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Performance Guide

Pellicon® Capsules with 100 and 300 kDa Ultracel® Membrane Performance Guide

Single-use tangential flow filtration devices offering high flux performance, reliable scalability, and enhanced operator safety for viral gene therapy applications.

MilliporeSigma is the U.S. and Canada
Life Science business of Merck KGaA,
Darmstadt, Germany.

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How to Use the Guide

This Performance Guide is a reference document to provide you with assistance in evaluating and validating Pellicon® Capsules with 100 and 300 kDa Ultracel® membrane for your ultrafiltration and diafiltration applications. Included in this guide are general guidelines on various performance aspects of Pellicon® Capsules and application studies that may be considered and evaluated by potential users. These studies have been included to provide you with a well-rounded overview of Pellicon® Capsules with 100 and 300 kDa Ultracel® membrane.

Results are intended as general examples and are not to be construed as product claims or specifications. The results included in this guide summarize outcomes and observations obtained in the specific application studies with the particular model stream and experimental conditions described. Therefore, all test results should be confirmed by the end user while using a feed stream and optimized conditions representative of their specific applications.

Introduction

Pellicon® Capsules are innovative single-use tangential flow filtration (TFF) devices for ultrafiltration and diafiltration of solutions that require single-use capabilities, including enhanced ease-of-use, process flexibility, fast product turnaround, and reduced operator exposure to harmful fluids. Pellicon® Capsules employ a self-contained, holderless design and come ready for processing within minutes. These single-use TFF filters are gamma sterilized with preservative-free reverse osmosis water, significantly reducing pre-use requirements. Offered with the C feed channel screen and Ultracel® membrane, Pellicon® Capsules are optimal for processes that require superior mass transfer and flux, including ultrafiltration and/or diafiltration of viral vectors, viral vaccines, mRNA, and plasmids. The capsules' design and automated manufacturing process provides performance consistency and linear scalability.

Scaling

Summary

Scaling between 50 cm² Pellicon® XL 50 cassette and different sizes of Pellicon® Capsule with 100 and 300 kDa Ultracel® membranes was demonstrated by comparing the flux in both pressure dependent and independent regions in transmembrane pressure (TMP) control mode. The average flux of all Pellicon® Capsules was within 20% of the average flux of the Pellicon® XL 50 cassette.

Method

Scaling studies for the primary TFF step (TFF-1 step showed in **Figure 1**) were performed using an AAV model feed that was produced in the same way as the AAV stream: with a detergent-lysed, depth filter-clarified, HEK293 cell culture. However, instead of transducing the HEK293, the clarified feed was spiked with a target 1 × 10^7 PFU/mL of a bacteriophage of similar size to AAV.

In this study, TMP control was used with a feed pump and free flow of permeate (**Figure 2**). Experiments were run in total recycle mode to characterize flux versus TMP.

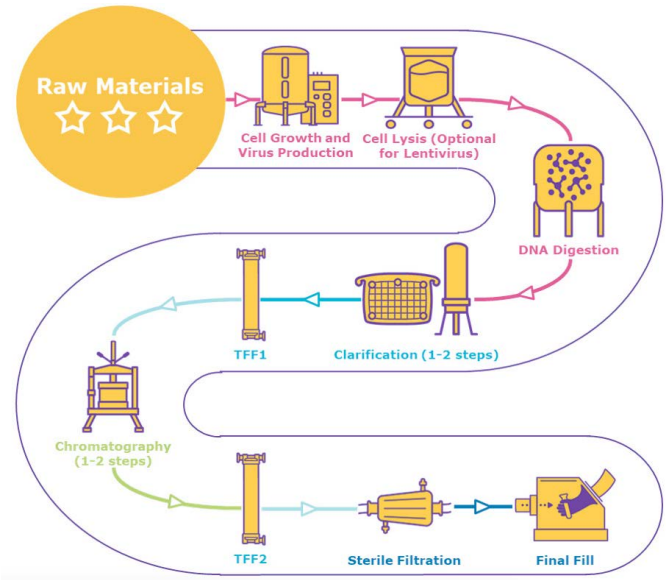

Figure 1.

AAV Manufacturing Template.

1. Flux stability for the TMP control system was demonstrated during a 15-minute total recycle of feed, where the flux declined less than 20%. Feed flow was set to give 5 L/min/m² (LMM) crossflow at the beginning of the flux stability test and the retentate valve throttled to give ~2 psi backpressure.
2. After flux stability was shown, a TMP excursion was run, where permeate flux was measured at 1-psi TMP increments by throttling the retentate valve (**Figure 3**).

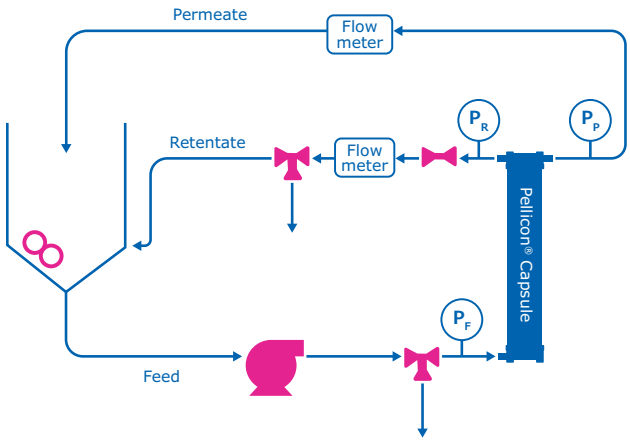

Figure 2.

TMP control system setup.

3. Following the TMP excursion, the target initial operating TMP was determined as the last point before the plateau (3 consecutive points of increasing TMP whose fluxes differ by less than 10%).

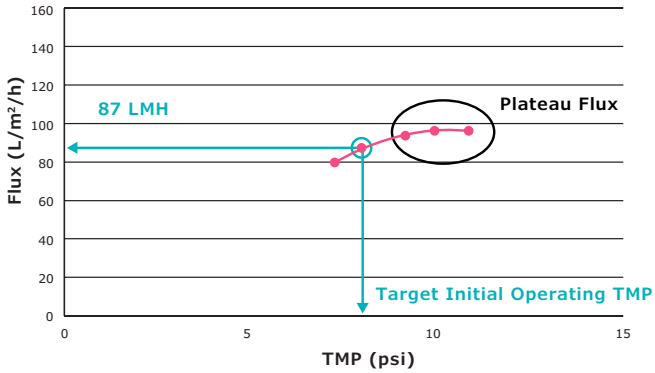

Figure 3.

TMP Excursion example.

Operating conditions were determined as described and target fluxes for all filters (listed in **Table 1**) were compared with the Pellicon® XL 50 to assess scaling of filters at the start of processing.

Table 1.

Filters Evaluated for Scaling.

Filter	Molecular Weight Cutoffs and Membrane	Membrane Area
Pellicon® XL 50 cassette	100 & 300 kDa, Ultracel®	50 cm²
Pellicon® Capsule	100 & 300 kDa, Ultracel®	0.1 m², 0.5 m², 1 m²* and 1.5 m²

*Manifold of 2 × 0.5 m² Pellicon® Capsules.

Results

The flux performance of Pellicon® XL 50 and Pellicon® Capsules for 100 and 300 kDa cut off membranes are shown in **Figure 4** and **Figure 5**. At 5 LMM feed flow, the fluxes of all capsule sizes fell within 20% of Pellicon® XL 50 in both the pressure dependent and pressure independent regions.

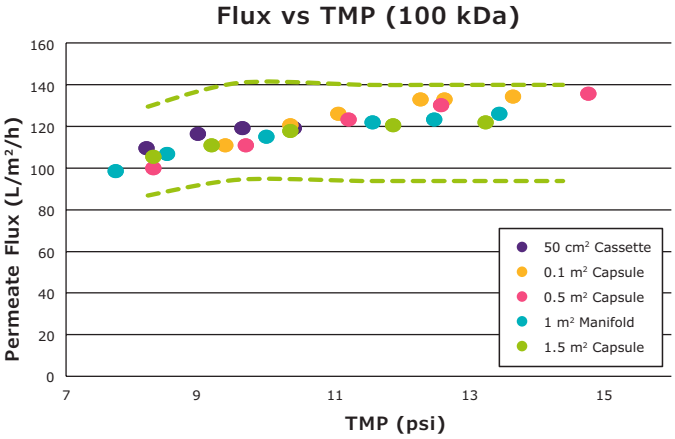

Figure 4.

TMP excursion of 100 kDa filters for processing AAV model feed.

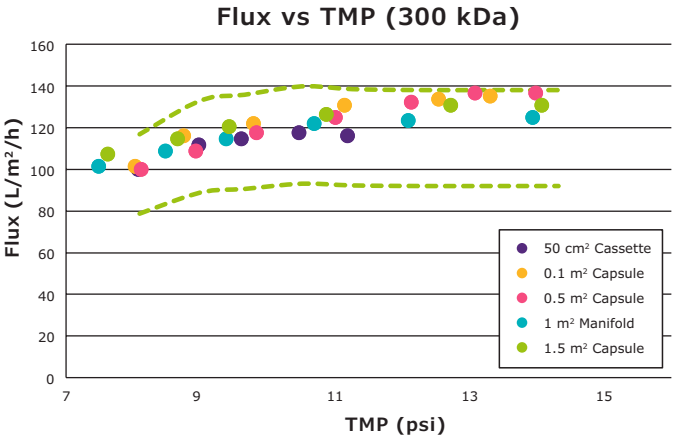

Figure 5.

TMP excursion of 300 kDa filters for processing AAV model feed.

Conclusion

The Pellicon® XL 50 cassette served as an effective scale-down tool for Pellicon® Capsules in viral vector processing. It was demonstrated that process development and optimization with 50 cm^2 cassette using 50–200 mL volume can be successfully scaled-up to 300-fold larger area device.

Capsule Flushing and Leachables

Objective

To evaluate and characterize flushing and leachables content of capsules.

Summary

Pellicon® Capsules are supplied gamma sterilized and with preservative-free reverse osmosis (RO) water, enabling reduced device preparation requirements; sanitization of capsules is not needed, and the storage water can be flushed out immediately after installation. Experiments were performed to evaluate flushing and leachables of capsules through measurement of Total Organic Carbon (TOC). After dynamic flushing with 20 L/m² RO water, the capsules exhibited ≤5 ppm TOC. Leachables were quantified after a subsequent mock product concentration process and 1-hour hold of the resultant product pool.

Method

Capsule flushing and leachables content were evaluated through measurement of TOC. The experimental design is summarized in **Table 1**.

Table 1.

Capsules and conditions used in this study.

Catalog No.	Flushing	Leachables
PCC100C01	Dynamic flushing with 20 L/m² RO water	10× mock UF processing, then 1-hour hold
PCC100C05		
PCC300C01		
PCC300C05		
PCC100C15C		
PCC100C45G		
PCC300C15C		
PCC300C45G		

Flushing

1. Capsules were flushed with 20 L/m² Milli-Q® water with the retentate and permeate lines directed into collection vessels. The feed flow rate was set to 2 L/min/m² and retentate pressure to 1–2 psi.
2. After 1-minute flow stabilization, retentate and permeate samples were collected. Additional samples were collected at ~1-minute intervals until the tank was empty.
3. Samples were analyzed for TOC content.

Leachables

1. After capsule flushing was completed, 20 L/m² Milli-Q® water were recirculated at a feed flow rate of 6 L/min/m² and retentate pressure at 1–2 psi to achieve a 10× feed volume reduction.
2. Once the 2-liter feed volume was achieved, the feed was recirculated in total recycle mode at feed flow rate of 2 L/min/m² and retentate pressure of 1–2 psi.
3. After 5 minutes, a sample from the tank was taken and Milli-Q® water of equivalent volume was added to the tank. A final sample was taken after 1 hour of total recirculation.
4. Both samples were analyzed for TOC content

Results

During the flushing procedure, samples were collected from the retentate effluent and then analyzed for TOC and plotted against feed volume. For capsule size 0.1 m² (**Figure 1**), the results show the average TOC at the end of the 20 L/m² flush to be less than ~1 ppm after quickly decreasing from initial TOC levels. A similar trend was observed for all capsule sizes, including the pre-assembled manifolds, by meeting the ≤5 ppm TOC target at ~10 L/m² flush volume (**Figures 2 and 3**).

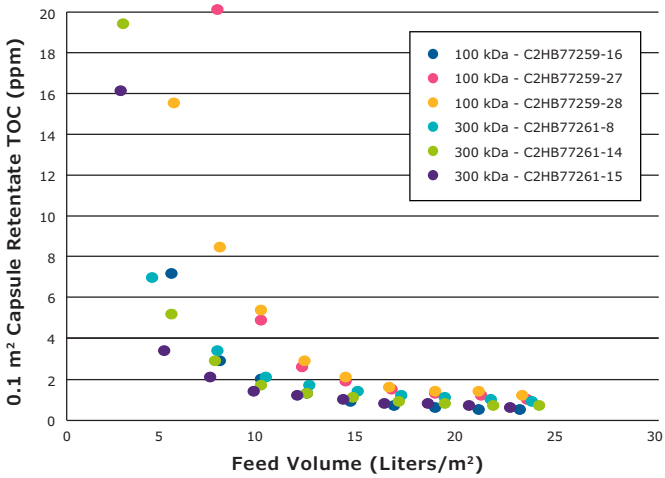

Figure 1.

Evaluation of retentate TOC by feed volume for capsule size 0.1 m².

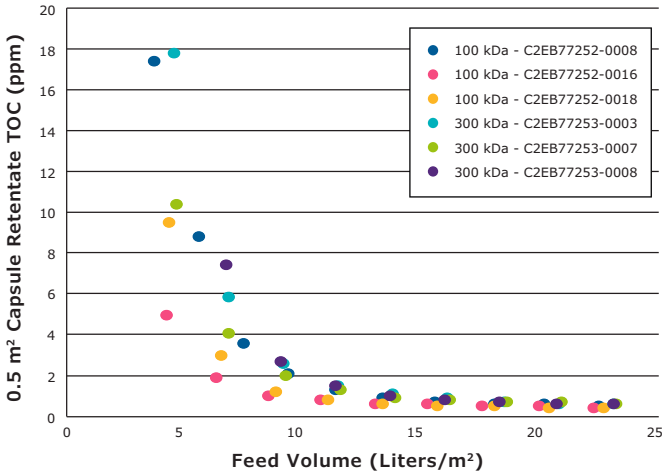

Figure 2.

Evaluation of retentate TOC by feed volume for capsule size 0.5 m².

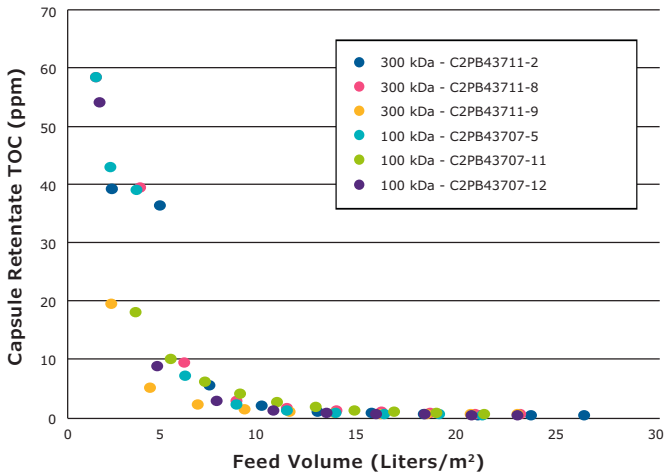

Figure 3.

Evaluation of retentate TOC by feed volume for 100 kDa and 300 kDa capsule sizes 1.5 m² and manifolds.

After the capsules were flushed with 20 L/m² water, a mock UF process was performed, consisting of concentrating 20 L/m² product pool of water to a final 2 L/m² product pool (10× concentration). Then, the retentate and permeate were recirculated (full recycle mode) to mimic a dynamic post-processing product hold. The product pool was sampled after the mock concentration procedure and after 1-hour total-recirculation hold for TOC analysis. The results after the 1-hour hold show TOC levels below 34 mg/m². The results shown in **Figures 4–6** were normalized to represent a 1 L/m² product pool.

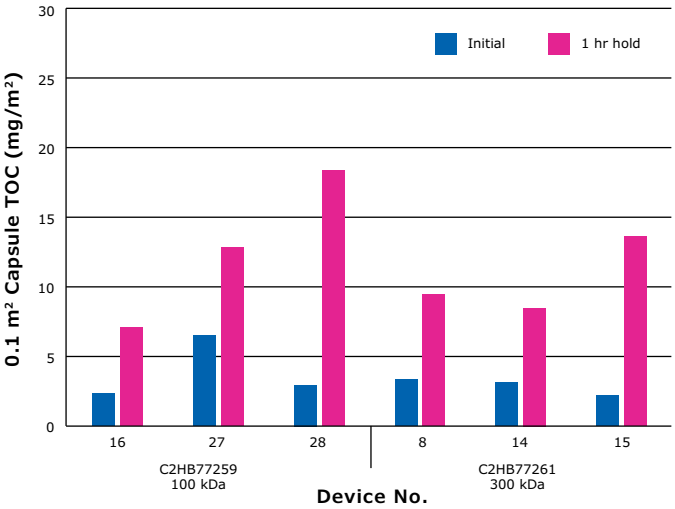

Figure 4.

Evaluation of product pool TOC before and after mock product hold for capsule size 0.1 m².

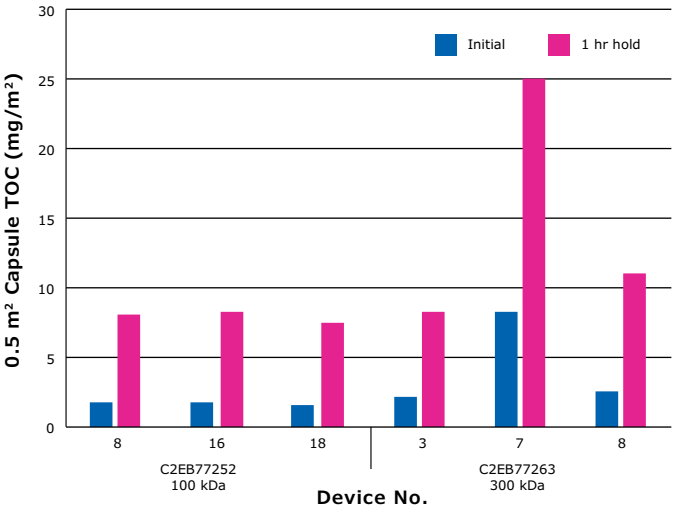

Figure 5.

Evaluation of product pool TOC before and after mock product hold for capsule size 0.5 m².

Conclusion

TOC levels for flushing and leachables in tested capsules are considered low after following the described procedures. The results support the benefit of using Pellicon® Capsules, which are supplied gamma sterilized and free of preservatives, in considerably reducing flushing volumes before product processing as well as leachables content during product processing.

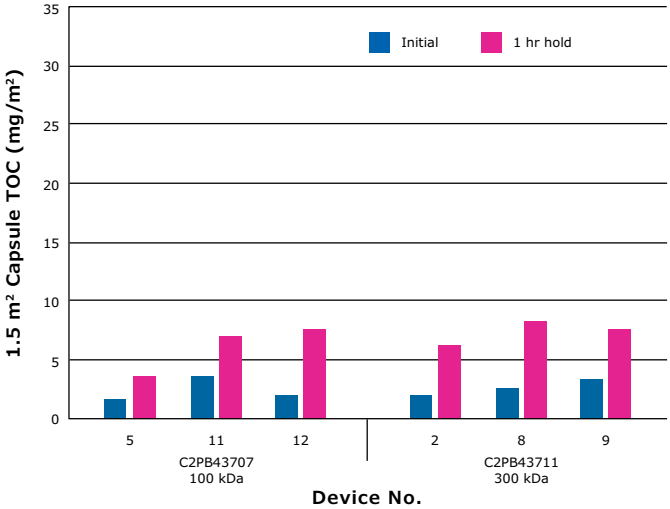

Figure 6.

Evaluation of product pool TOC before and after mock product hold for 100 kDa and 300 kDa capsule sizes 1.5 m² and manifolds.

Hold-Up Volume of Capsules

Objective

To determine the hold-up volume of capsules.

Summary

Experiments were performed to characterize the hold-up volumes of capsules 0.1, 0.5, 1.5 m^2, as well as the tubesets used in the Pellicon® Capsule manifolds. Hold-up volumes in the feed channel were measured to indicate the recoverable volume within the feed channel. The total hold-up volume of capsules and manifold tubesets was measured to help the user determine the minimum working volumes required to operate their systems.

Method

Capsule Devices

Hold-up volumes for Pellicon® Capsule device sizes 0.1, 0.5, and 1.5 m^2 were evaluated according to the procedure outlined below. Three capsules of each size were used for testing.

1. Reverse osmosis water was recirculated through the capsule for 5 minutes at a feed pressure of 20 psi, retentate pressure of 15 psi, and permeate pressure of 10 psi.
2. The feed, retentate, and permeate ports of the capsule were capped, making sure water inside the capsule was not lost.
3. The Initial Wet Capsule Weight was weighed and recorded.
4. The caps from the feed and retentate ports were removed and compressed air was blown down the feed channel at 10 psi for 3 minutes.
5. The caps were placed back onto the feed and retentate ports of the capsule to weigh and record the Post Feed Channel Blow Down Weight.
6. The cap from the permeate port was removed and the capsule was inverted. The capsule was shaken to remove as much water as possible from the permeate channel.
7. The cap was placed back onto the permeate port of the capsule to weigh and record the Post Permeate Channel Blow Down Weight.
8. The caps from the feed, retentate, and permeate ports were removed and compressed air was blown down through the capsule at 10 psi for ≥12 hours.
9. The caps were placed back onto the feed, retentate, and permeate ports to weigh and record the Final Dry Capsule Weight. The Initial and Final Dry Capsule Weights were compared to ensure that the capsule was completely dry.

Capsule Manifolds

Following the measurement of device hold-up volumes, manifold tubeset hold-up volumes were determined according to the procedure below.

1. An empty, dry tubeset was weighed and recorded to obtain the Dry Tubeset Weight.
2. Reverse osmosis water was circulated through the tubesets. The tubeset ports were capped to ensure water was not lost and all air was removed.
3. The Wet Tubeset Weight was weighed and recorded.
4. The Dry Tubeset Weight was then subtracted from the Wet Tubeset Weight to obtain the hold-up volume of the tubeset.

After measuring the hold-up volumes of the tubesets, the hold-up volumes of fully pre-assembled Pellicon® Capsule manifolds were calculated by adding the corresponding tubeset hold-up volumes to the hold-up volume of capsule devices used in the assembly configuration.

Results

All weights were converted to volumes, assuming one gram of water equals one milliliter of water.

1 g H_20 = 1 mL H_20

Calculations for hold-up volume of individual capsule devices were as follows:

Feed Channel Hold-up Volume = Initial Wet Capsule Weight – Post Feed Channel Blow Down Weight

Permeate Channel Hold-up Volume = Post Feed Channel Blow Down Weight – Final Dry Capsule Weight

Total Capsule Hold-up Volume = Feed Channel Hold-up Volume + Permeate Channel Hold-up Volume

Manifold hold-up volumes were calculated by adding the hold-up volumes of each individual capsule and tubeset in an assembly unit. For example, a 3 m^2 manifold consists of two 1.5 m^2 capsule devices and three tubesets (feed, retentate, permeate). Calculations for hold-up volume of manifolds were as follows:

Tubeset Hold-up Volume = Wet Tubeset Weight – Dry Tubeset Weight

Manifold Assembly Feed Channel Hold-up Volume = (Number of Capsules in Manifold × Feed Channel Hold-up Volume of Capsule) + (2× Tubeset Hold-up Volume)

Manifold Permeate Channel Hold-up Volume = (Number of Capsules in Manifold × Permeate Channel Hold-up Volume of Capsule) + Tubeset Hold-up Volume

All Calculated volumes for device and manifold sizes are presented in **Table 1**.

Table 1.

Hold-up volume results.

Catalog No.	Membrane Area (m^2)	Feed Channel Hold-up Volume (mL)	Permeate Channel Hold-up Volume (mL)	Total Device Hold-up Volume (mL)
Pellicon® Capsule Devices				
PCC100C01	0.1	27	60	87
PCC100C01C		39	66	105
PCC300C01		28	60	88
PCC300C01C		40	66	106
PCC100C05	0.5	118	133	251
PCC100C05C		130	139	269
PCC300C05		119	128	247
PCC300C05C		131	134	265
PCC100C15C	1.5	451	727	1178
PCC300C15C		456	741	1197
Pellicon® Capsule Manifolds				
PCC100C10G	1	276	286	562
PCC300C10G		278	276	554
PCC100C30G	3	1100	1553	2653
PCC100C30L		1120	1563	2683
PCC100C30E		1280	1643	2923
PCC300C30G		1110	1581	2690
PCC300C30L		1130	1591	2720
PCC300C30E		1290	1671	2960
PCC100C45G	4.5	1653	2331	3984
PCC100C45L		1671	2340	4011
PCC100C45E		1845	2427	4272
PCC300C45G		1667	2373	4040
PCC300C45L		1685	2382	4244
PCC300C45E		1859	2469	4328

Conclusion

Hold-up volumes in the feed channel of Pellicon® Capsules were characterized to indicate their recoverable volume. The total capsule hold-up volume indicates the volume contained within the device during a TFF process and is provided to help the user determine the minimum working volume required to operate their systems.

Further Information

1. Pellicon® Capsule Datasheet. Lit. No. DS1285EN.
2. Pellicon® Capsule User Guide. Lit. No. UG1549EN.
3. Ultracel® Membranes Data Sheet. Lit No. PF1401EN00.
4. Evaluation of TFF Operating Control Strategies and Scalability for Viral Vector Process Development. Lit. No. TB11669EN.

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