



Scientific Brief

From Cell Culture to Target Protein: Exploring the Benefits of the 24-Well Filter Plate Workflow

Introduction

Over the last 5 years, the use of 24-well cell culture plates in laboratory applications has substantially increased. This market segment has expanded at an estimated 14% compound annual growth rate (CAGR)— a pace that is expected to continue in coming years.

The popularity of 1 L benchtop bioreactors has also helped intensify the demand for 24-well filter plates. With their greater throughput, these automated bioreactors have sped up cell culture, enabling researchers to perform process development before selecting a final strain of interest. The 24-well filter plate bridges the gap between laboratories processing high-throughput 96-well plates and those moving up to benchtop reactors. This explosion in the use of 24-well cell culture plates have fueled laboratory interest in migrating to total 24-well filter plate workflows.

Pall Laboratory has met this growing market need with a comprehensive line of AcroPrep™ 24-well filter plates that offers total workflow solutions. The new line incorporates the same membranes found in Pall's 96-well filter plate formats, making scale-up much easier and less time-consuming. By using the same manufacturer and the same membranes across all filter plate portfolios, Pall envisioned significant savings in processing time, labor, and efficiency.

This paper explores Pall's evaluation of the potential applications for its new AcroPrep 24-well filter plate line, plus the time savings it can achieve in protein purification workflows.

Multiple Applications of Interest

Pall's AcroPrep 24-well filter plate line comes in a variety of membranes and pore sizes that make it useful for a vast number of applications. Protein purification workflows include the following:

- **AcroPrep filter plate with Omega™ membrane ultrafiltration:** this filtration uses a polyethersulfone membrane specifically modified to minimize protein and nucleic acid binding. It is designed for concentration and purification of peptides, proteins, oligonucleotides, DNA, and RNA; cleanup of labeling and PCR reactions; desalting and buffer exchange; and fractionation based on size exclusion.
- **AcroPrep filter plate with Supor® membrane:** Supor is a low protein-binding polyethersulfone (PES) membrane that is optimized for biological, pharmaceutical, and sterilizing filtration requirements. Supor membranes have extensive drug and chemical compatibility, making them ideal for different applications such as protein purification, lysate clearance, general sample preparation, multiplexing assays, mycoplasma reduction, and sterile and aqueous filtration. These AcroPrep 24-well filter plates enable processing that was previously very difficult at higher volumes.
- **AcroPrep cell clarification and sterile filter plates:** These multi-membrane plates perform cell clarification and sterile filtering in one step for protein purification workflows. They work well when partnered with Omega 24-well ultrafiltration plates (see above) in protein and nucleic acid purification workflows.

In addition, Pall's AcroPrep 24-well filter plate line includes plates with polyethylene/polypropylene (PE/PP) membranes. This filtration is ideal for laboratories engaged in plant and cannabis research due to its large pore size. It is designed for heavy, particulate-laden samples associated with grinding up plant materials before further processing.

Defining the AcroPrep 24-Well Filter Plate Protein Purification Workflow

In the past, laboratories growing cells in 24-well culture plates could not perform the protein purification workflow without transferring samples into new plate formats or using manual steps. Today, by processing cells entirely in 24-well plates, they can eliminate these steps — offering time-savings and workflow streamlining benefits for the laboratory.

24-well filter plates simplify protein purification

Protein purification from cells grown in 24-well filter plates is a multi-format, manually driven process. Following cell culture, laboratory users manually move their samples to a centrifuge for clarification. After centrifugation, the user has to recover the clarified supernatant from each sample and filter the protein product of interest through use of a sterile 0.2 µm syringe filter. In addition, because centrifugation will not always fully clarify a sample, some laboratories will process the samples through a 0.45 µm syringe filter first to ensure the 0.2 µm sterilizing-grade membrane does not clog. This process produces a sterile-filtered protein. But in many assays, the protein may be diluted or in spent media, requiring concentration, desalting, and/or buffer exchange for downstream processing. These steps would require the user to take the cover off a spin column, manually apply the sample, close the cover, put each of 24 columns in the centrifuge, and spin them. After removing the columns from the centrifuge, the samples are pipetted into a new plate (being careful not to puncture the filter membrane).

Incorporating Pall's AcroPrep 24-well filter plate line across the protein purification workflow eliminates these labor-intensive steps. The AcroPrep 24-well clarification and sterile filter plate combine two or more separate processes (clarification and sterilization) into one workflow step – permitting the user to go from cell culture to sterile protein in a single plate format. It eliminates the need to manually load samples in a centrifuge for clarification and then sterile filter proteins through individual syringe filters.

If downstream processing is required, the AcroPrep 24-well filter plate with Omega membrane rapidly performs clean-up and protein concentration versus manually loading 24 spin columns. The Omega membrane works on the premise of size exclusion, where samples are fractionated by molecular weight. Larger molecules can be separated from smaller molecules by selecting the appropriate molecular weight cut-off (MWCO) and filtering the sample through these plates by either centrifugation, vacuum, or positive pressure.

24-well filter plate workflows enable larger sample volumes

For laboratories using Pall's 96-well filter plates, the new AcroPrep 24-well filter plates are available with the same membranes. That means these laboratories can process increased sample volumes for their applications and workflows in less time. Where previously they might put the same sample in multiple wells of a 96-well filter plate, or use a spin device or syringe filter, the user now can process up to 7 mL of sample in each well of a 24-well filter plate.

Results

Pall Laboratory expects applying AcroPrep 24-well filter plates across laboratory workflows will achieve significant throughput increases, plus substantial time and labor savings.

For example, incorporating the filter plates across the entire 24-well protein purification workflow will provide the following benefits:

- The AcroPrep 24-well clarification and sterile filter plate will save up to 35 minutes versus manually processing a 24-well plate with syringe filters. With fewer steps, it can reduce the risk of contamination and errors. Plus, the dual-purpose filter plate produces seven times less plastic consumable waste.
- When additional processing is necessary, AcroPrep 24-well filter plates with Omega membrane offer at least a 25% reduction in set-up time over spin tubes. Using a multichannel pipette, the AcroPrep filter plate can be filled in six steps versus 24 steps for spin tubes. Other processing methods, such as using 24 individual syringe filters, would require more time than spin tubes. And splitting samples in multiple wells of a 96-well filter plate would demand considerably more labor and complexity.

- Processing time in a spin column depends on the filter's MWCO. The smaller the pore size, the longer the processing time. While the same is true for the AcroPrep filter plate, its capability to be spun or put in a vacuum manifold makes the impact of MWCO much less significant (see Table 1). In addition, the user has more control over the filter pressure, which speeds up processing.
- The filter plates enhance throughput since 24 samples can be processed in one step. By loading four plates in a centrifuge, 96 samples can be clarified or cleaned up at one time. This represents a significant improvement over 15 mL spin-column processing, particularly if growing cells in multiple plates.
- The AcroPrep filter plates typically recover about $\geq 90\%$ of the target molecules, performance commensurate with spin columns and syringe filters.

The AcroPrep filter plates with Omega membrane offer very low hold-up volumes that ensure minimal sample loss. In addition, the plates' predictable processing times make it easy for users to set their workflow protocols (see Table 1).

Table 1

Typical Hold-Up Volumes and Processing Times

AcroPrep 24-Well Filter Plate with Omega Membrane.

Typical hold-up volume by processing method

Hold-Up Volume (µL)	Centrifuge	26 µL
	Vacuum	75 µL
	Positive Pressure	71 µL

Typical processing times by MWCO (kDa)

		1 kDa	3 kDa	10 kDa	30 kDa	50 kDa
Processing Time (min)	Centrifuge	170	135	70	60	60
	Vacuum	165	135	85	60	60
	Positive Pressure	155	70	45	50	55

Conclusion

As research laboratories embrace 24-well filter plate formats for cell culture and protein purification applications, the demand for 24-well filter plate processing will intensify. The AcroPrep 24-well filter plate line meets this demand with a variety of membrane types and pore sizes that perform clarification, sterile filtration, desalting, clean up, and buffer exchange. Now, laboratories can create entire 24-well plate workflows that reduce labor and time and provide higher-throughput processing than spin columns and syringe filters. In some cases, the filter plates offer workflow solutions that were previously unavailable. The benefits of 24-well filter plate processing come with hold-up volume, target molecule capture, and protein binding performance that is commensurate or better than conventional laboratory workflows.



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