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.

36. A composition according to Claim 35, 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.