An Improved Affinity Chromatography Process for AAV6

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Introduction

Improved methods for purifying rAAV vectors can greatly facilitate manufacturing scale-up, while reducing costs. The present work focuses on improving recovery of AAV6 capsids from the affinity capture step and demonstration of resin reuse using sodium hydroxide. Capsid recovery at pH 3 can be improved using AVIPure® AAV6 resin, compared to other affinity resins.

Table 1: Performance metrics for AVIPure AAV6 resin

Performance attribute	AVIPure® AAV6
Capacity ($DBC_{10\%}$)	7.0E+14 vp/mL_{resin} at 4-min residence time 3.3E+14 vp/mL_{resin} at 1-min residence time
CIP agent	0.1 – 0.5 M NaOH
Cycling	> 60 cycles using 0.1 M NaOH (15 min/cycle) ~8 cycles using 0.5 M NaOH
% Yield	> 90%
Elution pH	pH 2 - 3

Cleanability with NaOH

AVIPure AAV resins are the only caustic stable affinity resins for AAV capture on the market. Cost savings with AVIPure AAV compared to other affinity resins is most evident for large scale production (Figure 1A).

To demonstrate caustic stability of the resin the $DBC_{10\%}$ was measured before and after a static hold in 0.1 M NaOH. A 0.3 x 2.5 cm column (0.177 mL CV) was loaded with 600 CVs of clarified AAV6 harvest, 9.5E+11 vp/mL, at a 1-minute residence time. Breakthrough curves in Figure 1B were determined by capsid ELISA of the flow through fractions.

AVIPure AAV6 maintained 85% of the initial $DBC_{10\%}$ after 15 hours exposure to 0.1 M NaOH.

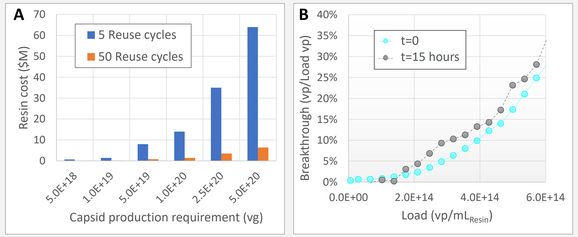


Figure 1: A) Cost of resin vs. production scale, assuming load of 100 L harvest per liter of resin and B) AAV6 breakthrough curves before and after 15 hours exposure to 0.1 M NaOH

Higher yields with pH 3 elution

AVIPure AAV6 gives nearly complete elution at pH 3 and significantly higher yield compared to a commercially-available resin. Resins were tested according to the protocol in Table 4 below with a load titer of 1E+12 vp/mL (ELISA) and elution at pH 3, pH 2.5, and finally pH 2. Yields from each column run are presented in Figure 2.

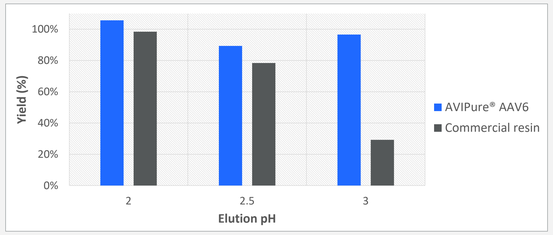


Figure 2: Effect of elution pH on yield for AVIPure® AAV6 and commercial Resin T

The purity of the AVIPure AAV6 elution at pH 3 is also noticeably better than the commercial resin with respect to HCP and HCDNA removal as shown in Table 3. Both resins deliver comparable performance with elution at pH 2.

Table 3: HCP and HCDNA results of resin comparison runs with elution at pH 2 and pH 3

Resin	Elution pH	HCP (ppm)	HCP (LRV)	DNA (ppm)	DNA (LRV)
AVIPure® AAV6	2.0	1600	4.1	1200	3.0
	3.0	2700	3.9	2300	2.7
Resin T	2.0	2100	4.0	1500	2.9
	3.0	9200	3.4	5100	2.4

Table 4: Process parameters for resin comparison runs using 0.3 x 2.5 cm packed columns

Step	Buffer	CV	Flow (mL/min)	Residence Time (min)
Pre-sanitize	0.1 M NaOH (AVIPure AAV6) 0.1 M phosphoric acid (Resin T)	10	0.2	1
Equilibration	20 mM Tris, 200 mM NaCl	20	0.2	1
Load	WT-AAV6 clarified lysate	200	0.2	1
Wash	20 mM Tris, 200 mM NaCl	20	0.2	1
Elution†	0.1 M glycine, 0.15 M NaCl, variable pH	20	0.05	4
Strip‡	0.1 M NaOH (AVIPure AAV6) 0.1 M phosphoric acid (Resin T)	10	0.2	1
Re-equilibration	20 mM Tris, 200 mM NaCl	20	0.2	1

†Elution fractions were collected in wells containing 20% of the fraction volume of 1 M Tris, pH 8
‡Strip fractions were collected in wells containing 50% of the fraction volume of 1 M Tris, pH 7

Product Cycling and Reuse

To demonstrate performance and reuse, 5 product cycles were conducted with clarified AAV6 lysate according to the parameters in Table 2 with a load titer of 4.9E+11 vp/ml and eluting at pH 3. During the strip phase of each cycle the column was held for 2.5 hours in 0.1 M NaOH. Consistent yield and purity were observed across all five cycles (Figure 3A and 3B).

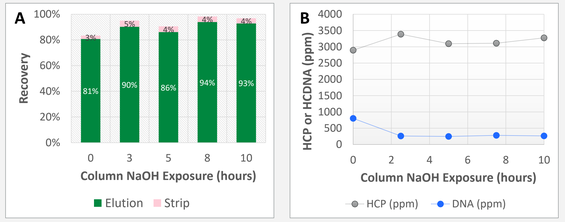


Figure 3: Performance of AVIPure AAV6 over multiple reuse cycles was monitored using A) yield by ELISA and B) HCP (ELISA) and HCDNA (PicoGreen) purity

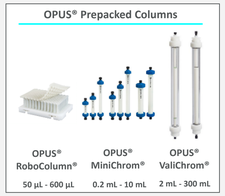
Pre-commercial AVIPure AAV6 resin available now

cGMP resin available expected Q4-2024

AVIPure AAV2, 5, 8 and 9 are in stock and available now

- Bulk resin: 10 – 1000 mL, < 5 days shipping
- OPUS prepacked formats, < 5 days shipping:

ID (cm)	H (cm)	Volume (mL)
0.5	5	1.0
1.13	5	5.0
0.8	10	5.0
RoboColumn – 8 rows		0.2
RoboColumn – 8 rows		0.6



Other configurations of pre-packed columns can be ordered directly through Repligen

Image 1: OPUS® Pre-packed Chromatography Columns provide linear scale-up from process validation to commercial and GMP manufacturing

AVIPure AAV2 ligand contacts 14 capsid residues

A low resolution cryo-EM structure of the AVIPure AAV2 ligand complexed with the WT-AAV2 capsid was determined (Figure 4). Based on the density map we observe that:

- AVIPure AAV2 ligand binds to VP3 residues 527, 530, 574-594
- These residues are common to VP1, 2, and 3
- Mutations to VP2 or VP3 specific residues are not expected to interfere with AVIPure AAV2 capture

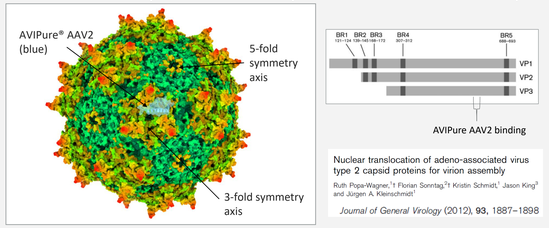
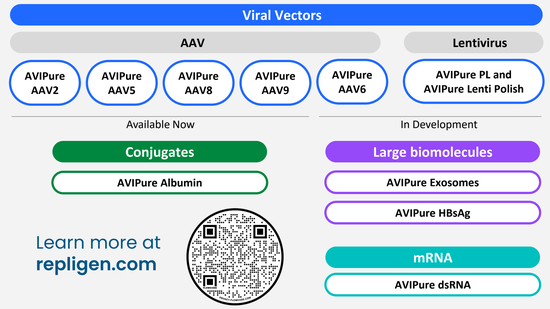


Figure 4: Cryo-EM structure of WT-AAV2 complexed with AVIPure AAV2 ligand

AVIPure Affinity Resin Portfolio and Pipeline

Repligen is building a best-in-class portfolio of catalog affinity products and offers unmatched timelines for custom affinity programs with prototype delivery within 3 months.



Acknowledgements

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