AVIPure® AAV Affinity Resins

User Guide

For use with:

- AVIPure AAV2 Affinity Resin
- AVIPure AAV5 Affinity Resin
- AVIPure AAV8 Affinity Resin
- AVIPure AAV9 Affinity Resin

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Abbreviations

AAV	Adeno-associated virus
As	Asymmetry
Bar	Equal to 100,000 Pascal
C	Celsius
CCCF	Clarified cell culture fluids
CF	Compression factor
cm	Centimeter
CV	Column volumes
DNA	Deoxyribonucleic acid
HCDNA	Host cell DNA
HCP	Host cell protein
HETP	Height equivalent to a theoretical plate
M	Molar
$MgCl_2$	Magnesium chloride
mL	Milliliter
mm	Millimeter
mM	Millimolar
MPa	Megapascal
NaCl	Sodium chloride
NaOH	Sodium hydroxide
pH	A measure of how acidic/basic a solution is
psi	Pounds per square inch
TMAC	Tetramethyl ammonium chloride
µm	Micrometer or Micron, a metric unit of measure for length equal to 0.001 mm

1. AVIPure® AAV Affinity Resins: AAV2, AAV5, AAV8, and AAV9

AVIPure AAV Affinity Resins incorporate alkali-tolerance for simple, one-step purification of serotype specific adeno-associated virus (AAV) vectors directly from clarified cell culture fluids (CCCF).

Table 1. Performance characteristics of AVIPure AAV Affinity Resins

Category	Description
Base matrix	Cross-linked agarose, spherical
Particle size (d_{50v})	~ 50 µm
Ligand	AAV2, AAV5, and AAV8: Alkali-tolerant recombinant protein (animal free) AAV9: Alkali-tolerant, peptide (synthetic)
Coupling chemistry	AAV2: Thiol AAV5: Epoxy AAV8 and AAV9: Carbamate
Binding capacity	>2 x 10^{14} vp/mL of resin at 1-minute residence time, and >7 x 10^{14} vp/mL of resin at 4-minute residence time depending upon capsid serotype, mutations, and composition of feed stock
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 stability	0.1 - 0.5 M NaOH
Pressure/flow[a]	3 bar at >300 cm/hr
Maximum pressure (ΔP)[a]	40 psi
Temperature stability	2 - 40 °C
Delivery conditions	2% benzyl alcohol (slurry), or 20% ethanol (pre-packed columns)
Storage	2 - 8 °C, 2% benzyl alcohol or 20% ethanol; do not freeze

[a] In a 2.6 x 20 cm column pressure packed at 4 bar.

Key performance attributes of AVIPure AAV Affinity Resins include:

- Binds AAV serotypes with high capacity in typical cell culture conditions
- Sanitize with up to 0.5 M NaOH
- Retain high capacity at residence times as short as 1 minute
- Reduce residual host cell protein (HCP) and host cell DNA (HCDNA)
- Use with standard bioprocess columns and relevant process flowrates

2. Process development recommendations

Optimal conditions for purification of AAV using AVIPure AAV Resins must be determined empirically for each AAV construct. Some general process development recommendations for identification of optimal process conditions are provided below and summarized in Table 2. For the most up to date application notes, please refer to www.repligen.com/resources.

Table 2. Recommended purification protocol for AVIPure AAV Affinity Resins to purify viral vectors from concentrated CCCF

Step	Column volumes	Residence time	Suggested buffer
Sanitization	3 - 5 cv	4 - 6 min	0.1 M NaOH
Equilibration	8 cv	4 min	20 mM Tris, 150-400 mM NaCl, pH 7.5 Generally, equilibration can be matched to lysis buffer
Load	Titer dependent cv	1 - 4 min	As an example, with a post-concentration titer of 1.0E+13 vp/mL, load 20 CV for 2.0E+14 vp/mLRES
Wash 1	5 cv	4 min	Equilibration buffer
Wash 2 (if needed)	5 cv	4 min	As an example, 20 mM Tris, 50 mM octanoic acid, 0.5 M urea, pH 8.0 **OR** 20 mM Tris, 0.5-1 M NaCl, pH 7.5 may improve product purity
Wash 3	2 cv	4 min	Equilibration buffer
Elution	5 cv	4 min	50 - 100 mM Glycine, 150 mM NaCl, pH 2.0[a] • Elute fractions into 1 M Tris, pH 9 neutralization buffer (10 - 20% fraction volume) • Upflow elution may improve yield/recovery • For most efficient elution, maintain a 4-minute residence time even if using shorter residence time for other steps.
Strip	2 cv	4 min	0.1 M NaOH or process specific (e.g., pH <2.0)
CIP[b]	5 or 1 cv	3 or 15 min	0.1 M NaOH
Re-equilibration	8 cv	4 min	Equilibration buffer

[a]See Table 3 for higher pH elution options.
[b]Total contact time for CIP should be 15 minutes. Can be used without separate strip step.

Table 3. Buffers demonstrated to give high recovery at pH 3

Serotype	Elution buffer
AAV2	50 - 100 mM glycine, 150 mM NaCl, pH 3
AAV5	
AAV8	
AAV9	30% ethylene glycol, 0.1 M glycine, 150 mM NaCl[a], pH 3

[a]High recoveries have been achieved for some AAV9 capsids without including NaCl in this buffer. Include NaCl initially to establish baseline performance.

The hardened agarose base bead enables use in typical bioprocess column diameters and bed heights (5 - 20 cm) and can be operated at short residence time, enabling **direct capture** from clarified lysate. The same protocol as shown above can be followed, but reducing the residence times for equilibration, washes, and re-equilibration to match the load residence time, which can be as short as 1 minute. For residence times less than two minutes, use of a shorter bed height (e.g., 5 cm) is recommended.

2.1 Equilibration and binding conditions

Binding of AAV viral vectors to AVIPure AAV Resins has been demonstrated with buffers at near-neutral pH (6 - 9) and over a wide range of ionic strength (100 - 400 mM NaCl). Salt concentrations greater than 400 mM NaCl have not been tested. Most conventional buffers (e.g., phosphate, citrate, acetate, Tris) may be used during equilibration and loading.

Note: *AVIPure AAV2 capsid variants can require significantly different process conditions, especially ionic strength. It is recommended to determine the salt sensitivity of each viral vector.*

2.2 Wash conditions

Optimized wash conditions ensure high purity AAV preparations. After loading the feed stock, washing unbound material with five column volumes (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, MgCl2): 0.2 - 1 M
- Octanoic (caprylic) acid: 25 - 100 mM
- Tetramethyl ammonium chloride (TMAC): 0.5 - 1 M
- Additional for AAV9:
  - Organic alcohols (e.g., propylene glycol, 1,6-hexanediol, ethanol): 5 - 20%
  - Osmoprotectants (e.g., trehalose, sucrose, glycine betaine): 5 - 20%

2.3 Elution conditions

Viral particles can be eluted from the affinity resin with low pH buffers. Due to the variability in vector tolerance to low pH across different AAV sub-serotypes, elution conditions must be optimized experimentally.

If elution above pH 3 is desired, citrate or acetate buffer systems at pH 3.0 - 4.5 with the following additives are recommended:

- Arginine: up to 1 M
- $MgCl_2$: up to 1 M (See note below)
- Ethylene glycol: up to 30%
- Propylene glycol: up to 20%

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.

2.4 Cleaning-In-Place and Sanitization conditions

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 example 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, for example with equilibration buffer.

AVIPure® AAV2, -AAV8, and -AAV9 can withstand 20 or more 30-minute exposures to 0.5 M NaOH, while AVIPure AAV5 can withstand five such exposures. The following sanitization protocol can be used for AVIPure AAV Resins.

1. Wash the column with 5 column volumes of equilibration buffer
2. Apply 5 column volumes of 0.5 M NaOH at a 6-minute residence time or perform a static hold for a total contact time of 30 minutes. NaOH contact time exposure should be empirically determined for each capsid and process.
3. Re-equilibrate the column with ≥5 column volumes of equilibration buffer.

3. Storage

AVIPure® AAV Resins are stored in 2% benzyl alcohol, packed columns in 20% ethanol. Keep unused resin in its original container and store at 2 - 8 °C. Do not freeze. After sanitization, store packed columns at room temperature (short term) or at 2 - 8 °C (long term) with an appropriate bacteriostatic agent such as 20% ethanol or 2% benzyl alcohol.

4. Column packing

AVIPure AAV is based on a 50 µm highly cross-linked rigid agarose base matrix developed for bioprocess applications. Pack in bioprocess column sizes with standard procedures developed for similar chromatography resins.

Pack laboratory scale and small-scale production columns according to the following instructions: The AVIPure® AAV storage solution should be exchanged with purified water or 100 mM NaCl before packing into a column. The packing solution should be filtered and then degassed prior to use.

Exchange of the storage solution can be done by either: (1) repeatedly settling the resin, decanting the buffer and re-mixing the resin in the packing buffer, or (2) pouring the resin slurry into the column, draining off the storage solution, and replacing it with the packing buffer. In either case, equilibrate the resin slurry temperature in the packing location prior to the buffer exchange procedure.

The recommended compression factor, the ratio of the settled bed height prior to column packing to the final bed height, is 1.20. If flow packing with buffer recycling, the minimum volume of packing buffer required is 3 - 4 times the packed bed volume.

If packing a column to more than 50% of the column hardware length, use an extension reservoir.
The following procedure can be used to pack a column:

1. Ensure the storage solution has been fully removed and the resin is in packing buffer at a slurry concentration between 45 and 60%. Magnetic stir bars are not recommended for slurry mixing due to potential damage to the resin from grinding against the container surface.
2. Calculate the slurry volume by dividing the column volume by the slurry concentration and multiplying the obtained ratio by the compression factor (CF).
3. Clean column hardware and frits.
4. Secure the column in a vertical and plumb position.
5. Wet the surface of the bottom frit with a small volume of packing solution.
6. Mix the packing slurry fully. Gently pour the slurry down the side of the column using care to ensure that air is not trapped in the slurry as the column is filled.
7. Fill the column with packing buffer completely. Remove air from the inlet flow adapter by flowing packing buffer through the tubing and frit. Stop the flow and attach the inlet fitting to the column ensuring that no air is trapped between the inlet frit and the slurry (hint: insert the top adapter at a 45° angle).
8. Confirm the expected compression factor when the resin has settled to the target bed heights.
9. For the Omnifit™ 10/100 column, follow steps 10 - 14. For HiScale™ 16 or 26 columns, follow steps 15 - 19.
10. OMNIFIT™ 10/100 COLUMNS
11. Open the column outlet and start packing buffer flow at 200 cm/h to remove air from the flow adaptor. Stop the flow and bring the top adaptor to approximately 1 mm above the bed formation. Restart the flow and connect the bottom tubing to the system.
12. Continue flowing and bring the adapter down to the target bed height. No further compression is needed.
13. Condition the packed column at 200 cm/h by flowing 3 column volumes of packing buffer upflow, followed by 3 column volumes downflow.
14. Repeat step 12 three times

Note: *check the pressure; for 5 cm bed height usually it is less than 3 bar = 0.3 MPa; if a gap has formed, lower the adapter and repeat the steps 12 - 13.*

15. The column is now ready to be tested.
16. HISCALE™ 16 OR 26 COLUMNS

17. Open the column outlet and start packing buffer flow at 300 cm/h.
18. Continue compressing the bed by flow for approximately 20 minutes.
19. Stop the flow and disconnect the tubing from the top of the column.
20. Manually compress the bed by adjusting the adaptor until the target bed height is reached.
21. The column is now ready to be tested.

5. Column integrity testing

Tested for mechanically correct packing by the application of either an acetone spike or high salt spike and recording the resultant peak. This test can also be used between runs to evaluate changes in bed integrity.

Evaluate the column packing efficiency by using a 2% CV plug injection of 1 - 2% acetone in packing buffer. This test should be conducted at a low linear velocity, typically around 30–60 cm/hr. Calculate the number of theoretical plates (N), the reduced plate height (h) from the plate height (HETP) and the peak asymmetry (As) by standard procedures described by the following equations:

$$N = 5.54 \times \left(\frac{Vr}{W_h}\right)^2$$

$$HETP = \frac{L}{N}$$

$$h = \frac{HETP}{d_{50v}}$$

$$As = \frac{b}{a}$$

Where V_r is volume eluted from the start of the sample application to the peak maximum, W_h is the width of the recorded peak at half of the peak height (V_r and W_h have the same units, e.g., CV, time, volume), L is bed height (cm), d_{50v} is mean particle size (cm; for AVIPure® AAV, d_{50v} = 0.005 cm), b and a are widths of descending and ascending parts of the peak measured at 10% of the peak height, respectively.

For a well packed AVIPure AAV Column, expected quality limits include:

- Asymmetry (As): 0.8 - 2
- Reduced height equivalent of a theoretical plate (h): <4

6. Ordering Information

Items listed here are available through the Repligen e-store (store.repligen.com) for most regions. You can also contact your sales representative or customer service for sales, or the email addresses for the regions listed below:
US: customerserviceUS@repligen.com
EU: customerserviceEU@repligen.com
China: customerserviceCN@repligen.com

Table 4. Product list – Resins

Resin	Item number	Item description
AVIPure® AAV2 Affinity Resin	100AAV2-10	AVIPure AAV2 Affinity Resin, 10 mL
	100AAV2-25	AVIPure AAV2 Affinity Resin, 25 mL
	100AAV2-50	AVIPure AAV2 Affinity Resin, 50 mL
	100AAV2-100	AVIPure AAV2 Affinity Resin, 100 mL
	100AAV2-250	AVIPure AAV2 Affinity Resin, 250 mL
	100AAV2-1000	AVIPure AAV2 Affinity Resin, 1 L
AVIPure® AAV5 Affinity Resin	100AAV5-10	AVIPure AAV5 Affinity Resin, 10 mL
	100AAV5-25	AVIPure AAV5 Affinity Resin, 25 mL
	100AAV5-100	AVIPure AAV5 Affinity Resin, 100 mL
	100AAV5-500	AVIPure AAV5 Affinity Resin, 500 mL
	100AAV5-1L	AVIPure AAV5 Affinity Resin, 1 L
	100AAV5-5L	AVIPure AAV5 Affinity Resin, 5 L
AVIPure® AAV8 Affinity Resin	100AAV8-10	AVIPure AAV8 Affinity Resin, 10 mL
	100AAV8-25	AVIPure AAV8 Affinity Resin, 25 mL
	100AAV8-50	AVIPure AAV8 Affinity Resin, 50 mL
	100AAV8-1000	AVIPure AAV8 Affinity Resin, 100 mL
	100AAV8-250	AVIPure AAV8 Affinity Resin, 250 mL
	100AAV8-1000	AVIPure AAV8 Affinity Resin, 1 L
AVIPure® AAV9 Affinity Resin	100AAV9-10	AVIPure AAV9 Affinity Resin, 10 mL
	100AAV9-25	AVIPure AAV9 Affinity Resin, 25 mL
	100AAV9-50	AVIPure AAV9 Affinity Resin, 50 mL
	100AAV9-100	AVIPure AAV9 Affinity Resin, 100 mL
	100AAV9-250	AVIPure AAV9 Affinity Resin, 250 mL
	100AAV9-1000	AVIPure AAV9 Affinity Resin, 1 L

Table 5. Product list – OPUS® Columns pre-packed with AVIPure® AAV Resins

Resin	Item number	Item description
AVIPure AAV2 Affinity Resin	23051008R	200 µL RoboColumn® - strip of 8 columns, 0.5 x 1 cm
	23051008R-30	600 µL RoboColumn - strip of 8 columns, 0.5 x 3 cm
	23051006*	1 mL Pre-packed MiniChrom® Column, 0.5 x 5 cm
	23051007*	5 mL Pre-packed MiniChrom Column, 1.13 x 5 cm
	23051004-100	5 mL Pre-packed MiniChrom Column, 0.8 x 10 cm
AVIPure AAV5 Affinity Resin	23051308R	200 µL RoboColumn - strip of 8 columns, 0.5 x 1 cm
	23051308R-30	600 µL RoboColumn - strip of 8 columns, 0.5 x 3 cm
	23051306*	1 mL Pre-packed MiniChrom Column, 0.5 x 5 cm
	23051307*	5 mL Pre-packed MiniChrom Column, 1.13 x 5 cm
	23051304-100	5 mL Pre-packed MiniChrom Column, 0.8 x 10 cm
AVIPure AAV8 Affinity Resin	23051108R	200 µL RoboColumn - strip of 8 columns, 0.5 x 1 cm
	23051108R-30	600 µL RoboColumn - strip of 8 columns, 0.5 x 3 cm
	23051106*	1 mL Pre-packed MiniChrom Column, 0.5 x 5 cm
	23051107*	5 mL Pre-packed MiniChrom Column, 1.13 x 5 cm
	23051104-100	5 mL Pre-packed MiniChrom Column, 0.8 x 10 cm
AVIPure AAV9 Affinity Resin	23051208R	200 µL RoboColumn - strip of 8 columns, 0.5 x 1 cm
	23051208R-30	600 µL RoboColumn - strip of 8 columns, 0.5 x 3 cm
	23051206*	1 mL Pre-packed MiniChrom Column, 0.5 x 5 cm
	23051207*	5 mL Pre-packed MiniChrom Column, 1.13 x 5 cm
	23051204-100	5 mL Pre-packed MiniChrom Column, 0.8 x 10 cm

*5 cm bed height is recommended for use with fast flow rates (i.e., 1-minute residence times)

Note: *Additional custom configurations are also available. Contact your sales representative for additional information.*

Table 6. Product list – Residual ligand assay kits

Item number	Item description
F1000	AVIPure AAV2 Residual Ligand Assay Kit
F1005	AVIPure AAV8 Residual Ligand Assay Kit
F970	AVIPure AAV9 Residual Ligand Assay Kit

Residual ligand assay kits for AVIPure AAV2, -AAV8, and -AAV9 Affinity Resins are available through Cygnus Technologies www.cygnustechnologies.com; 1-910-454-9442; orders@cygnustechnologies.com.

Residual ligand assay kit for AVIPure AAV5 will be available through Repligen.

7. Index

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