

# AVIPure® AAV Affinity Resins

## User Guide

For use with:

- AVIPure® AAV2 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 <sub>2</sub>	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, 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 <sub>50v</sub> )	~ 50 µm
Ligand	AAV2 and AAV8: Alkali-tolerant recombinant protein (animal free) AAV9: Alkali-tolerant, peptide (synthetic)
Coupling chemistry	AAV2: Thiol AAV8 and AAV9: Carbamate
Binding capacity	>2 x 10 <sup>14</sup> vp/mL of resin at 1 minute residence time, and >7 x 10 <sup>14</sup> 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 <sup>a</sup>	3 bar at >300 cm/hr
Maximum pressure (ΔP) <sup>a</sup>	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

<sup>a</sup> 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
- Clean with 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 <https://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 (min)	Suggested buffer
Sanitization	3–5	4–6	0.5 M NaOH
Equilibration	8	4	50 mM Tris, 400 mM NaCl, pH 7.5 Generally, equilibration can be matched to lysis buffer
Load	Titer dependent	4	-
Wash 1	5	4	Equilibration buffer
Wash 2 (if needed)	5	4	50 mM Tris, 50 mM octanoic acid, 0.5 M urea, pH 8.0
Wash 3	2	4	Equilibration buffer
Elution	5	4	50 mM Glycine, 150 mM NaCl, pH 2.0; upflow elution may improve yield/recovery (Neutralize collected fractions with 1 M Tris, pH 9)
Strip	2	4	Process specific (e.g., pH <2.0)
CIP <sup>a</sup>	5 or 1	6 or 30	0.5 M NaOH
Re-equilibration	8	4	Equilibration buffer

<sup>a</sup>Total contact time for CIP should be 30 minutes.

The recommended protocol for dilute feed streams is the same as that shown in Table 2 for concentrated feed streams, but residence times for equilibration, load washes, and re-equilibration steps may be reduced to match the residence time of the load. The agarose base bead enables use in typical bioprocess column diameters and bed heights (5–20 cm). For short residence times, use of a shorter bed height (e.g., 5 cm) is recommended.

For higher pH elution conditions, refer to section 2.3.

### 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 for AVIPure® AAV2:** 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, MgCl<sub>2</sub>): 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 (e.g., pH 2.0–3.0). Due to the variability in vector tolerance to low pH across different AAV sub-serotypes, elution conditions must be determined experimentally. A low pH elution buffer of 50 mM glycine, 150 mM NaCl, pH 2.0–3.0 can be used as a recommended starting point. If elution at higher pH 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<sub>2</sub>: up to 1 M (See note below)
- Propylene glycol: up to 70%

**Note:** MgCl<sub>2</sub> and NaOH will form precipitates. If using MgCl<sub>2</sub> 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 conditions

The alkaline tolerance of AVIPure® AAV supports the use of 0.5 M NaOH. A cleaning-in-place (CIP) process of 0.5 M NaOH exposure for 30 minutes per cycle can help maintain consistent chromatographic performance for 30 cycles of CCCF challenge. A robust CIP process can help maintain the consistency of key process parameters across multiple cycles, which include flow properties, residual HCP and DNA levels and binding capacity. Upflow based CIP may be used. The following CIP protocol represents a starting point for most 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 Concentration and 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.

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:

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

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

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

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

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](https://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](mailto:customerserviceUS@repligen.com)

EU: [customerserviceEU@repligen.com](mailto:customerserviceEU@repligen.com)

China: [customerserviceCN@repligen.com](mailto:customerserviceCN@repligen.com)

**Table 3. Product list – resins**

Product	Item number	Item description
AVIPure® AAV 2 Affinity Resin	100AAV2-10	AVIPure® AAV 2 Affinity Resin, 10 mL
	100AAV2-25	AVIPure® AAV 2 Affinity Resin, 25 mL
	100AAV2-50	AVIPure® AAV 2 Affinity Resin, 50 mL
	100AAV2-100	AVIPure® AAV 2 Affinity Resin, 100 mL
	100AAV2-250	AVIPure® AAV 2 Affinity Resin, 250 mL
	100AAV2-1000	AVIPure® AAV 2 Affinity Resin, 1 L
AVIPure® AAV 8 Affinity Resin	100AAV8-10	AVIPure® AAV 8 Affinity Resin, 10 mL
	100AAV8-25	AVIPure® AAV 8 Affinity Resin, 25 mL
	100AAV8-50	AVIPure® AAV 8 Affinity Resin, 50 mL
	100AAV8-1000	AVIPure® AAV 8 Affinity Resin, 100 mL
	100AAV8-250	AVIPure® AAV 8 Affinity Resin, 250 mL
	100AAV8-1000	AVIPure® AAV 8 Affinity Resin, 1 L
AVIPure® AAV 9 Affinity Resin	100AAV9-10	AVIPure® AAV 9 Affinity Resin, 10 mL
	100AAV9-25	AVIPure® AAV 9 Affinity Resin, 25 mL
	100AAV9-50	AVIPure® AAV 9 Affinity Resin, 50 mL
	100AAV9-100	AVIPure® AAV 9 Affinity Resin, 100 mL
	100AAV9-250	AVIPure® AAV 9 Affinity Resin, 250 mL
	100AAV9-1000	AVIPure® AAV 9 Affinity Resin, 1 L

**Table 4. Product list – OPUS® columns pre-packed with AVIPure® AAV resins**

Resin	Item number	Item description
AVIPure® AAV 2 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® AAV 8 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® AAV 9 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

Note that additional custom configurations are also available. Contact your sales representative for additional information.

**Table 5. 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® AAV affinity resins are available through Cygnus Technologies (<https://www.cygnustechnologies.com/>); 1-910-454-9442; [orders@cygnustechnologies.com](mailto:orders@cygnustechnologies.com).

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