

Instruction Manual

PurProX AAVEasy rapid to purify Spin Column-U2

PurProX[®] AAVEasy Rapid Purification Spin Column U2

Product Instruction Manual & AAV DSP Scalability Guide

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". At the heart of this technology, the ~50 µm custom pore structures of POROS beads maximize mass transfer and binding capacity, ensuring high performance and efficiency even at high flow rates.

The PurProX[®] AAVEasy Spin Column redefines laboratory-scale purification through a unique Embedded Column Design that is fully compatible with standard 50mL centrifuge tubes. This allows researchers to bypass expensive FPLC systems or tedious, time-consuming ultracentrifugation and complete purification in <30 minutes using a standard benchtop centrifuge.

By enabling complete AAV purification in under 30 minutes, the spin column easily facilitates high-throughput processing, making it highly advantageous for applications like AAV capsid engineering and vector screening. It is an accessible, highly efficient solution perfectly suited for academic laboratories, R&D departments, biotech startups, and any facility looking to streamline their viral vector workflows without heavy equipment investments.

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.



**1000× GMXbuffer01
(5 mL)**
Concentrated buffer
additive.

Buffer Preparation

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 1000 $\times$ 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 1000 $\times$ 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 1000 $\times$ GMX Buffer 01. Adjust to pH 8.8 with anhydrous HCl; bring to 100 mL with ddH ₂ O.
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 :

1. All buffers must be filtered through a 0.22 μ m filter before use.
2. Perform the entire purification process at room temperature.
3. Do not tighten the cap of the purification tube.
4. Do not pour liquid directly out of the purification tube to avoid resin loss.
5. AAVEasy-U2: yields maximum of 4.28E+13 capsids.
6. When instructed to stir, use a 10 mL sterile syringe needle to gently stir the mixture 5-10 times.

Protocol

Step A. Sample Preparation

1. **Centrifugation:** Add 1000x GMXBuffer01 to the AAV crude extract (cell lysate after Benzonase treatment) at a ratio of 1:1000 (v/v). Then, centrifuge at 8,000 x g for 10 minutes at room temperature.
2. **Filtration:** Collect the AAV supernatant and filter it through a 0.45 μ m filter.
3. **Dilution:** Add Binding Buffer to the filtered sample to reach a final volume of 10 mL. (The sample will be used in Step D.)

Note: 1. Ensure total AAV does not exceed 1.0E+14 capsids before purification.

2. If the volume is insufficient, dilute to 10 mL with Binding Buffer before filtration.

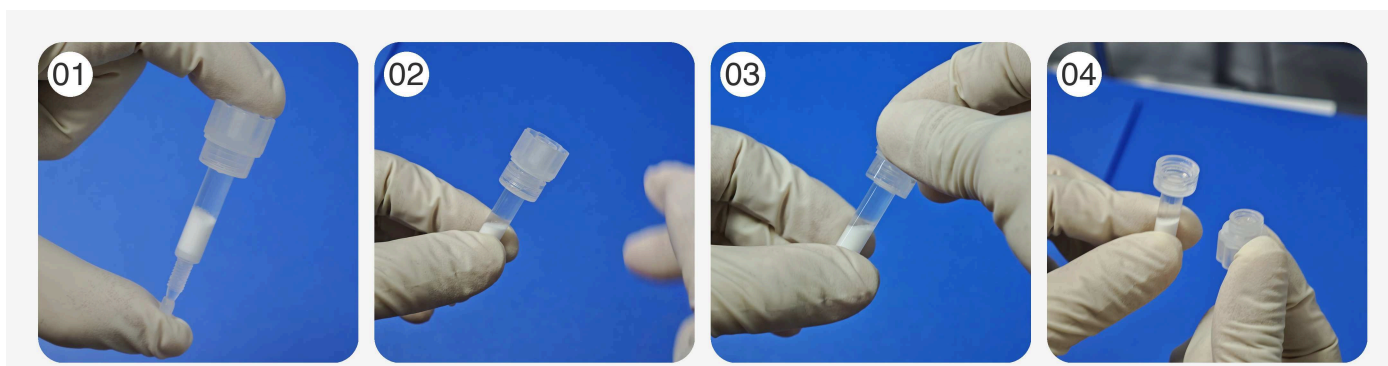
Step B: Column Assembly

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

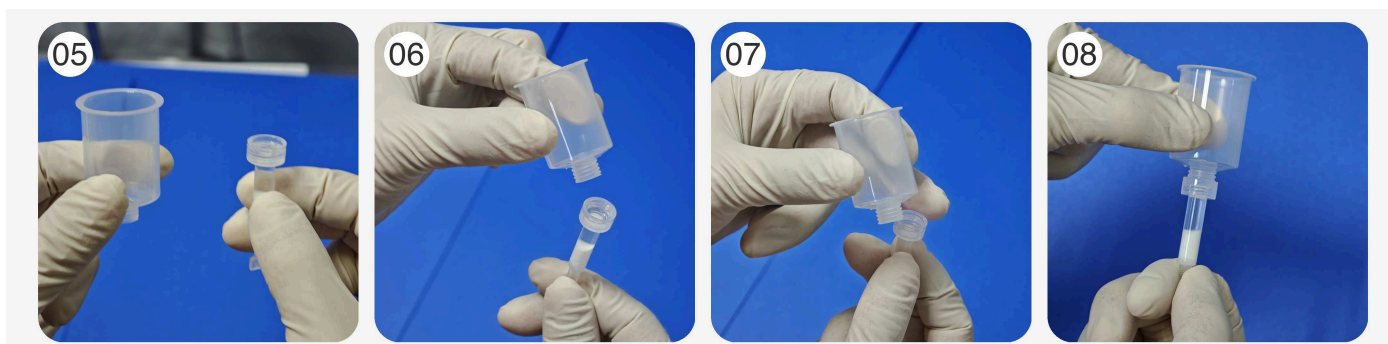


Note: Do not fully tighten the cap of the purification tube.

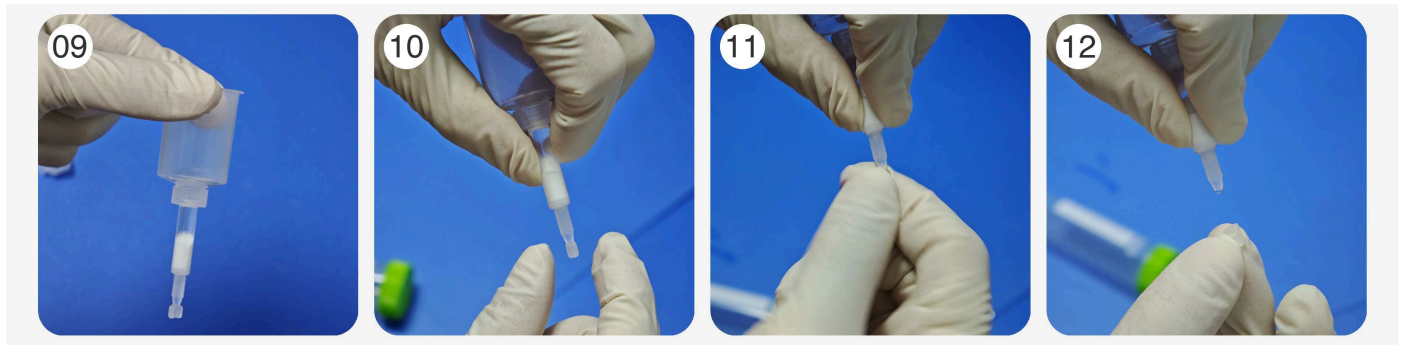
2. Remove the upper cover of the resin tube.



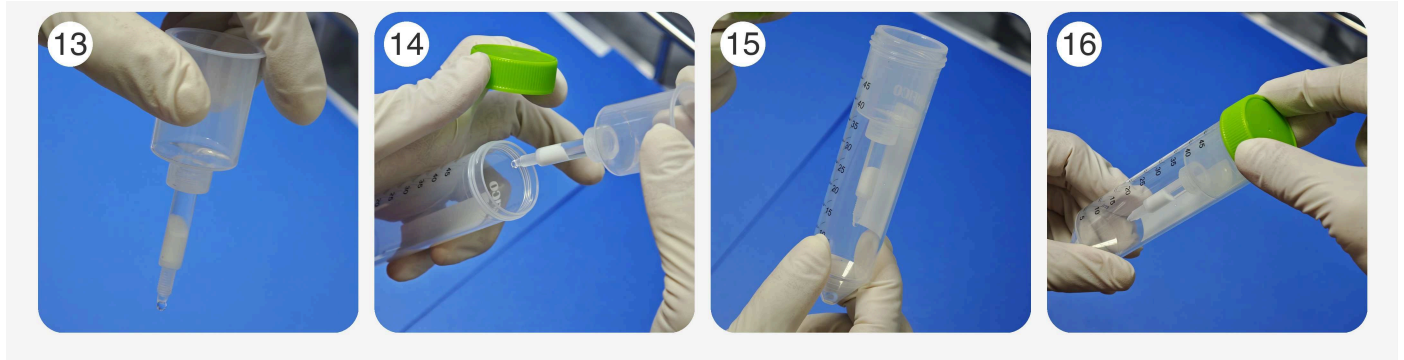
3. Securely attach the resin tube to the sample tube.



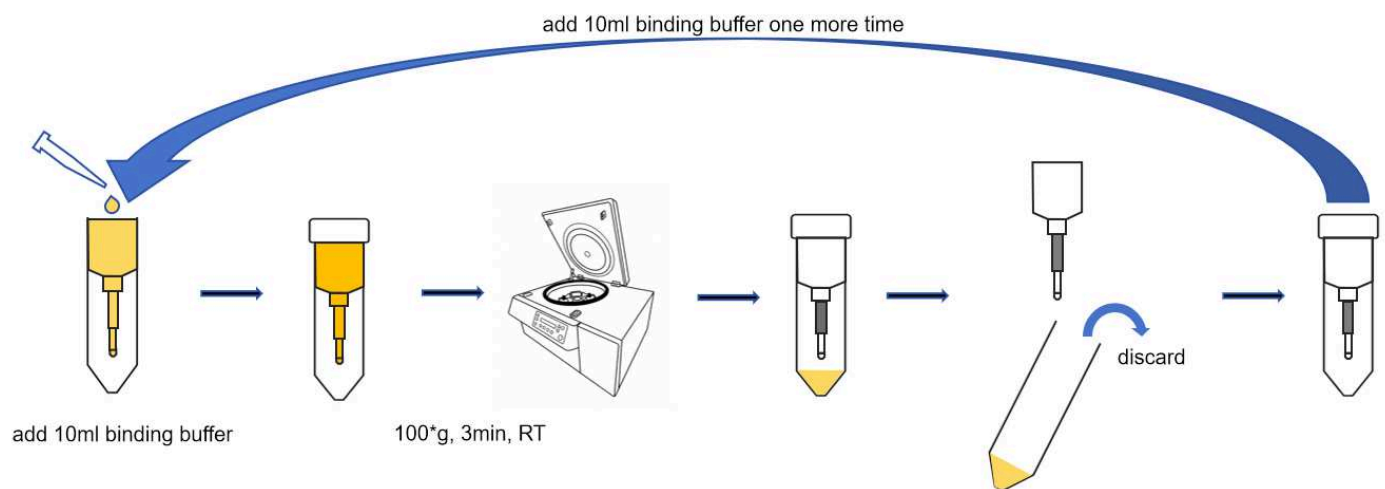
4. Snap off the plastic tail at the bottom of the resin tube.



5. Prepare the purification tube by placing into a 50 ml centrifuge tube



Step C: Equilibration



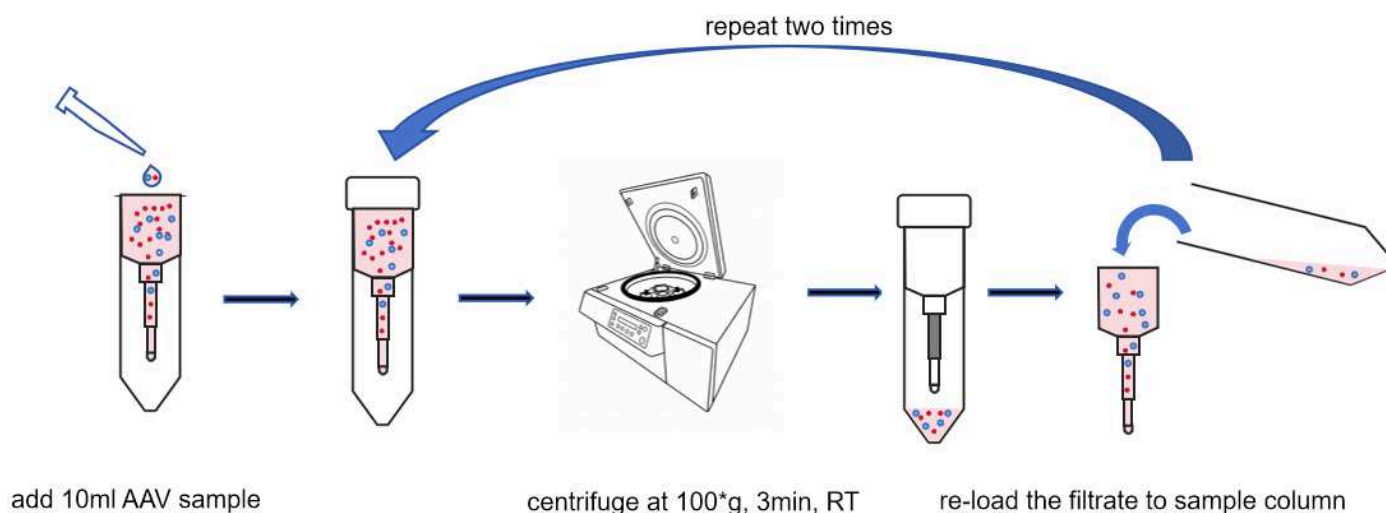
1. Add 10 mL of Binding Buffer to the column.
 2. Centrifuge at 100×g for 3 minutes.
 3. Repeat this step once.
- Note: If liquid remains in the column, centrifuge again at 100 × g for 1 minute (or longer) until all the buffer has completely drained.

Step D: Sample loading & Binding

1. Gently add the 10 mL prepared AAV sample from step A to the column.

2. Centrifuge at 100×g for 3 minutes.
3. Re-binding: Collect the flow-through from the sample tube and reload it onto the column to maximize binding.
4. Centrifuge again at 100 × g for 3 minutes. Ensure all liquid has passed through.
5. Repeat this cycle (step 3 and step 4) 3 times total to maximize binding.

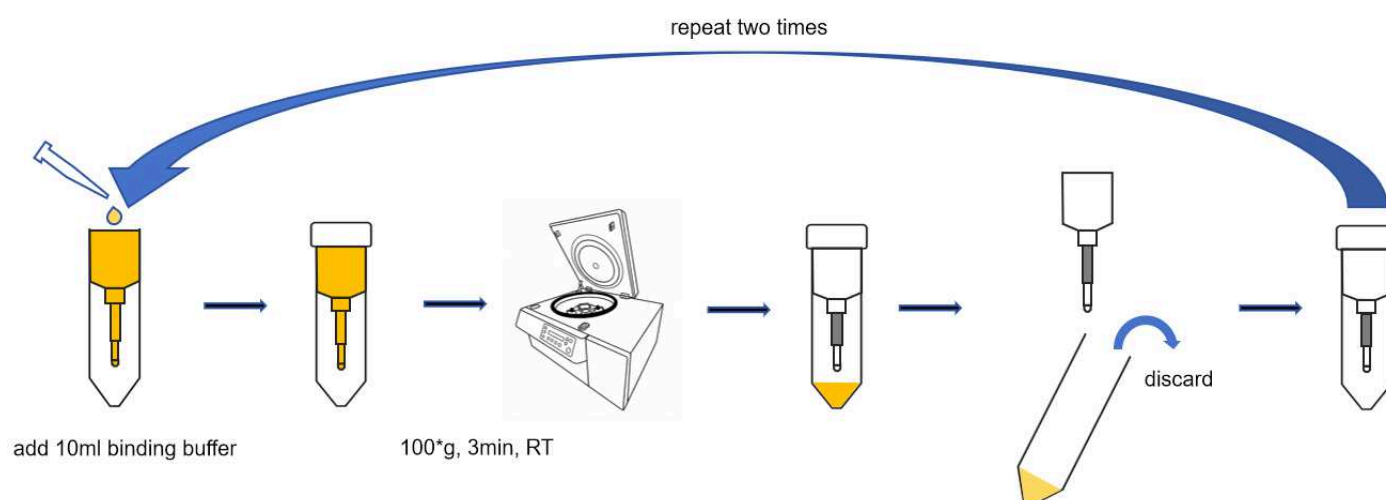
Note: If liquid remains in the column, centrifuge again at 100 × g for 1 minute (or longer) until all the buffer has completely drained.



Step E: Washing

1. Gently add 10 mL of Binding Buffer to the column.
2. Centrifuge at 100×g for 3 minutes.
3. Repeat this wash twice. Ensure all liquid has passed through.

Note: If liquid remains in the column, centrifuge again at 100 × g for 1 minute (or longer) until all the buffer has completely drained.



Step F: Elution

Choose the plan that fits your downstream needs:

Plan 1

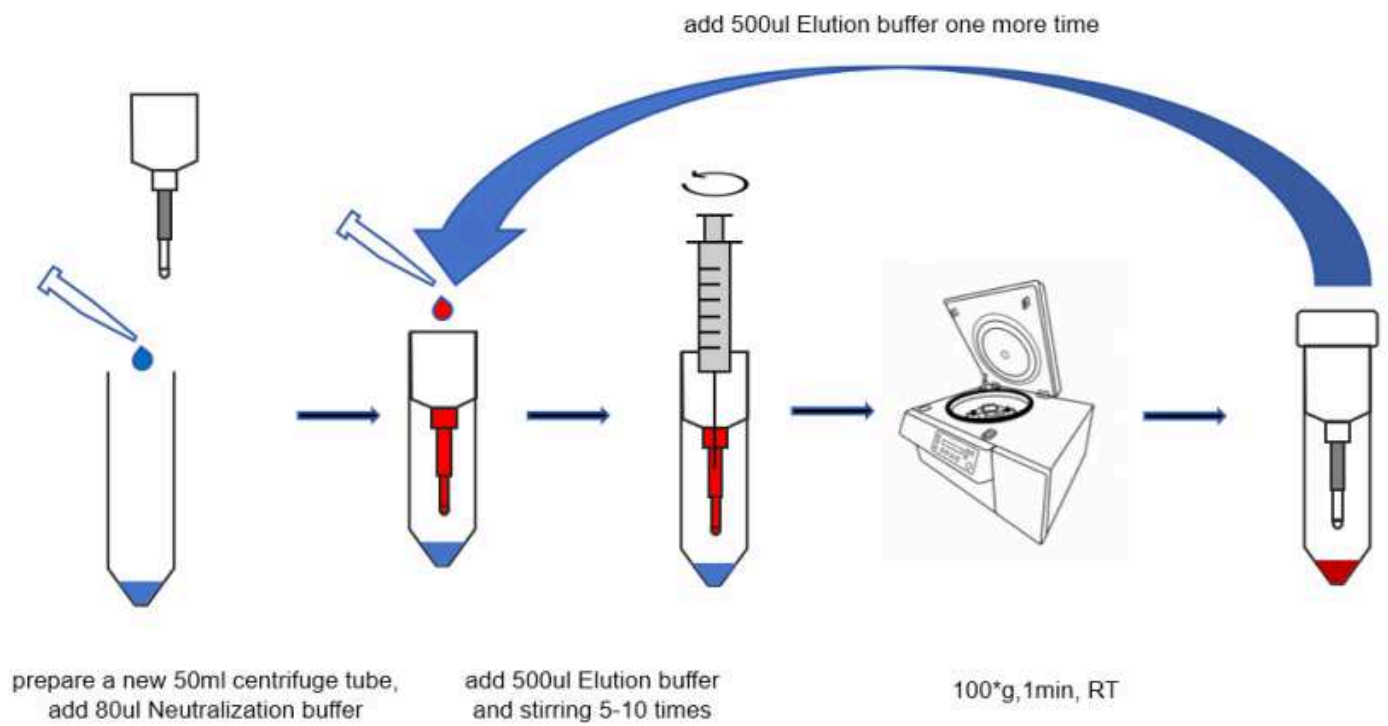
1. After the final wash, centrifuge the purification column at $200 \times g$ for 1 minute to remove residual buffer.
2. Transfer the resin tube to a new centrifuge tube pre-filled with $\sim 80 \mu\text{L}$ of Neutralization Buffer.
3. Slowly add 0.5 mL of Elution Buffer to the inner wall of the sample tube to wash all resin down to resin tube.
4. **Agitation:** Immediately use a sterile 10 mL syringe needle to gently stir the resin 5–10 times.
5. Centrifuge at $100 \times g$ for 1 minute.
6. Add another 0.5 mL of Elution Buffer, stir 5–10 times with the needle, and centrifuge again at $100 \times g$ for 1 minute. And then, centrifuge the purification column at $200 \times g$ for 1 minute to remove residual Elution Buffer.
7. Quickly mix the elution, and transfer to a 1.5 mL centrifuge tube.
8. Use pH paper to detect the pH of elution, if it is not neutral. Adjust the pH to neutral with 1M Tris (pH 8.8) or Elution Buffer.

Note: Although the eluted AAV is initially neutralized with Neutralization Buffer at step F-2, you must verify that the final elution pH is neutral. Adjust the pH as needed using additional 1M Tris (pH 8.8) or Elution Buffer. *Prolonged exposure to low or high pH will degrade capsids and destroy their infectivity.*

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

1. After the final wash, centrifuge the purification column at $200 \times g$ for 1 minute to remove residual buffer.
2. Transfer the resin tube to a new centrifuge tube pre-filled with 400 μL of Neutralization Buffer.
3. Slowly add 0.5 mL of Elution Buffer to the inner wall of the sample tube to wash all resin down to resin tube.
4. Agitation: Immediately use a sterile 10 mL syringe needle to gently stir the resin 5–10 times.
5. Centrifuge at $100 \times g$ for 1 minute.
6. Add another 0.5 mL of Elution Buffer, stir 5–10 times with the needle, and centrifuge again at $100 \times g$ for 1 minute. And then, centrifuge the purification column at $200 \times g$ for 1 minute to remove residual Elution Buffer.
7. Quickly mix the elution, and transfer to a 2 mL centrifuge tube.
8. Use pH paper to detect the pH of elution, if it is not neutral. Adjust the pH to neutral with 1M Tris (pH 8.8) or Elution Buffer.

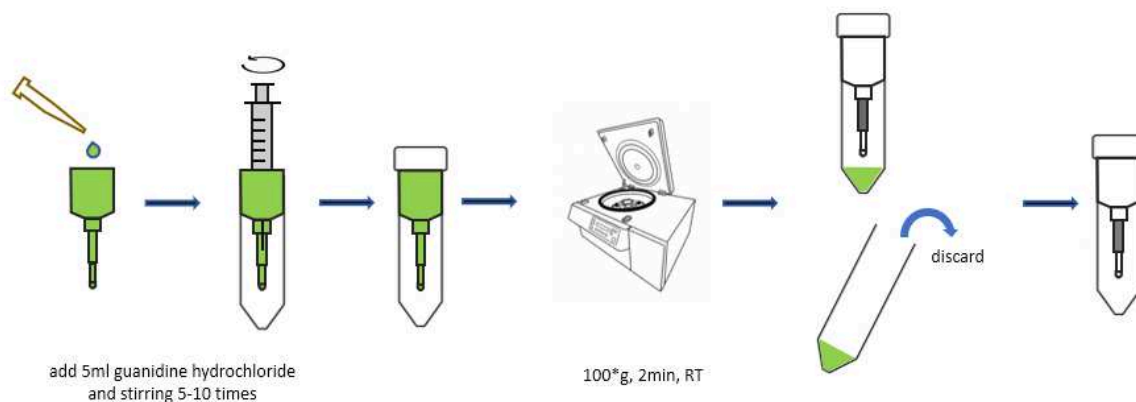
Note: Although the eluted AAV is initially neutralized with Neutralization Buffer at step F-2, you must verify that the final elution pH is neutral. Adjust the pH as needed using additional 1M Tris (pH 8.8) or Elution Buffer. *Prolonged exposure to low or high pH will degrade capsids and destroy their infectivity.*



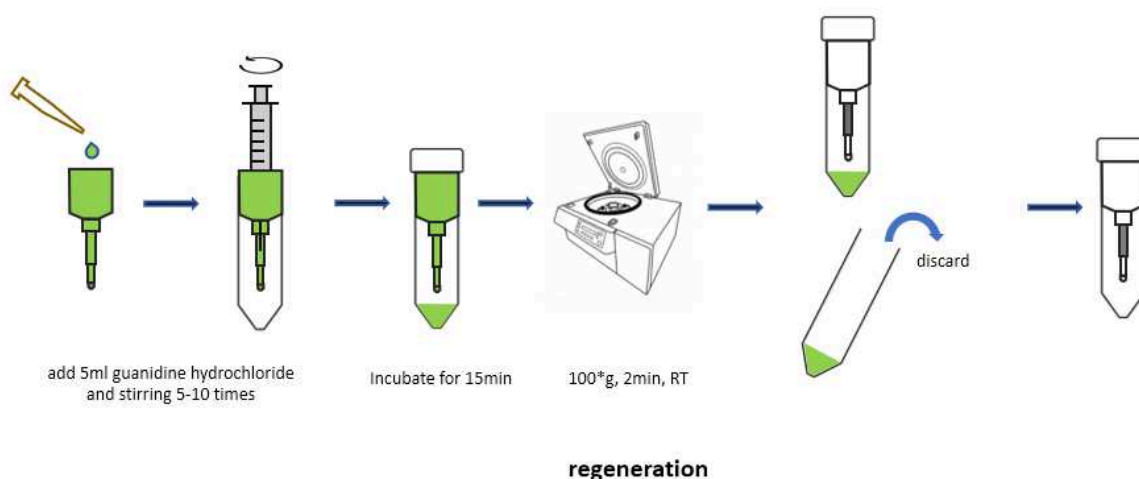
- Note: If liquid remains in the column, centrifuge again at $100 \times g$ for 1 minute (or longer) until all the buffer has completely drained.

Step G: Resin Regeneration

Step 1



Step 2



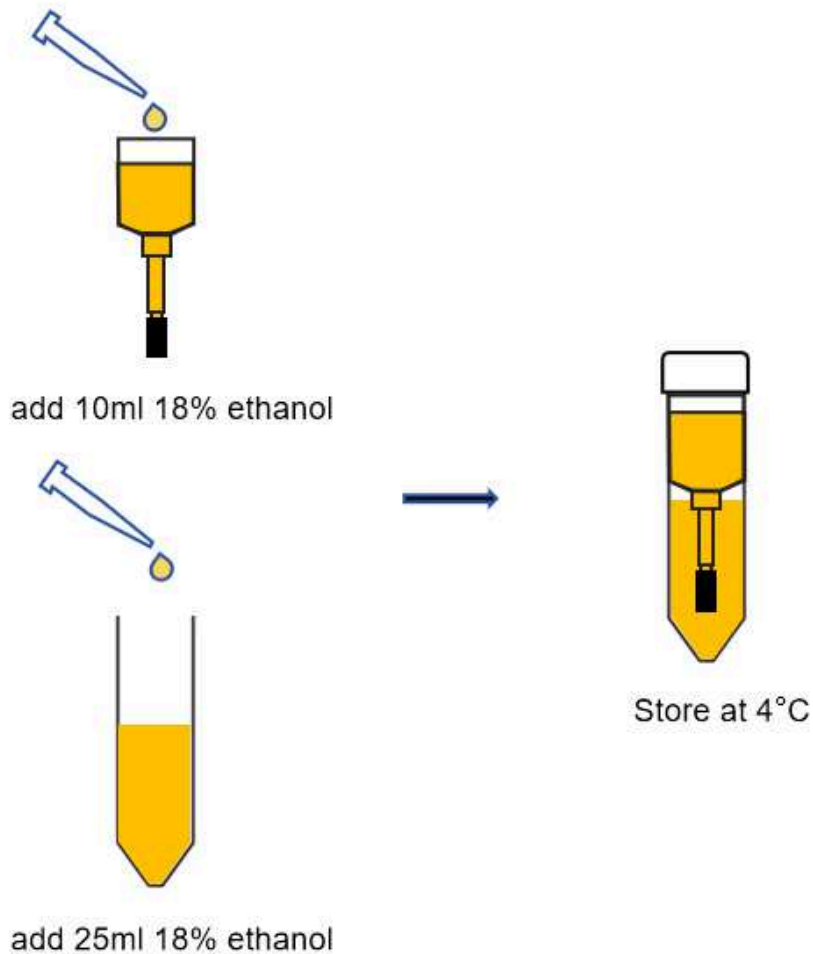
To ensure the longevity and effectiveness of the resin, follow these steps for regeneration:

- 1. Initial Wash:** Gently add 5 mL of 6 M Guanidine Hydrochloride (Regeneration Buffer) to the column.
- 2. Stirring:** Use a sterile 10 mL syringe needle to gently stir the resin 5 to 10 times.
- 3. Centrifugation:** Centrifuge at 100 x g for 2 minutes and discard the flow-through.
- 4. Incubation:** Add another 5 mL of 6 M Guanidine Hydrochloride and stir 5 to 10 times with the needle. Allow the buffer to drip through the resin by gravity for 15 minutes.
- 5. Final Centrifugation:** Centrifuge at 100 x g for 2 minutes and discard the flow-through.
- 6. 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.
- 7. Repeat:** Repeat the re-equilibration step (Step 7) twice more to ensure all regeneration buffer is removed.

Note: If liquid remains in the column, centrifuge again at 100 x g for 1 minute (or longer) until all the buffer has completely drained.

Step H: Resin Tube Storage

Proper storage is essential to maintain resin stability between uses:



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

- 1. Ethanol Wash:** Gently add 5 mL of 18% ethanol to the purification column.
- 2. Centrifugation:** Centrifuge at 100 x g for 3 minutes and discard the flow-through.
- 3. Repeat Wash:** Repeat the ethanol wash and centrifugation once more.
- 4. Sealing:**

After use and cleaning, follow these steps for storage:

- Ensure the internal resin tube is filled with 18% ethanol and gently stirred to remove any air bubbles.
- Verify that the lower plug is tightly secured to prevent leakage or evaporation.
- Add 10 mL of 18% ethanol to the sample tube.
- Place the sample tube into a new 50 mL centrifuge tube containing 25 mL of 18% ethanol.

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

Note:

- 1.** 6-month stability testing has been successfully completed; long-term stability evaluation is currently ongoing.
- 2.** Always ensure the resin is completely submerged in 18% ethanol before long-term storage.

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 $4.28\text{E}+13$ 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/ÄKTA screening) or bulk AAVPure Resins. The chemistry is identical; only the format changes.

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

CRITICAL NOTE:

Standard qPCR is highly susceptible to systemic errors caused by residual extra-viral nucleic acids, variable capsid lysis efficiency, and amplification biases from complex AAV ITR secondary structures. To prevent misinterpretation of this kit's purification efficacy due to inherent methodological limitations, we strongly advise against relying solely on qPCR for AAV titration. For a rigorous and accurate evaluation, we recommend utilizing ddPCR for absolute quantification or orthogonal validation via ELISA.

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.