

CLAIMS

What is claimed is:

1. A method of preparing a high purity virus stock solution, wherein the virus stock solution comprises a protein concentration of less than 100 fg/TCID₅₀,
5 the method comprising the sequential steps of:
 - (a) obtaining a viral sample prepared under low protein conditions;
 - (b) concentrating the viral sample in a suitable buffer; and
 - (c) precipitating the virus in the viral sample, wherein the precipitated virus comprises a protein concentration of less than 100 fg/TCID₅₀;
10 thereby preparing a high purity virus stock solution.
2. The method of Claim 1 further comprising processing of the precipitated virus to remove trace impurities.
- 15 3. The method of Claim 1, wherein the viral sample prepared under low protein conditions is a serum-free cell culture or defined media cell culture preparation of virus.
4. The method of Claim 1, wherein concentrating the viral sample in a suitable
20 buffer comprises ultrafiltration, tangential flow filtration, dialysis, or a combination thereof.
5. The method of Claim 1, wherein the suitable buffer is Tris-NaCl-EDTA (TNE).
25
6. The method of Claim 1, wherein precipitating the virus in the viral sample comprises ultracentrifugation, chemical-induced precipitation,
30 chromatographic binding, polymer-induced aggregation, or a combination thereof.

7. The method of Claim 6, wherein chemical-induced precipitation is polyethylene glycol (PEG)-induced precipitation.
8. The method of Claim 1, wherein the virus stock solution has a protein
5 concentration of less than about 60 fg/TCID₅₀.
9. The method of Claim 8, wherein the virus stock solution has a protein concentration of less than 60 fg/TCID₅₀ and a DNA concentration of less than 15 fg/TCID₅₀.
10
10. The method of Claim 2, wherein the further processing comprises flow-through chromatography, membrane absorption, a bead-based chromatography, size-exclusion processing, or hydrophobicity-based processing.
15
11. The method of Claim 10, wherein the further processing comprises cation exchange flow-through chromatography.
12. The method of Claim 1, wherein the virus is a mammalian virus or a
20 bacteriophage, or a combination thereof.
13. The method of Claim 1, wherein the virus is a parvovirus or a xenotropic murine leukemia virus (X-MuLV).
- 25 14. The method of Claim 13, wherein the parvovirus is a mouse minute virus (MVM).
15. A virus stock solution produced by the method of Claim 1.

16. A method of purifying a virus to a concentration of at least 10^6 TCID₅₀/ml and a protein concentration of less than 100 fg/TCID₅₀, the method comprising the sequential steps of:
- 5 (a) obtaining a viral sample prepared under low protein conditions;
- (b) concentrating the viral sample in a suitable buffer; and
- (c) precipitating the virus in the viral sample, wherein the precipitated virus has a virus concentration of at least 10^6 TCID₅₀/ml and a protein concentration of less than 100 fg/TCID₅₀;
- 10 thereby purifying a virus to a concentration of at least 10^6 TCID₅₀/ml and a protein concentration of less than 100 fg/TCID₅₀.
17. The method of Claim 16, further comprising processing of the precipitated virus to remove trace impurities.
- 15 18. The method of Claim 16, wherein the viral sample prepared under low protein conditions is a serum-free cell culture or defined media cell culture preparation of virus.
19. The method of Claim 16, wherein concentrating the viral sample in a suitable buffer comprises ultrafiltration, tangential flow filtration, dialysis, or a combination thereof.
- 20 20. The method of Claim 16, wherein the suitable buffer is Tris-NaCl-EDTA (TNE).
- 25 21. The method of Claim 16, wherein precipitating the virus in the viral sample comprises ultracentrifugation, chemical-induced precipitation, chromatographic binding, polymer-induced aggregation, or a combination thereof.
- 30

22. The method of Claim 21, wherein chemical-induced precipitation is polyethylene glycol (PEG)-induced precipitation.
23. The method of Claim 16, wherein the virus stock solution has a protein
5 concentration of less than about 60 fg/TCID₅₀.
24. The method of Claim 23, wherein the virus stock solution has a protein concentration of less than 60 fg/TCID₅₀ and a DNA concentration of less than 15 fg/TCID₅₀.
- 10 25. The method of Claim 16, wherein the further processing comprises flow-through chromatography, membrane absorption, a bead-based chromatography, size-exclusion processing, or hydrophobicity-based processing.
- 15 26. The method of Claim 25, wherein the further processing comprises cation exchange flow-through chromatography.
27. The method of Claim 16, wherein the virus is a mammalian virus or a
20 bacteriophage, or a combination thereof.
28. The method of Claim 16, wherein the virus is a parvovirus or a xenotropic murine leukemia virus (X-MuLV).
- 25 29. The method of Claim 28, wherein the parvovirus is a mouse minute virus (MVM).
30. A virus purified by the method of Claim 16.

31. A method of evaluating a virus clearance process, the method comprising:
- (a) spiking a sample with a virus solution, wherein the virus solution comprises: at least 10^6 TCID₅₀/ml of virus; a protein concentration of less than 100 fg/TCID₅₀; and a DNA concentration of less than 50 fg/TCID₅₀, thereby producing a test sample;
- (b) testing a scaled virus clearance process using the test sample; and
- (c) analyzing the results of the virus clearance process,
- wherein the amount of virus clearance determines the virus removal capability of the virus clearance process, thereby evaluating the virus clearance process.
32. The method of Claim 31, wherein the test sample comprises at least 10^5 TCID₅₀/ml of virus.
33. The method of Claim 31, wherein the virus clearance process comprises virus removal, virus inactivation, or a combination thereof.
34. The method of Claim 33, wherein the virus removal process comprises a filter, chromatography media, and wherein the virus inactivation process comprises low pH, high pH, ultraviolet (UV) irradiation, gamma irradiation, or temperature inactivation.
35. The method of Claim 31, wherein the virus is a mammalian virus or a bacteriophage, or a combination thereof.
36. The method of Claim 31, wherein the virus is a parvovirus or a xenotropic murine leukemia virus (X-MuLV).
37. The method of Claim 36, wherein the parvovirus is a mouse minute virus (MVM).

38. A method of evaluating a virus clearance process, the method comprising:
- (a) spiking a sample with a virus solution, wherein the virus solution is prepared by a method comprising flow-through chromatography, thereby producing a test sample;
 - 5 (b) testing a scaled virus clearance process using the test sample; and
 - (c) analyzing the results of the virus clearance process,
- wherein the amount of virus clearance determines the virus removal capability of the virus clearance process, thereby evaluating the virus clearance process.
- 10 39. The method of Claim 38, wherein the flow-through chromatography comprises cation exchange flow-through chromatography on a membrane or bead-based matrix.
- 15

CLAIMS

What is claimed is:

1. A method of preparing a high purity virus stock solution, wherein the virus stock solution comprises a protein concentration of less than 100 fg/TCID₅₀, the method comprising the sequential steps of:
 - (a) harvesting the culture supernatant of virus-infected cells grown under low serum conditions, wherein the culture supernatant is harvested after virus-induced cell lysis, thereby obtaining a viral sample;
 - (b) concentrating the viral sample by ultrafiltration, tangential flow filtration, dialysis, or a combination thereof and exchanging the culture supernatant for a suitable buffer; and
 - (c) precipitating the virus in the viral sample by ultracentrifugation, chemical-induced precipitation, chromatographic binding, polymer-induced aggregation, or a combination thereof, wherein the precipitated virus comprises a protein concentration of less than 100 fg/TCID₅₀,
thereby preparing a high purity virus stock solution.
2. The method of Claim 1, further comprising subjecting the precipitated virus to chromatographic polishing to remove trace impurities.
3. The method of Claim 1, wherein the low serum conditions are a serum-free cell culture or defined media cell culture preparation of virus.
4. The method of Claim 1, wherein concentrating the viral sample in a suitable buffer comprises ultrafiltration and wherein precipitating the virus in the viral sample comprises ultracentrifugation.
5. The method of Claim 1, wherein the virus stock solution has a protein concentration of less than about 60 fg/TCID₅₀.
6. The method of Claim 5, wherein the virus stock solution has a protein concentration of less than 60 fg/TCID₅₀ and a DNA concentration of less than 15 fg/TCID₅₀.

7. The method of Claim 2, wherein the chromatographic polishing comprises cation exchange flow-through chromatography.
8. The method of Claim 1, wherein the virus is a mammalian virus or a bacteriophage, or a combination thereof.
9. The method of Claim 1, wherein the virus is a parvovirus or a xenotropic murine leukemia virus (X-MuLV).
10. The method of Claim 9, wherein the parvovirus is a mouse minute virus (MVM).
11. A virus stock solution produced by the method of Claim 1.
12. The method of Claim 1, wherein the virus stock solution comprises a concentration of at least 10^6 TCID₅₀/ml.
13. The method of Claim 1, wherein the virus stock solution has a protein concentration of less than about 20 fg/TCID₅₀.
14. The method of Claim 13, wherein the virus stock solution has a protein concentration of less than 20 fg/TCID₅₀ and a DNA concentration of less than 15 fg/TCID₅₀.
15. A method of evaluating a virus clearance process, the method comprising:
 - (a) spiking a sample with a virus solution, wherein the virus solution is prepared by the method of Claim 1, thereby producing a test sample;
 - (b) testing a scaled virus clearance process using the test sample; and
 - (c) analyzing the results of the virus clearance process,wherein the amount of virus clearance determines the virus removal capability of the virus clearance process, thereby evaluating the virus clearance process.
16. The method of Claim 15, wherein the virus clearance process comprises virus removal, virus inactivation, or a combination thereof.

17. The method of Claim 16, wherein the virus clearance process comprises a filter and chromatography media, and wherein the virus inactivation process comprises low pH, high pH, ultraviolet (UV) irradiation, gamma irradiation, or temperature inactivation.
18. A method of evaluating a virus clearance process, the method comprising:
 - (a) spiking a sample with a virus solution, wherein the virus solution is prepared by a method comprising chromatographic polishing, thereby producing a test sample;
 - (b) testing a scaled virus clearance process using the test sample; and
 - (c) analyzing the results of the virus clearance process,wherein the amount of virus clearance determines the virus removal capability of the virus clearance process, thereby evaluating the virus clearance process.
19. The method of Claim 18, wherein the chromatographic polishing comprises cation exchange flow-through chromatography on a membrane or bead-based matrix.