

Implementation of a Process-Scale Adenovirus Purification with a Single-Use Platform⁽¹⁾

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1. Introduction

Adenovirus vectors are finding increasing application within the vaccine and gene therapy industries. Companies developing adenovirus-based biopharmaceuticals will benefit from single-use process platforms that are quick and easy to install and have been demonstrated previously to produce purified adenovirus.

We have assembled a platform from existing and proven technologies, available from lab to production scale, that meet the quality requirements of the vaccine industry (Fig. 1). The platform includes clarification, ultrafiltration | diafiltration, chromatography and sterile filtration steps.

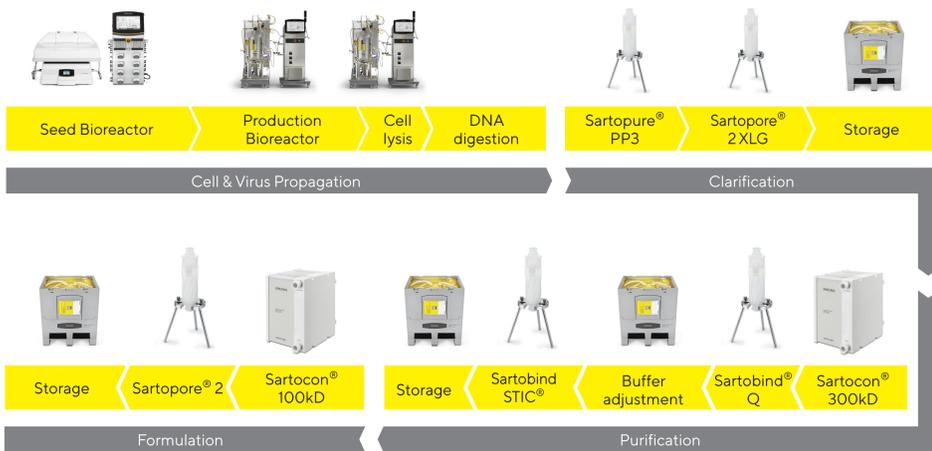


Figure 1: Single-use platform process for adenovirus production

2. Optimization of clarification at 1 L scale⁽²⁾

The process starts in the bioreactor with the infection of HEK293 cells, grown in a serum-free media, by adenovirus serotype 5 (Ad5). The cell concentration at infection was 1×10^6 cells/mL and a multiplicity of infection of five. Performing a cell lysis step at the end of the cell culture maximizes the yield of virus particles from the bioreactor but also releases large quantities of DNA. Adding an endonuclease to the bioreactor, digests the released DNA and facilitates subsequent purification operations. The turbidity of the cell culture broth before filtration was 24.6 NTU.

First, the Ad5 suspension was filtered with three different filters: (i) Sartopure® PP3 0.45 µm, (ii) Sartopure® 2 XLG and (iii) benchmark 1 membrane filter. In a subsequent experiment the filtrate from Sartopure® PP3 0.45 µm was processed with two filters: (i) Sartopure® 2 XLG and (ii) benchmark 2 membrane filter. The filterability trial was performed under constant pressure conditions using a pressure vessel. The total throughput results are shown in Figures 2 and 3, respectively.

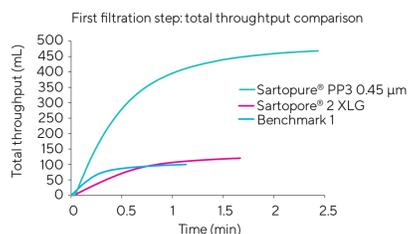


Figure 2: Total throughput with Sartopure® PP3 0.45 µm (Teal), Benchmark 1 filter (Cyan) and Sartopure® 2 XLG (Magenta)

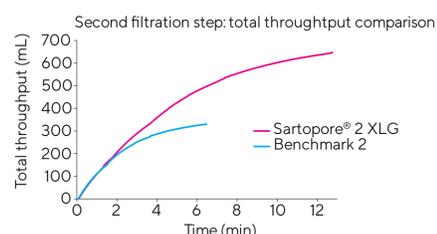


Figure 3: Total throughput with Benchmark 2 filter (Cyan) and Sartopure® 2 XLG (Magenta)

The analytical assays reveal a product recovery of 100% and some contaminants removal. When using Sartopure® PP3 0.45 µm as a prefilter to protect a Sartopure® 2 XLG downstream filter allow a 5-fold reduction in pre-filter area and a 2-fold reduction in sterilizing-grade filter area when compared to the benchmark filters. The recommended filter train for 100 L production is a 10" Maxicaps® Sartopure® PP3 0.45 µm with 0.4 m² followed by size 9 Midicaps® Sartopure® 2 XLG with 0.26 m².

3. Optimization of UF | DF at 1 L scale⁽³⁾

The objective of this experiment was to compare two cassettes, each with a 300kDa membrane cut-off, for the concentration and diafiltration of a clarified Ad5 suspension. The cassettes were installed within the Sartoflow® Smart tangential flow filtration system (Fig. 4).

Higher permeate flow rates were achieved with the PESU 300 kDa cassettes than with the Hydrosart® membrane. The PESU cassettes also allowed for a greater removal of contaminating DNA and protein (resp. 88% and 95%) than the Hydrosart cassette (resp. 56% and 90%). Nevertheless, Hydrosart® membranes are easier to clean compared to PESU, meaning that in the long term they are more suitable for multi-use applications.

Taking into account the results obtained with the PESU 300 kDa and Hydrosart® 300 kDa, the scale-up to 20 L (10× concentration and diafiltration volume of 5×) could be performed using the parameters shown in Table 1.



Figure 4: Sartoflow® Smart tangential flow filtration system

Table 1: Requirement for scale-up to 20 L

Cassette	Average flow (LMH)	Process time (h)	Filtration area (m ²)
PESU 300 kDa	73	1.2	0.4
Hydrosart® 300 kDa	60	1.5	0.4

4. Optimization of chromatography steps⁽⁴⁾

An adenovirus capture step was developed with the Sartobind® Q membrane adsorber operated in a bind-and-elute mode.

The binding capacity at 10% breakthrough was determined to be 6.26×10^{12} VP/mL of membrane. The virus recovery was 75% and the DNA level reduced more than 15-fold from 176 to 10.5 ng/dose. The capture step was performed within 30 minutes. The chromatogram for the capture step is shown in Fig. 5.

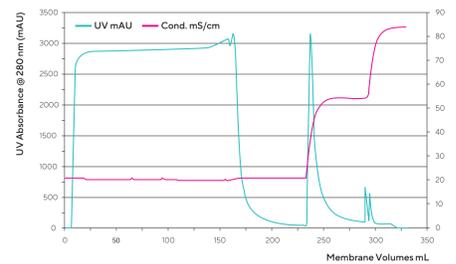


Figure 5: Typical elution chromatogram profile of Ad5 using Sartobind® Q membrane adsorber for primary purification step (Equilibration: 50 mM Hepes, 200 mM NaCl, pH 7.5; Wash: 50 mM Hepes, 200 mM NaCl, pH 7.5; Isocratic elution: 65% (v/v) of 50 mM Hepes pH 7.5, 1M NaCl. Strip: 50 mM Hepes, pH 7.5, 1M NaCl)

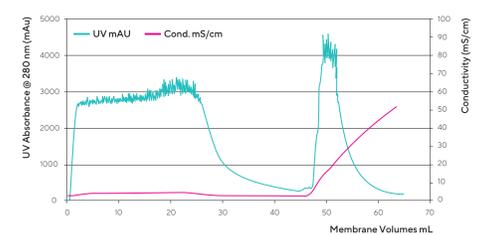


Figure 6: Chromatogram for the polishing of Ad5 using a Sartobind STIC® PA pico 0.08 mL membrane adsorber; Load without phosphate buffer and elution by linear gradient up to 1M phosphate.

Residual nucleic acids can be removed from the Sartobind® Q elution pool with a subsequent Sartobind STIC® PA polishing step in flowthrough mode. Sartobind STIC® PA is a weak anion exchanger with primary amine ligands. The results indicated that a buffer containing 50 mM HEPES, 250 mM sodium phosphate, 200 mM NaCl should be used to achieve recoveries of almost 100% of the loaded virus (Fig. 6). This step reduced the DNA concentration of the feed stream to 0.7 ng DNA/dose, far below the target of 10 ng DNA/dose.

5. Scalability to 20 L

Purification conditions determined at the 1 L scale were used to purify virus at the 20 L scale. Following the polishing step with Sartobind STIC® a final diafiltration step was performed prior to filtration with a sterilizing-grade filter. Table 2 shows the virus particle recoveries and increase in purity achieved through each purification step in the process.

Table 2: Process performance targets achieved with a single-use viral vector platform at 20 L scale

Unit Operation	Step Yield (%)
Endonuclease treatment	100
Clarification	98
Ultrafiltration	99
Bind & Elute Purification	78
Contaminant Capture Purification	85
Final Concentration & Diafiltration and filtration	86
Overall Process Performance	
Yield	55%
Final host cell protein concentration	< 20mg/mL
Final DNA concentration	< 10 ng/dose
Final Endonuclease concentration	< 5 ng/mL

6. Summary

In conclusion, a single-use platform is available for the purification of viral vaccines and Sartorius has demonstrated its application in an Adenovirus manufacturing process at the 20 L scale. It allows the complete purification of the vaccine in one day. It is flexible and can be scale-up to 2,000 L in a single-use format. The platform is a preconfigured set of unit operations for which Sartorius can recommend suitable operating conditions.

The platform requires little additional development work or capital expenditure, but it will allow drug developers to establish manufacturing processes quickly and reduce the time it takes to reach the market with new viral vaccine products.

7. References

- (1) Boulais, A., Hutchinson, N., Linz, F.: Enabling viral vaccine production, GEN, November 15, 2016.
- (2) Application Note Sartorius Stedim Biotech: Clarification of Adenovirus Serotype 5: Robust protection of Downstream processing steps, Order No.: 85037-560-72, November 2017
- (3) Application Note Sartorius Stedim Biotech: Ultrafiltration and diafiltration of Adenovirus serotype 5 with Sartocoon® Slice cassettes installed within a Sartoflow® Smart benchtop tangential flow filtration, Order No.: 85037-560-49, November 2017
- (4) Application Note Sartorius Stedim Biotech: Optimizing Adenovirus Purification Processes, Order No. 85037-558-81, April 2017