PurProX™ AAVPure Pre-packed Column

AAV2, AAV5, AAV6, AAV8, AAV7m8, AAV-DJ

Product Instruction Manual

Technical Parameters

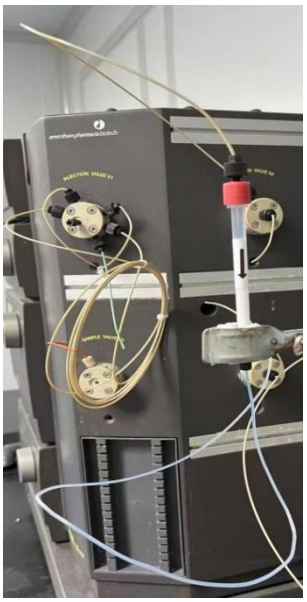
Classification	Description
Catalog number	GMV-PurProX-AAVPure-U-1-P0.5ml
Product name	PurProX™ AAVPure Pre-packed Column
Support matrix	Polymer resin
Immobilized ligand	VHH
Serotype affinity	Tested-Binding: AAV2, AAV5, AAV6, AAV8, AAV7m8, AAV-DJ
Binding capacity	~3E+14 vp per mL resin
Shipping solvent	20% ethanol
Average particle size	50 μm
Recommended flow rate	≥3-minute residence time
Mechanical resistance	100 bar (10 MPa)
pH Range (all ligands)	2–10
Operating temperature	RT
Storage instruction	Do not freeze

Usage Instructions

Buffer Preparation for Purification

Buffer	Component
Binding buffer	1x PBS + 0.35 M Nacl + 0.01% F-68 (pH 7.4)
Wash buffer	1x PBS + 0.35 M Nacl + 0.01% F-68 (pH 7.4)
Elution buffer	0.1 M Glycine + 2mM $MgCl_2$ + 0.01% F-68 (pH 2.5)
Neutralizing Buffer	1 M Tris + 0.01% F-68
Stripping buffer	0.1 M Phosphoric Acid + 0.01% F-68 (pH 2.0)
Note： *All solutions must be filtered using a 0.22um filter prior to the purification of AAV.*	

Operation Steps



1. **Prepare and Set Up the Column:** Equilibrate the required buffers to room temperature. Set up the column to AKTA protein purification system as indicated in image, with arrows representing the direction of liquid flow. Turn on the AKTA and set the flow rate to 1mL/min with 20% ethanol. Slowly unscrew the upper cap of the purification column. Allow 20% ethanol from the AKTA tube to drip into the upper adapter until it is fully filled with liquid. Then, slowly screw on the upper Luer adapter without tightening it fully.
2. **Connect the Column:** Slowly unscrew the bottom cap of the column to allow liquid to drain out. Later, connect the column to AKTA by tightening the bottom luer adapter. Finally, tighten the upper luer adapter.
3. **Column equilibration:** Switch to the Binding buffer at a flow rate of 1 mL/min, until conductivity stabilizes (approximately 5–10 CVs).
4. **Sample loading:** Treat the cell lysate with Benzonase, centrifuge to remove cell debris, and filter through 0.22µm filter. Load the sample on to the AKTA system at a flow rate of 0.8 mL/min.
5. **Washing:** Switch to the Wash buffer at a flow rate of 1 mL/min untill the A280 baseline stabilizes (5–10 CVs)
6. **Elution:** Switch to Elution buffer at a flow rate of 0.8 mL/min. Collect eluted fractions immediately after the peak appears, for 5–10 CVs. Add Neutralizing Buffer to adjust to neutral pH (Elution : Neutralizing buffer = 15 : 1, test the pH if necessary) and mix well. Proceed to the next step immediately after elution.
7. **Cleaning and regeneration:** Wash with Wash buffer at 1 mL/min until conductivity stabilizes. Then, wash with Stripping buffer at 1 mL/min until conductivity stabilizes (3–5 CVs). Follow with Binding buffer at 1 mL/min until conductivity stablizes (5–10 CVs). Finally, wash with 20% ethanol for 5-10 CVs to replace all column liquid with 20% ethanol.

> ***Note from experience**: When cleaning with Stripping buffer at a flow rate of 1mL/min, switch to Wash buffer once there is a significant decrease in conductivity. Continue cleaning with Wash buffer at 1mL/min until the conductivity stabilizes.*

8. **Remove the column**: Maintain 20% ethanol at 1 mL/min. Slowly unscrew the bottom luer adapter, then seal it with the cap (do not over-tighten). Unscrew the top AKTA adapter completely (ensuring the column interface remains filled with 20% ethanol), then seal with the upper cap.

> ***Note**: CV refers to column volume, approximately 1.3mL.*

Storage

Conditions: Store at 2–8°C.
Shelf life: At least 6 months (stability tested for 6 months; longer-term stability is is currently being evaluated).

Notes

All operations must be conducted at room temperature.

Purification Examples

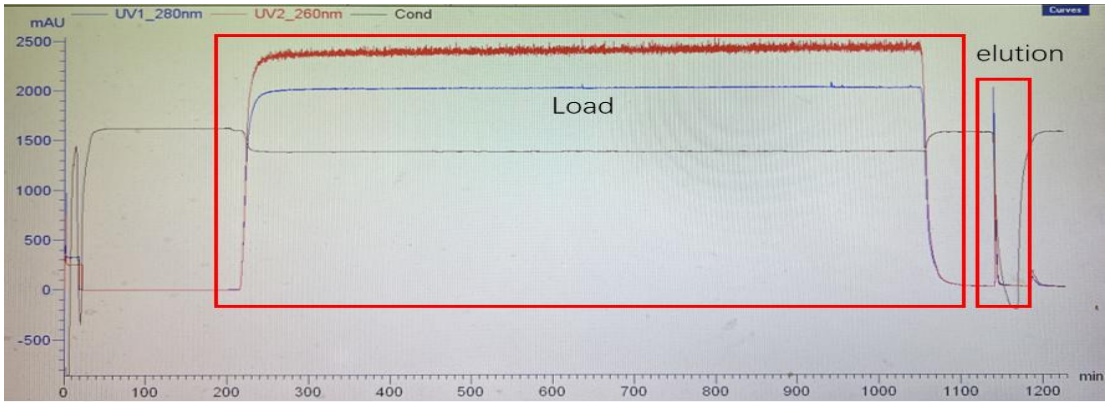


Fig.1 AAV8 purified with AAV affinity resin by AKTA

Chromatogram illustrating the purification process of AAV8 serotype using the AAV affinity resin on the ÄKTA system. The x-axis represents time, while the y-axis shows absorbance (A280 for protein, A260 for nucleic acid) and conductivity. Peaks indicate sample loading, washing, and elution phases.

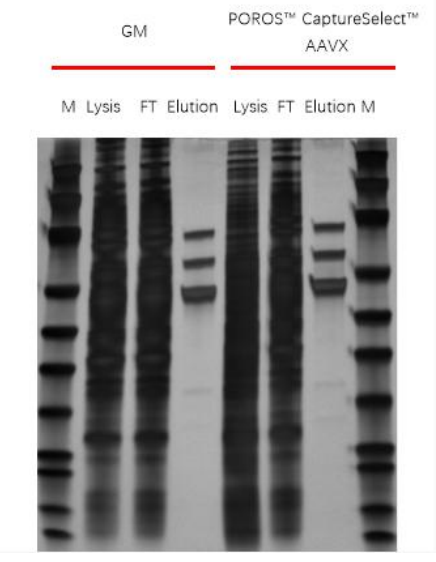


Figure. 2: Silver staining of AAV8 purification

Silver-stained SDS-PAGE gel showing protein bands from AAV8 purification fractions. Lanes include molecular weight markers, lysate, flow-through (FT), and elution fractions. Capsid proteins (VP1, VP2, VP3) are visible in the elution lane at ~60-80 kDa.

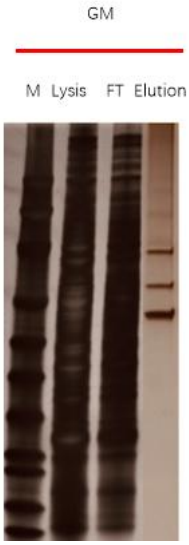


Fig.3 Silver staining of AAV6 purification

Silver-stained SDS-PAGE gel depicting protein bands from AAV6 purification fractions. Lanes show markers, lysate, flow-through (FT), and elution, with AAV6 capsid proteins (VP1, VP2, VP3) prominent in the elution lane.

Table.1 Recovery of several serotypes of purified AAV

Serotype	sample	Vg	recovery rate %
AAV2	Load	5.72E+12	/
	Elution	5.19E+12	90.73
AAV5	Load	3.36E+13	/
	Elution	3.13E+13	93.15
AAV6	Load	8.28E+12	/
	Elution	7.73E+12	93.36
AAV8	Load	1.33E+13	/
	Elution	1.24E+13	93.67