AVIPure® HiPer™ AAV Affinity Resin

User Guide

REPLIGEN

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Abbreviations

AAV	adeno-associated virus
BH	bed height
C	celsius
CCCF	clarified cell culture fluid
CIP	clean-in-place
cm	centimeter
CV	column volumes
d_{50v}	median particle size
hcDNA	host cell DNA
HCCF	harvest cell culture fluid
HCP	host cell protein
Hepes	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
ID	internal diameter
M	molar
$MgCl_2$	magnesium chloride
mL	milliliter
mM	millimolar
NaCl	sodium chloride
NaOH	sodium hydroxide
PAB	buffer containing phosphoric acid, acetic acid, and benzyl alcohol
PBS	phosphate buffered saline
psi	pounds per square inch
TMAC	tetramethyl ammonium chloride
µm	micrometer or micron

1. Introduction

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.

2. About This Document

This manual uses several different phrases. Each phrase should draw the following level of attention:

Table 1. Explanation of User Attention Phrases

Phrase	Description
Note:	Points out useful information.
IMPORTANT	Indicates information necessary for proper instrument operation.
PRECAUTION	Cautions users of potential physical injury or equipment damage if the information is not heeded.
WARNING!	Warns users that serious physical injury can result if warning precautions are not heeded.

3. Safety Precautions

Table 2. Safety Precautions

Symbol	Description
WARNING 	Wear standard laboratory personal protective equipment (PPE), including lab coat, protective eye wear, and gloves.
WARNING 	This product is for laboratory and manufacturing production use only. Not for administration to humans.
IMPORTANT 	This product is shipped in an 18.0 ±1% ethanol solution, a recognized bacteriostatic agent. It is flushed from the resin during equilibration and preparation for use. Follow all local regulations for safe disposal.
IMPORTANT 	Dispose of contents/container in accordance with local/regional/national/ international regulations.
IMPORTANT 	For a full list of precautionary statements, please read the Safety Data Sheet (SDS).

4. Key Performance Attributes

Key performance attributes of AVIPure HiPer AAV Affinity Resin include:

- High capacity
- Fast operating flow rates (30 – 60 second residence times)
- High yield (>80% yield)
- High purity (HCP ≥2 log reduction)
- Complete elution at pH 3
- Clean with 0.5 M NaOH

Table 3. Performance Characteristics of AVIPure HiPer AAV Affinity Resin

Category	Description
Base matrix	Cross-linked polymethacrylic polymer
Particle size (d_{50v})	50 µm
Ligand	Alkali-tolerant peptide or recombinant protein (animal-free)
Coupling chemistry	Epoxide
Binding capacity	≥7.0 x 10^{14} vp/mL_{Res} at 30 seconds residence time
Buffer compatibility	Stable to all commonly used aqueous buffers, including 8 M urea, 6 M guanidine hydrochloride, ethylene glycol, and detergents
Solvent compatibility	Water, alcohol (0 – 20% v/v), acetonitrile, 1 – 2 M acetic acid, other common organic solvents
pH stability	1 – 13
Cleaning-in-place (CIP) stability	0.1 – 0.5 M NaOH
Pressure/flow[a]	>600 cm/h at 2 bar
Maximum packing pressure (ΔP)[a]	2.8 – 3 bar
Temperature stability	2 – 40 °C
Delivery conditions	18% – 20% ethanol
Storage	2 – 8°C, 18% – 20% ethanol; do not freeze

[a]In a 10 x 10 cm OPUS® column

5. Process Development

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 4. 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 4. 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 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.

5.1 Equilibration and Binding

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.

5.2 Wash Conditions

Optimized wash conditions ensure high purity AAV preparations. After loading the feed stock, washing unbound material with five CV of equilibration buffer is recommended. An additional intermediate wash step can further increase final purity. Screening diverse wash buffers (pH 4 – 9) can help reduce host cell proteins (HCP). Wash additives shown to reduce HCP include:

- Arginine: 50 – 250 mM
- Chaotropic agents (e.g., urea, guanidine): 0.25 – 1 M
- High salt (e.g., NaCl, $MgCl_2$): 0.2 – 1 M
- Octanoic (caprylic) acid: 25 – 100 mM
- Tetramethyl ammonium chloride (TMAC): 0.5 – 1 M
- Organic alcohols (e.g., propylene glycol, 1,6-hexanediol, ethanol): 5% – 20%
- Osmoprotectants (e.g., trehalose, sucrose, glycine betaine): 5% – 20%

5.3 Elution

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 strip/CIP step. Addition of other excipients such as arginine, magnesium salts, or propylene glycol may also improve recovery, possibly without reducing pH.

- Arginine: up to 1 M
- $MgCl_2$: up to 1 M (See note below)
- Propylene glycol: up to 50% (high viscosity buffers require reduced flow rates to avoid overpressure)

Note: *$MgCl_2$ and NaOH will form precipitates. If using $MgCl_2$ in the elution buffer, be sure to include an intermediate wash between elution and CIP/strip steps that use NaOH to prevent precipitation.*

Combinations of additives can act synergistically for elution and should be evaluated for higher pH elution. Step elution can achieve high product concentrations; product typically elutes in two to three column volumes. Immediate pH neutralization of the elution fraction can help maintain product integrity.

5.4 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.

5.5 Cleaning-In-Place and Sanitization

AVIPure HiPer AAVis an alkali-tolerant resin 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.

A robust CIP process can help maintain the consistency of key process parameters such as flow properties, binding capacity, and clearance of HCP and DNA across multiple cycles. To identify the most desirable CIP conditions for a specific process scenario, concentration and contact time of NaOH exposure should be empirically determined to suit individual process requirements. Optimizing the CIP regime can provide an ideal balance of chromatographic performance, product quality, and resin lifetime.

An CIP protocol could entail:

1. Sanitize with 0.5 M NaOH prior to first use.
2. Clean-in-place with 0.1 M NaOH exposure for 15 – 30 minutes after each cycle.
3. Sanitize with 0.5 M NaOH for 30 minutes exposure every 10th cycle, or prior to long term storage. Before storing the column in storage solution (e.g., 20% ethanol or 2% benzyl alcohol), the column should be neutralized with, for example, equilibration buffer.

AVIPure HiPer AAV Affinity Resins can withstand up to 10 hours exposure to 0.5 M NaOH without significant loss of capacity.

5.6 Storage

AVIPure HiPer AAV Affinity Resins are shipped stored in 18% – 20% ethanol. Keep unused columns at 2 – 8°C. Do not freeze. Following sanitization and neutralization, store columns at 2 – 8°C (long term) with an appropriate bacteriostatic agent, such as 20% ethanol.

6. Polish step

Repligen HiPer QA resin can be used for rapid, high resolution empty/full capsid separation. Contact your account manager or regional customer service for more information.

7. Ordering Information

AVIPure HiPer AAV Affinity Resins can be ordered in OPUS pre-packed columns with a maximum bed height of 10 cm. Contact your account manager or your regional customer service using the email addresses below to place an order:

US: customerserviceUS@repligen.com
EU: customerserviceEU@repligen.com
China: customerserviceCN@repligen.com

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