Practical Guide: Selecting the Optimal Resins for Adenovirus Process Purification

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On the Quest to Purify a Large Complex Virus

The number of gene therapy–based treatments has grown significantly since they first appeared nearly three decades ago. This has created profound optimism about our potential to develop a cure for diseases such as cancer and AIDS. One of the most effective contributors to the success of gene therapy is the ability to use viruses as vehicles for delivering genes to their targets. Initially, murine retroviruses were recruited for this purpose, but more recently, adenovirus (Ad) and adeno-associated virus (AAV) have become the vehicles of choice. In fact, Ad and AAV studies account for over 25% of all ongoing gene therapy trials. However, producing sufficient quantities of pure clinical-grade virus, which is required to ensure biosafety, is not an easy task.

One of the main barriers to achieving high purity levels is the size and complexity of the adenovirus. One intact virus particle contains more than 2,700 protein subunits, has a mass of ~165 MDa, and has a diameter of ~0.1 µm. This complexity renders traditional virus purification methods such as filtration, density gradients, and ultracentrifugation inefficient. In addition, the virus has thousands of charge variants, making it difficult to establish well-defined binding and elution conditions. Therefore, purification protocols and strategies used for small and mid-sized viruses also fall short when it comes to adenoviruses. Furthermore, adenoviruses can be acid-labile, which further increases purification challenges. These hurdles call for alternate strategies for achieving efficient adenoviral purification. In response, column chromatography has gained popularity over the past two decades as a way to overcome the challenges of process adenoviral purification and the limitations of traditional purification methods (Huyghe et al. 1995).

Bio-Rad has provided a progressive selection of chromatography resins for process-scale purification of viruses for more than 50 years. After screening five different chromatography resins, we developed a two-column capture and polish cGMP-ready purification strategy for a recombinant adenovirus. We show that this process yields an active concentrated product with purity, host cell protein (HCP), and DNA contamination levels comparable to other clinical-grade products. Additionally, the process is readily scalable and sufficiently simple, rapid, and efficient for the production of clinical-grade viral vectors for gene therapy–based treatments. This guide provides a brief snapshot of the various resins considered, the reasons behind the selection of the final two resins, and the results from our study.

Design of Experiment (DoE) for the Process Purification of Adenoviruses

Initial Screening with Five Bio-Rad Resins

Four ion exchange (IEX) and one mixed-mode (MM) resins were initially screened to determine which had the potential to be used for mass capture of adenoviruses. As shown in Table 1, use of two cation exchange (CEX) resins, UNOsphere™ S and Nuvia™ S, left the majority of the virus in the flowthrough and/or wash samples. This makes them unsuitable for both bind-and-elute and flow-through modalities relative to the three other resins — Nuvia™ cPrime™, UNOsphere™ Q, and Nuvia™ Q — whose use resulted in the majority of the virus remaining in the eluate. Nuvia cPrime is a MM resin that offers a unique balance between hydrophobic and charged characteristics. It is built on a mechanically and chemically stable, rigid, macroporous base matrix with particle size optimized to provide exceptional flow properties, fast mass transfer, and stability (bulletin 6242). Both Nuvia Q and UNOsphere Q are anion exchange (AEX) resins. Nuvia Q Resin, with its high binding capacity, delivers excellent performance for polishing applications. It can significantly improve productivity while contributing to reduced capital costs, space requirements, and cycle time for downstream purification (bulletin 6129).

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UNOsphere Q Resin has large-diameter pores and a large surface area to maximize capture speed, macromolecule capacity, recovery, and productivity (bulletin 2724). It shows a high binding capacity of 125–180 mg/ml bovine serum albumin (BSA) at a flow rate of 150–1,200 cm/hr.

Table 1. Results from the initial resin screening.

Column type	Virus in flowthrough/ wash	Virus in eluate	Notes/Implications
UNOsphere S (CEX)	+++	++	Poorly suited for virus purification in both bind-and-elute and flow-through modalities
Nuvia S (CEX)	+++	++	Poorly suited for virus purification in both bind-and-elute and flow-through modalities
Nuvia cPrime (MM)	—	++++	Partial elution in 125 mM NaCl, pH 6.5; hence, dilution of crude harvest required prior to column loading
UNOsphere Q (AEX)	—	++++	Could be considered for direct mass capture
Nuvia Q (AEX)	—	++++	Could be considered for direct mass capture

Selection of the Mass Capture Resin

Of the three resins with potential for use in the mass capture process, Nuvia cPrime was selected for the following reasons. The main anticipated impurity in the feed is serum albumin. Nuvia Q and UNOsphere Q bind to albumin, decreasing the effective binding capacity for the virus. Nuvia Q also binds host cell DNA and other negatively charged impurities like lipopolysaccharides (LPS). Therefore, they were not good candidates for the mass capture step. On the other hand, albumin and the negatively charged impurities come out in the flowthrough with Nuvia cPrime. In addition, the use of Nuvia cPrime requires a smaller column and decreases the potential for column fouling. Hence, Nuvia cPrime was better suited for the mass capture step. A representative chromatogram from this mass capture is shown in Figure 1.

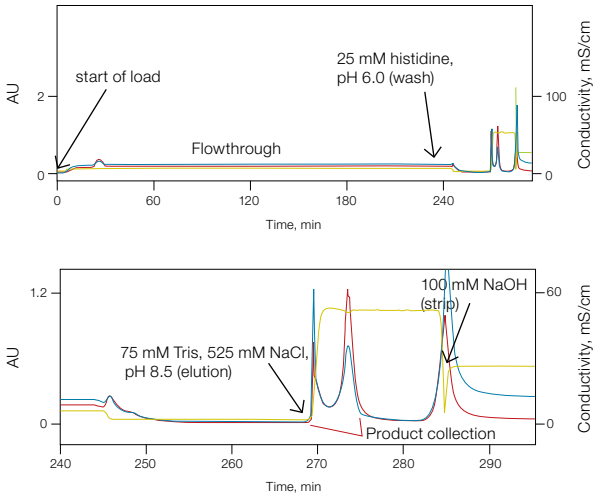

Fig. 1. Representative chromatogram from the Nuvia cPrime mass capture step. OD 260 (—); OD 280 (—); conductivity (—). AU, absorbance units.

Selection of the Polish Resin

The eluate from the Nuvia cPrime capture step had an NaCl concentration of ~500 mM. This rendered the Nuvia Q Resin more suitable for the polish purification step because it is able to adsorb virus at high NaCl concentrations. A representative chromatogram from this polish step is shown in Figure 2.

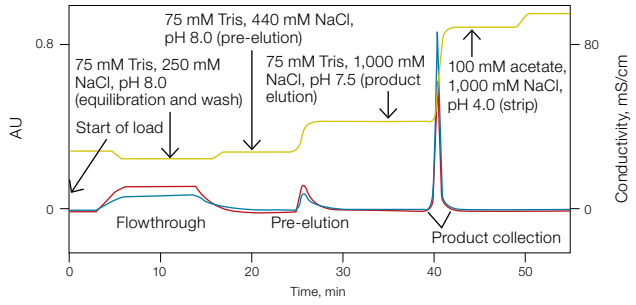

Fig. 2. Representative chromatogram from the Nuvia Q polish purification step. OD 260 (—); OD 280 (—); conductivity (—). AU, absorbance units.

Results

The initial capture purification of the recombinant adenovirus with Nuvia cPrime achieved a tenfold reduction in the processing volume and a significant reduction in feedstream contaminants (Figure 3, lanes 2–4). The final polish purification step with Nuvia Q achieved an additional twofold reduction of product volume along with a significant improvement in product purity (Figure 3, lanes 4–7). The five most prominent viral proteins, hexon, penton, core (V), hexon (VI), and core (VII) are readily visible in the final purified product (Figure 3, lane 7), whereas nonviral proteins are essentially absent.

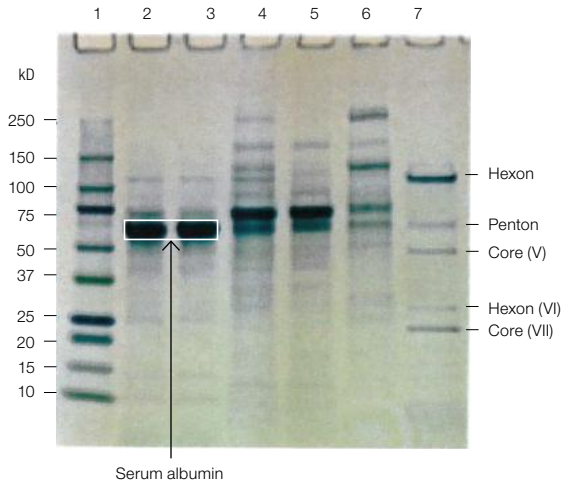

Fig. 3. SDS-PAGE analysis of the intermediates and the final product. Lane 1, MW marker; lane 2, Nuvia cPrime load; lane 3, Nuvia cPrime flowthrough; lane 4, Nuvia cPrime elution/Nuvia Q load; lane 5, Nuvia Q flowthrough; lane 6, Nuvia Q pre-elution; lane 7, final product.

This process yields an active, concentrated virus product with purity, HCP, and DNA levels comparable to clinical-grade products (Table 2). In addition, this protocol is less laborious and time consuming than other purification methods.

Table 2. Viral particle recovery and impurity clearance.

Sample	Total virus ($x10^{11}$ particles)	Impurity levels ($ng/10^{10}$ particles)	
		DNA	HCP
Bulk harvest	30.6	3,144	n/d
Nuclease-treated harvest	31.8	30	3,022
Nuvia cPrime eluate	18.4	n/d	58
Nuvia Q eluate	16.4	<0.02	2

n/d, not determined.

The resins and conditions used for each step in your purification process will have to be optimized based on your adenovirus feed. If you are interested in purifying small to mid-sized viruses, such as dengue virus, poliovirus, or Japanese encephalitis virus, a different mixed-mode media — CHT™ Ceramic Hydroxyapatite — would be the ideal choice to begin with. The details of such virus purifications are described in bulletin 6790 and bulletin 6549.

The information provided here can help you get started on your adenovirus purification strategy. For technical/product support or to request a quote, email your regional Bio-Rad representative at process@bio-rad.com or contact our customer service at 1-800-4-BIORAD (1-800-424-6723).

References

Huyghe BG et al. (1995). Purification of a type 5 recombinant adenovirus encoding human p53 by column chromatography. Hum Gene Ther 6, 1403–1416.

Explore our extensive selection of process-scale chromatography resins and their performance characteristics and applications (bulletin 6713). For process optimization of your adenovirus purification, request a sample.

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