Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Millipore Express® Ace 0.2 µm Membrane

An innovative sterilizing-grade membrane for efficient filtration of biologics

Introduction

Membrane-based sterile filtration is a crucial component of aseptic drug manufacturing; it removes microbial contaminants and assures a sterile drug product that is safe for patient administration. Sterilizing-grade membrane filters are used to filter a wide variety of fluid streams from simple buffers with water-like characteristics, to more complex fluids including cell culture media and process intermediates that may contain components that can prematurely plug the membrane pores and limit filter capacity. Furthermore, in final sterile filtration steps during the fill finish process, sterilizing-grade filters must efficiently process high concentration fluids while minimizing adsorption of protein or excipients to the membrane surface.

Millipore Express® Ace 0.2 µm membrane filters are the most recent addition to our Millipore Express® filter family and deliver exceptional sterile filtration performance for a range of bioprocessing applications. These filters contain an innovative, optimized pore size gradient polyethersulfone (PES) single-layer membrane structure, with proprietary low protein binding surface chemistry that enables more membrane area in standard filter formats as compared to other commercially available filters. This advancement enables a sterilizing-grade filter with exceptional flux and capacity, minimizing the footprint during both warehousing and the production process.

Adapting to evolving global regulations, Millipore Express® Ace 0.2 µm filters are manufactured without intentional addition of per- and polyfluoroalkyl substances (PFAS) in materials of construction, thereby accelerating sustainability for future-ready processing.

The application note summarizes key performance attributes of this innovative membrane and highlights the benefits for specific bioprocessing applications.

High Flow Rates Enable Faster Processing

Membrane filtration at high flow rates improves efficiency and enables faster processing; it can also reduce the impact of filter fouling, extending filter life. The optimized pore size gradient of Millipore Express® Ace 0.2 µm membrane was designed to maximize filter flow rates. This study compares water flow rates of Millipore Express® Ace 0.2 µm filters with those of other commercially available sterilizing-grade filters.

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Materials & Methods

The water flow rates of several commercially available 10-inch filters were measured at 10 psi (**Table 1**).

Table 1.

Sterilizing-grade filters tested to assess water flow rates and filter capacity. Sartopore® and Supor™ filters are manufactured by Sartorius and Cytiva respectively.

Filter	Filtration area per 10-inch device [m²]	Membrane pore size [µm]	Membrane composition	Membrane layers
[1]**Millipore Express® Ace 0.2 µm**	1.38	0.2	PES	1
Sartopore® Platinum	1.0	0.45 / 0.2	PES	2
Sartopore® 2	0.6	0.45 / 0.2	PES	2
Sartopore® 2 XLG	0.8	0.8 / 0.2	PES	2
Sartopore® 2 XLI	0.8	0.8 / 0.2	PES	2
Supor™ EX ECV	1.04	[2]X / 0.2	PES	2
Supor™ Prime	1.34	[2]X / 0.2	PES	2

[1] Prototype 10-inch filtration devices.
[2] Pore size of top membrane layer not specified.

Results & Discussion

Figure 1 shows the water flow rates of 10-inch Millipore Express® Ace 0.2 µm filters benchmarked against other commercially available sterilizing-grade filters.

Millipore Express® Ace 0.2 µm filters had the highest flow rate of all filters tested with flow rates that were 1.6× higher than those of Supor™ Prime filters.

There are advantages for plugging streams too: higher initial flow rates (or lower initial pressures for constant flux operations) means you can reach your filtration end point sooner than if you were to use filters with lower flow rates.

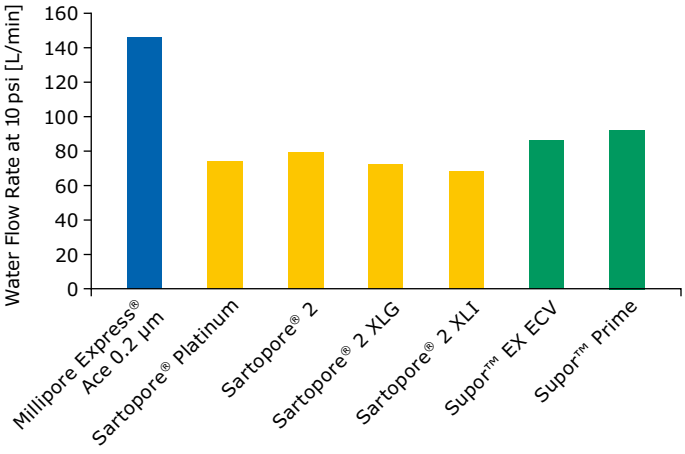


Figure 1.

Water flow rates at 10 psi measured for Millipore Express® Ace 0.2 µm filters and other commercially available 10-inch filters.

These results show that Millipore Express® Ace 0.2 µm membrane filters have higher flow rates than other commercially available sterilizing-grade membrane filters. Higher flow rates provide advantages for both plugging and non-plugging streams: faster processing, reduced time to reach filtration endpoint and improved overall process efficiency.

High Filter Capacity Improves Efficiency

Filter capacity determines filtration area requirements and impacts overall process economics. Process streams that contain higher levels of plugging components, such as complex cell culture media or process intermediates, can reduce filter capacity, leading to oversized filters, higher costs and a larger footprint. Millipore Express® Ace 0.2 µm membrane filters were optimized for high throughput and filter capacity, resulting in more favorable process economics.

Cell culture media is generally filter sterilized to prevent bioreactor contamination. Process intermediates are filtered through bioburden reduction or sterilizing-grade filters to control bioburden and minimize the risk of contaminating costly chromatography columns. These downstream process intermediates vary widely in impurity composition and protein concentration which impacts filter capacity.

The study below benchmarks the relative performance of Millipore Express® Ace 0.2 µm filters with other commercially available filters designed to efficiently filter cell culture media or process intermediates (PI).

Materials & Methods

Three complex process streams were evaluated:

- Cell culture medium. The average throughput of these two media is shown.
  - EX-CELL® Advanced CHO Fed-batch Medium.
  - EX-CELL® Advanced HD Perfusion Medium.
- Post-clarification PI: 1–5 NTU.
- Post-chromatography PI: 4 g/L, 2.5 NTU.

Filterability of these three complex streams was evaluated on Millipore Express® Ace 0.2 µm filters and other commercially available filters listed in **Table 1**. Selected comparisons are presented based on the marketed application of competitor filters and the limitations of process fluids.

Water flow rates of all test membranes were measured in OptiScale® 25 (3.5 cm^2 filtration area) devices at 10 psi constant pressure and compared to flow rates in 10-inch devices to determine the water scaling factor.[1] OptiScale® 25 devices containing the test membranes were then evaluated with the various fluid streams and the throughput capacity for 10-inch devices was calculated based on respective filtration areas (**Table 1**) and a scaling factor. For these plugging streams, the scaling factor was an interpolated value between the water scaling factor and 1, depending on how fouled the membrane was at the filtration end point. These adjusted throughput values were then benchmarked relative to that of the Millipore Express® Ace 0.2 µm prototype 10-inch device.

Results & Discussion

Figure 2 shows the results of throughput studies designed to assess throughput capacity of Millipore Express® Ace 0.2 µm filters with cell culture media and two process intermediates. All commercially available filters tested were reported to enable high throughput and capacity for for their marketed applications.

For both cell culture medium and process intermediates, Millipore Express® Ace 0.2 µm filters outperformed the other filters with much higher throughput capacity per 10-inch device. Absolute throughput ranged from 2,700–17,500 L dependent on the process fluid. Millipore Express® Ace 0.2 µm filters showed improved filter capacity of 1.4×–2.5× when compared to the tested commercially available filters shown in **Figure 2**.

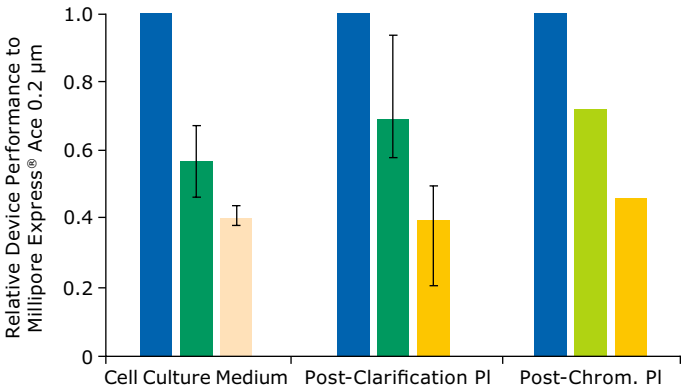


Figure 2.

Relative throughput of cell culture media and two process intermediate feeds on Millipore Express® Ace 0.2 µm and other commercially available sterilizing filters. Values reflect predicted performance of 10-inch devices normalized to Millipore Express® Ace 0.2 µm (blue). Supor™ EX ECV (green), Supor™ Prime (light green), Sartopore® Platinum (yellow), and Sartopore® 2 XLI (light yellow). The error bars represent the range of values for each stream tested and the average is represented by the bars.

Results demonstrate that Millipore Express® Ace 0.2 µm membrane filters provide excellent throughput for complex and plugging streams, performing significantly better than other commercially available filters. Higher throughput translates to higher filter capacity, reduced manufacturing footprint and improved process efficiency.

1. Water scaling factor: Device permeability/membrane permeability.

Cell Culture Media Filtration: Excellent Cell Growth & Productivity

Hydrophilic membrane filters are essential for ensuring sterility and preventing bacterial contamination of upstream cell culture processes. Sterilizing-grade filters should meet the ASTM F838 standard for quantitative retention of *Brevundimonas diminuta* without removing vital components for cell growth from the complex cell culture media. Any filter for cell culture media filtration should support excellent cell culture performance including viable cell density, viability and productivity, while also meeting filtration flux, capacity and filtration area targets as these affect process economics. This study explores cell culture performance following media filtration over Millipore Express® Ace 0.2 µm filters as compared to other commonly used cell culture media filters. Filter capacity was reported in the previous section.

Materials & Methods

Performance of cell lines was assessed following filtration of cell culture media and feeds over Millipore Express® Ace 0.2 µm and other sterilizing-grade filters listed in **Table 2**.

Table 2.

Membrane filters and their characteristics.

Filter	Membrane Pore Size [µm]	Membrane Composition	Membrane layers	Membrane Symmetry
Millipore Express® Ace 0.2 µm	0.2	PES	one	Asymmetric
Millipore Express® SHC	0.5/0.2	PES	two	Asymmetric
Durapore® 0.22 µm	0.22	PVDF*	one	Symmetric

* PVDF = Polyvinylidene fluoride.

The medium and feed for each cell line were supplied by MilliporeSigma and prepared according to the manufacturer's protocol on the same day they were filtered.

Table 3.

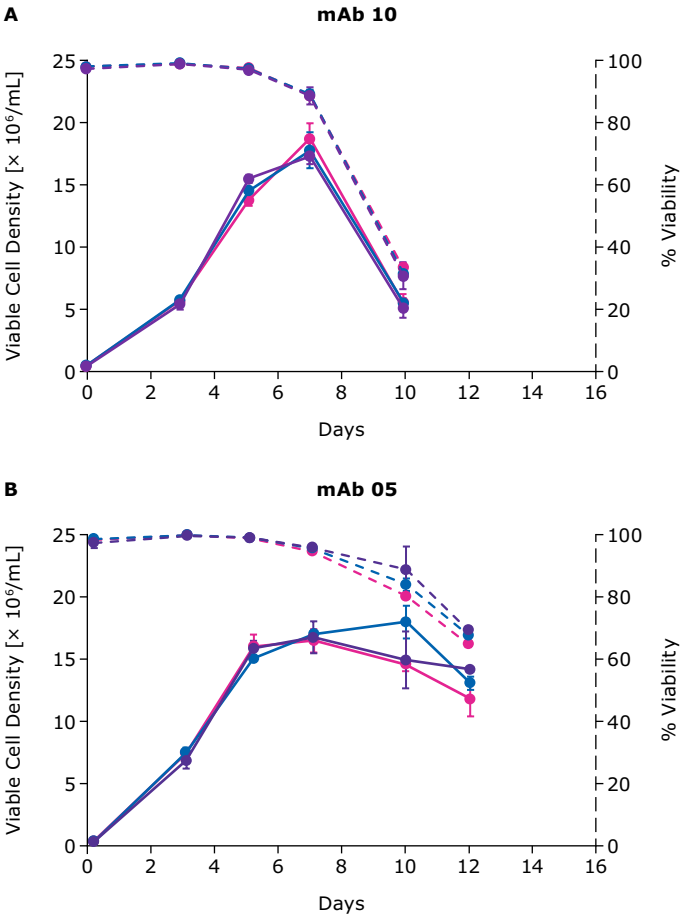
Cell lines with corresponding media/feed.

Cell Line	Media	Catalog #	Feed	Catalog #
mAb 10	EX-CELL® Advanced CHO Fed-batch Medium	24366C	EX-CELL® Advanced CHO Feed 1 (without glucose)	24368C
mAb 05	Cellvento® 4CHO Comp	103795	Cellvento® 4Feed Comp	103796

All filtration tests were run using prototype OptiScale® 25 devices that were presterilized and flushed before filtration at 10 psi constant pressure. Two recombinant Chinese hamster ovary (CHO) cell lines were thawed and passaged; cell lines were centrifuged and resuspended in a small volume of fresh filtered media and inoculated into triplicate spin tube bioreactors under simulated fed-batch process conditions. Cell culture performance was monitored over the duration of the culture.

Results & Discussion

Figure 3 summarizes the results and shows cell growth, viability and titer of two cell lines following media filtration over Millipore Express® Ace 0.2 µm, Millipore Express® SHC or Durapore® 0.22 µm membrane filters. Growth curves were typical for each of the two cell lines. No significant differences were observed in cell growth or viability (**Figure 3A–B**) or antibody titer (**Figure 3C–D**) between the cultures, indicating that the membrane filters did not negatively impact cell growth or titer.



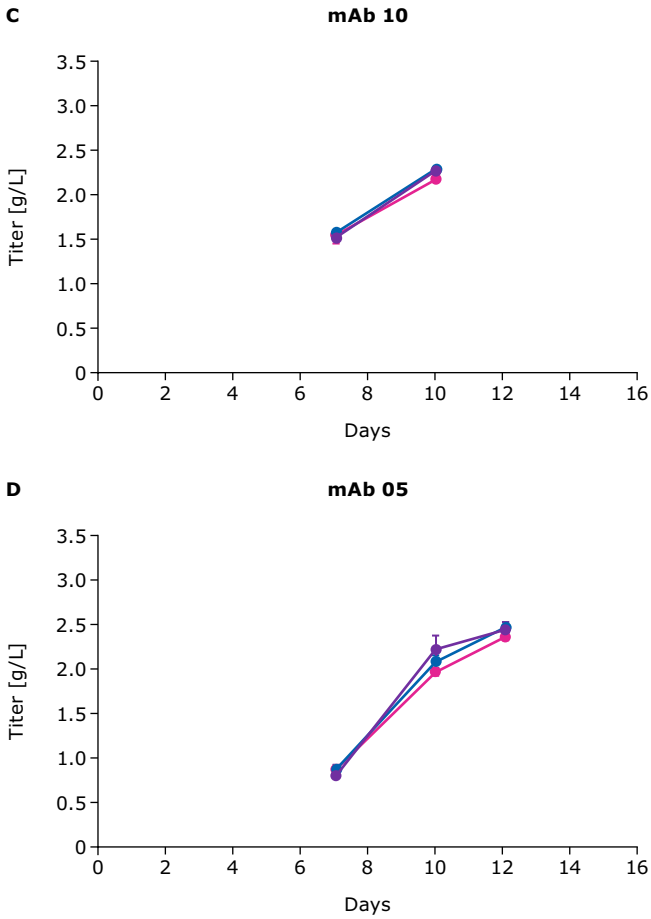


Figure 3.

(A–B) Viable cell density (solid lines) and percent viability of each cell line (dotted lines, secondary y-axis), (C–D) antibody titer after media filtration with Millipore Express® Ace 0.2 µm (blue), Millipore Express® SHC (pink), and Durapore® 0.22 µm (purple) membrane filters. Each data point is the average of measurements from triplicate cultures; error bars represent the standard deviation.

These results confirm that filtration with Millipore Express® Ace 0.2 µm filters did not negatively impact the composition or quality of the cell culture media, and that these filters enabled equivalent cell culture performance to Millipore Express® SHC or Durapore® 0.22 µm filters.

Final Filtration: Low Protein and Excipient Binding

Final sterilizing filtration is the last step in downstream processing before fill finish and assures the sterility of the drug product for administration to patients. Any filtration membrane and device used for final filtration must meet the demands of this critical step without compromising the quality of the drug product. Drug products often contain low levels of excipients such as polysorbate or poloxamer, which improve their stability, absorption and shelf life. However, these excipients add complexity to filtration: their chemical composition may affect fluid viscosity, interact with the filter membrane, or cause premature filter fouling.

Durapore® 0.22 µm PVDF membrane filters have long been used for final filtration due to their non-reactivity and exceptionally low binding of proteins and excipients. However, PES filters, including Millipore Express® SHF and Millipore Express® SHC filters are increasingly being implemented throughout biomanufacturing for enhanced speed and economics. Millipore Express® Ace 0.2 µm filters, with proprietary surface-modified membrane chemistry, offer an advanced PES membrane with the ideal performance characteristics for final sterile filtration.

The studies below evaluated protein and excipient binding on Millipore Express® Ace 0.2 µm membranes and benchmarked performance with select commercially available filters used for final filtration applications, **Table 1**.

Materials & Methods

Protein binding was assessed by soaking three 13 mm discs of each test membrane filter in a goat gamma globulin solution (1 mg/mL) spiked with approximately 10^6 cmp of ^{125}I-goat anti rabbit IgG labeled protein in a PBS (pH 7.4) solution. Protein binding was expressed as µg/cm^2 for each membrane.

Binding of the excipients polysorbate 20, and poloxamer 188 in 10 mM acetate buffer (pH 5.5) was assessed with the various membranes in OptiScale® 25 small scale devices at constant flux of 130 L/m^2/hr (LMH), equivalent to 0.75 mL/min flow rate. Excipient levels were measured in each feed and in filtrate samples that were collected throughout the runs. All samples were analyzed by high-performance liquid chromatography (HPLC)-charged aerosol detector (CAD). The ratio of excipient concentration in the filtrate and corresponding feeds were used to assess binding throughout the runs.

The impact of flushing Millipore Express® Ace 0.2 µm membrane with a polysorbate 20 containing buffer before filtering drug product was evaluated. Studies were performed with mAb 10 (3.54 mg/mL) in 10 mM acetate buffer pH 5.5 containing 0.05% (v/v) polysorbate 20 and mAb concentration was determined using Protein A HPLC.

Results & Discussion

Excipients such as polysorbate 20, polysorbate 80, and poloxamer 188 are commonly used in biologics formulation to improve drug product stability, absorption or shelf life. The changing regulatory landscape and increasing use of PES membrane filters in bioprocessing focused efforts on developing a PES membrane with minimal protein and excipient binding while providing the essential performance characteristics for critical final filtration steps.

Figure 4 summarizes results of protein binding studies with the Millipore Express® Ace 0.2 µm, Durapore® 0.22 µm membrane and membrane from two commercially available filters. Millipore Express® Ace 0.2 µm showed slightly lower protein binding than the benchmark Durapore® 0.22 µm membrane and at least 3–5× lower protein adsorption than the other PES membranes tested.

For any final filtration application, this lower binding will minimize product loss to the membrane surface area and help maximize drug product yield.

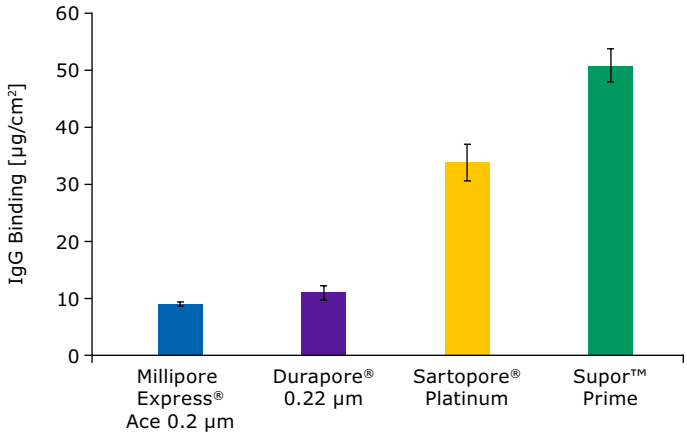


Figure 4.

Average IgG protein binding (n=3) on test membranes; error bars show the standard deviation.

Figure 5A shows the results of filtration studies with 0.05% (v/v) polysorbate 20 and these same membrane filters.

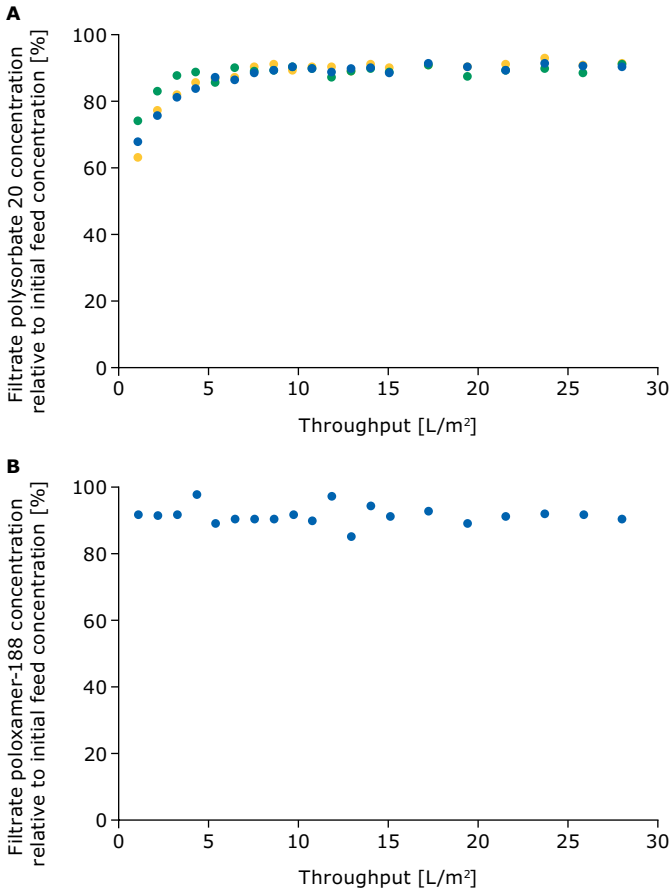


Figure 5.

Filtrate concentration over throughput of (A) 0.05% polysorbate 20 and (B) poloxamer-188 in 10 mM acetate buffer for Millipore Express® Ace 0.2 µm (blue) Sartopore® Platinum (yellow) and Supor™ Prime (green).

Millipore Express® Ace 0.2 µm membrane showed a similar polysorbate 20 profile as the other membranes tested: measurable reduction was observed, indicating polysorbate 20 adsorption to the membrane but by 4–10 L/m^2, levels in the filtrate matched those of the unfiltered fluids. A similar adsorption profile, but with slightly lower levels of binding, was observed for polysorbate 80 on all three membrane filters (data not shown).

The excipient adsorption for these PES membranes is similar based on throughput (L/m^2) of the filters. However, if the increased filter capacity of Millipore Express® Ace 0.2 µm filters relative to other PES filters is considered, then the product loss to excipient binding would be proportionally less on Millipore Express® Ace 0.2 µm filters due to the reduced filtration area required for similar throughput performance.

Figure 5B shows Millipore Express® Ace 0.2 µm filters with 0.05% poloxamer-188. In contrast to the polysorbates, poloxamer-188 surfactant did not appear to measurably adsorb to the PES membrane.

For any final filtration step, the throughput needed to reach the target excipient level in the filtered drug product will depend on the excipient, excipient concentration, membrane filter, and individual process specifications.

One option for minimizing excipient binding to any PES membrane is to preflush the filters with excipient containing buffers during pre-use post sterilization integrity testing (PUPSIT) before product filtration. **Figure 6** highlights the benefits of this approach. Without preflushing, binding of 0.01% polysorbate 20 requires product throughput of 28 L/m^2 to accommodate the adsorption losses to the membrane; this drug product (35 mg) would not be correctly formulated and would likely be excluded from the batch (**Figure 6A**). However, if the filter is preflushed with polysorbate containing buffer before product filtration, the throughput needed to fill these excipient binding sites is minimized and product losses are reduced to 4 mg (**Figure 6B**).

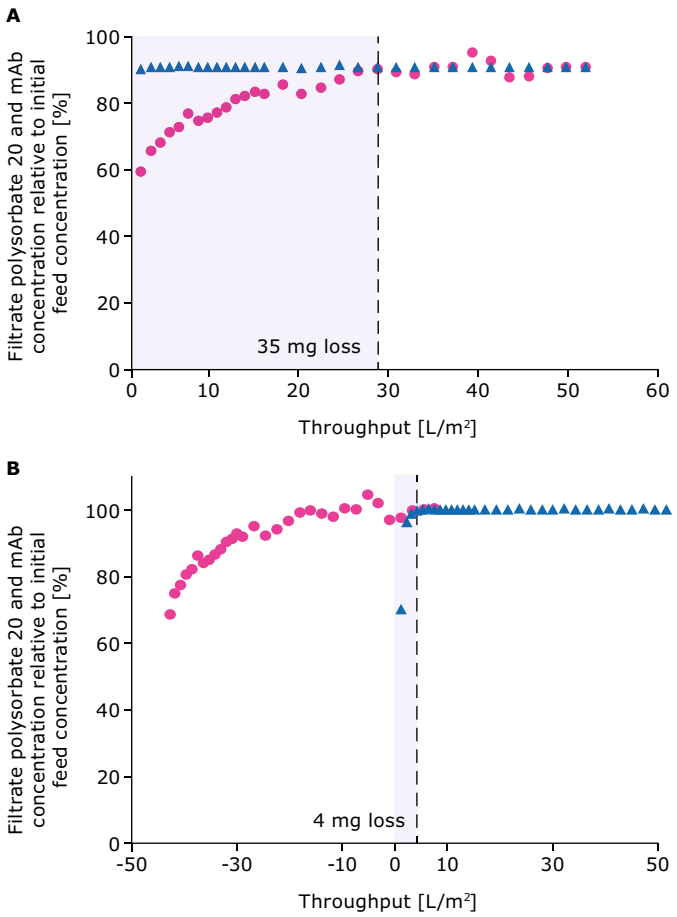


Figure 6.

Relative polysorbate concentration (pink circles) in filtrate relative to initial feed concentration with increasing throughput. Protein concentration shown in blue triangles (A) No preflushing performed. Product containing 3.5 mg/mL mAb and 0.01% polysorbate 20 filtered with no preflush (B) Filter preflushed with 50 L/m^2 0.01% polysorbate 20 in 10 mM acetate buffer, then integrity tested after the flush before introducing mAb product. The cumulative protein loss for each condition is listed. The black dotted lines represent when the filtrate samples contain 100% polysorbate 20 and the shaded purple area represents the filtrate samples that contain (A) less than 100% of the polysorbate concentration and (B) less than 100% of the mAb concentration and therefore discarded.

These results demonstrate that Millipore Express® Ace 0.2 µm membrane shows lower protein binding than other commercially available PES filters for final filtration but the adsorption profile for excipients such as polysorbate 20 and polysorbate 80 is similar. However, as Millipore Express® Ace 0.2 µm filters provide higher capacities than these PES filters and less filtration area is required, the product loss associated with excipient binding would be expected to be proportionally less. Their combined high capacity and low protein binding position these PES membrane filters as an ideal option for critical final filtration applications.

Conclusions

Millipore Express® Ace 0.2 µm membrane filters were designed to deliver exceptional sterile filtration performance for a range of bioprocessing applications. These filters feature an innovative, optimized pore size gradient PES single-layer membrane with a proprietary low protein binding surface chemistry, enabling superior throughput and capacity for both water-like and more complex streams than other commercially available filters.

When used for cell culture media filtration, this novel membrane does not measurably impact cell culture performance and enables optimal cell growth and mAb production. For process intermediates and more complex plugging process fluids, Millipore Express® Ace 0.2 µm membrane filters have much high filter capacity than other commercially available sterilizing-grade filters. This will reduce filter footprint and improve processing efficiency.

Most importantly, when used in final filtration, protein binding is low – comparable to Durapore® 0.22 µm – and excipient binding can be mitigated, offering an improved PES membrane option for this critical sterile filtration step.

This advanced sterilizing-grade membrane filter with exceptional flux and capacity, made without the intentional addition of PFAS, is ready to meet the needs of future-ready bioprocessing.

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