AAVidity™ Resin

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 (> $2 \cdot 10^{14}$ vp/mL at 2 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	Equilibration and Wash Buffers
Matrix	Polymethyl methacrylate beads (65 µm)
Ligand	AAVidity™ peptide
Binding capacity	> $2 \cdot 10^{14}$ vp per mL of resin
Mechanical stability	Up to 10 MPa
Storage conditions	20% v/v ethanol (aq), store at 2–8°C.

Serotypes	Equilibration and Wash Buffers
AAV 2, 3, 6, 9, and rh.10	10 mM Bis-Tris, 20 mM NaCl, pH 7.0 0.01% v/v Pluronic F68
AAV 1, 5, 7, and 8	50 mM Sodium Acetate, 2 mM $MgCl_2$, pH 5.0 0.01% v/v Pluronic F68
	Elution Buffer
AAV 2, 3, 6, 9, and rh.10	Elution 1:* 10 mM Bis-Tris, 400 mM $MgCl_2$, pH 6.0, 0.01% v/v Pluronic F68 (10 CVs) Elution 2: 10 mM Bis-Tris, 1 M $MgCl_2$ pH 6.0, 0.01% Pluronic F68 (10 CVs)
AAV 1, 5, 7, and 8	Elution 1:* 10 mM Bis-Tris, 20 mM NaCl, pH 7.0, 0.01% Pluronic F68 (10 CVs) Elution 2: 10 mM Bis-Tris, 400 mM $MgCl_2$, pH 6.5, 0.01% Pluronic F68 (10 CVs)
Regeneration	10 mM Phosphate, 150 mM NaCl, pH 2.0 0.01% Pluronic-F68

2. General Recommendations

1. Resin preparation: prior to the first use, wash the resin with 10 column volumes (CVs) of 20% v/v ethanol, 10 CVs of DI water, 10 CVs of Equilibration Buffer, 10 CVs of 10 mM phosphate buffer at pH 2, and 10 CVs of Equilibration Buffer. All steps should be conducted at the resident time of 1 min.

2. Feed material preparation: adjust the conductivity of the feedstock to ≤ 5 mS/cm (ideal 3.5 mS/cm) via diafiltration/tangential flow filtration (TFF) or dilution with Equilibration Buffer. Feed conditioning using a high nominal molecular weight cut-off filter (MWCO ≥ 50 kDa) is recommended to remove surfactants, such as Tween 20, which may reduce the binding capacity of the resin.

3.* Load and column wash: equilibrate the resin with 10 CVs of Equilibration Buffer at the resident time of 1 min (or until achieving constant pH and conductivity of the effluent). Load the sample at 3 minutes residence time in *down-flow mode*.

Serotypes	Feed material pH
AAV2, AAV3, AAV6, AAV9, and AAV10rh	pH 7.0
AAV1, AAV5, AAV7, and AAV8	pH 5.0

Note: Conducting a dynamic binding capacity (DBC) study is highly recommended. Underloading the column leads to lower AAV recovery.
Wash the column with 20 CVs of Equilibration/Wash Buffer.

3.* Elution of "Full" capsids: elute the bound AAVs in *up-flow mode* at the residence time of 1 min. Conducting Elution 1 step as:

- Step elution provides high yield of all bound AAVs
- Linear gradient elution triggers the release of "full" capsids as the first AAV to elute from the resin

Regenerate the resin with 10 CVs of Regeneration Buffer. Finally, wash the resin in Equilibration Buffer in preparation for a subsequent purification run *OR* store the resins in 20% v/v ethanol (aq).