

High Productivity and Cost-Effective AAV9 Manufacturing Enabled by Advanced Macroporous HiPer™ Resins

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Application Note

Introduction

Adeno-associated virus (AAV) vectors are a leading modality for in vivo gene therapy, but commercial adoption remains constrained by manufacturing productivity, product quality requirements, process complexity, and cost. Improving vector recovery, impurity clearance, and empty/full capsid separation while reducing processing time remains a critical objective for scalable and economically viable AAV manufacturing.

This application note describes a high-productivity two-step AAV9 purification process combining AVIPure® HiPer™ AAV9 Affinity Resin capture with AVIPure HiPer QA Resin anion exchange polishing. The macroporous HiPer Resin platform utilizes large convection-flow pores (0.8 – 1.6 μm) to enable rapid mass transfer, high binding capacity, and operation at short residence times. The process was designed to support direct loading of clarified lysate, minimize process complexity, maintain high vector recovery, and achieve effective full-capsid enrichment. The resulting workflow demonstrated high throughput, strong impurity clearance, and robust empty/full capsid separation while offering a path toward improved manufacturing productivity and reduced downstream processing costs.

Materials and Methods

A clarified HEK293 lysate containing AAV9 vector particles was used as the starting material for affinity capture. The AAV9 vector contained a single-stranded DNA genome encoding enhanced green fluorescent protein (eGFP). The feedstock volume was 7.7 L, with 1.3×10^{12} AAV9 viral particles (VP)/mL and 4.0×10^{11} vector genomes (VG)/mL, corresponding to 44% full capsids. Affinity capture was performed using a 10 mL AVIPure HiPer AAV9 column (1.6 cm internal diameter (ID) x 5 cm bed height (BH)) operated at 0.5 min residence time. The load capacity was 5×10^{14} VP/mL_{resin}, and the 7.7 L clarified feedstock, corresponding to approximately 1×10^{16} total VP, was processed using two loading cycles lasting just over 3 hours each.

Table 1. AVIPure HiPer AAV9 Affinity Capture Method

Step	Buffer	Residence Time (min)	Column Volume (CV)
Equilibration	125 mM NaCl, 0.01% Poloxamer 188 (P-188), 20 mM Tris, pH 7.5	0.5	10
Load	Clarified HEK293 lysate	0.5	383
Chase	125 mM NaCl, 0.01% P-188, 20 mM Tris, pH 7.5	0.5	5
Wash 1	1 M NaCl, 0.01% P-188, 20 mM Tris, pH 7.5	0.5	5
Wash 2	125 mM NaCl, 0.01% P-188, 20 mM Tris, pH 7.5	0.5	2
Elution	0.01% P-188, 50 mM glycine, pH 3.0	0.5	5
Strip	0.1 M phosphoric acid	0.5	5

For anion-exchange chromatography (AEX) polishing, post-affinity material was diluted with 25 mM bis-tris propane (BTP), pH 9.0, and loaded onto HiPer QA columns at 0.5 min residence time.

A 1 mL (0.5 cm ID x 5 cm BH) linear gradient scouting experiment was first performed at 5×10^{13} VP/mL_{resin} to map the conductivity of empty and full capsid elutions. Following identification of 3.7 mS/cm as the empty capsid elution conductivity and 6.3 mS/cm as the full capsid elution conductivity, two 10 mL (1.6 cm ID x 5 cm BH) step elution strategies were compared at 5×10^{13} VP/mL_{resin}.

The first strategy (Table 2, Column A) used a 3.7 mS/cm load to favor empty capsid removal in the flow-through, followed by a single 6.3 mS/cm full capsid elution step. This approach was designed to favor final product purity but carried a risk of lower full capsid yield.

The second strategy (Table 2, Column B) used a lower 2.5 mS/cm load conductivity to promote complete binding of full capsids, followed by sequential elution at 3.7 mS/cm for empty capsids and 6.3 mS/cm for full capsids; this approach was designed to favor full capsid recovery while maintaining high purity.

A robustness run (Table 2, Column C) was then performed by repeating the selected two-step sequential elution strategy on a 10 mL HiPer QA column at a doubled load capacity of 1×10^{14} VP/mL_{resin}.

Analytical testing included ddPCR for quantification of vector genome titer (VG/mL) and ELISA for quantification of viral particle titer (VP/mL). SEC-MALS was used for analysis of HiPer QA fractions as an alternative or complementary method to ddPCR and ELISA, enabling assessment of empty/full capsid composition and fraction specific recovery.

Table 2. Summary of HiPer QA AEX Polishing Experiments and Key Operating Conditions

Step/Parameter	Linear-gradient scouting	(A) Single-step elution strategy	(B) Two-step sequential elution strategy	(C) High-load robustness run
Column	1 mL HiPer QA (0.5 cm ID x 5 cm BH)	10 mL HiPer QA (1.6 cm ID x 5 cm BH)		
Load conductivity (mS/cm)	1.7	3.7	2.5	
Load capacity (VP/mL _{resin})	5 x 10 ¹³			1 x 10 ¹⁴
Empty-capsid elution (mS/cm)	N/A	3.7 (during load)	3.7 (15 CV elution)	
Full-capsid elution (mS/cm)	N/A	6.3 (15 CV elution)		
Elution format	50 CV linear NaCl gradient (2.3 to 24.3 mS/cm)	Single-step elution	Two-step sequential elution	
Purpose	Identify conductivity for empty and full capsid elution	Empty capsids removed in the flow-through. Risk of full-capsid loss	Yield-favoring strategy with stronger full capsid binding before elution	Robustness run at doubled load capacity

Results and Discussion

Affinity capture enabled direct-load, high-productivity purification

The AVIPure HiPer AAV9 affinity step processed 7.7 L of clarified HEK293 lysate directly, eliminating the need for an upstream concentration step prior to capture (Figure 1). The process achieved 84% VP recovery while operating at a short 30-second residence time and delivered a specific productivity of 1.2×10^{14} VP/mL_{resin}/h (Table 3). In addition to high recovery, the affinity step provided substantial impurity removal, achieving 4.1 log reduction value (LRV) host-cell protein clearance and 2.7 LRV host-cell DNA clearance (Table 3). These results demonstrate that the macroporous HiPer architecture enables high-capacity, high-throughput processing while simplifying the overall purification workflow.

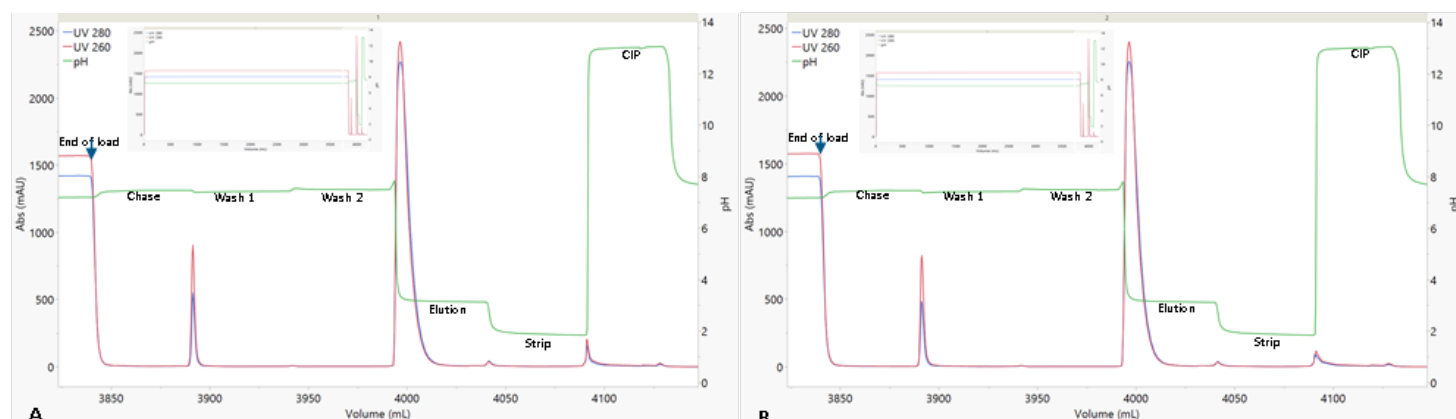


Figure 1. AVIPure HiPer AAV9 affinity capture chromatograms from two processing cycles (A: cycle 1; B: cycle 2).

Table 3. Performance of the AVIPure HiPer AAV9 Affinity Capture Step

Parameter	Value
Feedstock processed	7.7 L clarified HEK293 lysate, $\sim 1 \times 10^{16}$ total VP (1.3×10^{12} AAV9 VP/mL) processed in 7 hours across 2 cycles on a 10 mL column (5×10^{14} VP/mL _{resin})
VP Yield (%) – VP recovered	84% – 8.4×10^{15} VP
Specific productivity	1.2×10^{14} VP/mL _{resin} /h
HCP clearance	4.1 LRV
HCDNA clearance	2.7 LRV

HiPer QA scouting identified operational windows for empty/full capsid separation

The linear gradient scouting experiment identified distinct conductivity regions associated with enriched empty and full capsid populations (Figure 2), establishing practical operating conditions for subsequent process development. Empty capsids preferentially eluted at approximately 3.7 mS/cm, while full capsids eluted at approximately 6.3 mS/cm, providing the foundation for scalable step elution strategies designed to balance product purity and recovery.

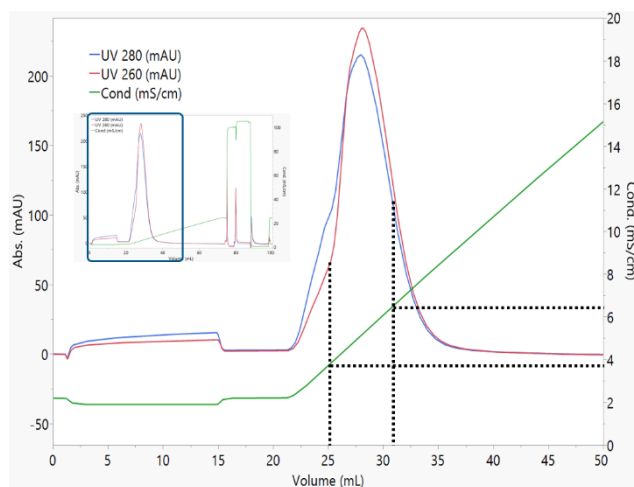


Figure 2. HiPer QA linear-gradient scouting chromatogram for empty/full AAV9 capsid separation. Conductivity mapping identified an empty-capsid elution step at 3.7 mS/cm and a full capsid elution step at 6.3 mS/cm.

Sequential elution improved recovery while maintaining high full capsid enrichment

The step elution approaches evaluated different process priorities. The single-step strategy favored operational simplicity and high final purity but resulted in substantial losses of vector genomes in the flow-through fraction (Figure 3A, Table 4). By comparison, the sequential elution strategy improved retention and recovery of full capsids while maintaining strong discrimination between empty

and full capsid populations ([Figure 3B](#), [Table 4](#)). SEC-MALS analysis demonstrated substantially higher recovery of vector genomes in the final product fraction, supporting selection of the sequential strategy for further evaluation.

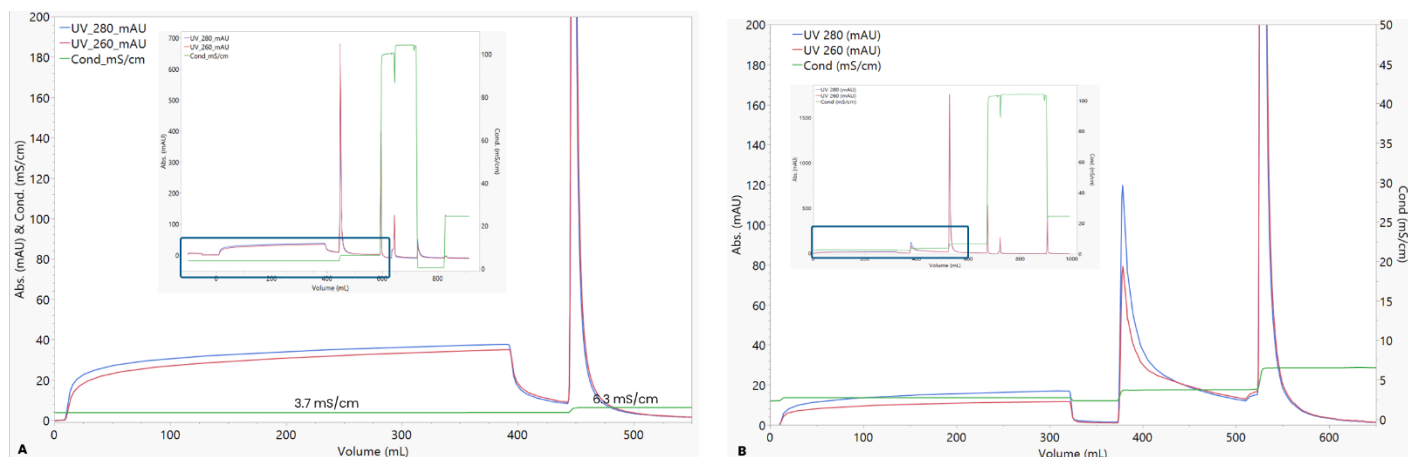


Figure 3. Chromatograms from HiPer QA single step (A) and sequential step elution (B) strategies.

Table 4. HiPer QA Polishing Performance at 5×10^{13} VP/mL_{resin} using Single and Sequential Elution, Measured by SEC-MALS.

Elution Approach	Fraction	Conductivity (mS/cm)	Full capsids* (%)	VG yield* (%)	VP yield* (%)
Single-step elution (A)	Flow-through	3.7	25	56	95
	Elution	6.3	91	32	15
Two-step sequential elution (B)	Flow-through	2.5	0	0	48
	Elution 1	3.7	14	5	15
	Elution 2	6.3	102	96	40

*Single or cumulative values above 100% reflect analytical uncertainty

High load capacity maintained process performance

When load capacity was doubled to 1×10^{14} VP/mL_{resin}, the sequential elution process maintained effective separation performance ([Figure 4](#)). No vector genomes were detected in the flow-through fraction, confirming efficient product capture under the selected loading conditions ([Table 5](#)). Although measured yield and full capsid percentages differed between analytical methods, both SEC-MALS and orthogonal ddPCR and ELISA analysis confirmed successful enrichment of full capsids in the final product fraction and demonstrated robust process performance at increased loading. These results support the scalability of the HiPer QA polishing workflow while maintaining short residence time operation.

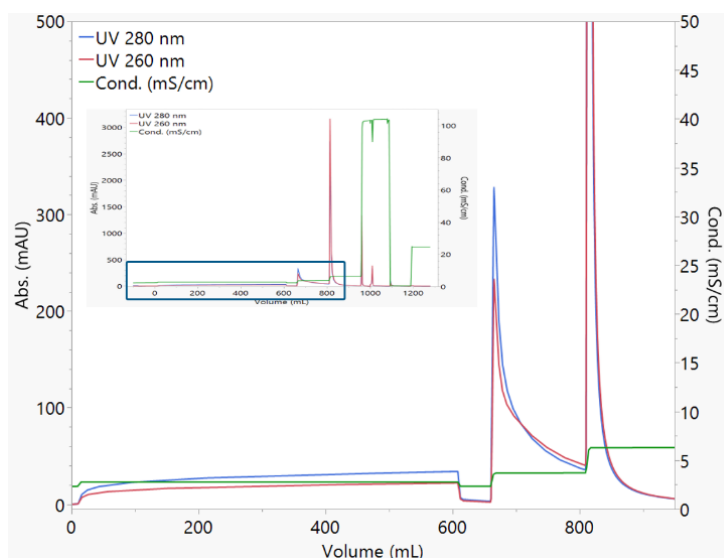


Figure 4. HiPer QA sequential elution at 1×10^{14} VP/mL_{resin}. The process used a 2.5 mS/cm load, a 3.7 mS/cm empty capsid elution, and a 6.3 mS/cm full capsid elution.

Table 5. HiPer QA Polishing Performance at 1×10^{14} VP/mL_{resin} Measured by SEC-MALS and ddPCR and ELISA.

Method	Fraction	Full capsids* (%)	VG yield* (%)	VP yield* (%)
SEC-MALS	Flow-through	0	0	41
	Elution 1	39	13	15
	Elution 2	102	96	43
ddPCR and ELISA	Flow-through	0	0	36
	Elution 1	22	11	11
	Elution 2	100	80	19

*Single or cumulative values above 100% reflect analytical uncertainty

The two-step process combined high recovery with strong impurity clearance

Combining AVIPure HiPer AAV9 affinity capture with HiPer QA polishing resulted in a streamlined two-step purification workflow delivering 66% overall yield, complete full capsid enrichment by ddPCR and ELISA analysis, and greater than 5-log reduction of both host cell proteins and host cell DNA (Table 6). Collectively, the data demonstrate that the two-step process can simultaneously address productivity, product quality, and impurity removal requirements within a simplified downstream workflow.

Table 6. Combined Two-Step Purification Performance

Step	Yield (%)	Full capsids (%)	HCP LRV	HCDNA LRV
AVIPure HiPer AAV9	84	44	4.1	2.7
HiPer QA	80	100	>1.0	>2.3
Two-step purification	66	100	>5.1	>5.0

Modeled 2,000 L scale analysis highlights productivity and cost advantages

The modeled 2,000 L manufacturing scenario was used to compare process productivity and consumable cost across three downstream purification workflows using the assumptions summarized in Figure 5. Under these model assumptions, the HiPer workflow delivered the highest productivity and the lowest consumable cost of the configurations evaluated. The improvement was primarily driven by elimination of the TFF concentration step, shorter chromatography residence times, reduced resin volume requirements using multiple cycles, and fewer overall processing hours.

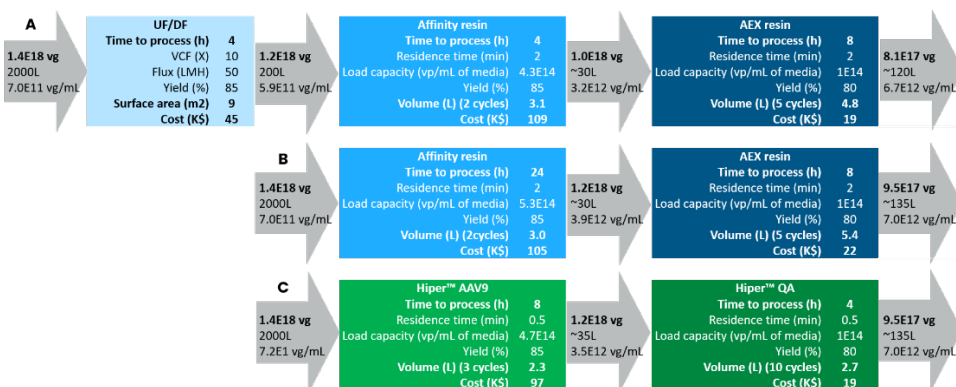


Figure 5. Modeled 2,000 L downstream purification workflows used for process productivity and consumable cost analysis. Workflow configurations include TFF volume reduction followed by affinity and AEX chromatography (A), affinity and AEX chromatography without prior TFF concentration (B), and AVIPure HiPer AAV9 affinity capture followed by HiPer QA polishing (C).

While the model is intended to illustrate relative process economics rather than predict manufacturing costs for a specific facility, the analysis demonstrates how combining direct-load affinity capture with high-capacity polishing can reduce downstream processing complexity while increasing manufacturing throughput (Figure 6).

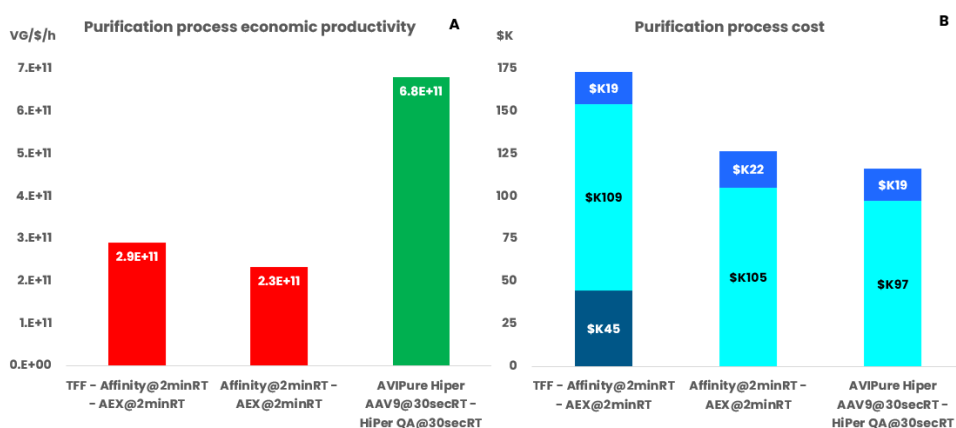


Figure 6. Modeled purification process economic productivity (A) and purification process consumable costs (B) for three 2,000 L AAV purification workflows: TFF volume reduction followed by affinity and AEX chromatography, affinity and AEX chromatography without TFF concentration, and AVIPure HiPer AAV9 affinity capture followed by HiPer QA polishing. Cost contributions from TFF modules, and affinity chromatography and AEX chromatography media are shown separately.

Conclusions

Advanced macroporous HiPer resins enabled a streamlined two-step AAV9 purification workflow that combined direct-load affinity capture with selective empty/full capsid polishing. AVIPure HiPer AAV9 affinity capture processed clarified lysate at high throughput while delivering 84% recovery and substantial HCP and hcDNA clearance. HiPer QA polishing provided effective empty/full capsid separation and generated highly enriched full capsid fractions at load capacities up to 1×10^{14} VP/mL_{resin}. The integrated process achieved 66% overall yield, complete full capsid enrichment by ddPCR and ELISA analysis, and greater than 5-log reduction of both HCP and hcDNA. In a modeled 2,000 L manufacturing scenario, the HiPer workflow delivered the highest productivity and lowest consumable costs among the workflows evaluated, highlighting the impact of direct-load processing, short residence times, and high-capacity chromatography on downstream manufacturing efficiency. Together, these results demonstrate a practical approach for increasing AAV manufacturing productivity, while reducing process complexity and downstream costs.



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