What is claimed:

1. A multi component buffer system for the purification of proteins by a series of chromatography steps, where the modes of chromatography are selected from the group consisting of affinity chromatography, anion exchange chromatography, cation exchange chromatography, and mixed-mode chromatography, wherein the modes of chromatography are operated in either bind-elute mode or flow-through mode, where the multi component buffer system comprises an organic acid, an alkaline metal or ammonium salt of the conjugate base of the organic acid, and an organic base and wherein the modes of chromatography are performed using buffers that are made without the addition of NaCl.

2. The system of claim 1, wherein the affinity chromatography is performed using a superantigen immobilized on a solid phase.

3. The system of claim 2 wherein the superantigen is selected from the group consisting of Protein A, Protein G, and Protein L.

4. The system of any preceding claim, wherein the organic acid is selected from the group consisting of formic acid, acetic acid, lactic acid, citric acid, malic acid, maleic acid, glycine, glycylglycine, succinic acid, TES (2-{[tris(hydroxymethyl)methyl]amino}ethanesulfonic acid), MOPS (3-(N-morpholino)propanesulfonic acid), PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid)), and MES (2-(N-morpholino)ethanesulfonic acid).

5. The system of any preceding claim, wherein the organic base is selected from the group consisting of tris base, Bis-Tris, Bis-Tris-Propane, Bicine (N,N-bis(2-hydroxyethyl)glycine), HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid), TAPS (3-{[tris(hydroxymethyl)methyl]amino}propanesulfonic acid), and Tricine (N-tris(hydroxymethyl)methylglycine).

6. The system of any preceding claim wherein the conjugate base of the organic acid is the sodium, potassium, or ammonium salt of the conjugate base of the organic acid.

7. The system of any preceding claim wherein the organic acid is acetic acid.

8. The system of any preceding claim wherein the organic base is tris base.

9. The system of any preceding claim wherein the organic acid is acetic acid and the conjugate base of acetic acid is the sodium salt.

10. The system of any preceding claim wherein the protein is an antigen binding protein.

11. The system of claim 10 wherein the antigen binding protein is an antibody of the IgG class.

12. The system of claim 10 wherein the antigen binding protein is a immunoglobulin single variable domain.

13. The system of any preceding claim wherein the sequence of chromatographic steps comprises protein A chromatography and flowthrough anion exchange chromatography.

14. The system of any preceding claim wherein the sequence of chromatographic steps comprises protein A chromatography, flowthrough anion exchange chromatography, and cation exchange chromatography.

15. The system of any preceding claim wherein the sequence of chromatographic steps comprises protein A chromatography performed in the presence of 55 mM tris base, 45 mM acetic acid, at about pH 7.5.

16. The system of any preceding claim wherein the sequence of chromatographic steps comprises flowthrough anion exchange chromatography performed in the presence of 55 mM tris base, 45 mM acetic acid, at about pH 7.5.

17. The system of any preceding claim wherein the sequence of chromatographic steps comprises cation exchange chromatography performed in the presence of 25 mM sodium acetate, 12.1 mM acetic acid, at about pH 5.0.

18. A method for purifying a protein from a contaminated solution thereof by Protein A chromatography comprising: (a) equilibrating a Protein A immobilized on a solid phase with a Protein A equilibration buffer comprising 55 mM tris base, 45 mM acetic acid, at about pH 7.5; (b) adsorbing the protein from the contaminated solution to the Protein A immobilized on the solid phase; (c) removing contaminants by washing the solid phase with a first Protein A wash buffer comprising 55 mM Tris Base, 45 mM Acetic acid, 300 mM Sodium Acetate, at about pH 7.5; and (d) recovering the protein from the solid phase with a Protein A elution buffer comprising 1.8 mM Sodium Acetate, 28.2 mM Acetic acid, at about pH 3.6, wherein all the buffers are made without the addition of NaCl.

19. The method of claim 18, further comprising the following step after step (c) and before step (d): removing contaminants by washing the solid phase with a second Protein A wash buffer comprising 55 mM Tris Base, 45 mM Acetic acid, at about pH 7.5, wherein the second Protein A wash buffer is made without the addition of NaCl.

20. The method of claim 18 or 19, further comprising the following steps after step (d): (e) titrating the solution containing the recovered protein to about pH 3.0 with 30 mM acetic acid, 100 mM HCl; (f) allowing the solution of step (e) to remain at about pH 3.0 for about 30 to about 60 minutes; and (g) adjusting the pH of the solution of step (f) to about pH 7.5 with 1 M Tris.

21. The method of claim 20 further comprising filtering the solution produced by step (g) of claim 18.

22. A method for purifying a protein from a contaminated solution thereof by flowthrough anion exchange chromatography comprising: (a) equilibrating an anion exchange matrix with an anion equilibration buffer comprising 55 mM tris base, 45 mM acetic acid, at about pH 7.5; (b) applying the contaminated solution to the anion exchange matrix and collecting the first flowthrough; and (c) applying an anion wash buffer comprising 55 mM

Tris Base, 45 mM Acetic acid, 300 mM Sodium Acetate, at about pH 7.5 to the anion exchange matrix and collecting the second flowthrough, wherein all the buffers are made without the addition of NaCl.

23. The method of claim 22 wherein the contaminated solution is the solution produced by step (g) of claim 18 or the filtered solution of claim 19.

24. The method of claim 22 or 23 wherein the first flowthrough and the second flowthrough are combined into a single combined flowthrough solution.

25. The method of claims 22, 23, or 24 wherein the pH of the first flowthrough, the second flowthrough, and the single combined flowthrough solution is adjust to about pH 5.0 with 30 mM acetic acid, 100 mM HCl.

26. A method for purifying a protein from a contaminated solution thereof by cation exchange chromatography comprising: (a) equilibrating a cation exchange matrix with a cation equilibration buffer comprising 25 mM sodium acetate, 12.1 mM acetic acid, at about pH 5.0; (b) adsorbing the protein from the contaminated solution to the cation exchange matrix; (c) removing contaminants by washing the solid phase with a first cation wash buffer comprising 25 mM sodium acetate, 12.1 mM acetic acid, at about pH 5.0; and (d) recovering the protein from the solid phase with a cation elution buffer comprising 175 mM sodium acetate, 75 mM acetic acid, at about pH 5.0, wherein all the buffers are made without the addition of NaCl.

27. The method of claim 26 wherein the contaminated solution is selected from the first flowthrough of claims 20 or 21, the second flowthrough of claims 20 or 21, the single flowthrough combined solution of claim 22, and the pH-adjusted flowthrough produced by claim 23.

CLAIMS

1. A multi component buffer system for the purification of proteins by a series of chromatography steps, where the modes of chromatography comprise affinity chromatography, and wherein the modes of chromatography are operated in either bind-elute mode or flow-through mode, where the multi component buffer system comprises an organic acid, an alkaline metal or ammonium salt of the conjugate base of the organic acid, and an organic base and wherein the modes of chromatography are performed using buffers that are made without the addition of NaCl.

2. The system of claim 1, wherein the affinity chromatography is performed using a superantigen immobilized on a solid phase.

3. The system of claim 2 wherein the superantigen is selected from the group consisting of Protein A, Protein G, and Protein L.

4. The system of any preceding claim, wherein: (a) the organic acid is selected from the group consisting of formic acid, acetic acid, lactic acid, citric acid, malic acid, maleic acid, glycine, glycylclycine, succinic acid, TES (2-{[tris(hydroxymethyl)methyl]amino}ethanesulfonic acid), MOPS (3-(N-morpholino)propanesulfonic acid), PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid)), and MES (2-(N-morpholino)ethanesulfonic acid);

(b) the organic base is selected from the group consisting of tris base, Bis-Tris, Bis-Tris-Propane, Bicine (N,N-bis(2-hydroxyethyl)glycine), HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid), TAPS (3-{[tris(hydroxymethyl)methyl]amino}propanesulfonic acid), and Tricine (N-tris(hydroxymethyl)methylglycine); or

(c) the conjugate base of the organic acid is the sodium, potassium, or ammonium salt of the conjugate base of the organic acid.

5. The system of any preceding claim wherein:
(a) the organic acid is acetic acid;

(b) the organic base is tris base; or

(c) the organic acid is acetic acid and the conjugate base of acetic acid is the sodium salt.

6. The system of any preceding claim wherein the protein is:

(a) an antigen binding protein; and/or

(b) an antibody of the IgG class; or

(c) an immunoglobulin single variable domain.

7. The system of any preceding claim wherein the sequence of chromatographic steps comprises:

(a) protein A chromatography and flowthrough anion exchange chromatography;

(b) protein A chromatography, flowthrough anion exchange chromatography, and cation exchange chromatography;

(c) protein A chromatography performed in the presence of 55 mM tris base, 45 mM acetic acid, at about pH 7.5;

(d) protein A chromatography, flowthrough anion exchange chromatography performed in the presence of 55 mM tris base, 45 mM acetic acid, at about pH 7.5; and/or

(e) protein A chromatography, cation exchange chromatography performed in the presence of 25 mM sodium acetate, 12.1 mM acetic acid, at about pH 5.0.

8. A method for purifying a protein from a contaminated solution thereof by Protein A chromatography comprising:

(a) equilibrating a Protein A immobilized on a solid phase with a Protein A equilibration buffer comprising 55 mM tris base, 45 mM acetic acid, at about pH 7.5;

(b) adsorbing the protein from the contaminated solution to the Protein A immobilized on the solid phase;

(c) removing contaminants by washing the solid phase with a first Protein A wash buffer comprising 55 mM Tris Base, 45 mM Acetic acid, 300 mM Sodium Acetate, at about pH 7.5; and

(d) recovering the protein from the solid phase with a Protein A elution buffer comprising 1.8 mM Sodium Acetate, 28.2 mM Acetic acid, at about pH 3.6, wherein all the buffers are made without the addition of NaCl.

9. The method of claim 8, further comprising the following step after step (c) and before step (d): removing contaminants by washing the solid phase with a second Protein A wash buffer comprising 55 mM Tris Base, 45 mM Acetic acid, at about pH 7.5, wherein the second Protein A wash buffer is made without the addition of NaCl.

10. The method of claim 8 or 9, further comprising the following steps after step (d): (e) titrating the solution containing the recovered protein to about pH 3.0 with 30 mM acetic acid, 100 mM HCl; (f) allowing the solution of step (e) to remain at about pH 3.0 for about 30 to about 60 minutes; and (g) adjusting the pH of the solution of step (f) to about pH 7.5 with 1 M Tris.

11. The method of claim 10 further comprising filtering the solution produced by step (g) of claim 10.

12. The method of claim 10 or 11 for purifying a protein from thesolution produced by step (g) of claim 10, or the filtered solution of claim 11 by flowthrough anion exchange chromatography comprising: (a) equilibrating an anion exchange matrix with an anion equilibration buffer comprising 55 mM tris base, 45 mM acetic acid, at about pH 7.5; (b) applying the contaminated solution to the anion exchange matrix and collecting the first flowthrough; and (c) applying an anion wash buffer comprising 55 mM Tris Base, 45 mM Acetic acid, 300 mM Sodium Acetate, at about pH 7.5 to the anion exchange matrix and collecting the second flowthrough, wherein all the buffers are made without the addition of NaCl.

13. The method of claim 12 wherein:
(a) the first flowthrough and the second flowthrough are combined into a single combined flowthrough solution; and/or

(b) the pH of the first flowthrough, the second flowthrough, and the single combined flowthrough solution is adjust to about pH 5.0 with 30 mM acetic acid, 100 mM HCl.

14. The method of any one of claims 10 to 13 for purifying a protein from the contaminated solution selected from the first flowthrough of claims 10 or 11, the second flowthrough of claims 10 or 11, the single flowthrough combined solution of claim 12, and the pH-adjusted

flowthrough produced by claim 13(a), by cation exchange chromatography comprising: (a) equilibrating a cation exchange matrix with a cation equilibration buffer comprising 25 mM sodium acetate, 12.1 mM acetic acid, at about pH 5.0; (b) adsorbing the protein from the contaminated solution to the cation exchange matrix; (c) removing contaminants by washing the solid phase with a first cation wash buffer comprising 25 mM sodium acetate, 12.1 mM acetic acid, at about pH 5.0; and (d) recovering the protein from the solid phase with a cation elution buffer comprising 175 mM sodium acetate, 75 mM acetic acid, at about pH 5.0, wherein all the buffers are made without the addition of NaCl.