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AVIPure® AAV2 Affinity Resin Process Development Guide

Introduction

Adeno-associated virus (AAV) is increasingly recognized as an ideal choice for delivery of gene therapy modalities. To realize the therapeutic benefits of AAV, an efficient manufacturing process that delivers AAVs sufficiently pure for use in a clinical setting is necessary. Current AAV production methods are characterized by low viral titers and high HCP levels; consequently, downstream purification processes must deliver high yields at high purity, and short processing times.

AVIPure® AAV2 Affinity Resin is characterized by high binding capacities, high yields and the ability to process lysate at high volumetric flow rates, hence enabling capture from dilute feed streams. In addition, the resin is alkali stable enabling the repeated use of 0.5 M NaOH for cleaning-in-place (CIP) and sanitization applications. These attributes assure that use of this resin will result in improved process economy at any scale of operation.

AVIPure® AAV2 Affinity Resin consists of a high affinity recombinant protein ligand coupled to a highly cross-linked agarose base matrix developed for bioprocess applications. Attachment of the ligand to the base matrix through a long flexible spacer ensures ligand accessibility and subsequently leads to high binding capacities. The engineered affinity of the ligand ensures highly specific binding of AAV2 at neutral pH, while enabling elution at low pH (*e.g.,* pH 2.0). Elution at higher pH can be possible, but additives will need to be used to assure reasonable yields. The inert agarose base matrix shows minimal nonspecific binding, leading to a high purity of recovered AAV2. Furthermore, the agarose base bead of the AVIPure® AAV2 Affinity Resin base matrix enables rapid processing of large volumes of lysate without excess pressure drop over the packed column.

This application note describes the recommended operating conditions and process optimization methodology for the resin. The methodology described can be applied to determine optimal capture conditions for dilute and concentrated feed, and for determination of preferred CIP conditions for the capture step using AVIPure® AAV2 Affinity Resin.

Recommended chromatographic conditions

Optimal conditions for purification of AAV2 using AVIPure® AAV2 Affinity Resin must be determined empirically for each AAV2 construct. However, as a starting point, the chromatography method summarized in Table 1 is recommended. To assure comparability of each cycle, it is also recommended to perform a sanitization step prior to the first use of the resin. The step should be equivalent to the CIP step used before resin storage.

Table 1. Recommended purification protocol for AVIPure® AAV2 Affinity Resin to purify viral vectors from concentrated lysate

Step	Column volumes	Residence time (min)	Suggested buffer
Equilibration	5	4	50 mM Tris, 250 mM NaCl, pH 8.3
Load	Titer dependent	1 - 8	-
Wash 1	5	4	Equilibration buffer
Elution	5	6 or 4[a]	50 mM Glycine, 150 mM NaCl, pH 2
Strip	2	4	Process specific (e.g., pH <2)
CIP	5	6	0.5 M NaOH
Re-equilibration	8	4	Equilibration buffer

[a] From the process efficiency perspective, an increase in residence time (reduction of flowrates) during elution step results in lower buffer consumption and more concentrated pools.

In the case of AAV2 purification from unconcentrated feeds, the same protocol as described in Table 1 can be used, but the residence time for load can be reduced to 1 minute.

The unprecedented quality of AVIPure® AAV2 Affinity Resin is exemplified in Figure 1, where chromatograms from three consecutive runs with a real feed are compared. The average reduction in HCP and DNA was 4.9 log and 3.4 log, respectively. Between the runs, the resin was stored in a storage solution at 5° C.

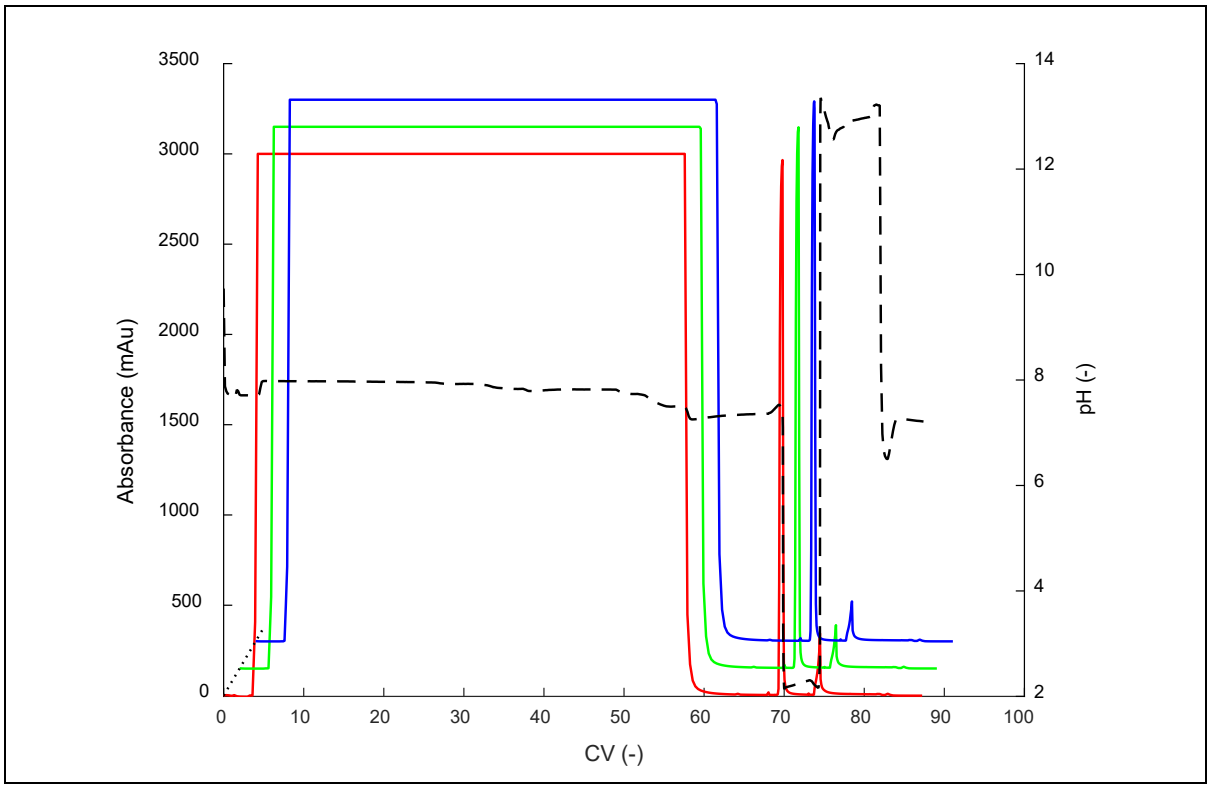

Figure 1. Chromatograms from consecutive purification cycles obtained with AVIPure® AAV2 Resin loaded with concentrated lysate containing AAV2 capsids. Load: 2.3 x 10^{14} vp/mL of resin at 1 minute residence time; Elution: 50 mM glycine, 150 mM NaCl, pH 2.0; CIP: 30 minutes with 0.5 M NaOH. Chromatograms are shifted diagonally for clarity. The black dashed line represents the pH trace from the first cycle.

As compared to other commercially available affinity resins for purification of AAV2 capsids, AVIPure® AAV2 Affinity Resin delivers the same process yields with improved product purity. Figure 2 shows SDS PAGE gel obtained during the head-to-head comparison against another agarose based affinity resin. The gel clearly shows a better reduction in HCPs achieved with AVIPure® AAV2 Affinity Resin. In fact, in this particular study the level of HCP and DNA were reduced by 7- and 2-fold, respectively. What is also remarkable is that the 4400 ppm[1] and 2400 ppm levels were obtained with just a standard equilibration buffer as the wash buffer. Further improvements in purity are expected when additives are used in the wash buffer.

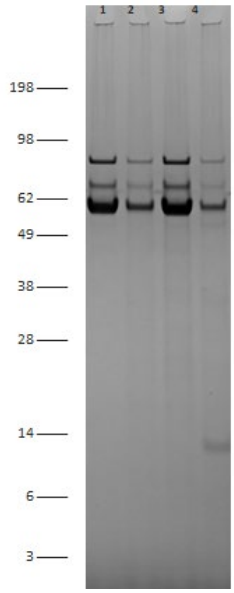

Figure 2. SDS PAGE of elution and CIP pools for Avipure® AAV2 and other AAV2 affinity resin.
Avipure® AAV2: lanes 1 (elution) and 2 (CIP). Other affinity resin: lanes 3 (elution) and 4 (CIP).

Effect of residence time and capsid concentration on dynamic binding capacity

Figure 3 shows partial breakthrough curves obtained when AVIPure® AAV2 Affinity Resin was loaded with purified AAV2 capsids. The breakthrough curves were obtained at 1- and 4-minute residence times and at two feed concentrations representing unconcentrated and concentrated feeds. Dynamic binding capacities at 10% breakthrough extracted from Figure 3 are listed in Table 2. These capacities are some of the highest reported for AAV affinity resins, varying from 5.0×10^{17} to almost 1×10^{18} vp/L of resin at 1- and 4-minute residence times for low and high concentration feeds, respectively. The high capacities at short residence times yield very high resin productivities, which makes the AVIPure® AAV2 Affinity Resin an attractive bioprocess resin for purification of AAV2 capsids at various process configurations and scales, including processing of unconcentrated feed material. Note that the results presented in Figure 3 represent data obtained with a specific variant of AAV2, and while the trends shown can be considered characteristic for all types of AAV2 capsids, the absolute values for binding capacities should always be determined for specific AAV2 variants.

[1] ppm = ng / 1 x 10^{14} vp

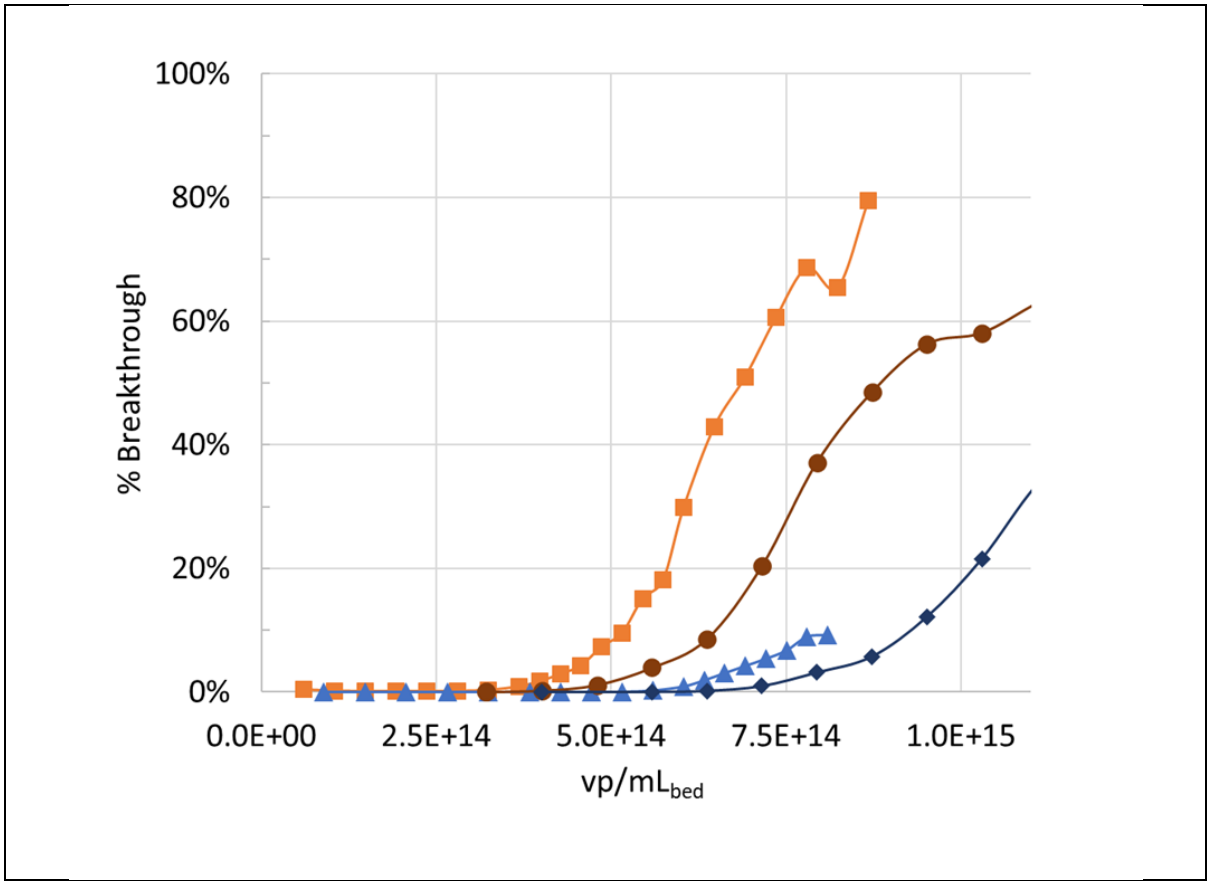

Figure 3. Effect of residence time and feed concentration on binding capacity of AVIPure® AAV2 Affinity Resin. Legend: (■) 1 min residence time at 5.0×10^{12} vp/mL, (●) 4 min residence time at 5.0×10^{12} vp/mL , (▲) 1 min residence time at 6.0×10^{13} vp/mL , (◆) 4 min residence time at 6.0×10^{13} vp/mL. The study was carried out at 0.2 mL scale using purified AAV2 capsids that were diluted to the desired concentration using 20 mM Tris, 400 mM NaCl, 0.01% poloxamer 188 at pH 7.5. The use of 0.01% poloxamer 188 is necessary at small scale to remove any non-specific adsorption of capsids to elements of chromatography system.

Table 2. Dynamic binding capacities at 10% breakthrough for AVIPure® AAV2 Affinity Resin obtained at various residence times

Residence time (min)	Feed concentration (vp/L)	$DBC_{10\%}$ (vp/L_{bed})
1	5.0×10^{15}	5.0×10^{17}
4		8.0×10^{17}
1	6.0×10^{16}	6.2×10^{17}
4		9.5×10^{17}

Upstream production of AAV viral vectors often results in low capsid concentration in the lysate. While the capsids can be concentrated and buffer exchanged prior to the capture step, direct loading of unconcentrated lysate may be preferred to eliminate a process step and the associated potential capsid loss. However, to process the large lysate volumes at low capsid titers, an affinity resin with high binding capacity at short residence times is required for efficient processing in a reasonable time.

The data in Figure 3 clearly demonstrate that the high DBC at low residence times and at low capsid concentrations of the AVIPure® AAV2 Affinity Resin can enable processing of lysate without a concentration step prior to affinity capture. The benefits of direct loading can include mitigation of potential yield losses during ultrafiltration and even shorter overall process times.

Elution conditions

Identification of most suitable wash and elution conditions will ensure high purity and high yields from the affinity step, respectively. If use of the recommended elution conditions for AVIPure® AAV2 Affinity Resin (50 mM glycine, 150 mM NaCl, pH 2) does not result in full elution of adsorbed capsids, a screening study can be performed either in a column or microtiter plate format. From the perspective of the amount of sample required, the initial screening in the microtiter plate format is preferable. It allows for a quick assessment of the most promising conditions that can later be optimized using columns.

Below, we present an example of a wash and elution buffer screening study in microtiter plate format that can be used as a template for identifying the optimum wash and elution conditions when working with AAV2 capsids. However, considering that purity after an affinity step is already very high, identifying elution conditions should be the primary focus, and optimization of wash conditions should be performed only if resulting purity in the eluted product using the standard conditions don't suffice.

The following protocol described here and shown in Figure 4 works well for screening of elution conditions for use with AVIPure® AAV2 Affinity Resin. Dispense five microliters of the resin into a well of a microtiter plate containing 100 µL of equilibration buffer. Filter the plate and add 250 µL of feed containing 1×10^{13} vp/mL. After 60 minutes incubation, filter the plate and add 100 µL of wash (equilibration) buffer. After 5 minutes of incubation, filter the plate and perform another wash cycle. Following filtration of the second wash cycle, add 100 µL of elution solution. Incubate for 5 minutes and filter the plate, collecting the filtrate. Perform another elution cycle and collect the filtrate separately. Neutralize elution fractions by adding 40 µL of 1 M Tris Base, 0.5 M NaCl, pH 9. Analyze the neutralized elution fractions for AAV capsids using AAV2 capsid ELISA. UV absorbance can also be used as it can provide guidance as to which samples to analyze by ELISA. For most efficient elution buffers, most of the capsids should be found in the first elution cycle. Note that the effectiveness of elution buffers varies between different types of capsids. Users are encouraged to determine the optimal elution conditions for each of their capsids.

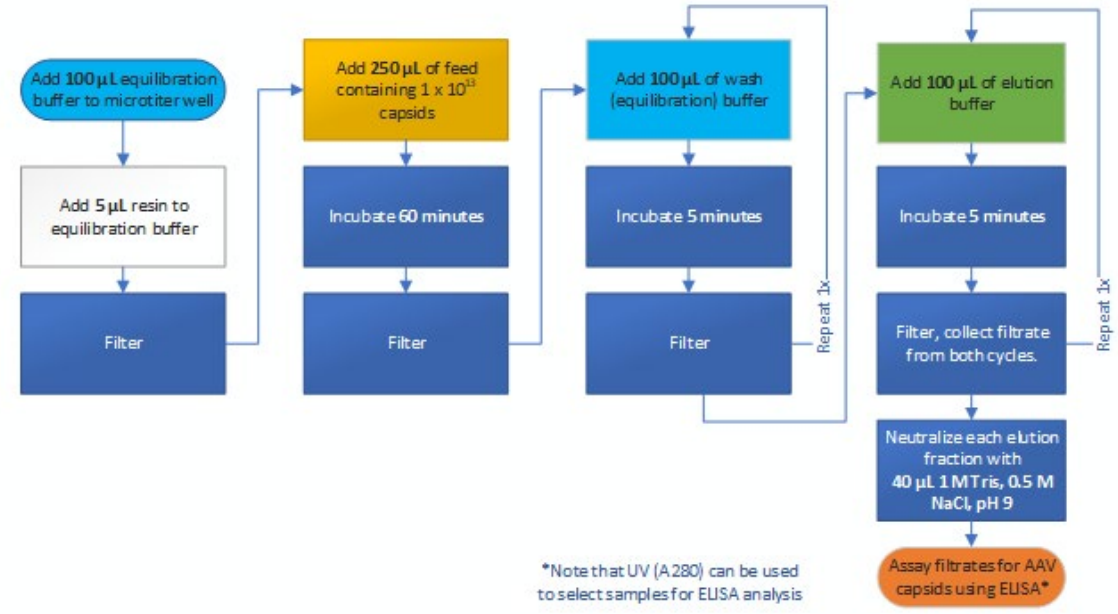

Figure 4. Visual representation of an elution condition screening protocol.

Pressure flow properties of the base beads

AVIPure® AAV2 Affinity Resin is based on a 50 µm highly cross-linked agarose matrix. While the rigidity of the base matrix enables process-relevant flowrates below equipment pressure limits, the compressible nature of agarose beads needs to be accounted for when designing the capture step with consideration for the column dimensions. The resin can be packed into large diameter columns (e.g., 30 cm in diameter), but the maximum allowable flow rate will need to be considered if the column is packed to higher bed heights. For instance, at 20 cm bed height the minimum residence time in larger diameter columns will be 5 minutes. Therefore, a wider, shorter bed is recommended if operating at faster flow rates. Such configurations can be accommodated with OPUS® Pre-packed Columns from Repligen.

Examples of pressure flow curves for bed heights between 5 and 20 cm are shown in Figure 5. The minimum recommended residence times for 5 and 20 cm bed heights are 1 and 5 minutes, respectively. Short residence times are recommended if viral particles are loaded without a prior concentration step.

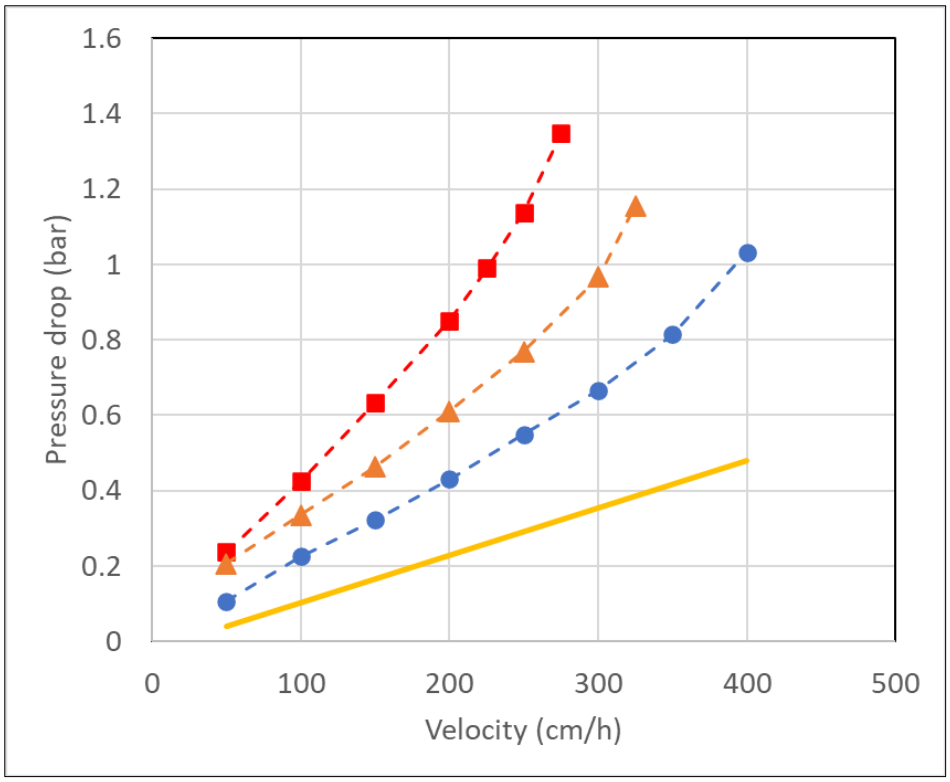

Figure 5. Pressure flow data for AVIPure® AAV2 Affinity Resin base bead in 30 cm diameter column packed to 20 cm (red squares), 15 cm (orange triangles), 10 cm (blue circles), and 5 cm (solid line, simulated data) bed heights at 1.15 compression factor. Data courtesy of Purolite® Ltd.

Development of an efficient cleaning in place (CIP) protocol

During CIP procedures cleaning solutions are applied to the column to remove precipitated or denatured material tightly bound to elements of the purification system such as resin, hardware, etc. From the resin reusability perspective, lack of stringent CIP at each cycle will lead to accumulation of undesired material, and increased probability of resin fouling and of endotoxin contamination, limited ligand accessibility, and increased backpressure during subsequent runs, eventually making the column unusable. Among various CIP agents, NaOH is preferred in bioprocess settings due to its low cost, ease of disposal, and ability to dissolve precipitated proteins, remove nucleic acids, saponify fats, and inactivate endotoxins. AVIPure® AAV2 Affinity Resin is an alkali-tolerant resin enabling the use of NaOH concentrations up to 0.5 M for CIP between cycles.

Below, a resin cycling study using real feed and 0.5 M NaOH as the CIP solution is described. The outline of the AVIPure® AAV2 Affinity Resin lifetime study is presented in Figure 6. The study was carried out at 0.35 mL scale using crude feeds containing AAV2 capsids. The following resin performance attributes were measured after each cycle: step yield defined as total capsids eluted compared with total capsids loaded, level of residual host cell protein (HCP), and level of residual host cell DNA (HCDNA). HCP and HCDNA were measured using ELISA and PicoGreen™, respectively. Total capsids were measured using Progen ELISA.

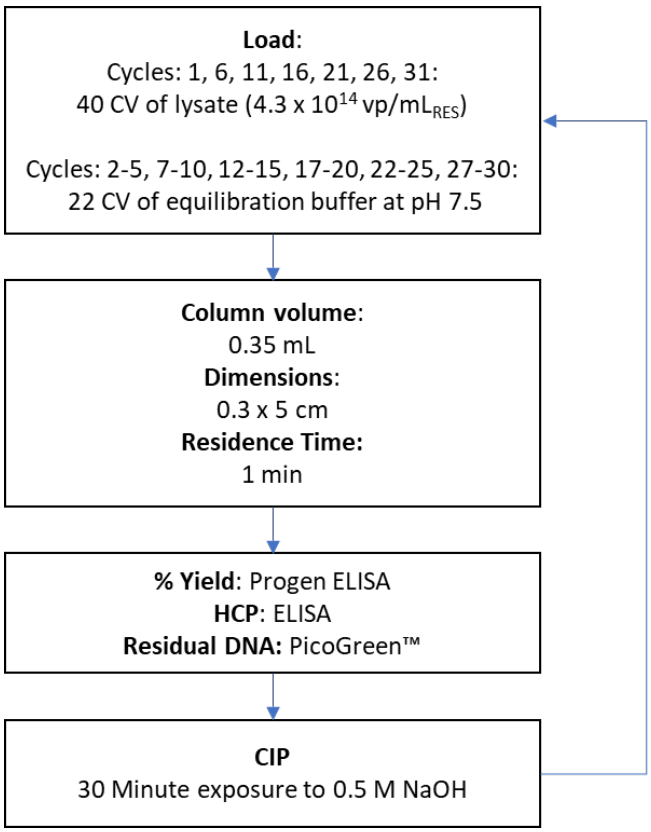

Figure 6. Outline of AVIPure® AAV2 lifetime studies.

Overlay of chromatograms from cycles run with the real feed is shown in Figure 7. Summary of the resin lifetime study is shown in Figure 8, where the resin performance attributes are plotted against the 0.5 M NaOH CIP cycle number. The study was performed by loading the column with real feed every 5th cycle; i.e., each load cycle was followed by four cycles consisting of only CIP and equilibration steps. In total, by the end of the study the resin was exposed to 0.5 M NaOH for 15 hours. The results clearly demonstrate the alkaline stability of AVIPure® AAV2 Affinity Resin. The resin retains ≥ 90% of its binding capacity after 30 CIP cycles with 30 minutes of exposure to 0.5 M NaOH per cycle. Removal of both the HCP and HCDNA is also unaffected by the extended NaOH exposure and remains at the average levels of 5- and 3-logs reduction for HCP and HCDNA, respectively. During the study, the column pressure drop was also monitored for each chromatography cycle, but no changes in the pressure drop were observed (results not shown).

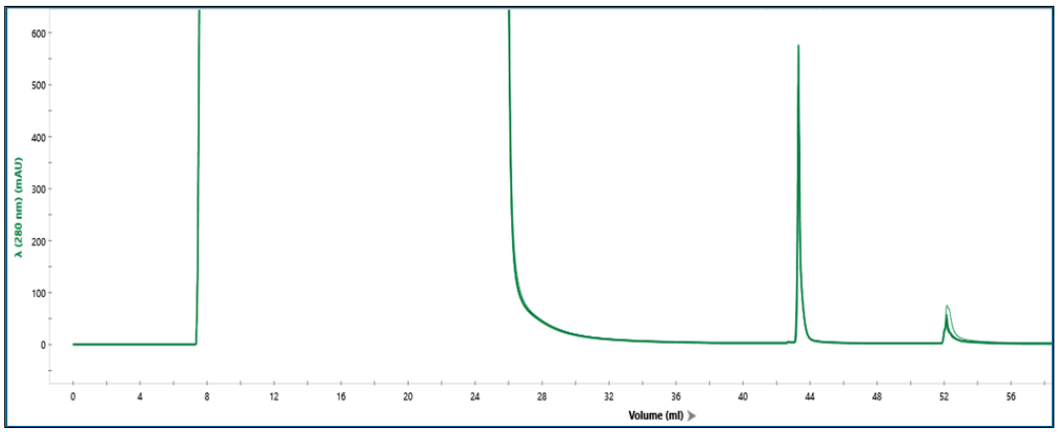

Figure 7. Overlay of chromatograms recorded for real feed cycles during the AVIPure® AAV2 Affinity Resin lifetime study. CIP regime used: 30-minute contact time with 0.5 M NaOH after each cycle. Feed: HEK293 clarified lysate.

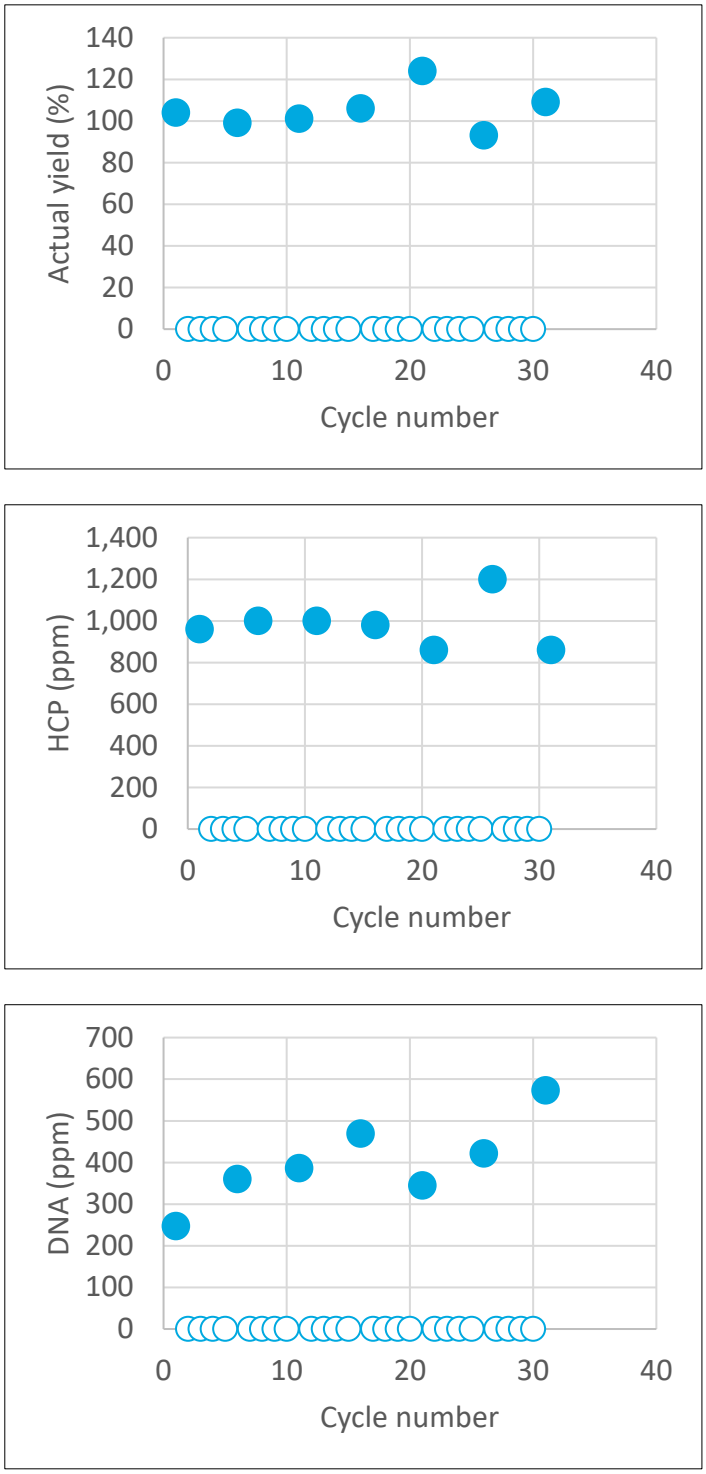

Figure 8. Effect of number of 30 minutes long 0.5M NaOH CIP cycles on relative yield, residual HCP, and residual HCDNA measured for real feed cycles (solid symbols). The average log HCP reduction and fold HCDNA reduction were 2.8 and 5.3 respectively. Open symbols represent CIP/Equilibration cycles and are presented for clarity.

The level of stability shown in Figure 8 is unparalleled for other commercial affinity resins for AAV2 purification. Based on the results obtained in this study, a 1.5 L column packed with AVIPure® AAV2 Affinity Resin can be used to purify not just one but *thirty* 2,000 L bioreactors, thus reducing the effective cost of the resin 30-fold.

A recommended CIP protocol for AVIPure® AAV2 Affinity Resin is provided below. It represents a good starting point for determination of the most efficient CIP protocol for the specific AAV2 purification process. With an optimized CIP protocol, the AVIPure® AAV2 Affinity Resin lifetime can be further increased even beyond the 30 cycles shown in the results presented above.

The following CIP protocol has been shown to work effectively for AVIPure® AAV2 Affinity Resin:

1. Wash the column with 5 column volumes of equilibration buffer.
2. Perform CIP step using 0.5 M NaOH:
    a. Apply 3 CV of CIP solution at 3 minutes residence time.
    b. Perform a static hold for a total contact time of 15 minutes.
    c. Apply 2 CV of CIP solution at 3 minutes residence time.
3. Re-equilibrate the column with at least 5 column volumes of equilibration buffer.

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. Depending on the nature of the feed stock, different CIP regimes may provide an optimal balance of chromatographic performance and resin lifetime. For example, 0.1 M NaOH exposure for 15 minutes every cycle, with a 0.5 M NaOH exposure for 30 minutes every 10^{th} cycle or at the end of each batch will further extend resin lifetime. CIP with 0.5 M NaOH is recommended before 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.

Conclusions

AVIPure® AAV2 Affinity Resin is an alkaline-stable affinity chromatography resin developed for simple, one-step purification of adeno-associated virus (AAV2) vectors directly from lysate. AVIPure® AAV2 ligand has been engineered for enhanced alkali stability, enabling the repeated use of 0.5 M NaOH for cleaning-in-place (CIP) and sanitization applications. Compared to existing AAV affinity chromatography resins outside of the AVIPure® family, AVIPure® AAV2 Affinity Resin delivers an order of magnitude increase in resin lifetime and promises to dramatically decrease resin costs.

Process development workflow followed a standard approach consisting of determination of dynamic binding capacity at 1- and 4-minute residence times and nonconcentrated and concentrated feeds, identification of optimum wash and elution conditions, and finally determination of efficient CIP protocol based on NaOH.

AVIPure® AAV2 Affinity Resin is available in bulk or in prepacked columns. A residual ligand kit ELISA assay is available from Cygnus Technologies (Part number F1000). Regulatory Support File for the AVIPure® AAV2 Affinity Resin is available upon request.

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

Bulk resin volume	AVIPure® AAV2
10 mL	100AAV2-10
25 mL	100AAV2-25
50 mL	100AAV2-50
100 mL	100AAV2-100
250 mL	100AAV2-250
1000 mL	100AAV2-1000

Column Type	ID [mm]	H [mm]	V [mL]	AVIPure® AAV2
OPUS® MiniChrom®	5	50	1.0	23051006
OPUS® MiniChrom®	11.3	50	5.0	23051007
OPUS® MiniChrom®	8	100	5.0	23051004-100

Column Type	Rows	V [µL]	AVIPure® AAV2
OPUS® RoboColumn®	8	200	23051008R
OPUS® RoboColumn®	8	600	23051008R-30

Description	AVIPure® AAV2
Residual Ligand ELISA Assay, available through Cygnus Technologies	F1005

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.