the load buffer comprises 94-96 mM sodium chloride.
3. The method according to claim 2, wherein the load buffer comprises 96-105 mM sodium chloride.
4. The method according to claim 2, wherein the load buffer comprises 94 mM sodium chloride.
5. The method according to claim 2, wherein the load buffer comprises 96 mM sodium chloride.
6. The method according to claim 2, wherein the load buffer comprises 98 mM sodium chloride.
7. The method according to claim 2, wherein the load buffer comprises 105 mM sodium chloride.
8. The method according to claim 1, wherein the load buffer comprises acetate.
9. The method according to claim 8, wherein the load buffer comprises acetate, pH 4.9-5.1.
10. The method according to claim 8, wherein the load buffer comprises acetate, pH 5.0 ± 0.05 to 5.0 ± 0.1 .
11. The method according to claim 8, wherein the load buffer comprises 100 mM acetate.
12. The method according to claim 1, wherein the load buffer comprises acetate, 94-105 mM sodium chloride.
13. The method according to claim 1, wherein at least one wash buffer comprises 94-105 mM sodium chloride.
14. The method according to claim 13, wherein at least one wash buffer comprises 94-96 mM sodium chloride.

15. The method according to claim 13, wherein at least one wash buffer comprises 96-105 mM sodium chloride.
16. The method according to claim 13, wherein at least one wash buffer comprises 94 mM sodium chloride.
17. The method according to claim 13, wherein at least one wash buffer comprises 96 mM sodium chloride.
18. The method according to claim 13, wherein at least one wash buffer comprises 98 mM sodium chloride.
19. The method according to claim 13, wherein at least one wash buffer comprises 105 mM sodium chloride.
20. The method according to claim 1, wherein at least one wash buffer comprises acetate.
21. The method according to claim 20, wherein at least one wash buffer comprises acetate, pH 4.9-5.1.
22. The method according to claim 20, wherein at least one wash buffer comprises acetate, pH 5.0 ± 0.05 to 5.0 ± 0.1 .
23. The method according to claim 20, wherein at least one wash buffer comprises 100 mM acetate.
24. The method according to claim 1, wherein at least one wash buffer comprises acetate, 94 mM–105 mM sodium chloride.
25. The method according to claim 1, wherein the method comprises at least one additional wash buffer.
26. The method according to claim 25, wherein at least one additional wash buffer is a second wash buffer.
27. The method according to claim 25, wherein at least one additional wash buffer comprises 0-26 mM sodium chloride.
28. The method according to claim 25, wherein at least one wash buffer comprises acetate, 94-105 mM sodium chloride, followed by at least one additional wash buffer comprising acetate, 0-25 mM sodium chloride.
29. The method according to claim 1, wherein at least one equilibration buffer comprises 94-105mM sodium chloride.

30. The method according to claim 29, wherein at least one equilibration buffer comprises 94-96 mM sodium chloride.
31. The method according to claim 29, wherein at least one equilibration buffer comprises 96-105 mM sodium chloride.
- 5 32. The method according to claim 29, wherein at least one equilibration buffer comprises 94 mM sodium chloride.
33. The method according to claim 29, wherein at least one equilibration buffer comprises 96 mM sodium chloride.
34. The method according to claim 29, wherein at least one equilibration buffer comprises 98 mM
10 sodium chloride.
35. The method according to claim 29, wherein at least one equilibration buffer comprises 105 mM sodium chloride.
36. The method according to claim 1, wherein the equilibration buffer comprises acetate.
37. The method according to claim 36, wherein the equilibration buffer comprises acetate, pH 4.9-
15 5.1.
38. The method according to claim 36, wherein the equilibration buffer comprises acetate, pH 5.0 ± 0.05 to 5.0 ± 0.1 .
39. The method according to claim 36, wherein the equilibration buffer comprises 100 mM acetate.
40. The method according to claim 1, wherein the composition is loaded at 10-27 g/L.
- 20 41. The method according to claim 40, wherein the composition is loaded at 15-27 g/L.
42. The method according to claim 1, wherein the multispecific protein is eluted from the cation exchange resin by a gradient.
43. The method according to claim 42, wherein the gradient is linear.
44. The method according to claim 42, wherein the gradient is a salt gradient.
- 25 45. The method according to claim 1, wherein the multispecific protein is a bispecific protein.
46. The method according to claim 1, wherein the multispecific protein is a bispecific antibody.
47. A purified, multispecific protein prepared by a method according to claim 1.
48. The method according to claim 1, wherein the cation exchange chromatography medium is a resin.

49. A method of reducing low pI impurities in the eluate from cation exchange chromatography, the method comprising
- equilibrating a cation exchange chromatography medium with an equilibration buffer comprising 94-105 mM Sodium chloride;
 - 5 loading the composition on to the cation exchange medium in a load buffer comprises 94-105 mM Sodium chloride;
 - washing the column with at least one wash buffer comprising 94-105 mM Sodium chloride;
 - and
 - eluting the multispecific protein from the cation exchange chromatography medium;
- 10 wherein the cation exchange chromatography eluate has reduced low pI impurities compared to the cation exchange chromatography eluate recovered in a corresponding method in which no sodium chloride is used in the equilibration, load, and wash steps.
50. The method according to claim 49, wherein the low pI impurity is a product-related impurity.
51. The method according to claim 50, wherein at least one product-related impurity is a half
- 15 antibody or 2X, 3X, or 4X light chain-mis-assembly.
52. A method of performing cation exchange chromatography under high salt loading conditions to reduce product-related impurities, the method comprising
- equilibrating a cation exchange chromatography medium with an equilibration buffer;
 - loading the composition on to the cation exchange medium in a load buffer;
 - 20 washing the column with a first and a second wash buffer; and
 - eluting the multispecific protein from the cation exchange chromatography medium;
- wherein the equilibration, loading and first wash buffers comprise 94-105 mM Sodium chloride.
53. The method according to claim 52, wherein the second wash buffer comprises 0-26 mM Sodium chloride.
- 25 54. A method of producing an isolated, purified, recombinant multispecific protein, the method comprising
- establishing a cell culture in a bioreactor with a host cell expressing the multispecific protein;
 - culturing the host cells to express the multispecific protein;
 - harvesting the recombinant multispecific protein;
 - 30 affinity purifying the harvested recombinant multispecific protein;

inactivating virus at low pH in the eluate pool from the affinity purification and neutralizing the pool;

equilibrating a cation exchange chromatography medium with an equilibration buffer comprising 94-105 mM Sodium chloride;

5 loading the neutralized affinity purified recombinant multispecific protein on to the equilibrated cation exchange medium in a load buffer comprises 94-105 mM Sodium chloride;

washing the cation exchange medium with a wash buffer comprising 94-105 mM Sodium chloride, followed by a second wash buffer comprising 0-26mM Sodium chloride;

eluting the multispecific protein from the cation exchange chromatography medium;

10 loading the cation exchange chromatography eluate comprising the recombinant multispecific protein onto a second chromatography resin in flow through mode; and

concentrating the purified recombinant multispecific protein in a formulation buffer.

55. The method according to claim 47, wherein the second chromatography resin is selected from an anion exchange chromatography resin, cation exchange chromatography resin, multi-modal
15 chromatography resin, hydrophobic interaction chromatography resin, and hydroxyapatite chromatography resin.

56. An isolated, purified, recombinant multispecific protein prepared by a method according to claim 55.

57. A pharmaceutical composition comprising the isolated, purified, recombinant multispecific
20 protein prepared by a method according to claim 55.