

# Ultrafiltration/Diafiltration (UF/DF) of Adeno-Associated Viruses (AAV)

With Pellicon® Capsules and Pellicon® XL 50 Cassettes

Adeno-associated virus (AAV) is one of the most common viral vectors used for gene therapies, often preferred for its safety and scalability. In downstream manufacturing of AAV, tangential flow filtration (TFF) is an essential operation—usually performed at two steps in the process (**Figure 1**)—that provides concentration, buffer exchange, and impurity reduction via ultrafiltration/diafiltration (UF/DF).

Filters that are reliable and provide process predictability help accelerate the path from development to commercialization. Part of the linearly scalable Pellicon® family of TFF filters, single-use Pellicon® Capsules and scale-down Pellicon® XL 50 cassettes provide reliability and reproducibility at any stage of your AAV process, from development to manufacturing.

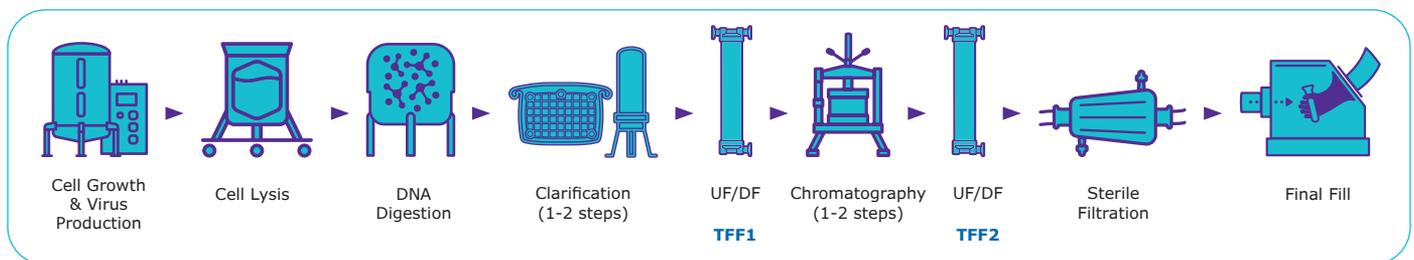


Figure 1. Typical adeno-associated virus (AAV) manufacturing process flow.

## Case Study: TFF of Clarified AAV2

### Materials and Methods

Scalability performance of Pellicon® Capsule and Pellicon® XL 50 cassette with 300 kDa Ultracel® membrane was evaluated using an in-house AAV2 feed that was detergent-lysed, treated with 100 U/mL of Benzonase® endonuclease and then clarified using Millistak+® HC Pro depth filter with D0SP media and sterile-filtered with Millipore Express® SHC 0.5/0.2 µm cartridge. The feed stream for Pellicon® XL 50 cassette was generated in a shake flask, while a bioreactor generated the feed for Pellicon® Capsule and resulted in a 3.6-fold higher AAV2 titer. The TFF process consisted of a 4× concentration step (UF1), followed by diafiltration (DF) with 5 diavolumes (DV) of HEPES buffer, and a final 2.5× concentration (UF2) to achieve total 10× concentration. A permeate-control TFF process (with permeate pump) was performed with flux for each filter controlled to 50% of its critical flux for UF1, and then 25% for DF and UF2. Membrane loading was 60 L/m<sup>2</sup> and crossflow rate was 5 L/min/m<sup>2</sup> (LMM).

### Results

Permeate flux for both capsule and cassette were similar and hence, processing time (Figure 2). Average and maximum transmembrane pressure (TMP) were slightly higher for the capsule compared to the scale-down cassette due to differences in AAV2 titer. AAV2 yield and impurity removal were similar between the 0.1 m<sup>2</sup> Pellicon® Capsule and Pellicon® XL 50 cassette (Figure 3). The results demonstrate that the Pellicon® XL 50 cassette is an excellent scaling tool for Pellicon® Capsule able to predict performance at scale-up.

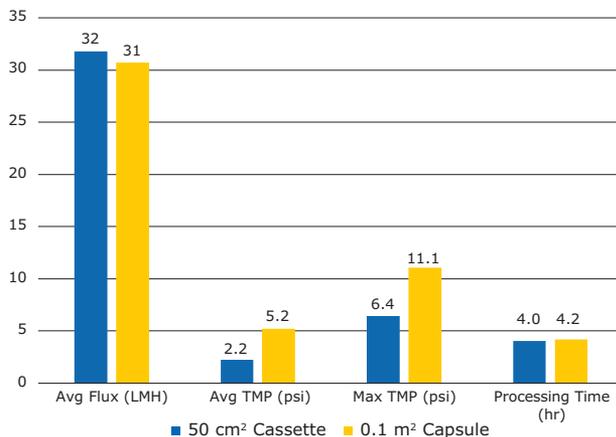


Figure 2. Flux, TMP and process time comparison.

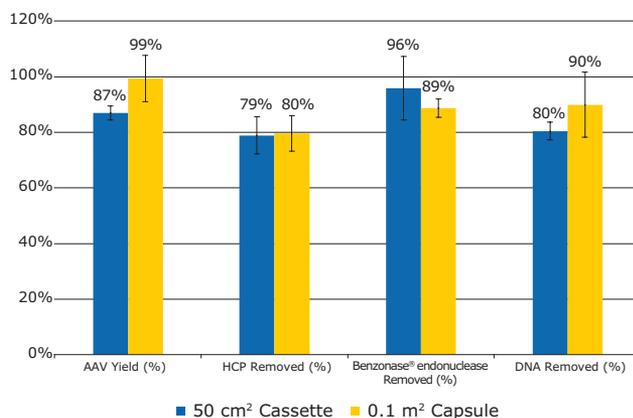


Figure 3. AAV2 yield and impurity reduction levels comparison.

### To place an order or receive technical assistance

In the U.S. and Canada, call toll-free 1-800-645-5476  
For other countries across Europe and the world, please visit: [EMDMillipore.com/offices](https://www.emdmillipore.com/offices)  
For Technical Service, please visit: [EMDMillipore.com/techservice](https://www.emdmillipore.com/techservice)

[EMDMillipore.com](https://www.emdmillipore.com)

We have built a unique collection of life science brands with unrivalled experience in supporting your scientific advancements.

Millipore® Sigma-Aldrich® Supelco® Milli-Q® SAFC® BioReliance®

MilliporeSigma  
400 Summit Drive  
Burlington, MA 01803

