

CLAIMS

What is claimed is:

1. A method of purifying an Fc region containing target protein from one or more impurities in a sample, the method comprising the steps of:
 - (a) contacting the sample with an affinity chromatography media;
 - (b) eluting the Fc region containing target protein from the affinity media;
 - (c) contacting the eluate with a cation exchange chromatography media;
 - (d) contacting the cation exchange chromatography media with a buffer having a pH lower than 4.0 for a period time suitable for viral inactivation, wherein the buffer conductivity is 5 to 50 mS;
 - (de) eluting the Fc region containing target protein from the cation exchange chromatography media;
 - (ef) contacting the eluate with an anion exchange chromatography media; and
 - (fg) recovering the Fc region containing target protein,wherein the method eliminates the need for a holding tank between steps (b) and (c) and steps (de) and (ef).

2. A method of purifying an Fc region containing target protein from one or more impurities in a sample, the method comprising the steps of:
 - (a) contacting the sample with an affinity chromatography media;
 - (b) eluting the Fc region containing target protein from the affinity media;
 - (c) contacting the eluate with a cation exchange chromatography media;
 - (d) contacting the cation exchange chromatography media with a buffer having a pH lower than 4.0 for a period time suitable for viral inactivation, wherein the buffer conductivity is 5 to 50 mS;
 - (de) eluting the Fc region containing target protein from the cation exchange chromatography media;

- (ef) contacting the eluate with an anion exchange chromatography media; and
 - (fg) recovering the Fc region containing target protein,
- wherein the method eliminates the need for a buffer exchange step between steps (b) and (c) and steps (de) and (ef).
3. A method of purifying an Fc region containing target protein from one or more impurities in a sample, the method comprising the steps of:
 - (a) contacting the sample with an affinity chromatography media;
 - (b) eluting the Fc region containing target protein from the affinity media;
 - (c) contacting the eluate with a cation exchange chromatography media;
 - (d) contacting the cation exchange chromatography media with a buffer having a pH lower than 4.0 for a period time suitable for viral inactivation, wherein the buffer conductivity is 5 to 50 mS;
 - (de) eluting the Fc region containing target protein from the cation exchange chromatography media;
 - (ef) contacting the eluate with an anion exchange chromatography media; and
 - (fg) recovering the Fc region containing target protein,

wherein the method eliminates the need for a holding tank and a buffer exchange step between steps (b) and (c) and steps (de) and (ef).
 4. The method of claim 3, wherein the method further comprises a pre-elution step between steps (a) and (b).
 5. The method of claim 3, wherein the Fc region containing target protein is selected from the group consisting of an antibody, an immunoadhesion molecule and an Fc fusion protein, and Fc containing fragments thereof.
 6. The method of claim 5, wherein the antibody is a monoclonal antibody.
 7. The method of claim 3, wherein step (b) comprises the use of a buffer having a pH ranging from 2.0 to 4.0.

8. The method of claim 4, wherein the pre-elution step comprises contacting the affinity media with a cation exchange chromatography buffer.
9. ~~The method of claim 1, further comprising a virus inactivation step between steps (c) and (d).~~
10. ~~The method of claim 9, wherein the virus inactivation step comprises contacting the cation exchange chromatography media with a buffer having a pH lower than 4.0 for a period time suitable for viral inactivation.~~
11. ~~The method of claim 10, wherein the period of time suitable for virus inactivation ranges from 15 through 60 minutes.~~
12. ~~The method of claim 10, wherein the buffer conductivity is 5 to 50 mS.~~
13. ~~The method of claim 3, wherein step (d) comprises the use of a buffer having a pH ranging from 5.0 to 9.0.~~
14. ~~The method of claim 3, wherein step (d) comprises the use of a buffer having a pH ranging from 6.0 to 8.0.~~
15. ~~The method of claim 3, wherein step (b) comprises the use of a buffer comprising a conductivity ranging from 5 to 50 mS.~~
16. ~~The method of claim 3, wherein step (b) comprises the use of a buffer comprising a conductivity ranging from 12 to 25 mS.~~
17. ~~The method of claim 3, wherein the affinity chromatography media comprises protein A or a functional variant thereof.~~
18. ~~A kit for purifying an Fe region containing target protein from one or more impurities in a sample, the kit comprising one or more of: an affinity chromatography media, a cation exchange chromatography media, an anion exchange chromatography media and one or more elution~~

~~buffers along with instructions for using the kit without the need for a holding tank and/or a buffer exchange step.~~

~~19. The kit of claim 18, wherein the one or more media are packed in a column.~~

~~20. The kit of claim 19, wherein the column is disposable.~~

~~21~~15. A connected process for purifying an Fc region containing target protein from one or more impurities in a sample, the method comprising the steps of:

- (a) contacting the sample with an affinity chromatography media;
- (b) eluting the Fc region containing target protein from the affinity media;
- (c) contacting the eluate with a cation exchange chromatography media;
- (d) contacting the cation exchange chromatography media with a buffer having a pH lower than 4.0 for a period time suitable for viral inactivation, wherein the buffer conductivity is 5 to 50 mS;
- ~~(de)~~ eluting the Fc region containing target protein from the cation exchange chromatography media;
- ~~(ef)~~ contacting the eluate with an anion exchange chromatography media; and
- ~~(fg)~~ recovering the Fc region containing target protein.

~~22~~16. The connected process of claim ~~21~~15, wherein the process eliminates the need for a holding tank and/or a buffer exchange step between steps (b) and (c) and between steps ~~(de)~~ and ~~(ef)~~.