AVIPure® HiPer™ AAV9 Affinity Resin

Data Sheet

Introduction

AVIPure® HiPer™ AAV9 Affinity Resin provides an innovative solution to long capture step processing times for AAV9 and most AAV9-derived capsids, utilizing Repligen's novel convective HiPer matrix to achieve over twice the binding capacity of other leading AAV affinity resins at a residence time as short as 30 seconds. The sodium hydroxide-stable AVIPure AAV9 ligand enables robust reusability, further improving resin productivity through unmatched cycling capability.

AVIPure HiPer AAV9 Affinity Resin provides:

- Fast operating flow rates (30 second residence times)
- Over twice the dynamic binding capacity (DBC) of other AAV affinity resins
- Repeated cleaning with up to 0.5 M NaOH
- High yield (>80%) even at low titers (e.g., 1 E+11 vp/mL) and loading to only 5% capacity
- Excellent impurity clearance (≥2 log HCP reduction)
- Low ligand leachate (<2 ppm)
- Streamlined purchasing of pre-packed OPUS® columns
- Elution at pH 3 without salt
- Companion HiPer QA resin to complete your purification toolbox

Figure 1. AVIPure HiPer AAV9 Affinity Resin Binding Capacity

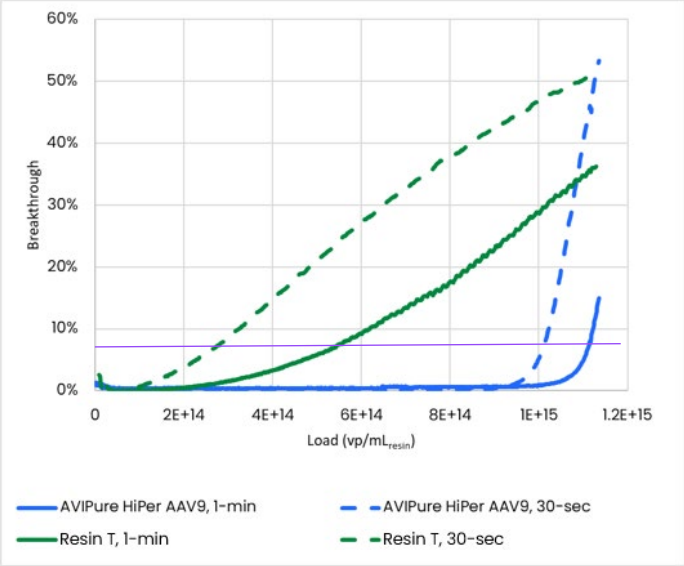

Table 1. DBC Comparison

Resin	$DBC_{5\%}$ 30 sec RT (vp/mL_{resin})	$DBC_{5\%}$ 1 min RT (vp/mL_{resn})
AVIPure HiPer AAV9	1.0×10^{15}	1.1×10^{15}
Resin T	2.3×10^{14}	4.8×10^{14}

HiPer Matrix

The HiPer matrix is a polymethacrylate, convective, spherical bead introduced in 2022 with duplicate Repligen production facilities in the US and Asia. The beads feature a nominal pore size of 1.6 µm and a 50 µm average diameter, making them ideal for processing large biologics.

The convective matrix enables high flow rates with low back pressure.

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Figure 2. Pressure vs. Flow

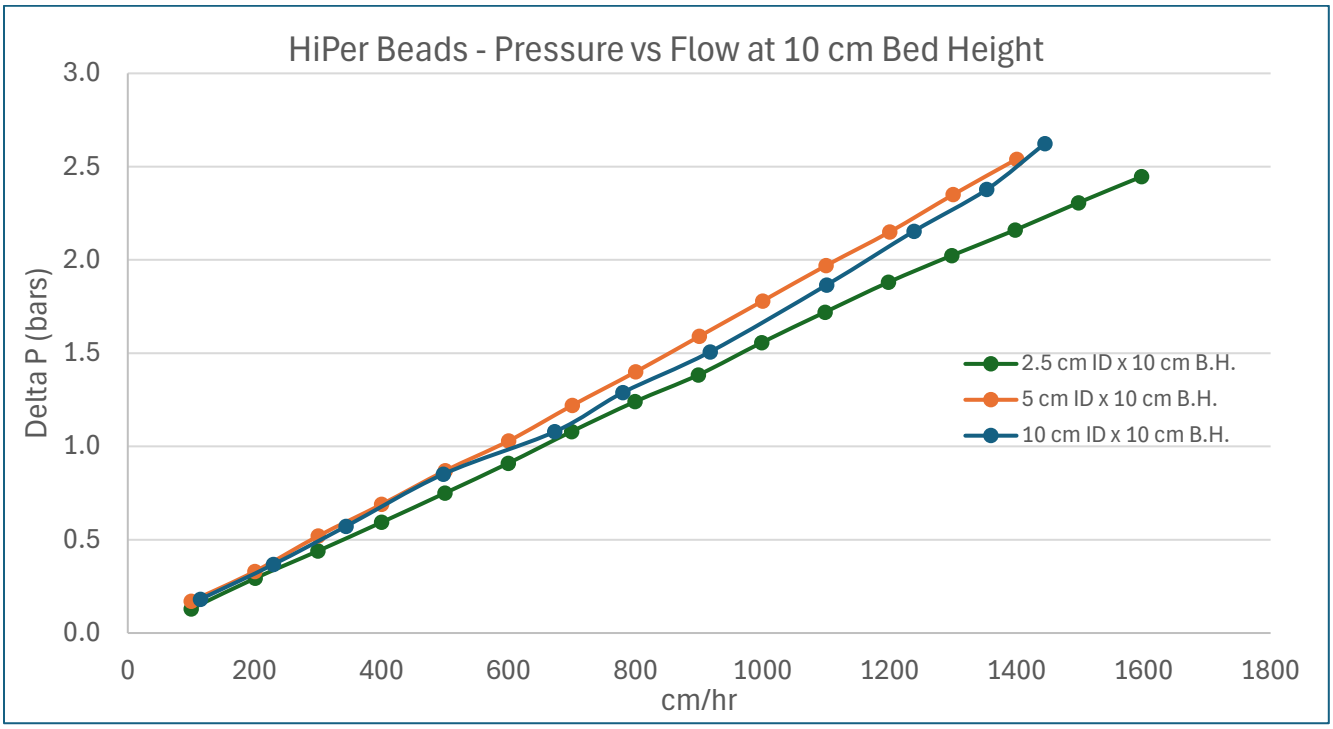
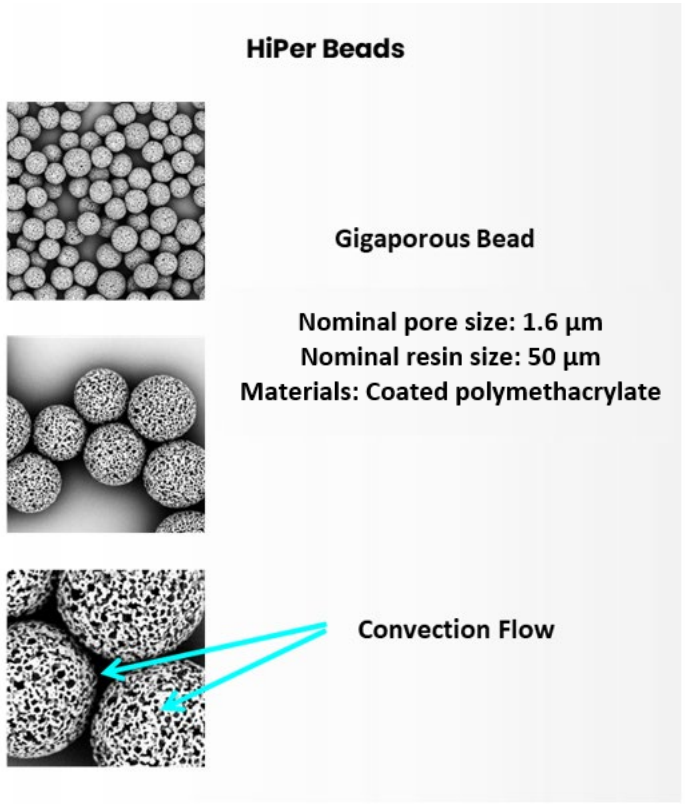


Figure 3. HiPer Resin Bead Specifications



AVIPure Ligands

AVIPure Ligands, amino acid-based and derived from a suite of libraries, are engineered for sodium hydroxide tolerance and exquisite selectivity. The AVIPure AAV9 ligand is a small, ~1.6 kDa synthetic peptide.

Repligen has collaborated with the University of Florida® to use cryo-electron microscopy (Cryo EM) to elucidate which residues of the AAV capsid bind with the AVIPure AAV9 ligand.

The AVIPure HiPer AAV9 ligand is highly specific to AAV9 and has a small binding footprint, enabling extensive capsid engineering with low risk to ligand binding, thus maintaining effective capture performance across many AAV9-derived capsids.

By identifying the specific residues involved in binding, it is possible to predict the likelihood of AVIPure HiPer AAV9 resin binding to engineered capsids or to protect critical regions during capsid engineering. Residues located within ~4 Å of the binding interface are highlighted (Figure 5). Modifications to these residues may impair binding, depending on the substitution.

Figure 4. AAV9 Capsid with Bound Ligand

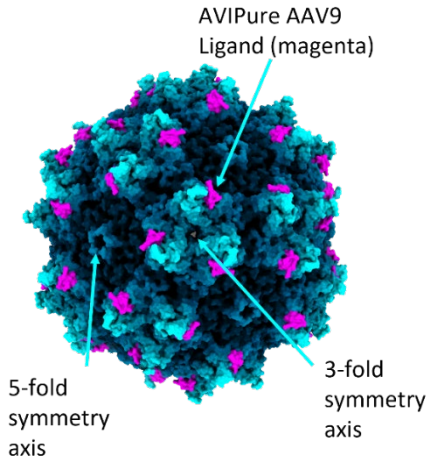


Figure 5. Residues Involved in Binding

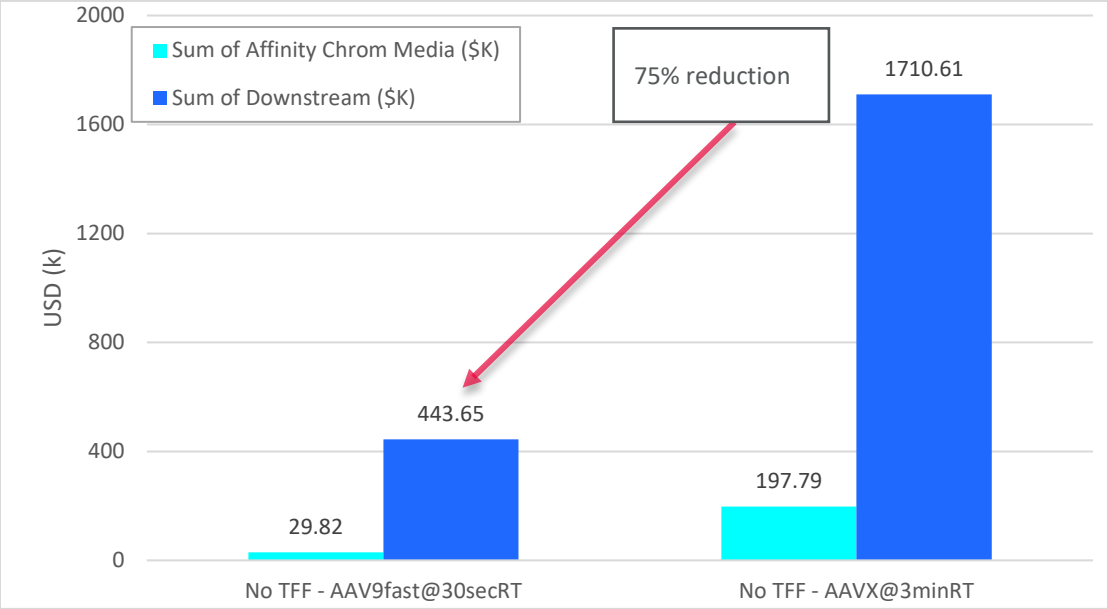
A webinar describing the cryo EM work can be viewed at https://www.insights.bio.

AVIPure HiPer AAV9 Supercharges Productivity

The higher capacity and faster processing enabled by AVIPure HiPer AAV9 resin, compared with other leading AAV affinity resins (Figure 6), reduce resin requirements and maximize facility utilization. For the purposes of the comparison below, several assumptions were made:

- Processing time: one shift (8 hr) and up to two cycles
- Bioreactor volume: 500 L
- Residence Time:
  - HiPer AAV9: 30 sec
  - Other AAV: 3 min
- Capacity:
  - HiPer AAV9: 1 x 10^{15} vp/mL
  - Other AAV: 6 x 10^{14} vp/mL
- Column size required:
  - HiPer AAV9: 10 x 9 cm (ID x BH; max 10 cm)
  - Other AAV: 20 x 18.5 cm (ID x BH; max 20 cm)
- Cost per liter based on 2026 list prices

Figure 6. Process Cost Comparison of HiPer AAV9 vs Competitor Media



In this example, AVIPure HiPer AAV9 costs are nearly 7x lower than the other AAV resin, and overall downstream costs are reduced by about 75%.

Resin Characteristics

Table 2. Resin Characteristics

Category	Description
Base matrix	Coated polymethacrylic polymer
Particle size (D_{v50})	50 µm
Ligand	Alkali-tolerant synthetic peptide (animal-free)
Coupling chemistry	Epoxide
Binding capacity (minimum)	≥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] (minimum)	> 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

Recommended Process Steps

Table 3. Process Steps

Step	Suggested Buffer	Column Volumes	Residence Time (min)	Notes
Sanitization (OPTIONAL)	Up to 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	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	Up to 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.

Robust Performance Over 20 Product Cycles using NaOH

NaOH provides the most robust cleaning-in-place option for AAV affinity resin and ensures resins can be reliably reused for numerous cycles. Published studies show that AAV affinity resins without NaOH stability cannot be fully cleaned, resulting in progressive fouling and carryover from run to run (Soni et al., Biotechnology and Bioengineering, 2025).

A cycling study was performed that shows that AVIPure HiPer AAV9 can be cycled with up to 0.5 M NaOH for consistent chromatographic performance (Figure 7), yield (Figure 8), and impurity clearance (Figure 9). For this study, a 0.5 x 5.6 cm (1.10 mL CV) column was loaded with HEK293 process-intensified AAV9 clarified harvest at 4.5 x 10^{12} vp/mL. This cycling study shows that leached ligand measured using AVIPure AAV9 assay kits from Cygnus, was no more than 1 ppm in any cycle (Figure 11).

A static hold study shows that AVIPure HiPer AAV9 maintains ≥ 80% of its initial binding capacity after 10 hours of exposure to 0.5 M NaOH (Figure 12). Capacity is further preserved with exposure to 0.1 M NaOH, with ≥95% binding retained after 10 hours exposure. Thus, if CIP with 0.1 M NaOH is effective for a given process, it can be used regularly with occasional CIP using 0.5 M NaOH (such as after a set number of cycles or prior to long term storage) to further extend the life of the resin.

Table 4. Cycling Study Process

Step	Buffer	Residence Time (min)	CV	Linear Velocity (cm/hr)
Equilibration	125 mM NaCl, 0.01% P-188, 20 mM Tris, pH 7.5	1	3	300
Load	HEK293 process-intensified AAV9 clarified harvest at 4.5 x 10^{12} vp/mL	1	178	300
Chase	125 mM NaCl, 0.01% P-188, 20 mM Tris, pH 7.5	1	5	300
Wash1	1 M NaCl, 0.01% P-188, 20 mM Tris, pH 7.5	1	5	300
Wash2	125 mM NaCl, 0.01% P-188, 20 mM Tris, pH 7.5	1	2	300
Elution[1]	0.01% P-188, 50 mM glycine, pH 3.0	1	5	300
Strip[2]	0.1 M phosphoric acid	1	5	300
CIP[3]	0.5 M sodium hydroxide	4	3.4	76
	0.5 M sodium hydroxide, 15-min static hold	N/A	0	0
Re-Equilibration	125 mM NaCl, 0.01% P-188, 20 mM Tris, pH 7.5	1	5	300

[1]Neutralized with 10% volume of 1 M Tris, pH 7.5
[2]Neutralized with 20% volume of 1 M Tris, pH 7.5
[3]Neutralized with 50% volume of 1 M Tris, pH 6.5

Table 5. Analytics

Measurement	Assay
Titer	Progen AAV9 XPress ELISA
Yield	Progen AAV9 XPress ELISA
HCP purity	Cygnus HEK293 HCP ELISA
HCDNA purity	ThermoFisher Quant-iT
Purity	Reduced SDS-PAGE, 7.5 µL of elution pool loaded
HMW aggregates	HP-SEC, Phenomenex Biozen dSEC-7, 700 Å, 3 µm, 150 x 4.6 mm

Figure 7. Chromatogram

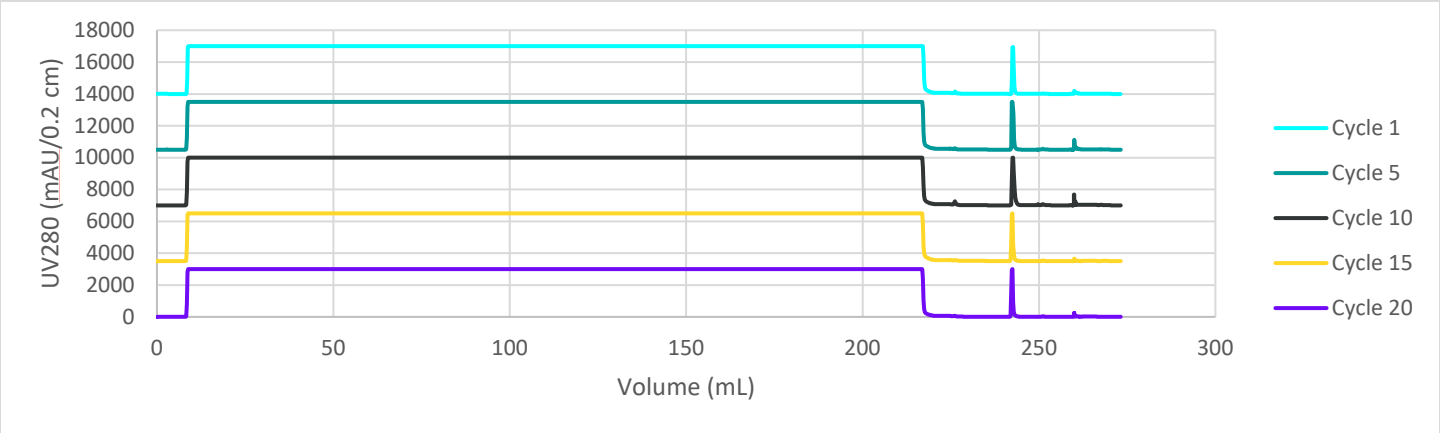


Figure 8. Yield

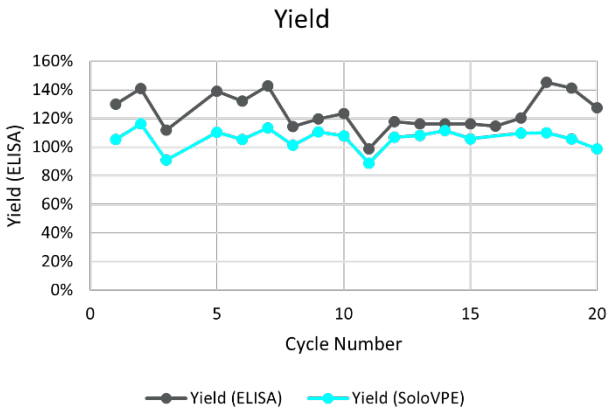


Figure 9. HCP/HCDNA Removal

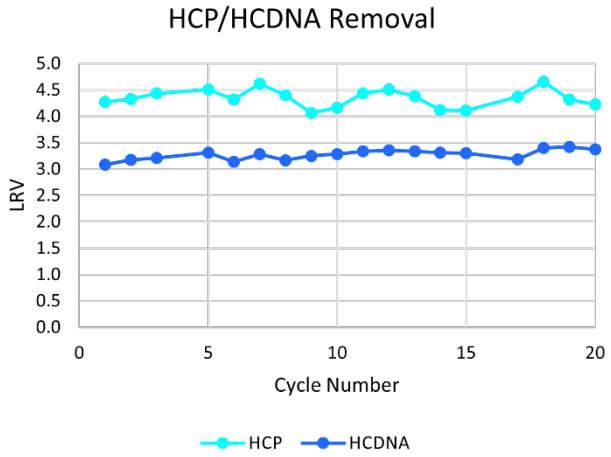


Figure 10. HP-SEC Purity

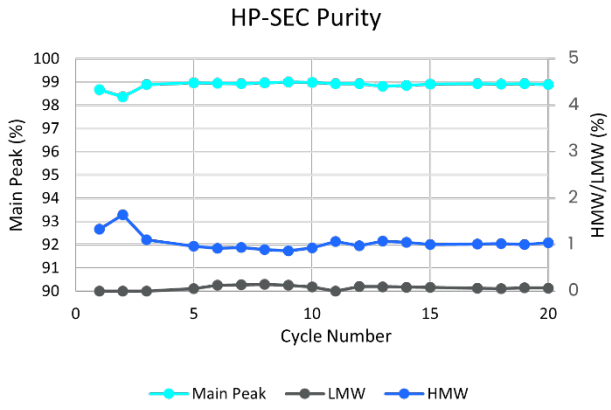


Figure 11. Residual Ligand

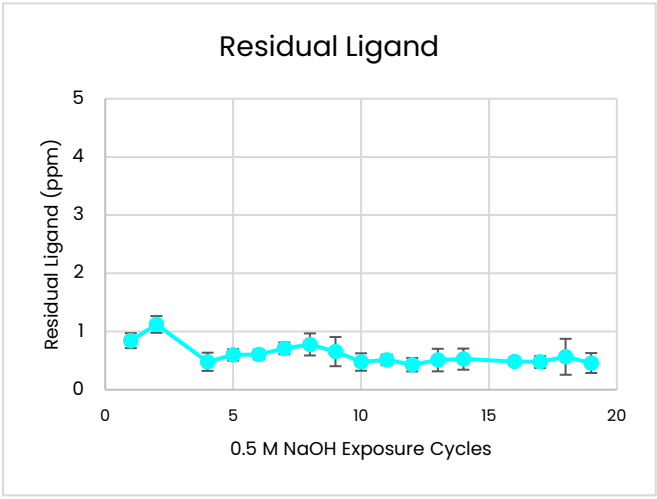
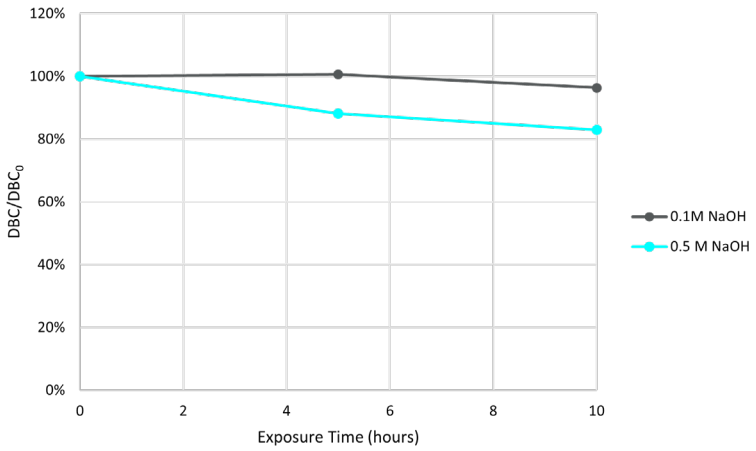


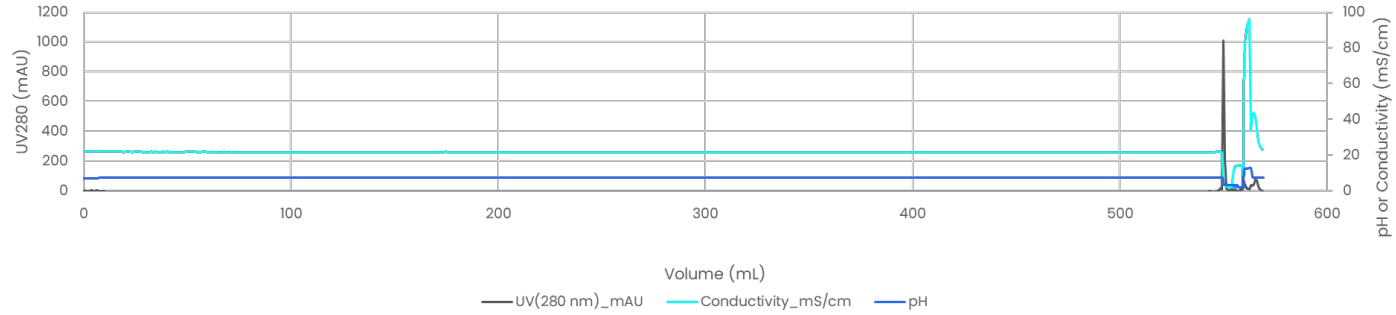
Figure 12. Dynamic Binding Capacity vs Exposure to NaOH



Performance with Low Titer Harvests and Underloaded Columns

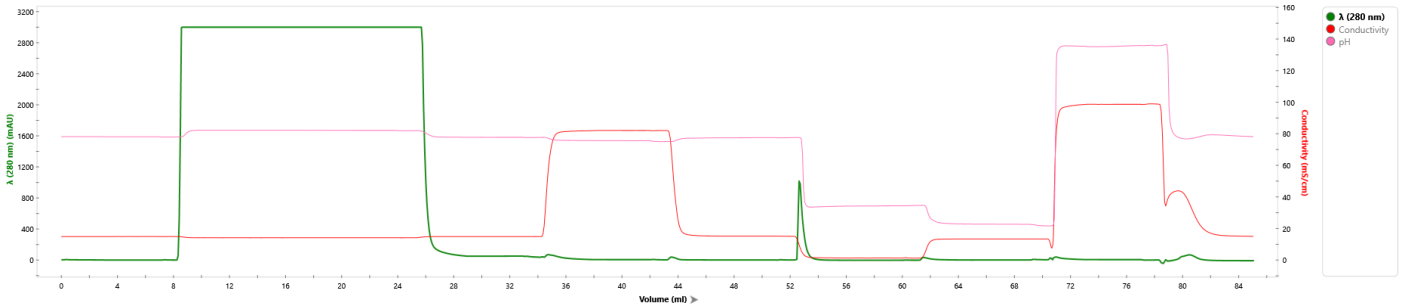
AVIPure HiPer AAV9 successfully captures capsids in a low titer harvest (1.1 x 10^{11} vp/mL). Coupled with short residence times enabled by the HiPer bead, this enables easy transition to a direct capture mode of purification whereby the pre-concentration TFF step can be eliminated. Figure 13 shows a 500 CV load on a 1 mL column completed in about two hours, resulting in 94% yield as measured by UV (PATsmart™ SoloVPE®) and 89% by total capsid ELISA.

Figure 13. Chromatogram of 540mL of Low Titer Harvest (Total Load of 5.9E13 vp)



A common challenge for AAV producers is achieving high yields if their affinity resin is not loaded close to capacity. Even when AVIPure HiPer AAV9 is loaded to 5.0E+13 vp/mLresin, less than 5% resin capacity, yields exceeding 80% are still achieved (121% by PATsmart SoloVPE and 87% by ELISA).

Figure 14. Chromatogram of High Recovery Underloaded Run



Polishing Step and Scale-up

HiPer QA ion exchange resin is recommended for the polishing step. This resin uses the same HiPer base bead and is also available through Repligen, your one stop shop for all your gene therapy needs. Contact your account manager to learn more and request a sample.

Resin Storage and Shelf Life

After CIP, neutralization, and re-equilibration, AVIPure HiPer AAV9 should be stored in 18 – 20% ethanol at 2 – 8 °C for long-term storage. Storing at refrigerated conditions minimizes risk of microbial growth. At time of launch, data are available to support a 2.5-year shelf life. Real time stability studies are ongoing.

OPUS PD (Process Development) and OPUS LS (Large-Scale) Pre-Packed Column Service

AVIPure HiPer AAV and HiPer QA resins are available exclusively in OPUS pre-packed columns, ensuring you get a properly packed column so every run is successful, and scientists can do the value-added work of running the process. AVIPure HiPer AAV and HiPer QA resins are available in sizes ranging from Robocolumns® at 50 µL to OPUS large scale columns up to 80 cm ID. OPUS columns arrive ready to run with a certificate of analysis. Off-the-shelf OPUS PD columns are shown below; custom configurations are available through the online configurator.

Table 6. Off-the-shelf AVIPure HiPer AAV9 OPUS PD Scale Pre-packed Columns

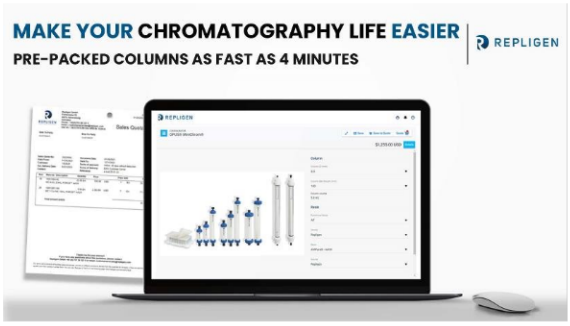
Column Volume (mL)	AVIPure HiPer AAV9 Part #	Format
8 x 200 µL	23051808R	Strip of 8 Robocolumns®
8 x 600 µL	23051808R-30	Strip of 8 Robocolumns
1 mL	23051806	0.5 x 5 cm MiniChrom column (1 mL)
5 mL	23051804-100	0.8 x 10 cm MiniChrom column (5 mL)
10 mL	23051807-100	1.13 x 10 cm MiniChrom column (10 mL)

Table 7. Off-the-shelf HiPer QA OPUS PD Scale Pre-packed Columns

Column Volume (mL)	HiPer QA Part #	Format
1 mL	23010106	0.5 x 5 cm MiniChrom column (1 mL)
5 mL	23010104-100	0.8 x 10 cm MiniChrom column (5 mL)
10 mL	23010107-100	1.13 x 10 cm MiniChrom column (10 mL)

OPUS PD Online Column Configurator

Easily configure and quote your OPUS pre-packed columns on Repligen.com



10 clicks
4 minutes

Ease of Use
Latest web-based technology
Intuitive user interface

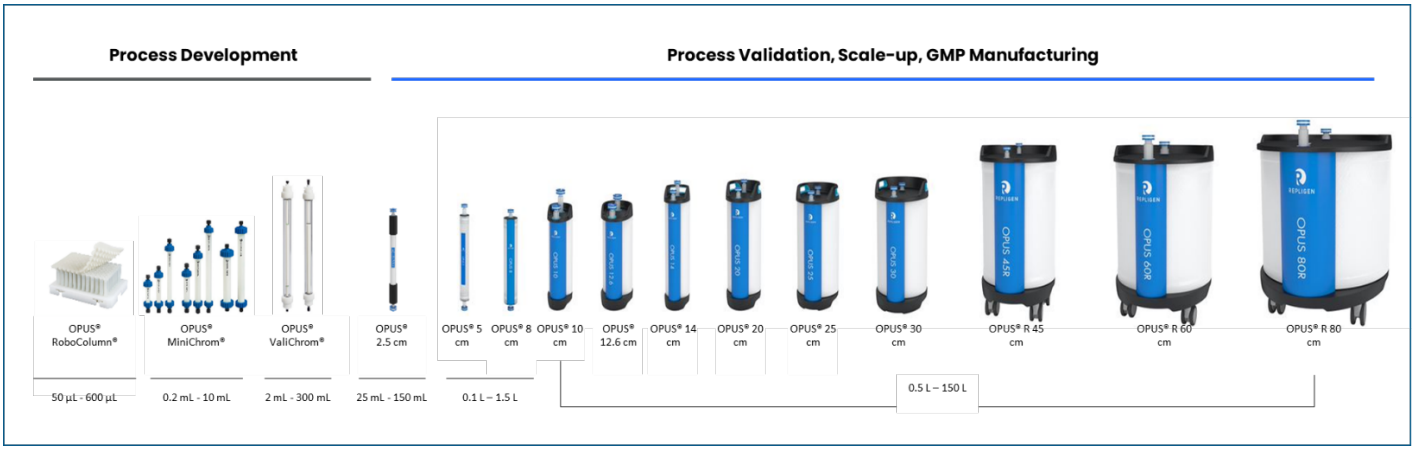
Configurable
Select column size and resin type to meet application-specific needs

Fully Automated
Remove manual steps
No prep time

Online Instructions
CLICK HERE!

https://www.repligen.com/all-styles/product-landing-page/opus-pd-configurator-campaign

Seamless scale up in OPUS pre-packed Columns from 50uL RoboColumns to 80cm diameter Large-scale GMP columns.



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