

CLAIMS

1. A method of purifying a recombinant adeno-associated viral (rAAV) vector by anion exchange chromatography (AEX), comprising:
 - i) diluting a solution comprising a rAAV vector to be purified with a dilution solution comprising a buffering agent, an amino acid, and a detergent, such that the rAAV vector binds to an AEX stationary phase;
 - ii) loading the diluted solution onto said AEX stationary phase under conditions sufficient for rAAV vector in said diluted solution to bind to said AEX stationary phase; and
 - iii) eluting the rAAV vector from said AEX stationary phase with an elution solution that increases in conductivity as elution proceeds.
2. The method of claim 1, wherein said solution comprising a rAAV vector to be purified is an affinity eluate.
3. The method of claim 1 or claim 2, wherein said solution comprising a rAAV vector to be purified is diluted at least 2-fold.
4. The method of any one of claims 1 to 3, wherein said dilution solution has pH 8 to 10, and comprises 10 mM to 500 mM of said buffering agent, 10 mM to 600 mM of said amino acid, and 0.005% to 1.5% of said detergent.
5. The method of any one of claims 1 to 4, wherein said buffering agent is Tris, BIS-Tris propane, diethanolamine, diethylamine, tricine, triethanolamine, or bicine.
6. The method of any one of claims 1 to 5, wherein said amino acid is histidine, arginine, glycine, or citrulline.
7. The method of any one of claims 1 to 6, wherein said detergent is poloxamer 188 (P188), Triton X-100, polysorbate 80 (PS80), Brij-35, or nonyl-phenoxypolyethoxylethanol (NP-40).
8. The method of any one of claims 1 to 7, wherein after the step of diluting, the pH and conductivity of said diluted solution is higher and lower, respectively,

compared to the pH and conductivity of said solution comprising a rAAV vector to be purified.

9. The method of any one of claims 1 to 8, wherein after the step of diluting, the pH of said diluted solution is 8.5 to 9.5
10. The method of any one of claims 1 to 9, wherein after the step of diluting, the conductivity of said diluted solution is 1.7 to 3.5 mS/cm.
11. The method of any one of claims 1 to 10, wherein said elution solution comprises a salt.
12. The method of claim 11, wherein said salt is sodium chloride, sodium acetate, or ammonium acetate.
13. The method of claim 11 or 12, wherein at the beginning of said step of eluting, said elution solution comprises 0 mM to 100 mM of said salt, and at the end of said step of eluting, said elution solution comprises 100 mM to 1 M of said salt.
14. The method of any one of claims 11 to 13, wherein at the beginning of said step of eluting, the conductivity of said elution solution is between 1.0 mS/cm and 2.5 mS/cm, and at the end of said step of eluting, the conductivity of said elution solution is between 20 mS/cm and 35 mS/cm.
15. The method of any one of claims 1 to 14, wherein said dilution solution has pH of about 8.5 to 9.5 and comprises about 100 mM to 300 mM Tris, about 100 mM to 300 mM histidine, and about 0.1% to 1.0% P188, and wherein said elution solution comprises sodium acetate.
16. The method of any one of claims 1 to 15, further comprising measuring the UV light absorbance of the eluate at 260 nm and 280 nm and collecting a fraction of said eluate when the A260/A280 ratio is at least 1.25.
17. The method of claim 16, wherein the proportion of full capsids in said eluate fraction is at least 50%.

18. The method of any one of claims 1 to 17, wherein said stationary phase is polystyrenedivinylbenzene particle with covalently bound quaternized polyethyleneimine disposed in a column.
19. The method of claim 18, wherein the concentration of rAAV vector in said diluted solution is 2.6×10^{12} to 6.8×10^{13} VG/mL of column volume.
20. The method of any one of claims 1 to 19, wherein said rAAV vector comprises an AAV capsid selected from the group consisting of AAV1, AAV2, AAV3, AAV3A, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAVrh10, AAVrh74, AAV12, AAV2i8, NP4, NP22, NP66, AAVDJ, AAVDJ/8, AAVDJ/9, AAVLK03, AAV1.1, AAV2.5, AAV6.1, AAV6.3.1, AAV9.45, RHM4-1 (SEQ ID NO:5 of WO 2015/013313), RHM15-1, RHM15-2, RHM15-3/RHM15-5, RHM15-4, RHM15-6, AAVhu.26, AAV1.1, AAV2.5, AAV6.1, AAV6.3.1, AAV9.45, AAV2i8, AAV29G, AAV2,8G9, AAV-LK03, AAV2-TT, AAV2-TT-S312N, AAV3B-S312N, AAVHSC1, AAVHSC2, AAVHSC3, AAVHSC4, AAVHSC5, AAVHSC6, AAVHSC7, AAVHSC8, AAVHSC9, AAVHSC10, AAVHSC11, AAVHSC12, AAVHSC13, AAVHSC14, and AAVHSC15.