

## AAVidity™ Prepacked Column

### 1. Product Description

AAVidity™ is a novel affinity resin designed to transform the purification of Adeno-Associated Viral vectors (AAVs) for gene therapy. The AAVidity™ peptide ligand selectively targets AAVs of all clinically-relevant serotypes (1 – 9 and rh.10) and isolates them from HEK293 and Sf9 cell lysates, returning high product yield, purity, and transduction activity.

Key features of the AAVidity™ include:

- High serotype-agnostic binding capacity ( $> 5 \cdot 10^{13}$  vp/mL at 3 min residence time)
- Mild elution conditions
- $> 250$ -fold reduction of host cell proteins
- Elution of “full” AAVs upon linear gradient elution with  $MgCl_2$
- Minimum resin lifetime  $> 20$  cycles

Parameter	Values
Matrix	Polymethyl methacrylate beads (65 $\mu$ m)
Ligand	AAVidity™ peptide
Binding capacity	$> 5 \cdot 10^{13}$ vp per mL of resin
Mechanical stability	Up to 10 MPa
Storage conditions	20% v/v ethanol (aq), store at 2–8°C.

Cell lysis conditions ( <i>note: the following protocol does not require DNase treatment</i> )	
<b>Lysis Buffer:</b> 5% w/v Cetyltrimethylammonium bromide (CTAB) and 1 M $MgSO_4$ in DI water <b>Step 1:</b> Mix the Lysis Buffer with the HEK293 cell culture harvest at a 1:20 volume ratio <b>Step 2:</b> Incubate for 2 hours under gentle stirring at 37°C <b>Step 3:</b> Remove the precipitate via centrifugation (4000g for 30 min) or depth filtration <b>Step 4:</b> Conduct tangential flow filtration (TFF) of the clarified lysate against 5 Diavolumes of:	
10 mM Bis-Tris buffer, 20 mM NaCl, 0.01% v/v Pluronic F68, pH 7.0	AAV serotypes 2, 3, 6, 9, and rh.10
50 mM Acetate buffer, 2 mM $MgCl_2$ , 0.01% v/v Pluronic F68, pH 5.0 (Acetate Buffer: mix <i>either</i> 2.762 g of sodium acetate anhydrous <i>or</i> 4.581 g of sodium acetate trihydrate <i>with</i> 980.7 mg of glacial acetic acid <i>and</i> add DI water to a final volume of 1 L)	AAV serotypes 1, 5, 7, and 8

Binding (residence time: 3 min) and washing	
AAV serotypes 2, 3, 6, 9, and rh.10	10 mM Bis-Tris buffer, 20 mM NaCl, 0.01% v/v Pluronic F68, pH 7.0
AAV serotypes 1, 5, 7, and 8	50 mM Acetate buffer, 2 mM $MgCl_2$ , 0.01% v/v Pluronic F68, pH 5.0 (Acetate Buffer: mix <i>either</i> 2.762 g of sodium acetate anhydrous <i>or</i> 4.581 g of sodium acetate trihydrate <i>with</i> 980.7 mg of glacial acetic acid <i>and</i> add DI water to a final volume of 1 L)

<b>Elution (residence time: 1 min)</b>	
AAV serotypes 2, 3, 6, 9, and rh.10	Elution 1 <sup>§</sup> (10 CVs): 10 mM Bis-Tris, 0.4 M MgCl <sub>2</sub> , 0.01% v/v Pluronic F68, pH 6.0 Elution 2 (10 CVs): 10 mM Bis-Tris, 1 M MgCl <sub>2</sub> , 0.01% v/v Pluronic F68, pH 6.0
AAV serotypes 1, 5, 7, and 8	Elution 1 <sup>§</sup> (10 CVs): 10 mM Bis-Tris, 20 mM NaCl, 0.01% v/v Pluronic F68, pH 7.0 Elution 2 (10 CVs): 10 mM Bis-Tris, 0.4 M MgCl <sub>2</sub> , 0.01% v/v Pluronic F68, pH 6.5
<sup>§</sup> Conducting AAV elution in two steps promotes the enrichment of “full” capsids in the “Elution 1” step.	
<b>Regeneration and Cleaning-in-Place (CIP)</b>	
<b>Step 1:</b> 10 CVs of Binding Buffer <b>Step 2:</b> 15 CVs of 0.5 M NaOH (optional add 2 M NaCl) and incubation for 15 minutes <b>Step 3:</b> 10 CVs of Binding Buffer <b>Step 4:</b> 15 CVs 10 mM sodium phosphate with 150 mM NaCl and 0.01% Pluronic-F68 at pH 2.0 <b>Step 5:</b> 10 CVs of Binding Buffer	

## **2. General Recommendations**

**2.1. Resin preparation:** prior to the first use with AAV, perform a blank run (load Binding Buffer) including washing, elution, and regeneration and CIP. Equilibrate the resin with 10 CVs of Equilibration Buffer at the residence time of 1 min (or until achieving constant pH and conductivity of the effluent).

**2.2. Loading ratio:** Measuring the binding capacity (DBC<sub>10%</sub>) of the AAV product is highly recommended, since product design (*e.g.*, transgene length) and capsid concentration can cause variations in DBC<sub>10%</sub>.

- Recommended Loading Ratio ~ 95% - 100% of DBC<sub>10%</sub>
- Note: underloading the column leads to lower AAV recovery.

### **2.3. Residence time:**

- Loading of cell lysate: 3 min in down-flow mode
- Elution: 1 min in up-flow mode
- Washing, Regeneration, and Cleaning-in-Place: 1 min