continued effort has focused on scalable manufacturing processes that can efficiently generate high titer quantities of rAAV vectors with high purity and potency.

[0005] Though the effort to design efficient, large-scale methods to purify an AAV product suitable for human administration has been great, there remains a need for better AAV purification methods. There are various other proteins and materials from the host cell culture matrix that could be more efficiently removed during the purification of AAV. AAV purification methods which include steps for removing host cell material from the final AAV product are therefore desired.

SUMMARY

[0006] A feature of AAV vector generation in cell culture is the formation of a complex matrix that comprises material from disrupted cells. In particular, host cell proteins, proteasomes, cell debris and potential virus-specific receptors are often present in the material from disrupted cells. The disclosed methods which include steps for removing host cell material from the final AAV product in conditions that result in greater purity at a physiologically applicable pH.

[0007] In one aspect, the present disclosure refers to a method of purifying an adeno-associated virus (AAV) comprising (a) loading an AAV containing solution onto an affinity resin targeted against AAV under conditions that allow binding between the AAV in the solution and the affinity resin; (b) undertaking at least three wash steps; and (c) eluting the AAV from the affinity resin; and (d) contacting the AAV containing solution with an anion exchanger and eluting the AAV containing solution from the anion exchanger prior to loading the AAV containing solution onto the affinity resin, wherein the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 mM to about 200 mM sodium acetate and from about 0.05% (w/w) to about 0.2% (w/w) polysorbate 80, and wherein the first buffer has a pH from about 5.5 to about 6.5; wherein the second wash step comprises applying to the affinity resin a second buffer comprising from about 10 mM to about 70 mM TrisHCl and from about 75 mM to about 250 mM salt, and wherein the second buffer has a pH from about 8.0 to about 9.0; and wherein the third wash step comprises applying to the affinity resin a

third buffer comprising from about 10 to about 70 mM TrisHCl and from about 30% (w/w) to about 75 % (w/w) ethylene glycol, and wherein the third buffer has a pH from about 8.0 to about 9.0; and wherein a support matrix of the affinity resin is a cross-linked poly[styrene-divinylbenzene]. In another example, there is ~~provided~~ described a method of purifying an adeno-associated virus (AAV) comprising (a) loading an AAV containing solution onto an affinity resin targeted against AAV under conductions that allow binding between the AAV in the solution and the affinity resin; (b) undertaking at least two wash steps; and (c) eluting the AAV from the affinity resin.

[0008] In some ~~embodiments~~examples, the method further comprises contacting the AAV containing solution with an anion exchanger and eluting the AAV containing solution from the anion exchanger prior to loading the AAV containing solution onto the affinity resin.

[0009] In some examples~~embodiments~~, at least one of the wash steps comprises applying to the affinity resin a buffer comprising an organic solvent or detergent. In some

examplesembodiments, the buffer comprises TrisHCl and a salt. In some examplesembodiments, the buffer comprises one or more of Histidine, Histidine-HCl, Arginine-HCl, Lysine-HCl, Glycine, Taurine, MES-Na, Bis-Tris, and N-acetyl-D,L-tryptophan. In some examplesembodiments, the salt is NaCl. In some examplesembodiments, the buffer comprises sodium acetate. In some examplesembodiments, the buffer comprises magnesium chloride. In some examplesembodiments, the buffer comprises TrisHCl and ethylene glycol. In some examplesembodiments, the buffer comprises Arginine-HCl and one of sucrose and glycerol. In some examplesembodiments, the buffer comprises Taurine and ethylene glycol. In some examplesembodiments, the buffer comprises Arginine-HCl, Lysine-HCl, and Histidine-HCl. In some examplesembodiments, the buffer comprises TrisHCl and DMSO.

[0010] In some examplesembodiments, at least three wash steps are performed. In some examplesembodiments, three wash steps are performed. In some examplesembodiments, the three wash steps are performed in succession.

[0011] In some examplesembodiments, the organic solvent or detergent is polysorbate 80, ethylene glycol, sorbitol, mannitol, xylitol, DMSO, sucrose, or trehalose. In some examplesembodiments, the detergent comprises one or more of Triton X100, polysorbate 80, and tri (n-butyl) phosphate (TNBP). In some examplesembodiments, the buffer comprises Bis-Tris.

[0012] In some examplesembodiments, the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 to about 2000 mM sodium acetate and from about 0.05 to about 0.2% polysorbate 80, and the first buffer has a pH from about 5.2 to about 6.8;

the second wash step comprises applying to the affinity resin a second buffer comprising from about 30 to about 200 mM TrisHCl and from about 75 to about 500 mM salt, and the second buffer has a pH from about 7.5 to about 9.2; and

the third wash step comprises applying to the affinity resin a third buffer comprising from about 30 to about 200 mM TrisHCl and from about 30 to about 75 vol% ethylene glycol, and the third buffer has a pH from about 7.3 to about 8.8.

[0013] In some examplesembodiments, a first wash step comprises applying to the affinity resin a first buffer comprising from about 50 to about 500 mM sodium salt of 2-(N-morpholino)ethanesulfonic acid (MES-Na), from about 3 to about 30 mM EDTA, and a solvent/detergent mixture comprising polysorbate 80, DMSO and tri(n-butyl)phosphate (TNBP), and the first buffer has a pH from about 5.2 to about 6.8;

the second wash step comprises applying to the affinity resin a second buffer comprising from about 30 to about 200 mM TrisHCl or Arginine-HCl and from about 75 to about 500 mM salt, and the second buffer has a pH from about 7.5 to about 9.2; and

the third wash step comprises applying to the affinity resin a third buffer comprising from about 20 to about 80 mM Arginine-HCl and from about 50 to about 200 mM salt, and the third buffer has a pH from about 7.3 to about 8.8.

[0014] In some examplesembodiments, the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 to about 200 mM taurine, and 0.2 to 1.5% PEG (e.g., PEG 6000), where the first buffer has a pH from about 5.2 to about 6.8;

the second wash step comprises applying to the affinity resin a second buffer comprising from about 30 to about 300 mM glycine, and the second buffer has a pH from about 7.5 to about 9.2; and

the third wash step comprises applying to the affinity resin a third buffer comprising from about 20 to about 150 mM taurine, from about 30 to about 75 vol% ethylene glycol, and from 0.05 to 0.2% octylglycopyranoside, and the third buffer has a pH from about 7.3 to about 8.8.

[0015] In some examplesembodiments, the first wash step comprises applying to the affinity resin a first buffer comprising from about 80 to about 400 mM Bis-Tris, and about 10 to about 20 grams of a solvent/detergent mixture comprising about Triton-X100, polysorbate 80 and TNBP in a ratio of about 11:3:3 (by weight), where the first buffer has a pH from about 5.2 to about 6.8;

the second wash step comprises applying to the affinity resin a second buffer comprising from about 5 to about 20 mmol sodium citrate, and where the second buffer has a pH from about 7.5 to about 9.2; and

the third wash step comprises applying to the affinity resin a third buffer comprising from about 50 to about 200 mM Arginine-HCl, from about 50 to about 200 mM Lysine HCl, from about 50 to about 200 mM Histidine-HCl, and from about 1mM to about 4 mM N-acetyl-D,L-tryptophan, and about 10% to about 40% (w/w) polysorbate 80, and where the third buffer has a pH from about 7.3 to about 8.8.

[0016] In some examples~~embodiments~~, the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 nM to about 200mM NaAcetate and from about 0.05 to about 0.2% Polysorbate80, where the first buffer has a pH of about 5.2 to about 6.8;

the second wash step comprises applying to the affinity resin a second buffer comprising from about 20 nM to about 100mM TrisHCl and from about 50 nM to about 200 nM of salt, where the second buffer has a pH of about 7.5 to about 8.8; and

the third wash step comprises applying to the affinity resin a third buffer comprising about 20mM to 100 mM TrisHCl, from about 40% to about 60%(w/w) ethylene glycol, and where the third buffer has a pH from about 7.5 to about 8.8.

[0017] In some examples~~embodiments~~, the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 nM to about 200mM NaAcetate and from about 0.05 to about 0.2% Polysorbate80, where the first buffer has a pH of about 5.2 to about 6.8;

the second wash step comprises applying to the affinity resin a second buffer comprising from about 20 nM to about 100mM TrisHCl and from about 50 nM to about 200 nM of salt, where the second buffer has a pH of about 7.5 to about 8.8; and

the third wash step comprises applying to the affinity resin a third buffer comprising about 20mM to 100 mM TrisHCl, from about 40% to about 60%(w/w) ethylene glycol, and the third buffer has a pH from about 7.5 to about 8.8.

[0018] In some examples~~embodiments~~, the salt is selected from NaCl, KCl, $MgCl_2$, $CaCl_2$, Sodium Citrate, LiCl, CsCl, Sodium Acetate, and a combination of one or more of NaCl, KCl, $MgCl_2$, $CaCl_2$, Sodium Citrate, LiCl, CsCl, and Sodium Acetate. In some examples~~embodiments~~, the salt is NaCl.

[0019] In some examples~~embodiments~~, the concentration of the salt does not exceed 500 mM. In some examples~~embodiments~~, the concentration of the salt does not exceed 200 mM. In some examples~~embodiments~~, the concentration of salt in the third buffer does not exceed 500 mM. In some examples~~embodiments~~, the concentration of salt in the third buffer does not exceed 200 mM.

[0020] In some examples~~embodiments~~, the method further comprises a fourth wash step that takes place before the first wash step and comprises applying to the affinity resin a fourth buffer comprising from about 10 to about 30 mM TrisHCl and from about 75 to about 250 mM NaCl, and the fourth buffer has a pH from about 6.5 to about 8.0.

[0021] In some examples~~embodiments~~, the first buffer comprises about 100 mM sodium acetate, about 0.1% polysorbate 80, and the first buffer has a pH of about 6.0. In some examples~~embodiments~~, the second buffer comprises about 50 mM TrisHCl and about 125 mM NaCl, and the second buffer has a pH of about 8.5. In some examples~~embodiments~~, the third buffer comprises about 50 mM TrisHCl and about 50 vol% ethylene glycol, and the third buffer has a pH of about 8.5.

[0022] In some examples~~embodiments~~, the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 to about 200 mM sodium acetate and from about 0.05 to about 0.2% polysorbate 80, and the first buffer has a pH from about 5.5 to about 6.5;

the second wash step comprises applying to the affinity resin a second buffer comprising from about 10 to about 70 mM TrisHCl and from about 75 to about 250 mM NaCl, and the second buffer has a pH from about 8.0 to about 9.0; and

the third wash step comprises applying to the affinity resin a third buffer comprising from about 10 to about 70 mM TrisHCl and from about 30 to about 75 vol%

ethylene glycol, and the third buffer has a pH from about 8.0 to about 9.0. In some ~~embodiments~~examples, the method further comprises a fourth wash step that takes place before the first wash step and comprises applying to the affinity resin a fourth buffer comprising from about 10 to about 30 mM TrisHCl and from about 75 to about 250 mM NaCl, and the fourth buffer has a pH from about 6.5 to about 8.0.

[0023] In some examples~~embodiments~~, the first buffer comprises about 100 mM sodium acetate and about 0.1% Polysorbate 80, and the first buffer has a pH of about 6.0. In some examples~~embodiments~~, the second buffer comprises about 50mM TrisHCl and about 125 mM NaCl, and the second buffer has a pH of about 8.5. In some examples~~embodiments~~, the third buffer comprises about 50 mM TrisHCl and about 50 vol% ethylene glycol, and the third buffer has a pH of about 8.0.

[0024] In some examples~~embodiments~~, an acidic component is removed. In some examples~~embodiments~~, the acidic component is host cell DNA, such as HEK293 DNA, and where the acidic component is reduced to a value below 250 pg per microgram of AAV antigen as measured by qPCR. In some examples~~embodiments~~, the acidic component is host cell DNA, such as HEK293 DNA, and where the acidic component is reduced to a value below 250 pg per microgram of AAV antigen as measured by ELISA.

[0025] In some examples~~embodiments~~, eluting comprises applying a continuous linear increase of the conductivity of an elution buffer by gradient elution. In some examples~~embodiments~~, eluting comprises applying continuous linear increase of the concentration of an organic solvent by gradient elution. In some examples~~embodiments~~, eluting comprises contacting the affinity resin with an elution buffer comprising ethylene glycol, a salt such as NaCl, and a buffer such as TrisHCl, where the pH is at least 7.0. In some examples~~embodiments~~, the salt concentration is about 750 mM, the buffer concentration is about 50 mM, and the ethylene glycol is 50-60% (w/w). In some examples~~embodiments~~, the pH is about 8.0. In some examples~~embodiments~~, the salt is NaCl and the buffer is TrisHCl.

[0026] In some examples~~embodiments~~, eluting comprises contacting the affinity resin with an elution buffer comprising about 750mM NaCl and 50-60% (w/w) ethylene glycol at a pH of at least 7.0. In some examples~~embodiments~~, the elution buffer comprises at least about 55%

(w/w) ethylene glycol. In some examples~~embodiments~~, eluting comprises contacting the affinity resin with an elution buffer comprising about 50 mM Tris HCl, about 750mM to about 1250 mM NaCl and about 60% (w/w) ethylene glycol at a pH of at least 7.8.

[0027] In some examples~~embodiments~~, eluting comprises contacting the affinity resin with an elution buffer comprising about 2 mM magnesium chloride, about 50 mM Arginine-HCl, about 750mM to about 1000 mM NaCl and at least about 55% (w/w) sucrose at a pH of at least about 8.0.

[0028] In some examples~~embodiments~~, eluting further comprises

(a) contacting the affinity resin with a fifth buffer comprising from about 20 to about 100 mM Arginine-HCl and from about 75 to about 250 mM NaCl, and the fifth buffer has a pH from about 7.5 to about 8.5; and

(b) contacting the affinity resin with a second elution buffer comprising from about 20 to about 100 mM Arginine-HCl, from about 40 to about 60% (w/w) glycerol, and from about 500 to 1000 mM salt, and the second elution buffer has a pH from about 7.5 to about 8.5.

[0029] In some examples~~embodiments~~, the steps are performed in succession.

[0030] In some examples~~embodiments~~, eluting comprises contacting the affinity resin with an elution buffer comprising about 2 mM magnesium chloride, about 50 mM Arginine-HCl, about 750mM to about 1000 mM NaCl and at least about 50% (w/w) glycerol at a pH of at least about 8.0.

[0031] In some examples~~embodiments~~, eluting comprises contacting the affinity resin with an elution buffer comprising about 2 mM magnesium chloride, about 50 mM Taurine, about 600mM to about 1000 mM NaCl, about 0.05 to about 0.2% octylglycopyranoside, and about 60% (w/w) ethylene glycol at a pH of at least about 7.8. In some ~~embodiments~~examples, eluting further comprises

(a) contacting the affinity resin with a fifth buffer comprising from about 20 to about 100 mM Tris-HCl and from about 75 to about 250 mM NaCl, and the fifth buffer has a pH from about 8.0 to about 8.8; and

(b) contacting the affinity resin with a second elution buffer comprising about 1 M ammonium sulfate, about 50 mM Tris HCl, and about 50% (v/v) ethylene glycol at a pH of at least about 6.8.

[0032] In some examplesembodiments, the steps are performed in succession.

[0033] In some examplesembodiments, eluting comprises contacting the affinity resin with an elution buffer comprising about 1 M ammonium sulfate, about 50 mM Tris HCl, and about 50% (v/v) ethylene glycol at a pH of at least about 6.8. In some examplesembodiments, eluting comprises contacting the affinity resin with an elution buffer comprising about 20% (w/w) sucrose, about 10% (w/w) sorbitol, about 5% (w/w) mannitol or about 5% (w/w) sucrose, about 15% (w/w) glycerol, about 50 mM Histidine, and about 750 to about 1000 mM NaCl at a pH of at least about 7.8.

[0034] In some examplesembodiments, eluting further comprises

(a) contacting the affinity resin with a fifth buffer comprising from about 20 to about 100 mM Histidine, from about 80 to about 120 mM NaCl, and the fifth buffer has a pH from about 8.0 to about 8.8; and

(b) contacting the affinity resin with a second elution buffer comprising from about 20 to about 100 mM Histidine, from about 600 to about 900 mM NaCl, and from about 5 to 60% (w/w) DMSO, and the fifth buffer has a pH from about 6.5 to about 8.5.

[0035] In some examplesembodiments, the steps are performed in succession. In some examplesembodiments, eluting comprises contacting the affinity resin with an elution buffer comprising about 100 mM Glycine-HCl, about 200 mM NaCl, at a pH of about 2.5.

[0036] In some examplesembodiments, the elution buffer is at a pH of about 8.0. In some embodiments, the elution buffer is at a pH of 8.0.

[0037] In some examples~~embodiments~~, eluting comprises a stepwise increase of a counter ion concentration. In some examples~~embodiments~~, eluting comprises a stepwise increase of an organic solvent concentration. In some examples~~embodiments~~, the salt in the elution buffer is selected from monovalent, divalent or polyvalent anions, such as chloride, acetate, sulfate, and citrate.

[0038] In some examples~~embodiments~~, the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 to about 200 mM sodium acetate, from about 0.05 to about 0.2% Polysorbate80, and the first buffer has a pH from about 5.2 to about 6.8;

the second wash step comprises applying to the affinity resin a second buffer comprising from about 25 to about 100 mM TrisHCl and from about 50 to about 200 mM NaCl, and the second buffer has a pH from about 7.5 to about 9.2; and

the third wash step comprises applying to the affinity resin a third buffer comprising from about 20 to about 100 mM TrisHCl and from about 75 to about 250 mM NaCl, and the third buffer has a pH from about 7.5 to about 8.8. In some ~~embodiments~~examples, the method further comprises elution by applying to the affinity resin purified water, followed by applying to the affinity resin from about 20 to about 50 mM HCl at a pH from about 1.7 to about 2.5. In some ~~embodiments~~examples, the method further comprises elution by applying a gradient of 0 to 100% 20-50mM Hydrochloric acid/800-1200mM NaCl in 0.5-2.0mM Hydrochloric acid.

[0039] In some examples~~embodiments~~, the AAV obtained from the eluting step has an HC impurity level of ≤ 99.9 %. In some examples~~embodiments~~, the AAV obtained from the eluting step has an HC impurity level of ≤ 99.0 %.

[0040] In some examples~~embodiments~~, the AAV is AAV8, the affinity resin is POROS™ CaptureSelect™ AAV8, and the elution buffer is acidic and does not comprise ethylene glycol. In some examples~~embodiments~~, the AAV is AAV9, the affinity resin is POROS™ CaptureSelect™ AAV9, and the elution buffer is acidic and does not comprise ethylene glycol.

[0041] In some examplesembodiments, the AAV is AAV8, and the affinity resin is an immune affinity resin consisting of an immobilized monoclonal antibody against AAV8 from type ADK8 or ADK8/9 immobilized on a chromatography matrix. In some examplesembodiments, the AAV is AAV9, and the affinity resin is an immune affinity resin consisting of an immobilized monoclonal antibody against AAV9 from type ADK9 or ADK8/9 immobilized on a chromatography matrix.

[0042] In some examplesembodiments, the method further comprises contacting the AAV containing solution with a filter comprising positively charged groups effective to deplete acidic charged contaminants from the AAV containing solution.

[0043] In some examplesembodiments, the method further comprises nanofiltration of an AAV fraction to remove viruses greater than 35 nm.

[0044] In some examplesembodiments, the method further comprises a polish step comprising performing AEX chromatography with a column comprising tentacle gel.

[0045] In some examplesembodiments, the method further comprises testing an AAV fraction via an AAV-specific ELISA.

[0046] In some examplesembodiments, the AAV specific ELISA is a sandwich ELISA specific for AAV.

[0047] In another aspect is providedexample, there is disclosed an AAV product produced by a method according to any one of claims 1 to 83as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] Figure 1 depicts a Western Blot showing substantially less Heat Shock Protein 70 kDa (HSP70) according to a purification protocol with multiple wash steps described herein (lane 4) as opposed to a comparative purification protocol without the wash steps (lane 6).

[0049] Figure 2 depicts an SDS-PAGE silver stain gel showing AAV8 proteins in lane 2, the AAV8-containing eluate from the purification protocol with multiple wash steps

WHAT IS CLAIMED IS:

1. A method of purifying an adeno-associated virus (AAV) comprising

(a) loading an AAV containing solution onto an affinity resin targeted against AAV under ~~conductions~~ conditions that allow binding between the AAV in the solution and the affinity resin;

(b) undertaking at least ~~two~~ three wash steps; and

(c) eluting the AAV from the affinity resin; and

(d) contacting the AAV containing solution with an anion exchanger and eluting the AAV containing solution from the anion exchanger prior to loading the AAV containing solution onto the affinity resin,

wherein the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 mM to about 200 mM sodium acetate and from about 0.05% (w/w) to about 0.2% (w/w) polysorbate 80, and wherein the first buffer has a pH from about 5.5 to about 6.5;

wherein the second wash step comprises applying to the affinity resin a second buffer comprising from about 10 mM to about 70 mM TrisHCl and from about 75 mM to about 250 mM salt, and wherein the second buffer has a pH from about 8.0 to about 9.0; and

wherein the third wash step comprises applying to the affinity resin a third buffer comprising from about 10 to about 70 mM TrisHCl and from about 30% (w/w) to about 75 % (w/w) ethylene glycol, and wherein the third buffer has a pH from about 8.0 to about 9.0; and

wherein a support matrix of the affinity resin is a cross-linked poly[styrene-divinylbenzene].

2. The method of claim 1, further comprising a wash step that takes place before the first wash step and comprises applying to the affinity resin a buffer comprising from

about 10 mM to about 30 mM TrisHCl and from about 75 mM to about 250 mM NaCl, and wherein the buffer has a pH from about 6.5 to about 8.0

~~2. The method of claim 1, further comprising contacting the AAV containing solution with an anion exchanger and eluting the AAV containing solution from the anion exchanger prior to loading the AAV containing solution onto the affinity resin.~~

~~3. The method of claim 1, wherein at least one of the wash steps comprises applying to the affinity resin a buffer comprising an organic solvent or detergent.~~

~~4. The method of claim 3, wherein the buffer comprises TrisHCl and a salt.~~

~~5. The method of claim 3, wherein the buffer comprises one or more of Histidine, Histidine-HCl, Arginine-HCl, Lysine-HCl, Glycine, Taurine, MES-Na, Bis-Tris, and N-acetyl-D,L-tryptophan.~~

~~6. The method of claim 4 or claim 5, wherein the salt is NaCl.~~

~~7. The method of claim 3, wherein the buffer comprises sodium acetate.~~

~~8. The method of claim 3, wherein the buffer comprises magnesium chloride.~~

~~9. The method of claim 3, wherein the buffer comprises TrisHCl and ethylene glycol.~~

~~10. The method of claim 3, wherein the buffer comprises Arginine-HCl and one of sucrose and glycerol.~~

~~11. The method of claim 3, wherein the buffer comprises Taurine and ethylene glycol.~~

~~12. The method of claim 3, wherein the buffer comprises Arginine-HCl, Lysine-HCl, and Histidine-HCl.~~

~~13. The method of claim 3, wherein the buffer comprises TrisHCl and DMSO.~~

14. The method of claim 1, wherein at least three wash steps are performed.

15. The method of claim 8, wherein three wash steps are performed.

16. The method of claim 9, wherein the three wash steps are performed in succession.

17. The method of claim 3 wherein the organic solvent or detergent is polysorbate 80, ethylene glycol, sorbitol, mannitol, xylitol, DMSO, sucrose, or trehalose.

18. The method of claim 17, wherein the detergent comprises one or more of Triton X100, polysorbate 80, and tri (n-butyl) phosphate (TNBP).

19. The method of claim 18, wherein the buffer comprises Bis-Tris.

20. The method of claim 15 or claim 16, wherein the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 to about 2000 mM sodium acetate and from about 0.05 to about 0.2% polysorbate 80, and wherein the first buffer has a pH from about 5.2 to about 6.8;
wherein the second wash step comprises applying to the affinity resin a second buffer comprising from about 30 to about 200 mM TrisHCl and from about 75 to about 500 mM salt, and wherein the second buffer has a pH from about 7.5 to about 9.2; and
wherein the third wash step comprises applying to the affinity resin a third buffer comprising from about 30 to about 200 mM TrisHCl and from about 30 to about 75 vol% ethylene glycol, and wherein the third buffer has a pH from about 7.3 to about 8.8.

21. The method of claim 15 or claim 16, wherein the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 to about 500 mM sodium salt of 2-(N-morpholino)ethanesulfonic acid (MES-Na), from about 3 to about 30 mM EDTA, and a solvent/detergent mixture comprising polysorbate 80, DMSO and tri(n-butyl)phosphate (TNBP), and wherein the first buffer has a pH from about 5.2 to about 6.8;

wherein the second wash step comprises applying to the affinity resin a second buffer comprising from about 30 to about 200 mM TrisHCl or Arginine-HCl and from about 75 to about 500 mM salt, and wherein the second buffer has a pH from about 7.5 to about 9.2; and

wherein the third wash step comprises applying to the affinity resin a third buffer comprising from about 20 to about 80 mM Arginine-HCl and from about 50 to about 200 mM salt, and wherein the third buffer has a pH from about 7.3 to about 8.8.

22. The method of claim 15 or claim 16, wherein the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 to about 200 mM taurine, and 0.2 to 1.5% PEG (e.g., PEG 6000) wherein the first buffer has a pH from about 5.2 to about 6.8;

wherein the second wash step comprises applying to the affinity resin a second buffer comprising from about 30 to about 300 mM glycine, and wherein the second buffer has a pH from about 7.5 to about 9.2; and

wherein the third wash step comprises applying to the affinity resin a third buffer comprising from about 20 to about 150 mM taurine, from about 30 to about 75 vol% ethylene glycol, and from 0.05 to 0.2% octylglycopyranoside, and wherein the third buffer has a pH from about 7.3 to about 8.8.

23. The method of claim 15 or claim 16, wherein the first wash step comprises applying to the affinity resin a first buffer comprising from about 80 to about 400 mM Bis-Tris, and about 10 to about 20 grams of a solvent/detergent mixture comprising about Triton-X100, polysorbate 80 and TNBP in a ratio of about 11:3:3 (by weight) wherein the first buffer has a pH from about 5.2 to about 6.8;

wherein the second wash step comprises applying to the affinity resin a second buffer comprising from about 5 to about 20 mmol sodium citrate, and wherein the second buffer has a pH from about 7.5 to about 9.2; and

wherein the third wash step comprises applying to the affinity resin a third buffer comprising from about 50 to about 200 mM Arginine-HCl, from about 50 to about 200 mM Lysine HCl, from about 50 to about 200 mM Histidine-HCl, and from about 1mM to

about 4 mM N-acetyl-D,L-tryptophan, and about 10% to about 40% (w/w) polysorbate 80, and wherein the third buffer has a pH from about 7.3 to about 8.8.

24. The method of claim 15 or claim 16, wherein the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 nM to about 200mM NaAcetate and from about 0.05 to about 0.2% Polysorbate80, wherein the first buffer has a pH of about 5.2 to about 6.8;
wherein the second wash step comprises applying to the affinity resin a second buffer comprising from about 20 nM to about 100mM TrisHCl and from about 50 nM to about 200 nM of salt, wherein the second buffer has a pH of about 7.5 to about 8.8; and
wherein the third wash step comprises applying to the affinity resin a third buffer comprising about 20mM to 100 mM TrisHCl, from about 40% to about 60%(w/w) ethylene glycol, and wherein the third buffer has a pH from about 7.5 to about 8.8.

25. The method of claim 15 or claim 16, wherein the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 nM to about 200mM NaAcetate and from about 0.05 to about 0.2% Polysorbate80, wherein the first buffer has a pH of about 5.2 to about 6.8;
wherein the second wash step comprises applying to the affinity resin a second buffer comprising from about 20 nM to about 100mM TrisHCl and from about 50 nM to about 200 nM of salt, wherein the second buffer has a pH of about 7.5 to about 8.8; and
wherein the third wash step comprises applying to the affinity resin a third buffer comprising about 20mM to 100 mM TrisHCl, from about 40% to about 60%(w/w) ethylene glycol, and wherein the third buffer has a pH from about 7.5 to about 8.8.

26. The method of any one of claims 20 to 25, wherein the salt is selected from NaCl, KCl, $MgCl_2$, $CaCl_2$, Sodium Citrate, LiCl, CsCl, Sodium Acetate, and a combination of one or more of NaCl, KCl, $MgCl_2$, $CaCl_2$, Sodium Citrate, LiCl, CsCl, and Sodium Acetate.

27. The method of claim 26, wherein the salt is NaCl.

~~28. The method of any one of claims 20 to 27, wherein the concentration of the salt does not exceed 500 mM.~~

~~29. The method of any one of claims 20 to 27, wherein the concentration of the salt does not exceed 200 mM.~~

~~30. The method of any one of claims 20 to 29, wherein the concentration of salt in the third buffer does not exceed 500 mM.~~

~~31. The method of any one of claims 20 to 29, wherein the concentration of salt in the third buffer does not exceed 200 mM.~~

~~32. The method of any one of claims 20 to 31, further comprising a fourth wash step that takes place before the first wash step and comprises applying to the affinity resin a fourth buffer comprising from about 10 to about 30 mM TrisHCl and from about 75 to about 250 mM NaCl, and wherein the fourth buffer has a pH from about 6.5 to about 8.0.~~

~~33.~~3. The method of ~~any one of claims 20 to 32~~claim 1 or 2, wherein the first buffer comprises about 100 mM sodium acetate, about 0.1% polysorbate 80, and wherein the first buffer has a pH of about 6.0.

~~34.~~4. The method of any one of claims ~~20~~ 1 to ~~33~~3, wherein the second buffer comprises about 50 mM TrisHCl and about 125 mM NaCl, and wherein the second buffer has a pH of about 8.5.

~~35.~~5. The method of any one of claims ~~20~~ 1 to ~~34~~4, wherein the third buffer comprises about 50 mM TrisHCl and about ~~50 vol%~~50% (w/w) ethylene glycol, and wherein the third buffer has a pH of about 8.5.

~~36. The method of claim 15 or claim 16, wherein the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 to about 200 mM sodium acetate and from about 0.05 to about 0.2% polysorbate 80, and wherein the first buffer has a pH from about 5.5 to about 6.5;~~

~~wherein the second wash step comprises applying to the affinity resin a second buffer comprising from about 10 to about 70 mM TrisHCl and from about 75 to about 250 mM NaCl, and wherein the second buffer has a pH from about 8.0 to about 9.0; and~~

~~wherein the third wash step comprises applying to the affinity resin a third buffer comprising from about 10 to about 70 mM TrisHCl and from about 30 to about 75 vol% ethylene glycol, and wherein the third buffer has a pH from about 8.0 to about 9.0.~~

~~37. The method of claim 36, further comprising a fourth wash step that takes place before the first wash step and comprises applying to the affinity resin a fourth buffer comprising from about 10 to about 30 mM TrisHCl and from about 75 to about 250 mM NaCl, and wherein the fourth buffer has a pH from about 6.5 to about 8.0.~~

~~38. The method of claim 36 or claim 37, wherein the first buffer comprises about 100 mM sodium acetate and about 0.1% Polysorbate 80, and wherein the first buffer has a pH of about 6.0.~~

~~39. The method of any one of claims 36 to 38, wherein the second buffer comprises about 50mM TrisHCl and about 125 mM NaCl, and wherein the second buffer has a pH of about 8.5.~~

~~40. The method of any one of claims 36 to 39, wherein the third buffer comprises about 50 mM TrisHCl and about 50 vol% ethylene glycol, and wherein the third buffer has a pH of about 8.0.~~

~~41. The method of claim 40, wherein an acidic component is removed.~~

~~42. The method of claim 41, wherein the acidic component is host cell DNA, such as HEK293 DNA, and wherein the acidic component is reduced to a value below 250 pg per microgram of AAV antigen as measured by qPCR.~~

~~43. The method of claim 41, wherein the acidic component is host cell DNA, such as HEK293 DNA, and wherein the acidic component is reduced to a value below 250 pg per microgram of AAV antigen as measured by ELISA.~~

~~44. The method of any one of claims 1 to 43, wherein eluting comprises applying a continuous linear increase of the conductivity of an elution buffer by gradient elution.~~

~~45. The method of any one of claims 1 to 44, wherein eluting comprises applying a continuous linear increase of the concentration of an organic solvent by gradient elution.~~

~~46.~~6. The method of any one of claims 1 to ~~45~~5, wherein eluting comprises contacting the affinity resin with an elution buffer comprising ethylene glycol, a salt ~~such as NaCl~~, and a buffer ~~such as TrisHCl~~, wherein the pH is at least 7.0.

~~47. The method of claim 46, wherein the salt concentration is about 750 mM, the buffer concentration is about 50 mM, and the ethylene glycol is 50-60% (w/w).~~

~~48. The method of claim 46 or claim 47, wherein the pH is about 8.0.~~

~~49.~~7. The method of ~~any one of claims 46 to 48~~claim 6, wherein ~~the~~ said salt is NaCl and/or the buffer is TrisHCl.

~~50. The method of any one of claims 1 to 49, wherein eluting comprises contacting the affinity resin with an elution buffer comprising about 750mM NaCl and 50-60% (w/w) ethylene glycol at a pH of at least 7.0.~~

~~51. The method of claim 50, wherein the elution buffer comprises at least about 55% (w/w) ethylene glycol.~~

~~52. The method of any one of claims 1 to 49, wherein eluting comprises contacting the affinity resin with an elution buffer comprising about 50 mM Tris HCl, about 750mM to about 1250 mM NaCl and about 60% (w/w) ethylene glycol at a pH of at least 7.8.~~

~~53. The method of any one of claims 1 to 49, wherein eluting comprises contacting the affinity resin with an elution buffer comprising about 2 mM magnesium chloride, about 50 mM Arginine-HCl, about 750mM to about 1000 mM NaCl and at least about 55% (w/w) sucrose at a pH of at least about 8.0.~~

~~54. The method of claim 53, wherein eluting further comprises~~

~~(a) contacting the affinity resin with a fifth buffer comprising from about 20 to about 100 mM Arginine-HCl and from about 75 to about 250 mM NaCl, and wherein the fifth buffer has a pH from about 7.5 to about 8.5; and~~

~~(b) contacting the affinity resin with a second elution buffer comprising from about 20 to about 100 mM Arginine-HCl, from about 40 to about 60% (w/w) glycerol, and from about 500 to 1000 mM salt, and wherein the second elution buffer has a pH from about 7.5 to about 8.5.~~

~~55. The method of claim 54, wherein the steps are performed in succession.~~

8. The method of claim 6 or claim 7, wherein the salt concentration is about 750 mM, the buffer concentration is about 50 mM, and the ethylene glycol from about 50-60% (w/w).

~~56.~~9. The method of any one of claims 1 to ~~49~~5, wherein eluting comprises contacting the affinity resin with an elution buffer comprising about 2 mM magnesium chloride, about 50 mM Arginine-HCl, about 750mM to about 1000 mM NaCl and at least about ~~50~~55% (w/w) ~~glycerol~~ sucrose at a pH of at least about 8.0.

~~57. The method of any one of claims 1 to 49, wherein eluting comprises contacting the affinity resin with an elution buffer comprising about 2 mM magnesium chloride, about 50 mM Taurine, about 600mM to about 1000 mM NaCl, about 0.05 to about 0.2% octylglycopyranoside, and about 60% (w/w) ethylene glycol at a pH of at least about 7.8.~~

~~58.~~10. The method of claim ~~57~~9, wherein eluting further comprises

(a) contacting the affinity resin with a ~~fifth~~ buffer comprising from about 20 mM to about 100 mM ~~Tris~~Arginine-HCl and from about 75 mM to about 250 mM NaCl, and wherein ~~the~~ a fifth buffer has a pH from about ~~8.0~~7.5 to about ~~8.8~~8.5; and

(b) contacting the affinity resin with a second elution buffer comprising from about 20 mM to about 100 mM Arginine-HCl, from about 40% (w/w) to about 60% (w/w) glycerol, and from about 500 mM to 1000 mM salt, and wherein the second elution buffer has a pH from about 7.5 to about 8.5~~contacting the affinity resin with a second elution buffer comprising about 1 M ammonium sulfate, about 50 mM Tris HCl, and about 50% (v/v) ethylene glycol at a pH of at least about 6.8~~.

~~59.~~11. The method of claim ~~58~~10, wherein the steps are performed in succession.

~~60. The method of any one of claims 1 to 49, wherein eluting comprises contacting the affinity resin with an elution buffer comprising about 1 M ammonium sulfate, about 50 mM Tris HCl, and about 50% (v/v) ethylene glycol at a pH of at least about 6.8.~~

12. The method of any one of claims 1 to 5, wherein eluting comprises contacting the affinity resin with an elution buffer comprising about 2 mM magnesium chloride, about 50 mM Arginine-HCl, about 750mM to about 1000 mM NaCl and at least about 50% (w/w) glycerol at a pH of at least about 8.0.

13. The method of any one of claims 1 to 5, wherein eluting comprises contacting the affinity resin with an elution buffer comprising about 2 mM magnesium chloride, about 50 mM Taurine, about 600mM to about 1000 mM NaCl, about 0.05 to about 0.2% octylglycopyranoside, and about 60% (w/w) ethylene glycol at a pH of at least about 7.8.

~~61.~~14. The method of any one of claims 1 to ~~49~~5, wherein eluting comprises contacting the affinity resin with an elution buffer comprising about 20% (w/w) sucrose, about 10% (w/w) sorbitol, about 5% (w/w) mannitol or about 5% (w/w) sucrose, about 15% (w/w) glycerol, about 50 mM Histidine, and about 750 mM to about 1000 mM NaCl at a pH of at least about 7.8.

~~62. The method of claim 61, wherein eluting further comprises~~

~~(a) contacting the affinity resin with a fifth buffer comprising from about 20 to about 100 mM Histidine, from about 80 to about 120 mM NaCl, and wherein the fifth buffer has a pH from about 8.0 to about 8.8; and~~

~~(b) contacting the affinity resin with a second elution buffer comprising from about 20 to about 100 mM Histidine, from about 600 to about 900 mM NaCl, and from about 5 to 60% (w/w) DMSO, and wherein the fifth buffer has a pH from about 6.5 to about 8.5.~~

~~63. The method of claim 62, wherein the steps are performed in succession.~~

~~64.~~15. The method of any one of claims 1 to ~~49~~5, wherein eluting comprises contacting the affinity resin with an elution buffer comprising about 100 mM Glycine-HCl, and about 200 mM NaCl, at a pH of about 2.5.

~~65. The method of any one of claims 50 to 63, wherein the elution buffer is at a pH of about 8.0.~~

~~66. The method of any one of claims 50 to 63, wherein the elution buffer is at a pH of 8.0.~~

~~67. The method of any one of claims 1 to 66, wherein eluting comprises a stepwise increase of a counter ion concentration.~~

~~68. The method of any one of claims 1 to 67, wherein eluting comprises a stepwise increase of an organic solvent concentration.~~

~~69. The method of any one of claims 1 to 68, wherein the salt in the elution buffer is selected from monovalent, divalent or polyvalent anions, such as chloride, acetate, sulfate, and citrate.~~

~~70. The method of claim 15 or claim 16, wherein the first wash step comprises applying to the affinity resin a first buffer comprising from about 50 to about 200 mM~~

~~sodium acetate, from about 0.05 to about 0.2% Polysorbate80, and wherein the first buffer has a pH from about 5.2 to about 6.8;~~

~~wherein the second wash step comprises applying to the affinity resin a second buffer comprising from about 25 to about 100 mM TrisHCl and from about 50 to about 200 mM NaCl, and wherein the second buffer has a pH from about 7.5 to about 9.2; and~~

~~wherein the third wash step comprises applying to the affinity resin a third buffer comprising from about 20 to about 100 mM TrisHCl and from about 75 to about 250 mM NaCl, and wherein the third buffer has a pH from about 7.5 to about 8.8.~~

~~71. The method of claim 70, further comprising elution by applying to the affinity resin purified water, followed by applying to the affinity resin from about 20 to about 50 mM HCl at a pH from about 1.7 to about 2.5.~~

~~72.~~16. The method of ~~claim 70~~any one of claims 1 to 5, further comprising elution by applying a gradient of 0 to 100% 20-50 mM ~~Hydrochloric acid~~HCl/800-1200 mM NaCl in 0.5-2.0 mM ~~Hydrochloric acid~~HCl.

17. The method of any one of claims 1 to 5, wherein eluting comprises contacting the affinity resin with an elution solution comprising about 1 mM HCl, at a pH of about 3.2.

18. The method of any one of claims 1 to 5, wherein elution comprises contacting the affinity resin with an elution buffer comprising from about 30 to about 70 mM TrisHCl, from about 700 to about 900 mM NaCl, and from 40% to 60% (w/w) DMSO, at a pH of about 7.5 to about 8.5.

19. The method of any one of claims 1 to 5, wherein eluting comprises contacting the affinity resin with an elution buffer comprising about 2M $MgCl_2$, and about 50 mM TrisHCl, at a pH of about 7.4.

20. The method of any one of claims 1 to 19, wherein

(a) the AAV is AAV8, the affinity resin is POROS™ CaptureSelect™ AAV8, and wherein the elution buffer is acidic and does not comprise ethylene glycol;

(b) the AAV is AAV9, the affinity resin is POROS™ CaptureSelect™ AAV9, and wherein the elution buffer is acidic and does not comprise ethylene glycol;

(c) the AAV is AAV8, and wherein the affinity resin is an immune affinity resin consisting of an immobilized monoclonal antibody against AAV8 from type ADK8 or ADK8/9 immobilized on a chromatography matrix; or

(d) the AAV is AAV9, and wherein the affinity resin is an immune affinity resin consisting of an immobilized monoclonal antibody against AAV9 from type ADK9 or ADK8/9 immobilized on a chromatography matrix.

~~73. The method of any one of claims 1 to 72, wherein the AAV obtained from the eluting step has an HC impurity level of ≤ 99.9 %.~~

~~74. The method of any one of claims 1 to 73, wherein the AAV obtained from the eluting step has an HC impurity level of ≤ 99.0 %.~~

~~75. The method of any one of claims 1 to 74, wherein the AAV is AAV8, the affinity resin is POROS™ CaptureSelect™ AAV8, and wherein the elution buffer is acidic and does not comprise ethylene glycol.~~

~~76. The method of any one of claims 1 to 74, wherein the AAV is AAV9, the affinity resin is POROS™ CaptureSelect™ AAV9, and wherein the elution buffer is acidic and does not comprise ethylene glycol.~~

~~77. The method of any one of claims 1 to 76, wherein the AAV is AAV8, and wherein the affinity resin is an immune affinity resin consisting of an immobilized monoclonal antibody against AAV8 from type ADK8 or ADK8/9 immobilized on a chromatography matrix.~~

~~78. The method of any one of claims 1 to 76, wherein the AAV is AAV9, and wherein the affinity resin is an immune affinity resin consisting of an immobilized monoclonal antibody against AAV9 from type ADK9 or ADK8/9 immobilized on a chromatography matrix.~~

~~79. The method of any one of claims 1 to 78, wherein the method further comprises contacting the AAV containing solution with a filter comprising positively charged groups effective to deplete acidic charged contaminants from the AAV containing solution.~~

~~80. The method of any one of claims 1 to 79, further comprising nanofiltration of an AAV fraction to remove viruses greater than 35 nm.~~

~~81. The method of any one of claims 1 to 80, further comprising a polish step comprising performing AEX chromatography with a column comprising tentacle gel.~~

~~82. The method of any one of claims 1 to 81, further comprising testing an AAV fraction via an AAV-specific ELISA.~~

~~83. The method of claim 82, wherein the AAV specific ELISA is a sandwich ELISA specific for AAV.~~

~~84. An AAV product produced by a method according to any one of claims 1 to 83.~~