Genemedi PurProX™AAV Affinity Resin

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

Product Instruction Manual

Technical Parameters

Classification	Description
Catalog number	GMV-PurProX-AAVPure-U-1
Product name	PurProX™ AAVPure Affinity Resin
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	2% Phenylmethanol
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, Do not freeze

Usage Instructions

Buffer Preparation for Purification

Buffer	Component
Binding buffer	1x PBS + 0.35 M Nacl + 1x GMXbuffer01 (pH 7.4)
Wash buffer	1x PBS + 0.35 M Nacl + 1x GMXbuffer01 (pH 7.4)
Elution buffer	0.1 M Glycine + 2mM $MgCl_2$ + 1x GMXbuffer01 (pH 2.5)
Neutralizing Buffer	1 M Tris + 1x GMXbuffer01
Stripping buffer	0.1 M Phosphoric Acid + 1x GMXbuffer01 (pH 2.0)

Note： *All solutions must be filtered using a 0.22um filter prior to the purification of AAV. GeneMedi provides 1000x GMXbuffer01.*

Operation Steps

1. Packing guidelines

1) Resins are supplied as approximately 50% slurry in 2% Phenylmethanol.
2) Do not gravity-settle resin in the column before packing.
3) Use standard 10–20 µm screens (frits)
4) Pack at a higher flow rate/pressure(~3bar). The top adapter position may need to be better seated in the packed resin bed to ensure that a headspace does not form.
5) Verify that there is no air in the column.

2. Equilibration/binding conditions

1) Execute at the flow rate that is defined for the intended unit operation, typically 150 cm/hour.
2) PBS pH 7.2 to 7.4 is a good starting buffer. However, other standard neutral pH buffers such as 10–50 mM sodium phosphate or Tris can be used. pH must be in the range of 6–8. Adding 0.1–0.5 M NaCl may prevent nonspecific adsorption due to protein/protein interactions.
3) After the load, wash unbound material from the column with the equilibration buffer. Generally a 5–10 CV wash is sufficient to remove all unbound proteins from the column. Samples with high impurity levels may require a longer wash to return to a stable baseline.

3. Elution conditions considerations

1) Because target molecules differ in their binding/elution behavior, the best elution conditions are determined experimentally.
2) To elute most target molecules, reduce the pH to the range of pH 2 to 3.
3) Use an elution buffer strength greater than the equilibration buffer strength to ensure a good pH transition.
4) Do not underload the column. A load significantly below the maximum binding capacity can hamper efficient release due to re- binding events during elution causing poor recovery.
5) Immediately neutralize the eluted pool to prevent denaturation of some molecules at low pH. When selecting buffer systems, consider molecule stability, binding optimization for the next step, and the ability of the buffer to control pH in the desired operating range.

4. A typical cleaning procedure

1) Strip with 0.1 M phosphoric acid (pH 2.0).
2) Clean with 6 M guanidine hydrochloride.
3) Re- equilibrate with neutral pH buffer such as PBS, pH 7.5. or store in buffered ethanol.
4) Resin storage
5) Store bulk resin at 2 to 8°C. Do not freeze.
6) Store packed columns at 2 to 8°C (long- term) or room temperature (short- term) after cleaning (described above) in a neutral- pH solution with a bacteriostatic agent such as 0.1 M sodium phosphate (pH 7.0) with 2% Phenylmethanol.

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

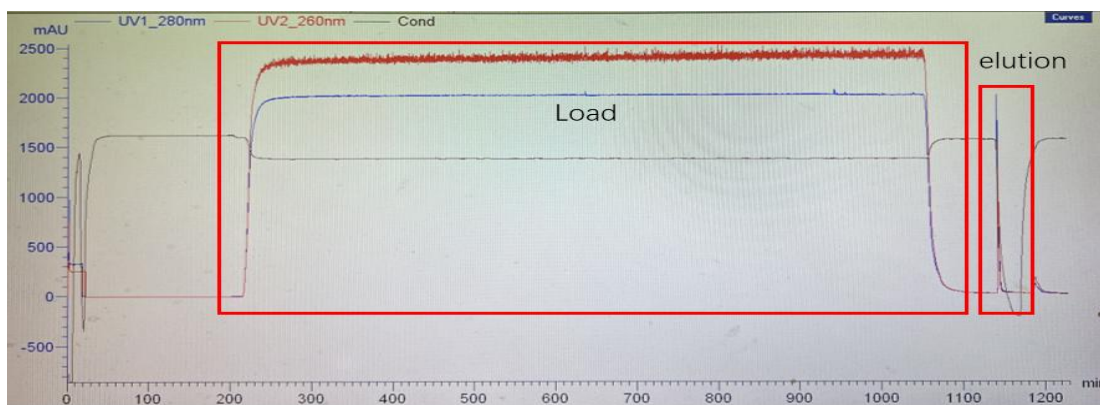

Fig.1 AAV8 purified with AAV affinity resin by AKTA

Chromatogram illustrating the purification process of AAV8 serotype using the AAV affinity resin (Cat. No----) 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.

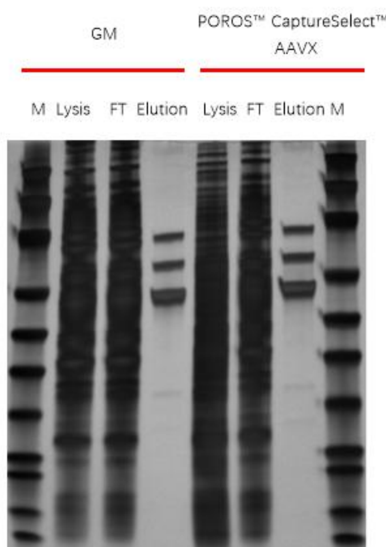

Figure. 2: Silver staining of AAV8 purification

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.

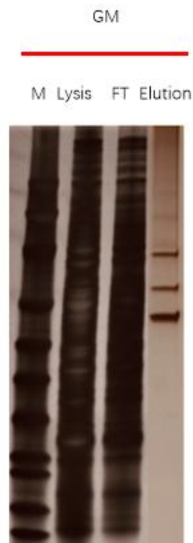

Fig.3 Silver staining of AAV6 purification

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