AVIPure® HiPer™ AAV Affinity Resins

Quick Start Guide

AVIPure® HiPer™ AAV Affinity Resins combine the specificity and caustic stability of AVIPure ligands with a macroporous, convective base bead for a high capacity, fast flow affinity resin to turbocharge capture step productivity.

Process Conditions

Optimal conditions for purification of AAV using AVIPure HiPer AAV Affinity Resin must be determined empirically for each source. Some general process development recommendations for identification of optimal process conditions are summarized in Table 1. For the most up to date application notes, please refer to repligen.com.

The resin should be operated at a flow velocity that maintains a pressure drop across the column lower than the maximum packing pressure (2.8 – 3 bar). Factors that influence maximum flow velocity include viscosity of the running buffer and turbidity of the load solution. The maximum flow velocity will vary depending on the feed stream. When loading feed material for columns with up to 12 cm internal diameter (ID) and a 10 cm bed height (BH), the evaluation should be started at 600 cm/h. Users can increase flow velocity provided the pressure drop over the column stays below maximum packing pressure.

Table 1. Recommended Process Steps

Step	Suggested Buffer	Column Volumes	Residence Time (min)	Notes
Sanitization (OPTIONAL)	0.5 M NaOH	5	1	A 15-minute static hold can also be used for this step.
Equilibration	Match clarified harvest load buffer or 20 mM Tris, 150 – 400 mM NaCl, pH 7.5	5	1	pH and conductivity return to baseline.
Load	Clarified harvest, pH 7 – 8	Titer dependent	1	For example, with a titer of 4.0 x 10^{12} vp/mL, load 100 column volumes (CV) for 4.0 x 10^{14} vp/mL of resin.
Wash 1	Equilibration buffer	2 – 5	1	Potential to remove during process development
Wash 2 (if needed)	1 M NaCl, 20 mM Tris, pH 7.5	2 – 5	1	
Wash 3	Equilibration buffer	2	1	Potential to remove during process development
Elution	0.1 M glycine, pH 3.0	5	1	Elution complete when A280 returns to baseline. Elute fractions into 1 M Tris base neutralization buffer (10% – 20% fraction volume).
Acid Strip (OPTIONAL)	0.1 M phosphoric acid	5	1	Other process-specific acid buffers can also be used (e.g., PAB).
Base Clean-in-Place (CIP)	0.5 M NaOH	5	1	A 15 min static hold can also be used for this step. Operate in upflow for best results.
Re-equilibration	Equilibration buffer	5	1	pH and conductivity return to baseline.
Long-term storage	18% – 20% ethanol	2 – 3	1	For long-term storage, store column/resin at 2 – 8°C.

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Equilibration and Binding Conditions

Generally, equilibration can be matched to load buffer pH and conductivity. For harvest cell culture fluid (HCCF) at pH 7.4, PBS or 20 mM Tris, 150 mM NaCl, pH 7.4 have been demonstrated as compatible equilibration buffers. Addition of 0.01% poloxamer-188 is recommended to reduce non-specific binding of capsids in the flow path of the chromatography system. Fractionate and collect the flow through during initial runs to understand capacity and breakthrough.

Wash and Conditions

To achieve the best HCP and hcDNA clearance, it is recommended to use a wash buffer comprised of 1 M NaCl, 20 mM Tris, pH 7.5. Empirical testing of other additives or buffers is recommended for further process optimization. Refer to the full user guide for more details.

Elution Conditions

AAV capsids can be eluted from the affinity resin with 0.1 M glycine, pH 3.0. Salt may be added to the elution buffer (e.g., 50 to 150 mM sodium chloride) if required for capsid stability.

Eluates are neutralized with 1 M Tris base at 10% – 20% of the fraction volume. These buffers have been demonstrated to provide effective elution of wild-type capsids. Other buffering agents (e.g., citrate, acetate, and sodium chloride concentrations) may provide improved recovery and should be tested empirically.

If poor recovery is observed, try lowering the pH of the elution buffer in increments of 0.5 until sufficient recovery is achieved with little-to-no peak observed in the acid strip. Addition of other excipients such as arginine, magnesium salts, or propylene glycol may also improve recovery, possibly without reducing pH.

Acid Strip Condition (Optional)

To remove material remaining on the column, an acid strip with 0.1 M phosphoric acid, pH 1.7 – 2, can be performed, if desired.

Clean-in-Place Conditions

AVIPure HiPer AAV are alkali-tolerant resins enabling the use of NaOH up to concentrations of 0.5 M. A cleaning-in-place (CIP) regime of 0.5 M NaOH exposure for 15 minutes per cycle ensures consistent chromatographic performance. For best results, operate the CIP in upflow. Neutralize prior to storage.

Storage

The neutralized resin can be stored in 18% – 20% ethanol at 2 – 8°C.

Customer Service

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