CLAIMS

What is claimed is:

1. A population of purified recombinantly expressed adeno associated virus (rAAV) particles, wherein the recombinantly expressed adeno associated virus particles are purified or isolated from a harvesting media by a process comprising:
    a. purifying/isolating a plurality of recombinantly expressed virus particles from the harvesting media via affinity chromatography to produce an eluate (affinity chromatography eluate) comprising the plurality of recombinantly expressed virus particles, wherein an elution buffer for affinity chromatography (affinity elution buffer) comprises a predetermined amount of glycine, optionally, the affinity elution buffer is substantially free of weak acids or salts thereof; and optionally, the affinity elution buffer comprises an amino acid that is not glycine;
    b. adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography, wherein the adjusted affinity eluate comprises a predetermined amount of a weak acid or salt thereof or the adjusted affinity eluate comprises a predetermined amount of an amino acid that is not glycine in addition to the predetermined amount of glycine already present in affinity eluate;
    c. purifying/isolating the plurality of recombinantly expressed virus particles from the adjusted eluate of affinity chromatography by anion exchange chromatography to produce a solution comprising a plurality of purified/isolated recombinantly expressed virus particles, wherein an equilibration buffer for anion exchange chromatography comprises a predetermined amount of a weak acid or a salt thereof; and, optionally,

    wherein: (i) the population of purified rAAV comprises less than about 35% empty viral capsids; and/or (ii) the purified rAAV has a particle to infectivity ratio less than 2×10^4 vg/TCID50; and/or (iii) a ratio of UV_{260} to UV_{280} in the anion exchange eluate is at least about 1.15X or higher than a ratio of UV_{260} to UV_{280} ratio in the adjusted affinity eluate.

2. The population of claim 1, wherein less than 5% of empty virus particles in the affinity eluate bind to anion exchange chromatography media.
3. The process of claim 2, wherein substantially no empty virus particles in the affinity eluate bind to anion exchange chromatography media.
4. The process of any one of claims 1-3, wherein the population of purified rAAV comprises less than about 10% empty viral capsids.

5. The process of claim 4, wherein the population of purified rAAV comprises less than about 5% empty viral capsids.
6. The process of claim 5, wherein the population of purified rAAV is substantially free of empty capsids.
7. The population of any one of claims 1-6, wherein the weak acid of 1(b) is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid , aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid , oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid , tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid.
8. The population of any one claims 1-7, wherein the affinity elution buffer comprises histidine at a concentration of at least about 1mM or more.
9. The population of any one claims 1-8, wherein the affinity elution buffer comprises histidine at a concentration from about 1mM to about 50mM.
10. The population of any one of claims 1-9, wherein the affinity elution buffer comprises glycine at a concentration of at least about 20mM or more.
11. The process of any one of claims 1-10, wherein the affinity elution buffer comprises glycine at a concentration of from about 25mM to about 100mM.
12. The population of any one of claims 1-11, wherein the affinity elution buffer comprises a salt.
13. The population of any one of claims 1-12, wherein the affinity elution buffer comprises a salt at concentration of at least about 5mM or more.
14. The population of any one of claims 1-13, wherein the affinity elution buffer comprises a salt concentration of from about 5mM to about 15mM.
15. The population of any one of claims 12-14, wherein the salt is a magnesium salt, a sodium salt, a potassium salt, an ammonium salt, a calcium salt, a copper salt, a cobalt salt, a manganese salt, a nickel salt or a zinc salt, optionally the salt is $MgCl_2$.
16. The population of any one of claims 1-15, wherein the affinity elution buffer comprises a polymer.

17. The population of any one of claims 1-16, wherein the affinity elution buffer comprises a polymer at a concentration of at least about 0.1% or more.
18. The population of any one of claims 1-17, wherein the affinity elution buffer comprises a polymer at a concentration of from about 0.1% to about 0.5%.
19. The population of any one of claims 16-18, the polymer is a non-ionic surfactant.
20. The population of any one of claims 1-19, wherein the affinity elution buffer has a low pH.
21. The population of any one of claims 1-20, wherein the affinity elution buffer has a pH lower than or equal to about 6.5.
22. The process of any one of claims 1-21, wherein the affinity elution buffer has a pH of from about 2.0 to about 3.0.
23. The population of any one of claims 1-22, wherein the affinity elution buffer comprises: about 75mM glycine.
24. The population of any one of claims 1-7 or 10-23, wherein the affinity elution buffer comprises a weak acid or a salt thereof.
25. The population of claim 24, wherein the affinity elution buffer comprises the weak acid or a salt thereof at a concentration of at least about 50mM or more.
26. The population of claim 24 or 25, wherein the affinity elution buffer comprises the weak acid or a salt thereof at a concentration of from about 50mM to about 100mM.
27. The population of any one of claims 24-26, wherein the weak acid is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid , aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid , oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid , tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, or, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid.
28. The population of any one of claims 24-27, wherein the weak acid is citric acid or a salt thereof.
29. The population of any one of claims 1-7, 10-22 or 24-28, wherein the affinity elution buffer comprises: about 50mM glycine, about 75mM citrate, about 10mM $MgCl_2$, about 0.3% (w/v) P188 and has a pH of about 3.0.

30. The population of any one of claims 1-29, wherein the affinity elution buffer has conductivity in a range from about 5 mS/cm to about 8 mS/cm, optionally the affinity elution buffer has conductivity in a range from about 5.5 mS/cm to about 7 mS/cm.
31. The population of any one of claims 1-30, wherein the affinity elution buffer has an osmolarity in a range from about 100 mOms to about 225 mOms.
32. The population of any one of claims 1-31, wherein the equilibration buffer for anion exchange chromatography comprises the weak acid or a salt thereof in a concentration of at least about 0.5mM.
33. The population of any one of claims 1-32, wherein the equilibration buffer comprises the weak acid or a salt thereof at a concentration from about 0.5mM to about 15mM.
34. The process of any one of claims 1-33, wherein the weak acid in the equilibration buffer is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid , oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid , tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, or, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid.
35. The population of any one of claims 1-34, wherein the weak acid or salt thereof in the equilibration buffer is citric acid or citrate.
36. The process of any one of claims claim 1-35, wherein adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography comprises adding the weak acid or a salt thereof to the affinity eluate.
37. The population of any one of claims 1-36, wherein the weak acid or a salt thereof is added to the affinity eluate to a final a concentration of at least about 0.5mM or higher.
38. The population of any one of claims 1-37, wherein the weak acid or a salt thereof is added to the affinity eluate to a final concentration from about 0.5mM to about 15mM.
39. The population of any one of claims 1-38, wherein the weak acid or a salt thereof added to the affinity eluate is citric acid or a salt thereof, optionally the salt is citrate.

40. The process of any one of claims claim 1-35, wherein adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography comprises adding an amino acid to the affinity eluate.

41. The population of any one of claims 1-35 or 40, wherein the amino acid is added to the affinity eluate to a final a concentration of at least about 15mM or higher.

42. The population of any one of claims 1-35 or 40-41, wherein the amino acid is added to the affinity eluate to a final concentration from about 15mM to about 35mM.

43. The population of any one of claims 1-35 or 40-42, wherein amino acid added to the affinity eluate is aspartate, glutamate, histidine, arginine, lysine, cysteine or tyrosine, optionally, the amino acid is histidine.

44. The population of any one of claims 1-43, wherein adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography comprises diluting the affinity eluate.

45. The population of any one of claims 1-44, wherein adjusting the affinity eluate for anion exchange chromatography comprises diluting the eluate by at least 2X or more.

46. The population of any one of claims 1-45, wherein adjusting the affinity eluate for anion exchange chromatography comprises diluting the affinity eluate with a dilution buffer (anion exchange dilution buffer).

47. The population of claim 46, wherein the dilution buffer comprises a weak acid or a salt thereof.

48. The population of claim 47, wherein the dilution buffer comprises the weak acid or a salt thereof at a concentration of at least about 0.5mM.

49. The population of any one of claims 47 or 48, wherein the dilution buffer comprises the weak acid or a salt thereof at a concentration from about 0.5mM to about 15mM.

50. The population of any one of claims 47-49, wherein the weak acid in the dilution buffer is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid , oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid , tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic

acid or 3-methylbutanoic acid, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid.

51. The population of any one of claims 47-50, wherein the weak acid or a salt thereof in the dilution buffer is citric acid or a salt thereof, acetic acid or a salt thereof, or succinic acid or a salt thereof, optionally the weak acid is citric acid or a salt thereof.

52. The population of any one of claims 46-51, wherein the dilution buffer comprises a buffering agent.

53. The population of claim 52, wherein the buffering agent is acetate, histidine, phosphate, citrate, propionate, tricine, borate, or tris(hydroxymethyl)aminomethane (tris), optinally the buffering agent is bris-tris propane (BTP).

54. The population of any one of claims 52-53, wherein the dilution buffer comprises the buffering agent at a concentration of at least about 25mM.

55. The population of any one of claims 52-54, wherein the dilution buffer comprises the buffering agent at a concentration of from about 25mM to about 175mM.

56. The population of any one of claims 46-55, wherein the dilution buffer comprises an amino acid.

57. The population of any one of claims 46-56, wherein the dilution buffer comprises an amino acid at a concentration of at least about 25mM.

58. The population of any one of claims 46-57, wherein the dilution buffer comprises an amino acid at a concentration of from about 25mM to about 175mM.

59. The process of any one of claims 56-58, wherein the amino acid is aspartate, glutamate, histidine, arginine, lysine, cysteine or tyrosine, optionally, the amino acid is histidine.

60. The population of any one of claims 46-59, wherein the dilution buffer comprises a viscosity modifier.

61. The population of claim 60, wherein the viscosity modifier is a polyol, optionally selected from the group consisting of hydrocarbons, monosaccharides, disaccharides, trisaccharides and any combinations thereof.

62. The population of claim 61, wherein the polyol is sorbitol, mannitol, glycerol, propylene glycol, polyethylene glycol, dulcitol, sucrose, lactose, maltose, trehalose, dextran or any combinations thereof, optionally the glycerol, sorbitol, mannitol, dulcitol, sucrose, lactose, maltose, trehalose and any combinations thereof

63. The population of any one of claim 61-62, wherein the polyol is sorbitol, mannitol, glycerol, propylene glycol, polyethylene glycol, dulcitol, sucrose, lactose, maltose, trehalose, dextran or any combinations thereof, optionally the polyol is glycerol.

64. The population of any one of claims 60-63, wherein the dilution buffer comprises the viscosity modifier at a concentration of at least about 0.5% (v/v or w/v) or higher.
65. The population of any one of claims 60-64, wherein the dilution buffer comprises the viscosity modifier at a concentration of from about 0.5% to about 9.5%, (v/v or w/v).
66. The population of any one of claims 46-65, wherein the dilution buffer comprises a non-ionic surfactant.
67. The population of any one of claims 46-66, wherein the dilution buffer comprises a non-ionic surfactant at a concentration of at least about 0.05% (v/v or w/v) or higher.
68. The population of any one claims 46-67, wherein the dilution buffer comprises a non-ionic surfactant at a concentration of about 0.05% to about 0.95%, (v/v or w/v).
69. The population of any one of claims 66-68, wherein the non-ionic surfactant is selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene alkylphenyl ethers, polyoxyethylene-polyoxypropylene block copolymers, alkylglucosides, alkylphenol ethoxylates, preferably polysorbates, polyoxyethylene alkyl phenyl ethers, and any combinations thereof.
70. The population of any one of claims 46-69, wherein the dilution buffer comprises a salt.
71. The population of any one of claims 46-70, wherein the dilution buffer comprises a salt at a concentration of at least about 0.1mM.
72. The population of any one of claims 46-71, wherein the dilution buffer comprises a salt at a concentration of from about 0.1mM to about 2mM.
73. The population of any one of claims 70-72, wherein the salt is a magnesium salt, a sodium salt, a potassium salt, an ammonium salt, a calcium salt, a copper salt, a cobalt salt, a manganese salt, a nickel salt or a zinc salt, optionally the salt is $MgCl_2$.
74. The population of any one of claims 46-73, wherein the dilution buffer has a high pH.
75. The population of any one of claims 46-74, wherein the dilution buffer has a pH greater than or equal to about 8.
76. The population of any one of claims 46 or 52-75, wherein the dilution buffer comprises: BTP, histidine, glycerol, PF68, $MgCl_2$ and has a high pH.
77. The population of any one of claims 46-76, wherein the dilution buffer comprises: BTP, histidine, PF68, $MgCl_2$, citric acid and has a high pH.
78. The population of any one of claims 1-77, wherein the dilution buffer has conductivity in a range from about 0.5 mS/cm to about 3 mS/cm.
79. The population of any one of claims 1-78, wherein the dilution buffer has an osmolarity of less than 900 mOsm.

80. The population of any one of claims 1-79, further comprising a step of removing or reducing amount of impurities (e.g., host cell DNA (hcDNA)) from the harvest media prior to affinity purification.

81. The population of claim 80, wherein said removing or reducing the amount of impurities comprises adding a cationic amine or nuclease to the harvest media.

82. The population of claim 80 or 81, wherein said removing or reducing the amount of impurities comprises adding a selective precipitation agent to harvest media.

83. The population of any one of claims 1-82, further comprising a step of lysing a host cell in the harvest media with a non-ionic surfactant prior purifying/isolating by affinity chromatography.

84. The population of claim 83, wherein the non-ionic surfactant is added to the harvest media to a final concentration of at least about 0.05% (v/v or w/v) or higher.

85. The population of claim 83 or 84, wherein the non-ionic surfactant is added to the harvest media to a final concentration of from about 0.05% to about 1% (v/v or w/v).

86. The population of any one of claims 83-85, wherein the non-ionic surfactant is mixed with the harvest media for a period of from about 15 minutes to about 2 hours.

87. The population of any one of claims 83-86, wherein the non-ionic surfactant is not Triton X-100.

88. The population of any one of claims 83-87, wherein the non-ionic surfactant is selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene alkylphenyl ethers, polyoxyethylene-polyoxypropylene block copolymers, alkylglucosides, alkylphenol ethoxylates, preferably polysorbates, polyoxyethylene alkyl phenyl ethers, and any combinations thereof.

89. The population of any one of claims 1-88, wherein the recombinant adeno associated virus particle comprise rAAV virion.

90. The population of any one of claims 1-89, wherein the purified rAAV has a particle to infectivity ratio less than 2×10^4 vg/TCID50.

91. The population of any one of claims 1-90, wherein the population of purified rAAV comprises less than about 10% empty viral capsids.

92. The population of any one of claims 1-91, wherein the wherein the rAAV is obtained by a method comprising transfecting a suspension mammalian cell line, and wherein the cells optionally are transfected in suspension.

93. The population any one of claim 92, wherein, the mammalian cell line is transfected in suspension with a) a nucleic acid sequence encoding helper proteins sufficient for rAAV replication; b) a nucleic acid sequence encoding rep and cap genes, and c) a close ended

linear duplexed rAAV vector nucleic acid comprising at least one ITR and a heterologous transgene operably linked to one or more regulatory elements.

94. The population claim 92 or 93, wherein the mammalian cell line is derived from a human embryonic cell line.

95. The population of any one of claims 92-94, wherein the human embryonic cell line is suspension adapted, serum free cell line derived from a human embryonic kidney cell line.

96. The population of any one of claims 92-95, wherein the purified recombinant adeno-associated virus (rAAV) lacks prokaryotic sequences.

97. The population of any one of claims 1-96, wherein the population is comprised in a composition comprising the population and wherein: (i) wherein the composition exhibits substantially no aggregation of the rAAV particles after two or more freeze thaw cycles; and/or (ii) the purified rAAV particle retains its $TCID_{50}$/ml by at least about 80% after two or more freeze thaw cycles; and/or (iii) the purified rAAV has a particle to infectivity ratio less than 2×10^4 vg/$TCID_{50}$; and/or (iv) the population of purified rAAV comprises less than about 35% empty viral capsids.

98. The population of claim 97, wherein the composition exhibits substantially no aggregation of the rAAV particles after two or more freeze thaw cycles.

99. The population of any one of claims 97-98, wherein the purified rAAV particle retains its $TCID_{50}$/ml by at least about 80% after two or more freeze thaw cycles.

100. The population of any one of claims 97-99, wherein the purified rAAV has a particle to infectivity ratio less than 2×10^4 vg/$TCID_{50}$.

101. The population of any one of claims 97-100, wherein the population of purified rAAV comprises less than about 10% empty viral capsids.

102. The population of any one of claims 97-101, wherein the composition comprises the purified rAAV are at a concentration of from about $1e^9$ vg/ml to about $1e^{15}$ vg/ml.

103. The population of any one of claims 97-102, wherein the composition comprises the purified rAAV are at a concentration of from about $1e^{12}$ vg/ml to about $1e^{15}$ vg/ml, optionally, the purified rAAV are at a concentration of from about $1e^{13}$ vg/ml to about $1e^{14}$ vg/ml.

104. The population of any one of claims 97-103, wherein the composition has a pH of about 6.5 to about 8, optionally, the composition has a pH of about 7 to about 8.

105. The population of any one of claims 97-104, wherein the composition comprises a buffer, a bulking agent and one or both of a non-ionic surfactant and a multivalent salt, and optionally: (i) the composition is substantially free of glycine; and/or (ii) the composition is substantially free of trehalose, trehalose dehydrate, Dextran T10 or Dextran T40; and/or

(iii) the composition is substantially free of sodium salts, ammonium salts or potassium salts; and/or (iv) the composition is substantially free of polysorbate-80 (PS80).

106. The population of claim 105, wherein the buffer is PBS, Tris.HCl, phosphate, citric acid, histidine, tromethamine, succinic acid, malic acid, α-ketoglutaric acid, carbonate, protein buffers or any combinations thereof, optionally, the buffer is PBS, Tris or histidine buffer.

107. The population of claims 105 or 106, wherein the bulking agent is a polyol or providone (PVP K24).

108. The population of claim 107, wherein the polyol is sorbitol, mannitol, glycerol, propylene glycol, polyethylene glycol, dulcitol, sucrose, lactose or maltose, optionally the polyol is sorbitol or mannitol.

109. The population of any one of claims 105-108, wherein the non-ionic surfactant is selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene alkyl phenyl ethers, polyoxyethylene-polyoxypropylene block copolymers, alkylglucosides, alkyl phenol ethoxylates, preferably polysorbates, polyoxyethylene alkyl phenyl ethers, and any combinations thereof.

110. The population of any one of claims 105-109, wherein the non-ionic surfactant is selected from the group of Brij 010, BRIJ 020, Brij Cl 0, Brij S20, BrijSl0, ECOSURF EH-14, ECOSURF EH-9, ECOSURF SA-15, IGEPAL CA-720, IGEPAL CO-630, IGEPAL CO-720, MERPOL HCS surfactant, MERPOL OJ surfactant, MERPOL SH surfactant, Pluronic 10R5, Pluronic 10R5, Pluronic F-68, Poloxamer P 188, Poloxamer P 338, Poloxamer P188, Poloxamer P407, Polyoxyethylene (12) tridecyl ether, TERGITOL 15-S-12, TERGITOL 15-S-7, TERGITOL 15-S-9, TERGITOL L-64, TERGITOL NP-10, TERGITOL NP-10, TERGITOL NP-11, TERGITOL NP-12, TERGITOL NP-8, TERGITOL NP-9, TERGITOL NP-9.5, TERGITOL NP-9.5, TERGITOL15-S-9, TERGITOLNP-13, TERGITOLNP-7, TWEEN 60 nonionic detergent, and any combinations thereof.

111. The population of any one of claims 105-110, wherein the multivalent salt is a calcium salt, citrate salt, sulfate salt or magnesium salt.

112. The population of any one of claims 105-111, wherein the composition has an osmolarity of less than 750 mOSm.

113. The population of any one of claims 105-112, wherein the composition has an osmolarity of from about 125 mOsm to about 500 mOsm.

114. The population any one of claims 105-113, wherein the composition is a pharmaceutical composition.

115. A process for purifying or isolating recombinantly expressed adeno associated virus particles from a harvesting media, the process comprising:

a. purifying/isolating a plurality of recombinantly expressed virus particles from the harvesting media via affinity chromatography to produce an eluate (affinity chromatography eluate) comprising the plurality of recombinantly expressed virus particles, wherein an elution buffer for affinity chromatography (affinity elution buffer) comprises a predetermined amount of glycine, optionally, the affinity elution buffer is substantially free of weak acids or salts thereof; and optionally, the affinity elution buffer comprises an amino acid that is not glycine;

b. adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography, wherein the adjusted eluate comprises a predetermined amount of a weak acid or a salt thereof or the adjusted affinity eluate comprises a predetermined amount of an amino acid in addition to the predetermined amount of glycine already present in affinity eluate; and

c. purifying/isolating the plurality of recombinantly expressed virus particles from the adjusted eluate of affinity chromatography by anion exchange chromatography to produce a solution comprising a plurality of purified/isolated recombinantly expressed virus particles, wherein an equilibration buffer for anion exchange chromatography comprises a predetermined amount of a weak acid or a salt thereof, optionally the weak acid is citric acid, acetic acid or succinic acid.

116. The population of claim 115, wherein less than 5% of empty virus particles in the affinity eluate bind to anion exchange chromatography media.

117. The process of claim 116, wherein substantially no empty virus particles in the affinity eluate bind to anion exchange chromatography media.

118. The process of any one of claims 115-117, wherein less than 10% of the virus particles in the eluate from the anion exchange are empty viral particles.

119. The process of any one of claims 115-118, wherein in less than 5% of the virus particles in the eluate from the anion exchange are empty viral particles.

120. The process of any one of claims 1, wherein the eluate from the anion exchange is substantially free of empty virus particles.

121. The process of any one of claims 1-103, wherein the recombinantly expressed virus particles are recombinant adeno associated virus (rAAV) particle.

122. The process of any one of claims 115-121, wherein the weak acid of 115(b) is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid,

glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid, oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid, tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid.

123. The process of any one claims 115-122, wherein the affinity elution buffer comprises histidine at a concentration of at least about 1mM or more.

124. The process of any one claims 115-123, wherein the affinity elution buffer comprises histidine at a concentration from about 1mM to about 50mM.

125. The process of any one of claims 115-124, wherein the affinity elution buffer comprises glycine at a concentration of at least about 20mM or more.

126. The process of any one of claims 115-128, wherein the affinity elution buffer comprises glycine at a concentration of from about 25mM to about 100mM.

127. The process of any one of claims 115-126, wherein the affinity elution buffer comprises a salt.

128. The process of any one of claims 115-127, wherein the affinity elution buffer comprises a salt at concentration of at least about 5mM or more.

129. The process of any one of claims 115-128, wherein the affinity elution buffer comprises a salt concentration of from about 5mM to about 15mM.

130. The process of any one of claims 127-129, wherein the salt is a magnesium salt, a sodium salt, a potassium salt, an ammonium salt, a calcium salt, a copper salt, a cobalt salt, a manganese salt, a nickel salt or a zinc salt, optionally the salt is $MgCl_2$.

131. The process of any one of claims 115-130, wherein the affinity elution buffer comprises a polymer.

132. The process of any one of claims 115-131, wherein the affinity elution buffer comprises a polymer at a concentration of at least about 0.1% or more.

133. The process of any one of claims 115-132, wherein the affinity elution buffer comprises a polymer at a concentration of from about 0.1% to about 0.5%.

134. The process of any one of claims 131-133, the polymer is a non-ionic surfactant.

135. The process of any one of claims 115-134, wherein the affinity elution buffer has a low pH.

136. The process of any one of claims 115-135, wherein the affinity elution buffer has a pH lower than or equal to about 6.5.

137. The process of any one of claims 115-136, wherein the affinity elution buffer has a pH of from about 2.0 to about 3.0.

138. The process of any one of claims 115-137, wherein the affinity elution buffer comprises: about 75mM glycine.

139. The process of any one of claims 115-122 or 125-138, wherein the affinity elution buffer comprises a weak acid or a salt thereof.

140. The process of claim 139, wherein the affinity elution buffer comprises the weak acid or a salt thereof at a concentration of at least about 50mM or more.

141. The process of claim 139 or 140, wherein the affinity elution buffer comprises the weak acid or a salt thereof at a concentration of from about 50mM to about 100mM.

142. The process of any one of claims 139-141, wherein the weak acid is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid, oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid, tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid.

143. The process of any one of claims 139-142, wherein the weak acid is citric acid or a salt thereof.

144. The process of any one of claims 115-122, 125-137 or 139-143, wherein the affinity elution buffer comprises: about 50mM glycine, about 75mM citrate, about 10mM $MgCl_2$, about 0.3% (w/v) P188 and has a pH of about 3.0.

145. The process of any one of claims 115-144, wherein the affinity elution buffer has conductivity in a range from about 5 mS/cm to about 8 mS/cm.

146. The process of any one of claims 115-145, wherein the affinity elution buffer has an osmolarity in a range from about 100 mOms to about 225 mOms.

147. The process of any one of claims 115-146, wherein the equilibration buffer for anion exchange chromatography comprises the weak acid or a salt thereof in a concentration of at least about 0.5mM.

148. The process of any one of claims 115-147, wherein the equilibration buffer comprises the weak acid or a salt thereof at a concentration from about 0.5mM to about 15mM.

149. The process of any one of claims 115-149, wherein the weak acid in the equilibration buffer is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid, oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid, tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid.

150. The process of any one of claims 115-149, wherein the weak acid or salt thereof in the equilibration buffer is citric acid or citrate.

151. The process of any one of claims claim 115-150, wherein adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography comprises adding the weak acid or a salt thereof to the affinity eluate.

152. The process of any one of claims 115-151, wherein the weak acid or a salt thereof is added to the affinity eluate to a final a concentration of at least about 0.5mM or higher.

153. The process of any one of claims 115-152, wherein the weak acid or a salt thereof is added to the affinity eluate to a final concentration from about 0.5mM to about 15mM.

154. The process of any one of claims 115-153, wherein the weak acid or a salt thereof added to the affinity eluate is citric acid or a salt thereof, optionally the salt is citrate.

155. The process of any one of claims claim 115-150, wherein adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography comprises adding an amino acid to the affinity eluate.

156. The process of any one of claims 115-150 or 155, wherein the amino acid is added to the affinity eluate to a final a concentration of at least about 15mM or higher.

157. The process of any one of claims 115-150 or 155-156, wherein the amino acid is added to the affinity eluate to a final concentration from about 15mM to about 35mM.

158. The process of any one of claims 115-150 or 155-157, wherein amino acid added to the affinity eluate is aspartate, glutamate, histidine, arginine, lysine, cysteine or tyrosine, optionally, the amino acid is histidine.

159. The process of any one of claims 115-158, wherein adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography comprises diluting the affinity eluate.

160. The process of any one of claims 115-159, wherein adjusting the affinity eluate for anion exchange chromatography comprises diluting the eluate by at least 2X or more.

161. The process of any one of claims 115-160, wherein adjusting the affinity eluate for anion exchange chromatography comprises diluting the affinity eluate with a dilution buffer (anion exchange dilution buffer).

162. The process of claim 161, wherein the dilution buffer comprises a weak acid or a salt thereof.

163. The process of claim 162, wherein the dilution buffer comprises the weak acid or a salt thereof at a concentration of at least about 0.5mM.

164. The process of any one of claims 162 or 163, wherein the dilution buffer comprises the weak acid or a salt thereof at a concentration from about 0.5mM to about 15mM.

165. The process of any one of claims 162-164, wherein the weak acid in the dilution buffer is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid, oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid, tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid.

166. The process of any one of claims 162-165, wherein the weak acid or a salt thereof in the dilution buffer is citric acid or a salt thereof, acetic acid or a salt thereof, or succinic acid or a salt thereof, optionally the weak acid is citric acid or a salt thereof.

167. The process of any one of claims 162-166, wherein the dilution buffer comprises a buffering agent.

168. The process of claim 167, wherein the buffering agent is acetate, histidine, phosphate, citrate, propionate, tricine, borate, or tris(hydroxymethyl)aminomethane (tris), optionally the buffering agent is bris-tris propane (BTP).

169. The process of any one of claims 167-168, wherein the dilution buffer comprises the buffering agent at a concentration of at least about 25mM.

170. The process of any one of claims 167-169, wherein the dilution buffer comprises the buffering agent at a concentration of from about 25mM to about 175mM.

171. The process of any one of claims 161-170, wherein the dilution buffer comprises an amino acid.

172. The process of any one of claims 161-171, wherein the dilution buffer comprises an amino acid at a concentration of at least about 25mM.

173. The process of any one of claims 161-172, wherein the dilution buffer comprises an amino acid at a concentration of from about 25mM to about 175mM.

174. The process of any one of claims 161-173, wherein the amino acid is aspartate, glutamate, histidine, arginine, lysine, cysteine or tyrosine, optionally, the amino acid is histidine.

175. The process of any one of claims 161-174, wherein the dilution buffer comprises a viscosity modifier.

176. The process of claim 175, wherein the viscosity modifier is a polyol, optionally selected from the group consisting of hydrocarbons, monosaccharides, disaccharides, trisaccharides and any combinations thereof.

177. The process of claim 176, wherein the polyol is sorbitol, mannitol, glycerol, propylene glycol, polyethylene glycol, dulcitol, sucrose, lactose, maltose, trehalose, dextran or any combinations thereof, optionally the glycerol, sorbitol, mannitol, dulcitol, sucrose, lactose, maltose, trehalose and any combinations thereof

178. The process of any one of claims 176-177, wherein the polyol is sorbitol, mannitol, glycerol, propylene glycol, polyethylene glycol, dulcitol, sucrose, lactose, maltose, trehalose, dextran or any combinations thereof, optionally the polyol is glycerol.

179. The process of any one of claims 175-178, wherein the dilution buffer comprises the viscosity modifier at a concentration of at least about 0.5% (v/v or w/v) or higher.

180. The process of any one of claims 175-179, wherein the dilution buffer comprises the viscosity modifier at a concentration of from about 0.5% to about 9.5%, (v/v or w/v).

181. The process of any one of claims 161-180, wherein the dilution buffer comprises a non-ionic surfactant.

182. The process of any one of claims 161-181, wherein the dilution buffer comprises a non-ionic surfactant at a concentration of at least about 0.05% (v/v or w/v) or higher.

183. The process of any one claims 161-182, wherein the dilution buffer comprises a non-ionic surfactant at a concentration of about 0.05% to about 0.95%, (v/v or w/v).

184. The process of any one of claims 181-183, wherein the non-ionic surfactant is selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene alkylphenyl ethers, polyoxyethylene-polyoxypropylene block copolymers, alkylglucosides, alkylphenol ethoxylates, preferably polysorbates, polyoxyethylene alkyl phenyl ethers, and any combinations thereof.

185. The process of any one of claims 161-184, wherein the dilution buffer comprises a salt.

186. The process of any one of claims 161-185, wherein the dilution buffer comprises a salt at a concentration of at least about 0.1mM.

187. The process of any one of claims 161-186, wherein the dilution buffer comprises a salt at a concentration of from about 0.1mM to about 2mM.

188. The process of any one of claims 185-187, wherein the salt is a magnesium salt, a sodium salt, a potassium salt, an ammonium salt, a calcium salt, a copper salt, a cobalt salt, a manganese salt, a nickel salt or a zinc salt, optionally the salt is $MgCl_2$.

189. The process of any one of claims 161-188, wherein the dilution buffer has a high pH.

190. The process of any one of claims 161-189, wherein the dilution buffer has a pH greater than or equal to about 8.

191. The process of any one of claims 161 or 167-190, wherein the dilution buffer comprises: BTP, histidine, glycerol, PF68, $MgCl_2$ and has a high pH.

192. The process of any one of claims 161-191, wherein the dilution buffer comprises: BTP, histidine, PF68, $MgCl_2$, citric acid and has a high pH.

193. The process of any one of claims 161-192, wherein the dilution buffer has conductivity in a range from about 0.5 mS/cm to about 3 mS/cm.

194. The process of any one of claims 161-193, wherein the dilution buffer has an osmolarity of less than 900 mOsm.

195. The process of any one of claims 115-194, wherein less than 5% of empty virus particles in the affinity eluate bind to anion exchange chromatography media.

196. The process of any one of claims 115-195, wherein less than 10% of the virus particles in the eluate from the anion exchange are empty viral particles.

197. The process of any one of claims 115-196, wherein the eluate from the anion exchange is substantially free of empty virus particles.

198. The process of any one of claims 115-197, wherein a ratio of UV_{260} to UV_{280} in the anion exchange eluate is at least 1.15X higher than a ratio of UV_{260} to UV_{280} ratio in the adjusted affinity eluate.

199. The process of any one of claims 115-198, wherein the recombinant adeno associated virus particle comprise rAAV virion.

200. The process of any one of claims 115-199, wherein the purified rAAV has a particle to infectivity ratio less than 2×10^4 vg/TCID50.

201. A population of recombinantly expressed virus particles purified or isolated by a method of any one of claims 115-200.

202. A composition comprising a population of recombinantly expressed virus particles purified or isolated by a method of any one of claims 115-200.

203. The composition of claim 202, wherein the composition is a pharmaceutical composition.

204. A population of purified recombinant adeno-associated virus (rAAV) lacking prokaryotic sequences, wherein, the purified rAAV has a particle to infectivity ratio less than 2×10^4 vg/TCID50, wherein the population of purified rAAV comprises less than about 10% empty viral capsids, and wherein the purified rAAV optionally is obtained by a method comprising transfecting a suspension mammalian cell line, and wherein the cells optionally are transfected in suspension.

205. The population of purified recombinant adeno-associated virus (rAAV) of claim 204, wherein, the population comprises less than about 5% empty viral capsids.

206. The population of purified recombinant adeno-associated virus (rAAV) of any one of claims 204-205, wherein the population is substantially devoid of empty viral capsids.

207. The population of purified recombinant adeno-associated virus (rAAV) any one of claims 204-206, wherein the mammalian cell line is derived from a human embryonic cell line.

208. The population of purified recombinant adeno-associated virus (rAAV) any one of claims 204-207, wherein the human embryonic cell line is suspension adapted, serum free cell line derived from a human embryonic kidney cell line.

209. A population of purified recombinant adeno-associated virus (rAAV) lacking prokaryotic sequences, wherein, the purified rAAV has a particle to infectivity ratio less than 2×10^4 vg/TCID50, and wherein the purified rAAV is obtained by a method comprising transfecting a suspension mammalian cell line.

210. The population of purified recombinant adeno-associated virus (rAAV) of claim 209, wherein, the mammalian cell line is transfected in suspension with a) a nucleic acid

sequence encoding helper proteins sufficient for rAAV replication; b) a nucleic acid sequence encoding rep and cap genes, and c) a close ended linear duplexed rAAV vector nucleic acid comprising at least one ITR and a heterologous transgene operably linked to one or more regulatory elements.

211. The population of purified recombinant adeno-associated virus (rAAV) of any one of claims 209-210, wherein the mammalian cell line is derived from a human embryonic cell line.

212. The population of purified recombinant adeno-associated virus (rAAV) of any one of claims 209-211, wherein the human embryonic cell line is suspension adapted, serum free cell line derived from a human embryonic kidney cell line.

213. A composition comprising a population of purified recombinant adeno-associated virus particles and a pH from about 6.5 to about 8.0, and wherein: (i) the purified rAAV has a particle to infectivity ratio less than 2 x 10^4 vg/TCID50; and/or (ii) the population of purified rAAV comprises less than about 10% empty viral capsids.

214. The composition of claim 213, wherein the composition comprises the purified rAAV are at a concentration of from about $1e^9$ vg/ml to about $1e^{15}$ vg/ml.

215. The composition of any one of claims 213-214, wherein the composition comprises the purified rAAV are at a concentration of from about $1e^{12}$ vg/ml to about $1e^{14}$ vg/ml, optionally, the the purified rAAV are at a concentration of from about $1e^{13}$ vg/ml to about $1e^{14}$ vg/ml.

216. The composition of any one of claims 213-215, wherein the composition has a pH of from about 6.5 to about 8.0, optionally, the composition has a pH of from about 7 to about 8.0.

217. The composition of any one of claims 213-216, wherein the composition comprises a buffer.

218. The composition of claim 217, wherein the buffer is selected from the group consisting of PBS, Tris.HCl, phosphate, citric acid, histidine, tromethamine, succinic acid, malic acid, α-ketoglutaric acid, carbonate, protein buffers, and any combinations thereof.

219. The composition of claim 217 or 218, wherein the buffer has a salt concentration of from about 20 mM to about 750 mM.

220. The composition of any one of claims 214-219, wherein the composition has an ionic strength of at least 100 mM.

221. The composition of claim 220, wherein the composition has an ionic strength from about 125 mM to about 750 mM.

222. The composition of any one of claims 214-221, wherein the composition has an osmolarity of less than about 600 mOsm.

223. The composition of claim 222, wherein the composition has an osmolarity from about 125 mOsm to about 500 mOsm.

224. The composition of any one of claims 214-223, wherein the composition comprises one or more ions and/or salts thereof.

225. The composition of claim 224, wherein the ion is selected from the group consisting of sodium, potassium, chroride, ammonium, carbonate, nitrate, chlorate, chlorite, and calcium.

226. The composition of any one of claims 214-225, wherein the composition comprises a bulking agent.

227. The composition of claim 226, wherein the bulking agent is a polyol or providone (PVP K24).

228. The composition of any one of claims 226-227, wherein the bulking agent is selected from the group consisting of polyhydroxy hydrocarbons, monosaccharides, disaccharides, and trisaccharides.

229. The composition of any one of claims 226-228, wherein the bulking agent is selected from the group consisting of sorbitol, mannitol, glycerol, propylene glycol, polyethylene glycol, dulcitol, sucrose, lactose, maltose, trehalose, and dextran.

230. The composition of any one of claims 226-229, wherein the composition comprises the bulking agentat a concentration from about 0.5 % (w/v) to about 10% (w/v).

231. The composition of any one of claims 214-230, wherein the composition comprises a non-ionic surfactant.

232. The composition of claim 231, wherein the non-ionic surfactant selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene alkyl phenyl ethers, polyoxyethylene-polyoxypropylene block copolymers, alkylglucosides, alkyl phenol ethoxylates, preferably polysorbates, polyoxyethylene alkyl phenyl ethers, and any combinations thereof.

233. The composition of claim 231 or 232, wherein the non-ionic surfactant is selected from the group consisting of TWEEN 60 nonionic detergent, PPG-PEG-PPG Pluronic 10R5, Pluronic F-68, Polyoxyethylene (18) tridecyl ether, Polyoxyethylene (12) tridecyl ether, MERPOL SH surfactant, MERPOL OJ surfactant, MERPOL HCS surfactant, Poloxamer P188, Poloxamer P407, Poloxamer P 338, IGEPAL CO-720, IGEPAL CO-630, IGEPAL CA-720, Brij S20, BrijSl0, Brij 010, Brij Cl0, BRIJ 020, ECOSURF EH-9 ,ECOSURF EH-14, TERGITOL 15-S-7, ECOSURF SA-15, TERGITOL15-S-9, TERGITOL 15-S-12, TERGITOL L-64, TERGITOLNP-7, TERGITOL NP-8, TERGITOL NP-9, TERGITOL

NP-9.5,TERGITOL NP-10, TERGITOL NP-11, TERGITOL NP-12, TERGITOLNP-13, polysorbate 20, and any combinations thereof.

234. The composition of any one of claims 231-233, wherein the composition comprises the non-ionic surfactant at a concentration from about 0.005% (w/v) to about 0.015% (w/v).

235. The composition of any one of claims 214-234, wherein the composition comprises one or more multivalent ions or salts thereof.

236. The composition of claim 235, wherein the multivalent ions are selected from the group consisting of citrate, sulfate, magnesium and phosphate.

237. The composition of any one of claims 235-236, wherein the composition comprises the multivalent ions at a concentration from about 5 mM to about 150 mM.

238. The composition of claim 237, wherein the composition comprises the multivalent ions at a concentration from about 10 mM to about 50 mM.

239. The composition of any one of claims 214-238, wherein the composition comprises calcium α-d-heptagluconate at a concentration from about 0.0001% (w/v) to about 0.01% (w/v).

240. The composition of any one of claims 214-239, wherein the population of purified rAAV comprises less than about 5% empty viral capsids.

241. The composition of any one of claims 214-240, wherein the population of purified rAAV is substantially devoid of empty viral capsids.

242. The composition of any one of claims 214-241, wherein the purified rAAV has a particle to infectivity ratio less than $1.5X10^4$ vg/TCID50.

243. The composition of any one of claims 214-242, wherein the composition exhibits substantially no aggregation after two or more freeze thaw cycles.

244. The composition of any one of claims 214-243, wherein the claims wherein the purified rAAV particle retains its $TCID_{50}$/ml by at least about 80% after two or more freeze thaw cycles.

245. The composition of any one of claims 214-244, wherein the population of purified recombinant adeno-associated virus particles is the population of purified rAAV of any one of claims 115-200.

CLAIMS

1. A population of purified recombinantly expressed adeno associated virus (rAAV) particles, wherein the recombinantly expressed adeno associated virus particles are purified or isolated from a harvesting media by a process comprising:

(a) purifying/isolating a plurality of recombinantly expressed virus particles from the harvesting media via affinity chromatography to produce an eluate (affinity chromatography eluate) comprising the plurality of recombinantly expressed virus particles, wherein an elution buffer for affinity chromatography (affinity elution buffer) comprises a predetermined amount of glycine, optionally, the affinity elution buffer is substantially free of weak acids or salts thereof; and optionally, the affinity elution buffer comprises an amino acid that is not glycine;

(b) adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography, wherein the adjusted affinity eluate comprises a predetermined amount of a weak acid or salt thereof or the adjusted affinity eluate comprises a predetermined amount of an amino acid that is not glycine in addition to the predetermined amount of glycine already present in affinity eluate;

(c) purifying/isolating the plurality of recombinantly expressed virus particles from the adjusted eluate of affinity chromatography by anion exchange chromatography to produce a solution comprising a plurality of purified/isolated recombinantly expressed virus particles, wherein an equilibration buffer for anion exchange chromatography comprises a predetermined amount of a weak acid or a salt thereof; and, optionally,

in which: (i) the population of purified rAAV comprises less than about 35% empty viral capsids; and/or (ii) the purified rAAV has a particle to infectivity ratio less than 2 x 10^4 vg/TCID50; and/or (iii) a ratio of UV_{260} to UV_{280} in the anion exchange eluate is at least about 1.15X or higher than a ratio of UV_{260} to UV_{280} ratio in the adjusted affinity eluate.

2. A population according to Claim 1, in which:

(a) less than 5% of empty virus particles, preferably substantially no empty virus particles, in the affinity eluate bind to anion exchange chromatography media;

(b) the population of purified rAAV comprises less than about 10% empty viral capsids, preferably less than about 5% empty viral capsids, preferably in which the population of purified rAAV is substantially free of empty capsids;

(c) the weak acid of Claim 1(b) is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid, oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid, tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid;

(d) the affinity elution buffer comprises histidine at a concentration of at least about 1mM or more, preferably about 1mM to about 50mM;

(e) the affinity elution buffer comprises glycine at a concentration of at least about 20mM or more, preferably from about 25mM to about 100mM; most preferably about 75mM glycine;

(f) the affinity elution buffer comprises a salt, preferably at concentration of at least about 5mM or more, preferably from about 5mM to about 15mM, preferably in which the salt is a magnesium salt, a sodium salt, a potassium salt, an ammonium salt, a calcium salt, a copper salt, a cobalt salt, a manganese salt, a nickel salt or a zinc salt, optionally the salt is $MgCl_2$;

(g) the affinity elution buffer comprises a polymer, preferably at a concentration of at least about 0.1% or more, preferably at a concentration of from about 0.1% to about 0.5%, preferably in which the polymer is a non-ionic surfactant; or

(h) the affinity elution buffer has a low pH, preferably a pH lower than or equal to about 6.5, preferably about 2.0 to about 3.0.

3. A population according to Claim 1 or 2, in which the affinity elution buffer comprises a weak acid or a salt thereof, preferably at a concentration of at least about 50mM or more, preferably from about 50mM to about 100mM, preferably in which the weak acid is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid, oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid, tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, or, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid, preferably citric acid or a salt thereof.

4. A population according to Claim 1, 2 or 3, in which the affinity elution buffer comprises: about 50mM glycine, about 75mM citrate, about 10mM $MgCl_2$, about 0.3% (w/v) P188 and has a pH of about 3.0.

5. A population according to any preceding claim, in which:

(a) the affinity elution buffer has conductivity in a range from about 5 mS/cm to about 8 mS/cm, optionally the affinity elution buffer has conductivity in a range from about 5.5 mS/cm to about 7 mS/cm;

(b) the affinity elution buffer has an osmolarity in a range from about 100 mOms to about 225 mOms;

(c) the equilibration buffer for anion exchange chromatography comprises the weak acid or a salt thereof in a concentration of at least about 0.5mM, preferably about 0.5mM to about 15mM;

(d) the weak acid in the equilibration buffer is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid,

glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid, oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid, tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, or, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid, preferably citric acid or citrate;

(e) adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography comprises adding the weak acid or a salt thereof to the affinity eluate, preferably to a final a concentration of at least about 0.5mM or higher, preferably about 0.5mM to about 15mM;

(f) the weak acid or a salt thereof added to the affinity eluate is citric acid or a salt thereof, optionally the salt is citrate; or

(g) adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography comprises adding an amino acid to the affinity eluate, preferably to a final a concentration of at least about 15mM or higher, preferably about 15mM to about 35mM.

6. A population according to any preceding claim, in which the amino acid added to the affinity eluate is aspartate, glutamate, histidine, arginine, lysine, cysteine or tyrosine, optionally, the amino acid is histidine.

7. A population according to any preceding claim, in which:

(a) adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography comprises diluting the affinity eluate;

(b) adjusting the affinity eluate for anion exchange chromatography comprises diluting the eluate by at least 2X or more; or

(c) adjusting the affinity eluate for anion exchange chromatography comprises diluting the affinity eluate with a dilution buffer (anion exchange dilution buffer).

8. A population according to Claim 7, in which:

(a) the dilution buffer comprises a weak acid or a salt thereof, preferably at a concentration of at least about 0.5mM, preferably about 0.5mM to about 15mM, in which preferably the weak acid in the dilution buffer is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid, oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid, tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid, preferably citric acid or a salt thereof, acetic acid or a salt thereof, or succinic acid or a salt thereof, optionally the weak acid is citric acid or a salt thereof;

(b) the dilution buffer comprises a buffering agent, preferably acetate, histidine, phosphate, citrate, propionate, tricine, borate, or tris(hydroxymethyl)aminomethane (tris), optionally the buffering agent is bris-tris propane (BTP), preferably at a concentration of at least about 25mM, preferably from about 25mM to about 175mM;

(c) the dilution buffer comprises an amino acid, preferably at a concentration of at least about 25mM, preferably from about 25mM to about 175mM, preferably in which the amino acid is aspartate, glutamate, histidine, arginine, lysine, cysteine or tyrosine, optionally, the amino acid is histidine;

(d) the dilution buffer comprises a viscosity modifier, preferably at a concentration of at least about 0.5% (v/v or w/v) or higher, preferably from about 0.5% to about 9.5%, (v/v or w/v), in which the viscosity modifier is preferably a polyol, optionally selected from the group consisting of hydrocarbons, monosaccharides, disaccharides, trisaccharides and any combinations thereof, preferably in which the polyol is sorbitol, mannitol, glycerol, propylene glycol, polyethylene glycol, dulcitol, sucrose, lactose, maltose, trehalose, dextran or any combinations thereof, optionally the glycerol, sorbitol, mannitol, dulcitol, sucrose, lactose, maltose, trehalose and any combinations

thereof, preferably in which the polyol is sorbitol, mannitol, glycerol, propylene glycol, polyethylene glycol, dulcitol, sucrose, lactose, maltose, trehalose, dextran or any combinations thereof, optionally the polyol is glycerol;

(e) the dilution buffer comprises a non-ionic surfactant, preferably at a concentration of at least about 0.05% (v/v or w/v) or higher, preferably about 0.05% to about 0.95%, (v/v or w/v), preferably in which the non-ionic surfactant is selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene alkylphenyl ethers, polyoxyethylene-polyoxypropylene block copolymers, alkylglucosides, alkylphenol ethoxylates, preferably polysorbates, polyoxyethylene alkyl phenyl ethers, and any combinations thereof;

(f) the dilution buffer comprises a salt, preferably at a concentration of at least about 0.1mM, preferably from about 0.1mM to about 2mM, preferably in which the salt is a magnesium salt, a sodium salt, a potassium salt, an ammonium salt, a calcium salt, a copper salt, a cobalt salt, a manganese salt, a nickel salt or a zinc salt, optionally the salt is $MgCl_2$; or

(g) the dilution buffer has a high pH, preferably a pH greater than or equal to about 8.

9. A population according to Claim 7 or 8, in which the dilution buffer comprises: BTP, histidine, glycerol, PF68, $MgCl_2$ and has a high pH.

10. A population of any of Claims 7, 8 or 9, in which the dilution buffer comprises: BTP, histidine, PF68, $MgCl_2$, citric acid and has a high pH.

11. A population according to any preceding claim, in which:

(a) the dilution buffer has conductivity in a range from about 0.5 mS/cm to about 3 mS/cm; or

(b) the dilution buffer has an osmolarity of less than 900 mOsm.

12. A population according to any preceding claim, in which:

(a) the method further comprises a step of removing or reducing amount of impurities (e.g., host cell DNA (hcDNA)) from the harvest media prior to affinity purification, preferably by adding a selective precipitation agent, preferably a cationic amine or nuclease, to harvest media; or

(b) the method further comprises a step of lysing a host cell in the harvest media with a non-ionic surfactant prior purifying/isolating by affinity chromatography, preferably in which the non-ionic surfactant is added to the harvest media to a final concentration of at least about 0.05% (v/v or w/v) or higher, preferably from about 0.05% to about 1% (v/v or w/v), preferably in which the non-ionic surfactant is mixed with the harvest media for a period of from about 15 minutes to about 2 hours, preferably in which the non-ionic surfactant is not Triton X-100, preferably in which the non-ionic surfactant is selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene alkylphenyl ethers, polyoxyethylene-polyoxypropylene block copolymers, alkylglucosides, alkylphenol ethoxylates, preferably polysorbates, polyoxyethylene alkyl phenyl ethers, and any combinations thereof.

13. A population according to any preceding claim, in which:

(a) the recombinant adeno associated virus particle comprise rAAV virion;

(b) the purified rAAV has a particle to infectivity ratio less than 2 x 10^4 vg/TCID50;

(c) the population of purified rAAV comprises less than about 10% empty viral capsids; or

(d) the rAAV is obtained by a method comprising transfecting a suspension mammalian cell line, and in which the cells optionally are transfected in suspension, preferably with (i) a nucleic acid sequence encoding helper proteins sufficient for rAAV replication; (ii) a nucleic acid sequence encoding rep and cap genes, and (iii) a close ended linear duplexed rAAV vector nucleic acid comprising at least one ITR and a heterologous transgene operably linked to one or more regulatory elements, preferably in which the mammalian cell line is derived from a human embryonic cell line, preferably a suspension adapted, serum free cell line derived from a human embryonic kidney cell line, preferably in which the purified recombinant adeno-associated virus (rAAV) lacks prokaryotic sequences.

14. A population according to any preceding claim, in which the population is comprised in a composition comprising the population and in which: (i) the composition exhibits substantially no aggregation of the rAAV particles after two or more freeze thaw cycles; and/or (ii) the purified rAAV particle retains its $TCID_{50}$/ml by at least about 80% after two or more freeze thaw cycles; and/or (iii) the purified rAAV has a particle to infectivity ratio less

than 2 x 10^4 vg/$TCID_{50}$; and/or (iv) the population of purified rAAV comprises less than about 35% empty viral capsids, preferably in which:

(a) the composition exhibits substantially no aggregation of the rAAV particles after two or more freeze thaw cycles;

(b) the purified rAAV particle retains its $TCID_{50}$/ml by at least about 80% after two or more freeze thaw cycles;

(c) the purified rAAV has a particle to infectivity ratio less than 2 x 10^4 vg/$TCID_{50}$;

(d) the population of purified rAAV comprises less than about 10% empty viral capsids;

(e) the composition comprises the purified rAAV at a concentration of from about $1e^9$ vg/ml to about $1e^{15}$ vg/ml;

(f) the composition comprises the purified rAAV at a concentration of from about $1e^{12}$ vg/ml to about $1e^{15}$ vg/ml, optionally, at a concentration of from about $1e^{13}$ vg/ml to about $1e^{14}$ vg/ml;

(g) the composition has a pH of about 6.5 to about 8, optionally, the composition has a pH of about 7 to about 8;

(h) the composition comprises a buffer, a bulking agent and one or both of a non-ionic surfactant and a multivalent salt, and optionally: (i) the composition is substantially free of glycine; and/or (ii) the composition is substantially free of trehalose, trehalose dehydrate, Dextran T10 or Dextran T40; and/or (iii) the composition is substantially free of sodium salts, ammonium salts or potassium salts; and/or (iv) the composition is substantially free of polysorbate-80 (PS80);

(i) the buffer is PBS, Tris.HCl, phosphate, citric acid, histidine, tromethamine, succinic acid, malic acid, α-ketoglutaric acid, carbonate, protein buffers or any combinations thereof, optionally, the buffer is PBS, Tris or histidine buffer;

(j) the bulking agent is a polyol or providone (PVP K24);

(k) the polyol is sorbitol, mannitol, glycerol, propylene glycol, polyethylene glycol, dulcitol, sucrose, lactose or maltose, optionally the polyol is sorbitol or mannitol;

(l) the non-ionic surfactant is selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene alkyl phenyl ethers, polyoxyethylene-polyoxypropylene block copolymers, alkylglucosides, alkyl phenol ethoxylates, preferably polysorbates, polyoxyethylene alkyl phenyl ethers, and any combinations thereof;

(m) the non-ionic surfactant is selected from the group of Brij 010, BRIJ 020, Brij Cl 0, Brij S20, BrijSl0, ECOSURF EH-14, ECOSURF EH-9, ECOSURF SA-15, IGEPAL CA-720, IGEPAL CO-630, IGEPAL CO-720, MERPOL HCS surfactant, MERPOL OJ surfactant, MERPOL SH surfactant, Pluronic 10R5, Pluronic 10R5, Pluronic F-68, Poloxamer P 188, Poloxamer P 338, Poloxamer P188, Poloxamer P407, Polyoxyethylene (12) tridecyl ether, TERGITOL 15-S-12, TERGITOL 15-S-7, TERGITOL 15-S-9, TERGITOL L-64, TERGITOL NP-10, TERGITOL NP-10, TERGITOL NP-11, TERGITOL NP-12, TERGITOL NP-8, TERGITOL NP-9, TERGITOL NP-9.5, TERGITOL NP-9.5, TERGITOL15-S-9, TERGITOLNP-13, TERGITOLNP-7, TWEEN 60 non-ionic detergent, and any combinations thereof;

(n) the multivalent salt is a calcium salt, citrate salt, sulfate salt or magnesium salt;

(o) the composition has an osmolarity of less than 750 mOSm;

(p) the composition has an osmolarity of from about 125 mOsm to about 500 mOsm; or

(q) the composition is a pharmaceutical composition.

15. A process for purifying or isolating recombinantly expressed adeno associated virus particles from a harvesting media, the process comprising:

(a) purifying/isolating a plurality of recombinantly expressed virus particles from the harvesting media via affinity chromatography to produce an eluate (affinity chromatography eluate) comprising the plurality of recombinantly expressed virus particles, in which an elution buffer for affinity chromatography (affinity elution buffer) comprises a predetermined amount of glycine, optionally, the affinity elution buffer is substantially free of weak acids or salts thereof; and optionally, the affinity elution buffer comprises an amino acid that is not glycine;

(b) adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography, in which the adjusted eluate comprises a

predetermined amount of a weak acid or a salt thereof or the adjusted affinity eluate comprises a predetermined amount of an amino acid in addition to the predetermined amount of glycine already present in affinity eluate; and

(c) purifying/isolating the plurality of recombinantly expressed virus particles from the adjusted eluate of affinity chromatography by anion exchange chromatography to produce a solution comprising a plurality of purified/isolated recombinantly expressed virus particles, in which an equilibration buffer for anion exchange chromatography comprises a predetermined amount of a weak acid or a salt thereof, optionally the weak acid is citric acid, acetic acid or succinic acid.

16. A process according to Claim 15, in which:

(a) less than 5% of empty virus particles in the affinity eluate bind to anion exchange chromatography media, preferably in which substantially no empty virus particles in the affinity eluate bind to anion exchange chromatography media;

(b) less than 10% of the virus particles in the eluate from the anion exchange are empty viral particles, preferably in which in less than 5% of the virus particles in the eluate from the anion exchange are empty viral particles, preferably in which the eluate from the anion exchange is substantially free of empty virus particles, preferably in which the recombinantly expressed virus particles are recombinant adeno associated virus (rAAV) particle;

(c) the weak acid of Claim 15(b) is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid, oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid, tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid;

(d) the affinity elution buffer comprises histidine at a concentration of at least about 1mM or more, preferably about 1mM to about 50mM;

(e) the affinity elution buffer comprises glycine at a concentration of at least about 20mM or more, preferably from about 25mM to about 100mM, preferably about 75mM glycine;

(f) the affinity elution buffer comprises a salt, preferably at a concentration of at least about 5mM or more, preferably from about 5mM to about 15mM, preferably in which the salt is a magnesium salt, a sodium salt, a potassium salt, an ammonium salt, a calcium salt, a copper salt, a cobalt salt, a manganese salt, a nickel salt or a zinc salt, optionally the salt is $MgCl_2$;

(g) the affinity elution buffer comprises a polymer, preferably at a concentration of at least about 0.1% or more, preferably at a concentration of from about 0.1% to about 0.5%, preferably in which the polymer is a non-ionic surfactant; or

(h) the affinity elution buffer has a low pH, preferably a pH lower than or equal to about 6.5, preferably about 2.0 to about 3.0.

17. A process according to Claim 15 or 16, in which the affinity elution buffer comprises a weak acid or a salt thereof, preferably at a concentration of at least about 50mM or more, preferably from about 50mM to about 100mM, preferably in which the weak acid is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid, oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid, tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid, preferably in which the weak acid is citric acid or a salt thereof.

18. A process according to Claim 15, 16 or 17, in which the affinity elution buffer comprises: about 50mM glycine, about 75mM citrate, about 10mM $MgCl_2$, about 0.3% (w/v) P188 and has a pH of about 3.0.

19. A process according to any of Claims 15 to 18, in which:

(a) the affinity elution buffer has conductivity in a range from about 5 mS/cm to about 8 mS/cm;

(b) the affinity elution buffer has an osmolarity in a range from about 100 mOms to about 225 mOms;

(c) the equilibration buffer for anion exchange chromatography comprises the weak acid or a salt thereof in a concentration of at least about 0.5mM, preferably about 0.5mM to about 15mM;

(d) the weak acid in the equilibration buffer is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid, oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid, tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid, preferably citric acid or citrate; or

(e) adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography comprises adding the weak acid or a salt thereof to the affinity eluate, preferably to a final a concentration of at least about 0.5mM or higher, preferably about 0.5mM to about 15mM.

20. A process according to any of Claims 15 to 19, in which the weak acid or a salt thereof added to the affinity eluate is citric acid or a salt thereof, optionally the salt is citrate.

21. A process according to any of Claims 15 to 20, in which:

(a) adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography comprises adding an amino acid to the affinity eluate;

(b) the amino acid is added to the affinity eluate to a final a concentration of at least about 15mM or higher, preferably about 15mM to about 35mM;

(c) the amino acid added to the affinity eluate is aspartate, glutamate, histidine, arginine, lysine, cysteine or tyrosine, optionally, the amino acid is histidine;

(d) adjusting the affinity chromatography eluate for subsequent purification through anion exchange chromatography comprises diluting the affinity eluate, preferably by at least 2X or more; or

(e) adjusting the affinity eluate for anion exchange chromatography comprises diluting the affinity eluate with a dilution buffer (anion exchange dilution buffer).

22. A process according to Claim 21, in which:

(a) the dilution buffer comprises a weak acid or a salt thereof, preferably at a concentration of at least about 0.5mM, preferably about 0.5mM to about 15mM, in which preferably the weak acid in the dilution buffer is citric acid, acetic acid, succinic acid, acetoacetic acid, adipic acid, alloxanic acid, ascorbic acid, aspartic acid, barbituric acid, boric acid, butanoic acid, butyric acid, carbonic acid, crotonic acid, diglycolic acid, dimethylmalonic acid, formic acid, fumaric acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glyceric acid, glycolic acid, hydroxyacetic acid, isocitric acid, itaconic acid, lactic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mesotartaric acid, methylsuccinic acid, methymalonic acid, oxalic acid, oxaloacetic acid, pentanic acid, pentanoic acid, phosphoric acid, m-phthalic acid, o-phthalic acid, p-phthalic acid, propionic acid, pyruvic acid, salicylic acid, tartaric acid, tartronic acid, terephthalic acid, trans-crotonic acid, trichloroacetic acid, uric acid, α-tartaric acid, 2 oxo-butanoic acid, 2-methylbutanoic acid, 2-oxoglutaric acid, 3-butenoic acid or 3-methylbutanoic acid, optionally the weak acid is citric acid, citrate, acetic acid or succinic acid, preferably citric acid or a salt thereof, acetic acid or a salt thereof, or succinic acid or a salt thereof, optionally the weak acid is citric acid or a salt thereof;

(b) the dilution buffer comprises a buffering agent, preferably acetate, histidine, phosphate, citrate, propionate, tricine, borate, or tris(hydroxymethyl)aminomethane (tris), optionally the buffering agent is bris-tris propane (BTP), preferably at a concentration of at least about 25mM, preferably from about 25mM to about 175mM;

(c) the dilution buffer comprises an amino acid, preferably at a concentration of at least about 25mM, preferably from about 25mM to about 175mM, preferably in which the amino acid is aspartate, glutamate, histidine, arginine, lysine, cysteine or tyrosine, optionally, the amino acid is histidine;

(d) the dilution buffer comprises a viscosity modifier, preferably at a concentration of at least about 0.5% (v/v or w/v) or higher, preferably from about 0.5% to about 9.5%, (v/v or w/v), in which the viscosity modifier is preferably a polyol, optionally selected from the group consisting of hydrocarbons, monosaccharides, disaccharides, trisaccharides and any combinations thereof, preferably in which the polyol is sorbitol, mannitol, glycerol, propylene glycol, polyethylene glycol, dulcitol, sucrose, lactose, maltose, trehalose, dextran or any combinations thereof, optionally the glycerol, sorbitol, mannitol, dulcitol, sucrose, lactose, maltose, trehalose and any combinations thereof, preferably in which the polyol is sorbitol, mannitol, glycerol, propylene glycol, polyethylene glycol, dulcitol, sucrose, lactose, maltose, trehalose, dextran or any combinations thereof, optionally the polyol is glycerol;

(e) the dilution buffer comprises a non-ionic surfactant, preferably at a concentration of at least about 0.05% (v/v or w/v) or higher, preferably about 0.05% to about 0.95%, (v/v or w/v), preferably in which the non-ionic surfactant is selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene alkylphenyl ethers, polyoxyethylene-polyoxypropylene block copolymers, alkylglucosides, alkylphenol ethoxylates, preferably polysorbates, polyoxyethylene alkyl phenyl ethers, and any combinations thereof;

(f) the dilution buffer comprises a salt, preferably at a concentration of at least about 0.1mM, preferably about 0.1mM to about 2mM, preferably in which the salt is a magnesium salt, a sodium salt, a potassium salt, an ammonium salt, a calcium salt, a copper salt, a cobalt salt, a manganese salt, a nickel salt or a zinc salt, optionally the salt is $MgCl_2$;

(g) the dilution buffer has a high pH, preferably a pH greater than or equal to about 8.

23. A process according to any of Claims 15 to 22, in which the dilution buffer comprises: BTP, histidine, glycerol, PF68, $MgCl_2$ and has a high pH.

24. A process according to any of Claims 15 to 22, in which the dilution buffer comprises: BTP, histidine, PF68, $MgCl_2$, citric acid and has a high pH.

25. A process according to any of Claims 15 to 24, in which:

(a) the dilution buffer has conductivity in a range from about 0.5 mS/cm to about 3 mS/cm; or

(b) the dilution buffer has an osmolarity of less than 900 mOsm.

26. A process according to any of Claims 15 to 25, in which:

(a) less than 5% of empty virus particles in the affinity eluate bind to anion exchange chromatography media, preferably less than 10% of the virus particles in the eluate from the anion exchange are empty viral particles, preferably the eluate from the anion exchange is substantially free of empty virus particles;

(b) a ratio of UV_{260} to UV_{280} in the anion exchange eluate is at least 1.15X higher than a ratio of UV_{260} to UV_{280} ratio in the adjusted affinity eluate;

(c) the recombinant adeno associated virus particle comprise rAAV virion; or

(d) the purified rAAV has a particle to infectivity ratio less than 2×10^4 vg/TCID50.

27. A population of recombinantly expressed virus particles purified or isolated by a method according to any of Claims 15 to 26.

28. A composition comprising a population of recombinantly expressed virus particles purified or isolated by a method according to any of Claims 15 to 26, preferably a pharmaceutical composition.

29. A population of purified recombinant adeno-associated virus (rAAV) lacking prokaryotic sequences, in which the purified rAAV has a particle to infectivity ratio less than 2×10^4 vg/TCID50, in which the population of purified rAAV comprises less than about 10% empty viral capsids, and in which the purified rAAV optionally is obtained by a method comprising transfecting a suspension mammalian cell line, and in which the cells optionally are transfected in suspension.

30. A population of purified recombinant adeno-associated virus (rAAV) according to Claim 29, in which:

(a) the population comprises less than about 5% empty viral capsids;

(b) the population is substantially devoid of empty viral capsids;

(c) the mammalian cell line is derived from a human embryonic cell line; or

(d) the human embryonic cell line is suspension adapted, serum free cell line derived from a human embryonic kidney cell line.

31. A population of purified recombinant adeno-associated virus (rAAV) lacking prokaryotic sequences, in which, the purified rAAV has a particle to infectivity ratio less than 2×10^4 vg/TCID50, and in which the purified rAAV is obtained by a method comprising transfecting a suspension mammalian cell line.

32. A population of purified recombinant adeno-associated virus (rAAV) according to Claim 31, in which the mammalian cell line is transfected in suspension with (a) a nucleic acid sequence encoding helper proteins sufficient for rAAV replication; (b) a nucleic acid sequence encoding rep and cap genes, and (c) a close ended linear duplexed rAAV vector nucleic acid comprising at least one ITR and a heterologous transgene operably linked to one or more regulatory elements.

33. A population of purified recombinant adeno-associated virus (rAAV) according to Claim 31 or 23, in which:

(a) the mammalian cell line is derived from a human embryonic cell line; or

(b) the human embryonic cell line is suspension adapted, serum free cell line derived from a human embryonic kidney cell line.

34. A composition comprising a population of purified recombinant adeno-associated virus particles and a pH from about 6.5 to about 8.0, and in which: (i) the purified rAAV has a particle to infectivity ratio less than 2×10^4 vg/TCID50; and/or (ii) the population of purified rAAV comprises less than about 10% empty viral capsids.

35. A composition according to Claim 34, in which:

(a) the composition comprises the purified rAAV at a concentration of from about $1e^9$ vg/ml to about $1e^{15}$ vg/ml;

(b) the composition comprises the purified rAAV at a concentration of from about $1e^{12}$ vg/ml to about $1e^{14}$ vg/ml, optionally, the purified rAAV are at a concentration of from about $1e^{13}$ vg/ml to about $1e^{14}$ vg/ml;

(c) the composition has a pH of from about 6.5 to about 8.0, optionally, the composition has a pH of from about 7 to about 8.0;

(d) the composition comprises a buffer, preferably selected from the group consisting of PBS, Tris.HCl, phosphate, citric acid, histidine, tromethamine, succinic acid, malic acid, α-ketoglutaric acid, carbonate, protein buffers, and any combinations thereof, preferably in which the buffer has a salt concentration of from about 20 mM to about 750 mM;

(e) the composition has an ionic strength of at least 100 mM, preferably about 125 mM to about 750 mM;

(f) the composition has an osmolarity of less than about 600 mOsm, preferably about 125 mOsm to about 500 mOsm;

(g) the composition comprises one or more ions and/or salts thereof, preferably in which the ion is selected from the group consisting of sodium, potassium, chroride, ammonium, carbonate, nitrate, chlorate, chlorite, and calcium.;

(h) the composition comprises a bulking agent, preferably a polyol or providone (PVP K24), preferably in which the bulking agent is selected from the group consisting of polyhydroxy hydrocarbons, monosaccharides, disaccharides, and trisaccharides, preferably in which the bulking agent is selected from the group consisting of sorbitol, mannitol, glycerol, propylene glycol, polyethylene glycol, dulcitol, sucrose, lactose, maltose, trehalose, and dextran, preferably at a concentration from about 0.5 % (w/v) to about 10% (w/v);

(i) the composition comprises a non-ionic surfactant, preferably selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene alkyl phenyl ethers, polyoxyethylene-polyoxypropylene block copolymers, alkylglucosides, alkyl phenol ethoxylates, preferably polysorbates, polyoxyethylene alkyl phenyl ethers, and any combinations thereof, preferably in which the non-ionic surfactant is selected from the group consisting of TWEEN 60 nonionic detergent, PPG-PEG-PPG Pluronic 10R5, Pluronic F-68, Polyoxyethylene (18) tridecyl ether, Polyoxyethylene (12) tridecyl ether,

MERPOL SH surfactant, MERPOL OJ surfactant, MERPOL HCS surfactant, Poloxamer P188, Poloxamer P407, Poloxamer P 338, IGEPAL CO-720, IGEPAL CO-630, IGEPAL CA-720, Brij S20, BrijSl0, Brij 010, Brij Cl0, BRIJ 020, ECOSURF EH-9, ECOSURF EH-14, TERGITOL 15-S-7, ECOSURF SA-15, TERGITOL15-S-9, TERGITOL 15-S-12, TERGITOL L-64, TERGITOLNP-7, TERGITOL NP-8, TERGITOL NP-9, TERGITOL NP-9.5,TERGITOL NP-10, TERGITOL NP-11, TERGITOL NP-12, TERGITOLNP-13, polysorbate 20, and any combinations thereof, preferably in which the composition comprises the non-ionic surfactant at a concentration from about 0.005% (w/v) to about 0.015% (w/v);

(j) the composition comprises one or more multivalent ions or salts thereof, preferably selected from the group consisting of citrate, sulfate, magnesium and phosphate, preferably at a concentration from about 5 mM to about 150 mM, preferably about 10 mM to about 50 mM;

(k) the composition comprises calcium α-d-heptagluconate at a concentration from about 0.0001% (w/v) to about 0.01% (w/v);

(l) the population of purified rAAV comprises less than about 5% empty viral capsids, preferably in which the population of purified rAAV is substantially devoid of empty viral capsids;

(m) the purified rAAV has a particle to infectivity ratio less than $1.5X10^4$ vg/TCID50;

(n) the composition exhibits substantially no aggregation after two or more freeze thaw cycles; or

(o) the purified rAAV particle retains its $TCID_{50}$/ml by at least about 80% after two or more freeze thaw cycles.

39. A composition according to any of Claims 35 to 38, in which the population of purified recombinant adeno-associated virus particles is the population of purified rAAV according to any of Claims 15 to 26.