

AAVidity Prepacked Columns

1. Description of Product

Parameter	Conditions for use
Equilibration and Washing buffer for AAVs: 2, 3, 6, 9, and 10rh	10 mM Bis-Tris, 20mM NaCl pH 7.0, 0.01% Pluronic-F68
Equilibration and Washing buffer for AAVs: 1, 5, 7, and 8	50 mM sodium acetate, 2 mM MgCl ₂ pH 5.0, 0.01% Pluronic-F68
Elution for AAVs: 2, 3, 6, 9, and 10rh	Elution 1: 10 mM Bis-Tris, 400 mM MgCl ₂ pH 6.0, 0.01% Pluronic-F68 (10 CVs) Elution 2: 10 mM Bis-Tris, 1 M MgCl ₂ pH 6.0, 0.01% Pluronic-F68 (10 CVs)
Elution for AAVs: 1, 5, 7, and 8	Elution 1: 10 mM Bis-Tris, 20mM 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)
Stripping/Regeneration	10 mM Phosphate, 150 mM NaCl, pH 2.0 0.01% Pluronic-F68

2. General Recommendations

1. Perform blank run by washing column with 10 column volumes (CVs) of 20% v/v ethanol, 10 CVs of MilliQ water, 10 CVs of equilibration buffer, 10 CVs of 10 mM phosphate pH 2, and 10 CVs of equilibration buffer. All steps should be conducted at 1 minute resident time.

2. Preparing feed material. Conduct tangential flow filtration (TFF) or dilution to reduce feed material conductivity to less than 5 mS/cm (ideal 3.5 mS/cm). Presence of surfactants, such as Tween 20 may reduce binding capacity, therefore, TFF is highly recommended.

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

3. Equilibrate column with 10 CVs of equilibration buffer or until constant pH and conductivity at 1 minute resident time.

4. Load the sample at 3 minutes residence time in down-flow mode.

Note: Conducting a DBC study is highly recommended. Underloading the column leads to lower AAV recovery.

5. Wash the column with 20 CVs of equilibration/washing buffer.

6. Elute with buffers described in the first table in up-flow mode at 1 minute resident.

7. Regenerate the resin with 10 CVs stripping buffer.

8. Equilibrate the resin in equilibration buffer in preparation for a subsequent purification run *OR* store the resins in 20% v/v ethanol.