AAV•Tek™

AEX Buffer Screening Kit Protocol

Our **AAV•Tek™ AEX Buffer Screening Kits** contain discrete buffer formulations designed to help you quickly identify the robust set needed for the full capsid enrichment of your AAV construct. Each serotype-specific kit contains paired sets of optimized equilibration and elution buffers in 1 L bottles. The buffers are intended to be applied in designated pairs (e.g.,1A + 1B, 2A + 2B) for AEX screening purposes. Once the optimal set has been identified, our team can help make further customizations as needed and assist with GMP-compliant manufacturing for scale up.

Sample Preparation

Prior to preparation, determine the appropriate AAV volume according to column binding capacity.

- Under/overloading the column can result in low recovery, irregular elution, and/or poor separation.

Allow the viral sample to equilibrate to room temperature. Dilute the viral sample with AEX Equilibration Buffer A (e.g., AEX Buffer 1A, 11A, 20A) until conductivity is below 5.50 mS/cm. Depending on incoming sample conditions, this may require a dilution factor of 17x to 20x or possibly more. After dilution, adjust pH to match the equilibration buffer (±0.10) by using hydrochloric acid/acetic acid and/or sodium hydroxide/Tris base.

- Adjusting the conductivity and pH improves overall recovery by decreasing the chance of early sample elution.
- Depending on the nature of the capture step, the amount of equilibration buffer required to dilute the sample for AEX load preparation can vary greatly. Affinity-captured AAV eluate may have a lower conductivity and elute over a narrower range, as compared to other capture step eluates of AAV, thereby requiring less dilution. Whereas the conductivity of CEX captured AAV eluate can vary due to the various binding interactions that different AAV serotypes exhibit.

Method

Set the multi-wavelength UV detector to measure absorbance at wavelengths (λ) of 280 nm and 260 nm during the run. The relative A280/A260 ratio of the AAV particles is used during analysis to indicate the full and empty AAV capsids. For more detail, see the example protocol displayed in Table 1 on the next page.

Column Performance (Optional):

Attach the column to your chromatography system and wash it with water to remove the storage solution. Next, prime the column with the equilibration buffer (#A), inject the tracer (e.g., 1 M NaCl), and then chase with the same equilibration buffer. Measure the asymmetry and HETP to evaluate the column performance.

Equilibration:

Equilibrate column by passing buffer A, the paired buffer B, and then buffer A again. Allow the column to reach pH and conductivity stability (i.e., signal plateau) respective of the buffer attributes at each of the three steps.

Sample Application:

Apply the sample. Wash column with buffer A until absorbances reach baseline or plateau, ensuring any loosely bound material is fully passed through. At a decreased flow rate, elute AAV using a linear gradient from 0% to 50% of buffer B over 50 column volumes. Collect eluate into fixed volume fractions.

- Smaller fraction volumes will allow a more precise collection of the “full” AAV samples.

Strip column with 100% of buffer B to remove more tightly bound material. For further column cleaning and/or regeneration, follow recommended protocols for your column.

Column Regeneration:

Wash column with water and follow with a CIP solution, water, 1 M Ammonium Acetate to neutralize column pH, water, and then re-equilibrate with buffer A. The column can then either be stored in vendor recommended storage buffer or solution (e.g., 20% Ethanol), or the process can be restarted from column performance if additional purification of sample(s) is needed.

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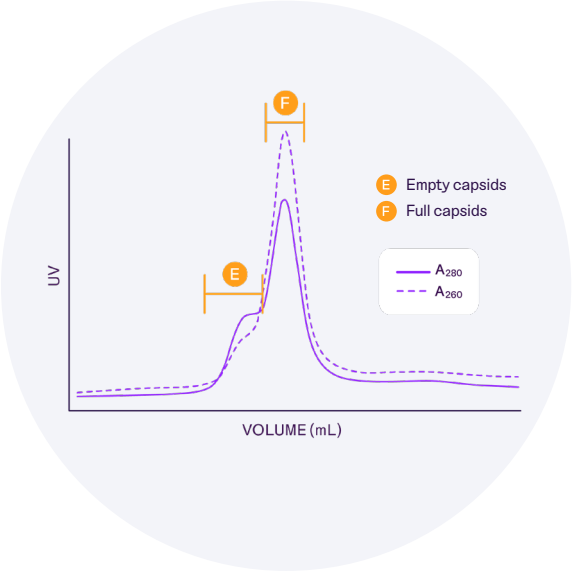
Sample Collection

For optimal enrichment, collect the full AAV particles based on the inversion/inflection of the UV 260 and UV 280 signals, as demonstrated below. Store your samples at 4°C–8°C for temporary storage, or -80°C for long-term storage.



Example Method

Method Phase	Buffer(s)	%B	Column Volumes (CV)
Column Wash	Water	-	10
Equilibration (1)	Buffer A	0%	10
Equilibration (2)	Buffer B	100%	10
Equilibration (3)	Buffer A	0%	15
Sample Application	AAV diluted in Buffer A	0%	-
Column Wash	Buffer A	0%	15
Elution*	Buffer A, Buffer B	0-50%	50
Strip	Buffer B	100%	10
Column Wash	Water	-	10
Clean-in-Place (CIP)	2 M NaCl, 1 M NaOH	-	10
Column Wash	Water	-	10
Neutralization Solution for Column Regeneration	1 M Ammonium Acetate	-	10
Column Wash	Water	-	10

Table 1. The information above is the recommended starting point for small (e.g., 1 mL CV) preparatory columns. More turbid samples may require an extended column wash to fully stabilize after sample application. Additionally, the optimal length of each method phase will vary according to resin type and size.

* **Note:** when purifying AAV6 with buffer AEX18, an extended linear elution gradient is recommended. For example, 0 - 75% B over 75 CV.

Step elution, isocratic hold, and scale-up

Once you've used the AEX Buffer Screening Kit to select the buffer that best fits your specific application, consult with our team to refine the formulation, and make further customizations as needed. Whether you need additional buffers to perform step elution or isocratic hold, or you are ready to scale up with GMP-compliant manufacturing, we're here to help.

Questions? Get in touch with us at research@teknova.com or call 1.800.209.4488.

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