

Product Instruction Manual & AAV DSP Scalability Guide

PurProX™ AAVEasy Rapid Purification Spin Column U2

Cat No	Product Name	Serotype Compatibility
GMV-PurProX-AAVEasy-U-2-10T	PurProX™ AAVEasy rapid-to-purify Spin Column-2	AAV9, and Serotypes developed based on AAV9, such as the AAV-PHP series.

Product Overview: The GM-PurProX™ DSP Solution

PurProX™ is GeneMedi's proprietary, end-to-end modular system for Adeno-Associated Virus (AAV) Downstream Processing (DSP). Engineered to bridge the "Process Gap" between benchtop discovery and GMP manufacturing, PurProX™ operates on the core philosophy of "Unified Chemistry & Linear Scalability".

The PurProX™ AAVEasy Spin Column redefines laboratory-scale purification through a unique Embedded Column Design compatible with standard 50mL centrifuge tubes. This allows researchers to bypass tedious ultracentrifugation and complete purification in <30 minutes using a standard benchtop centrifuge.

Serotype Specificity (U1 vs. U2):

AAVEasy-U2: Equipped with a ligand for high-efficiency binding of AAV9 and its variants.

(Note: For other multiple AAV serotypes, we recommend the AAVEasy-U1 equipped with a broad-spectrum ligand for high-efficiency binding of AAV2, AAV5, AAV6, AAV8, AAV-DJ, and AAV-7m8).

Kit Contents

Each kit contains materials for 10 purifications:

AAV Purification Kit Components



Resin Tubes (10)

Pre-filled with universal AAV-binding resin.



Lower Plugs (10)

For sealing the column during storage.



Sample Tubes (10)

12 mL maximum capacity.



1000x GMXbuffer01 (5 mL)

Concentrated buffer additive.

Buffer Preparation

Buffer Preparation Note: All buffers must be filtered through a 0.22 μ m filter before use. Perform the entire purification process at room temperature

Component	Target Volume	Required pH	Preparation Instructions
Binding buffer	500	7.4	Mix 500 mL of 1X PBS (pH 7.4) with 10.5 g NaCl and 500 μ L 1000x GMX Buffer 01.
Elution buffer	100	2.0	Mix 90 mL ddH ₂ O, 0.75 g glycine, 500 μ L 0.2 M MgCl ₂ , and 100 μ L 1000x GMX Buffer 01. Adjust to pH 2.0 with anhydrous HCl; bring to 100 mL with ddH ₂ O.
Neutralization buffer	100	8.8	Mix 90 mL ddH ₂ O, 12.1 g Tris, and 100 μ L 1000x GMX Buffer 01. Adjust to pH 8.8 with anhydrous HCl; bring to 100 mL with ddH ₂ O.

Component	Target Volume	Required pH	Preparation Instructions
Regeneration buffer	500	N/A	Dissolve 286.6 g Guanidine hydrochloride in 250 mL ddH ₂ O using a 37°C water bath. Bring to 500 mL with ddH ₂ O.

Note :

Perform the entire purification process at room temperature.

Do not tighten the cap of the purification tube.

Do not pour liquid directly out of the purification tube to avoid losing the resin.

AAVEasy-U2: yields maximum of 4.28E+13 capsids.

Use a 10 mL sterile syringe to gently stir the mixture 5-10 times.

Protocol

Step A. Sample Preparation

Centrifugation: Centrifuge the AAV crude extract (cell lysate after Benzonase treatment) at 8,000 x g for 10 minutes at room temperature.

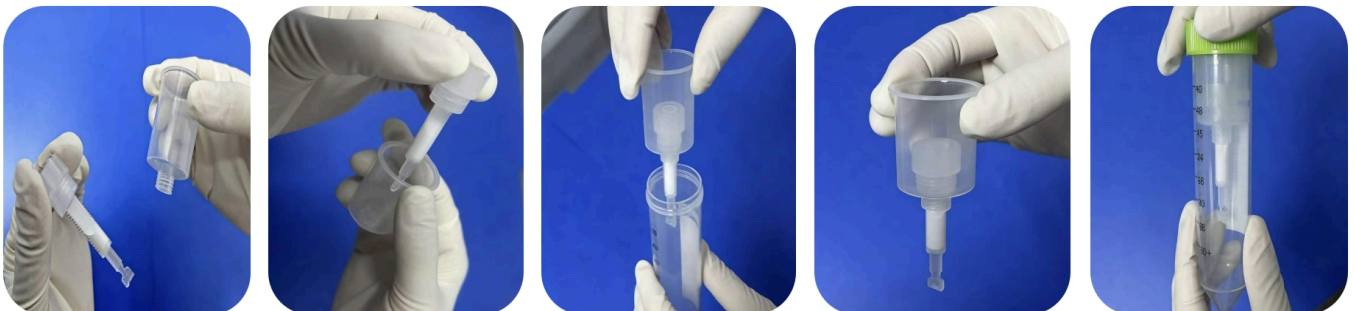
Filtration: Collect the AAV supernatant and filter it through a 0.45 µm filter.

Dilution: Add Binding Buffer to the filtered sample to reach a final volume of 10 mL.

Note: Ensure total AAV titer does not exceed 1.0E+14 capsids/mL before purification.

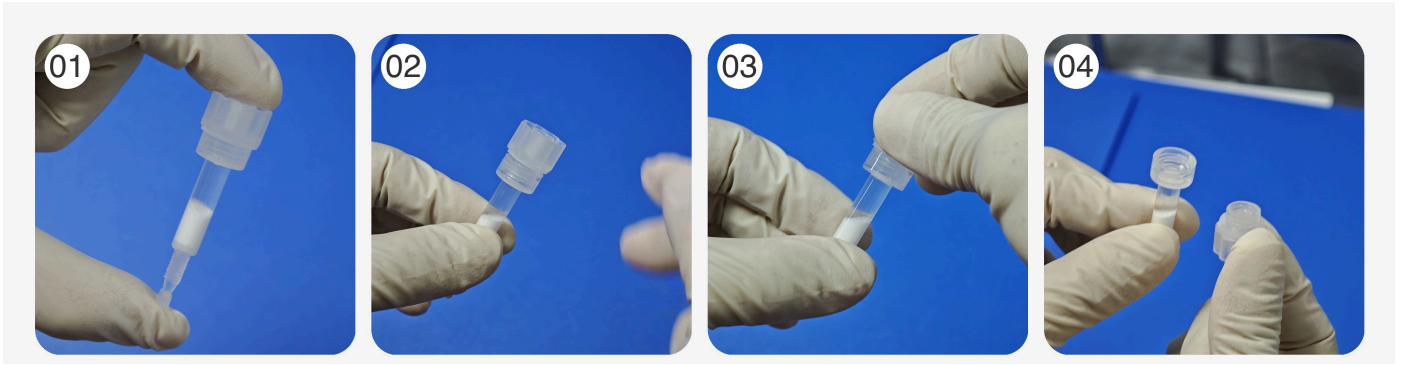
Step B: Column Assembly

Place the unopened resin tube into a sample tube and centrifuge at 300 x g for 3 minutes.

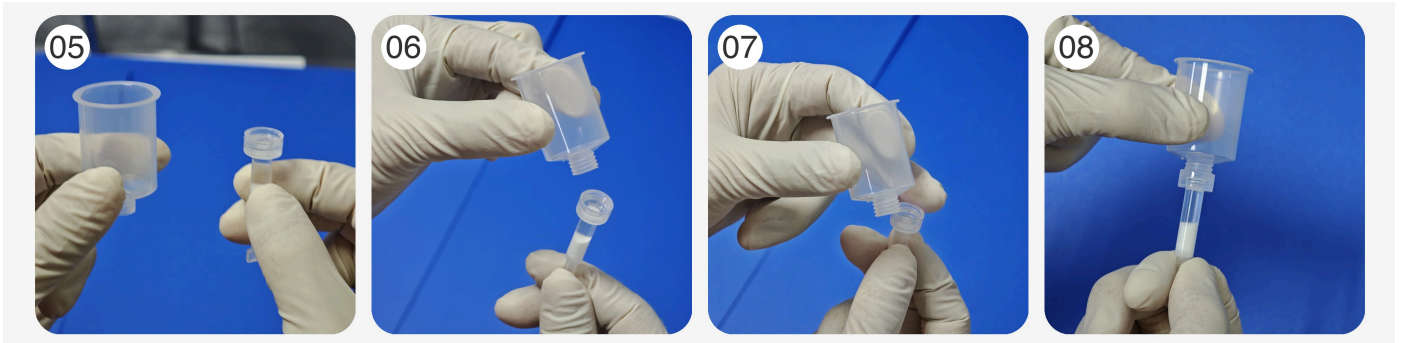


Note: Do not fully tighten the cap of the purification tube.

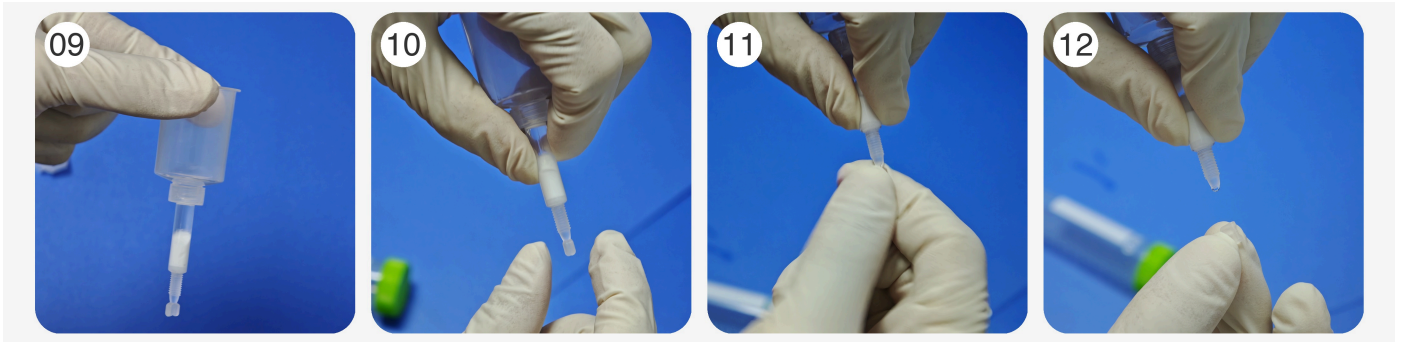
Remove the upper cover of the resin tube.



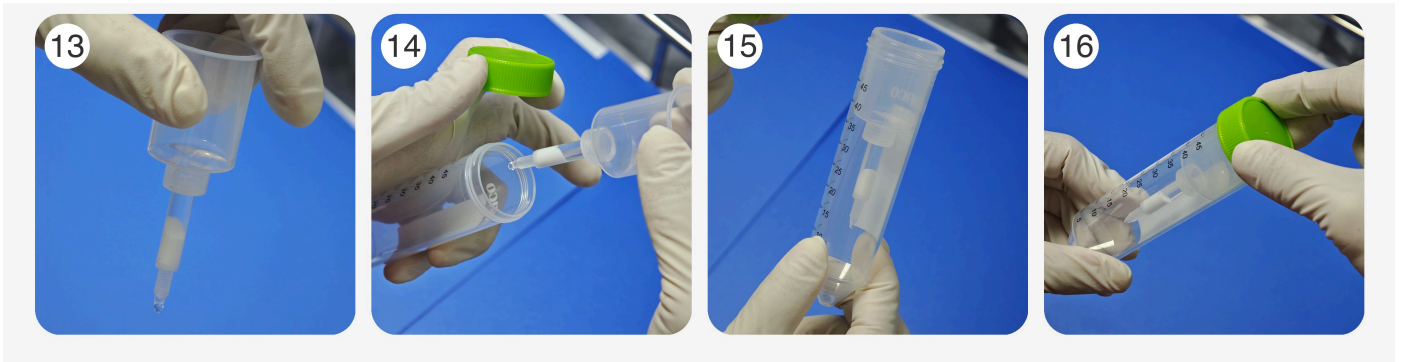
Securely attach the resin tube to the sample tube.



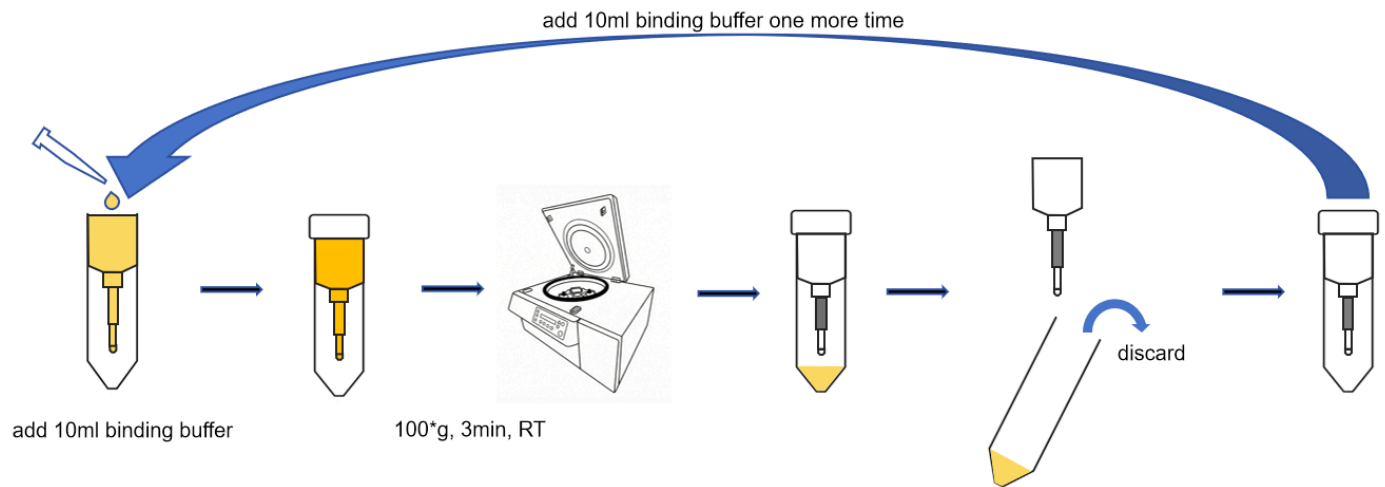
Snap off the plastic tail at the bottom of the resin tube.



Prepare the purification tube



Step C: Equilibration



Add 10 mL of Binding Buffer to the column.

Centrifuge at 100×g for 3 minutes.

Repeat this step once.

Note: If liquid remains in the column, centrifuge again at 100 × g for 1 minute

Step D: Sample loading & Binding

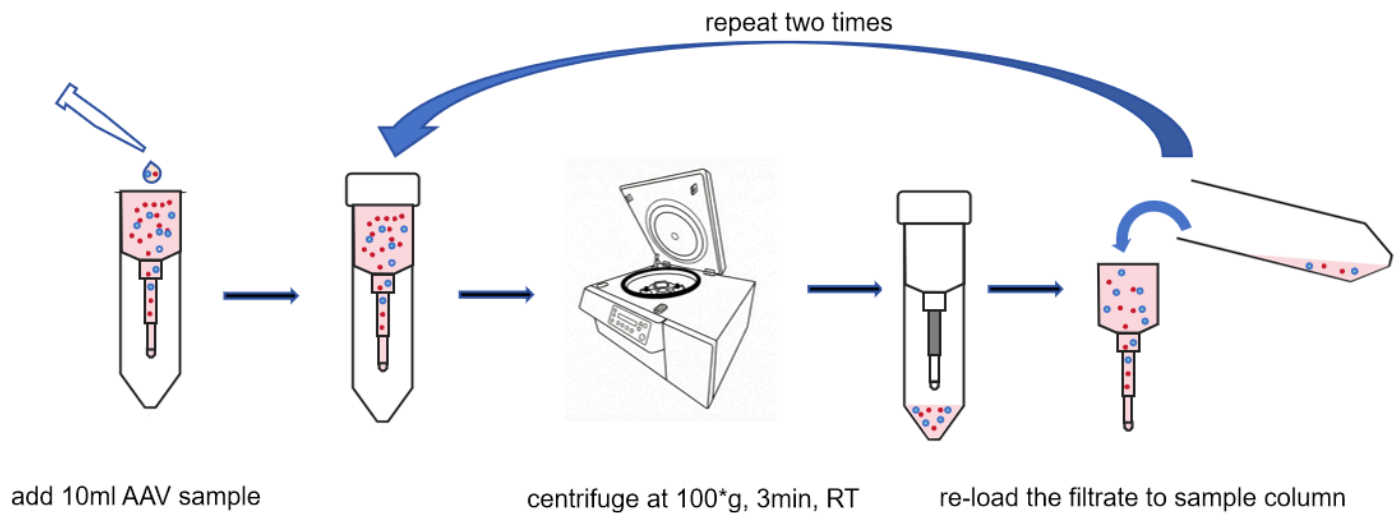
Gently add the 10 mL prepared AAV sample to the column.

Centrifuge at 100×g for 3 minutes.

Re-binding: Collect the flow-through from the sample tube and reload it onto the column to maximize binding.

Centrifuge again at 100 × g for 3 minutes. Ensure all liquid has passed through.

Repeat step 3 and step 4.

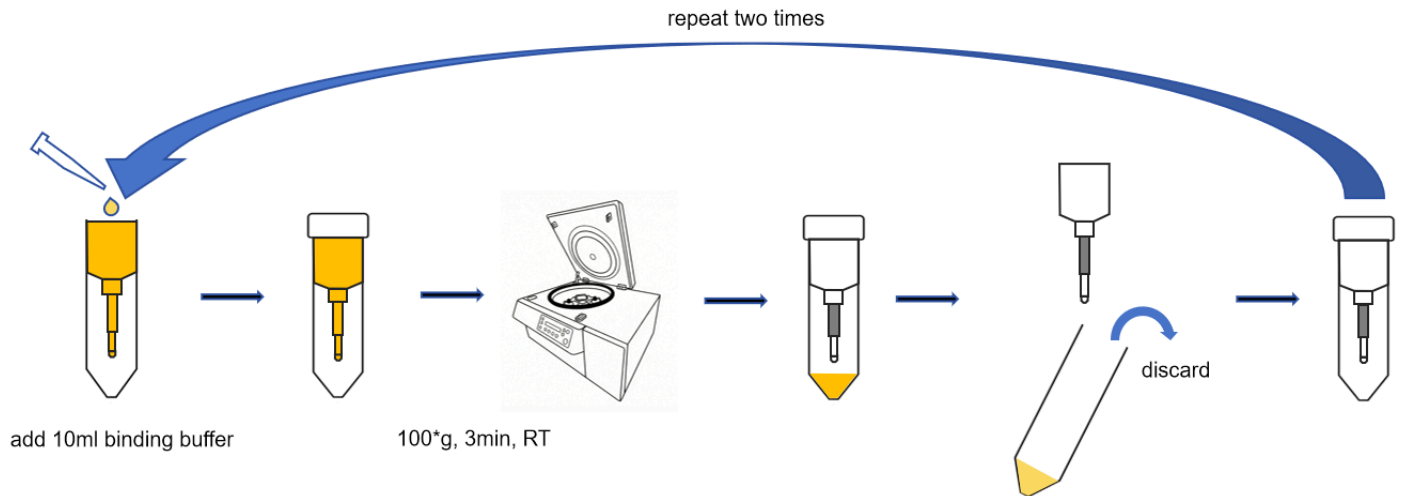


Step E: Washing

Gently add 10 mL of Binding Buffer to the column.

Centrifuge at 100×g for 3 minutes.

Repeat this wash step twice



Step F: Elution

Plan 1

After the final wash, centrifuge the purification column at 200 × g for 1 minute to remove residual buffer.

Transfer the resin tube to a new centrifuge tube pre-filled with ~80 µL of Neutralization Buffer.

Slowly add 0.5 mL of Elution Buffer to the inner wall of the sample tube to wash all resin down to resin tube.

Agitation: Immediately use a sterile 10 mL syringe needle to gently stir the resin 5–10 times.

Centrifuge at 100 × g for 1 minute.

Add another 0.5 mL of Elution Buffer, stir 5–10 times with the needle, and centrifuge again at 100 × g for 1 minute.

After the final elute, centrifuge the purification column at 200 × g for 1 minute to remove residual Elution Buffer.

Quickly mix the elution, and transfer to a 1.5 mL centrifuge tube

Plan 2 (High Recovery Elution, requires buffer exchange, to be used with ultrafiltration products)

After the final wash, centrifuge the purification column at 200× g for 1 minute to remove residual buffer.

Transfer the resin tube to a new centrifuge tube pre-filled with 400 µL of Neutralization Buffer.

Slowly add 0.5 mL of Elution Buffer to the inner wall of the sample tube to wash all resin down to resin tube.

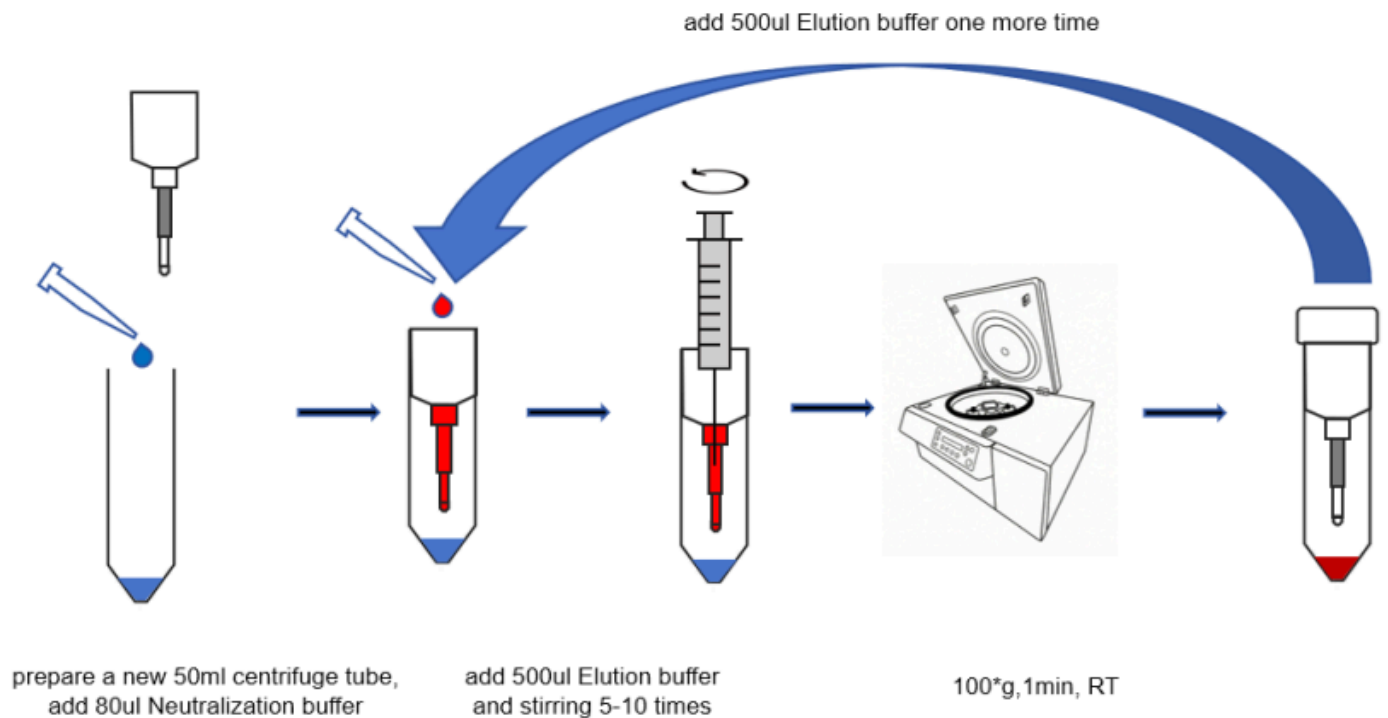
Agitation: Immediately use a sterile 10 mL syringe needle to gently stir the resin 5–10 times.

Centrifuge at 100 × g for 1 minute.

Add another 0.5 mL of Elution Buffer, stir 5–10 times with the needle, and centrifuge again at 100 × g for 1 minute.

After the final elute, centrifuge the purification column at 200 × g for 1 minute to remove residual Elution Buffer.

Quickly mix the elution, and transfer to a 2 mL centrifuge tube.



Note: After adding the Neutralization Buffer (1M Tris pH 8.8), use pH test paper to verify that the final pH is neutral. If it is not neutral, adjust the pH as needed using additional 1M Tris (pH 8.8) or Elution Buffer.

Step G: Resin Regeneration

add 5ml guanidine hydrochloride one more time, no incubation, centrifuge directly

To ensure the longevity and effectiveness of the resin, follow these steps for regeneration:

Initial Wash: Gently add 5 mL of 6 M Guanidine Hydrochloride (Regeneration Buffer) to the column.

Stirring: Use a sterile 10 mL syringe needle to gently stir the resin 5 to 10 times.

Note: Inadequate stirring may result in incomplete regeneration and reduce the resin's lifespan.

Centrifugation: Centrifuge at 100 x g for 1 minute and discard the flow-through.

Incubation: Add another 5 mL of 6 M Guanidine Hydrochloride and stir 5 to 10 times with the needle.

Gravity Drip: Allow the buffer to drip through the resin by gravity for 15 minutes.

Final Centrifugation: Centrifuge at 100 x g for 2 minutes and discard the flow-through.

Re-equilibration: Gently add 10 mL of Binding Buffer and stir the resin 5 to 10 times with the needle. Centrifuge at 100 x g for 3 minutes and discard the flow-through.

Repeat: Repeat the re-equilibration step (Step 7) twice more to ensure all regeneration buffer is removed.

Step H: Resin Tube Storage

Proper storage is essential to maintain resin stability between uses:

Ethanol Wash: Gently add 5 mL of 18% ethanol to the purification column.

Centrifugation: Centrifuge at 100 x g for 1 minute and discard the flow-through.

Repeat Wash: Repeat the ethanol wash and centrifugation once more.

Sealing: After use and cleaning, ensure the internal resin tube is filled with 18% ethanol, gently stirred to remove air bubbles, and that the lower plug is tightly secured to prevent leakage or evaporation. Add 10 mL of 18% ethanol to the sample tube, then place it into a new 50 mL centrifuge tube containing 25 mL of 18% ethanol.

Final Storage: Securely tighten the lower plug to seal the column.

Conditions: Store the column at 4°C.

Note: Always ensure the resin is completely submerged in 18% ethanol before long-term storage.

Resin Tube Storage and Stability

To maintain the integrity and binding capacity of the AAV-binding resin, please adhere to the following storage guidelines:

Storage Temperature: Store the resin tubes at 2–8°C . Do not freeze.

Storage Medium: Ensure the resin is completely submerged in 18% ethanol to prevent dehydration and maintain sterility.

Sealing: After use and cleaning, ensure the internal resin tube is filled with 18% ethanol, stirred to remove air bubbles, and the lower plug is tightly secured to prevent leakage or evaporation.

Shelf Life: The product has a guaranteed shelf life of at least 6 months when stored under the recommended conditions.

Note: 6-month stability testing has been successfully completed; long-term stability evaluation is currently ongoing.

Process Optimization & Troubleshooting

1. Capacity, Yield, and the "Impossible Triangle"

To set accurate expectations for your AAVEasy run, it is crucial to understand the relationship between input load and recovery rate:

Maximum Capacity Mode

The U2 column capacity is $4.28E+13$ capsids. Pushing the column to its limits with highly concentrated crude lysates will generate massive absolute yields, but the percentage recovery will naturally decrease as the resin reaches saturation.

Seamless Scale-Up

If your process requires larger volumes or maximum recovery at high titers, seamlessly transition to our PurProX™ AAVPure Pre-packed Columns (for FPLC/ÅKTA screening) or bulk AAVPure Resins. The chemistry is identical; only the format changes.

2. Critical Pre-Purification QC: VP vs. VG

CRITICAL NOTE:

Always quantify your AAV titer before and after purification using Viral Particle (VP) assays, NOT Vector Genome (VG) assays like qPCR or ddPCR.

The Science

PurProX™ ligands are computationally designed via our GM-LIBRA™ AI-Driven Platform to specifically target and capture the topological domains of the intact AAV structural shell (capsid), regardless of its genetic payload.

The VG Pitfall

Crude lysates contain vast amounts of "empty" capsids. qPCR/ddPCR only measures the DNA payload (VG). Relying on VG to calculate affinity recovery leads to severe miscalculations and underreports the resin's true binding efficiency.

The Standardized Solution

We mandate using the TrueX™ - ONE Universal AAV Titration Kit (ELISA) or the TrueX™ - UNIQ Serotype-Specific Kit to establish accurate VP baselines and objectively measure your recovery rate.

3. Downstream Polishing: Solving the Full/Empty Challenge

Affinity purification isolates all intact capsids. To achieve clinical-grade full-capsid ratios without the 20+ hour bottleneck of iodixanol ultracentrifugation, incorporate the PurProX™ AAVFull Enrichment Filter into your workflow.

This polishing specialist simulates industrial Anion Exchange (AEX) chromatography principles.

Simply pass your AAVEasy eluent through the AAVFull filter. It exploits subtle charge differences to effectively remove empty capsids and residual host cell impurities (HCP/HCD), dramatically enriching your percentage of full capsids.

4. Important Disclaimer on Process Variability

AAV production is a highly complex biological process. Variations in serotype biology, packaging plasmids, host cell lines, transfection efficiency, and upstream metabolic conditions all fundamentally impact the specific productivity, full/empty ratio, and impurity profile of your crude lysate.

While the GeneMedi scientific team has rigorously validated the PurProX™ AAVEasy system across a vast array of common scenarios to ensure broad compatibility, it is impossible to account for every bespoke upstream variable. Differences in individual lab protocols may lead to variations in final recovery and purity. We are deeply committed to understanding your specific application scenarios; if you encounter unexpected results, we encourage you to optimize your upstream lysis conditions and rigorously rely on TrueX™ VP Titration to locate the bottleneck. Our technical support team is always available to collaborate on continuous process optimization.

5. The GeneMedi Upstream Ecosystem

Your downstream purity is heavily dictated by upstream processing. For optimal AAVEasy performance, we highly recommend standardizing your upstream CMC process:

Recommend Products

Plasmids

Source sequence-verified constructs from the TarMart AAV Premade Library. For scalable manufacturing, utilize the GeneMedi INDKan™ Industry-Used AAV Vector System.

Note: Industrial CMC standards favor Kanamycin resistance over Ampicillin to avoid beta-lactam regulatory risks

Transfection

Maximize packaging titers in HEK293T suspension or adherent cells using our Lipogene™ Transfection Reagent.

Lysis Tip

While freeze-thaw cycles are standard, industrial users often utilize gentle detergent lysis (e.g., 0.5% Triton X-100 or NP-40) to minimize shear stress on capsids. We encourage empirical optimization to find what best suits your construct.