Scientific Brief

Efficient Protein Purification Strategies Using Multi-Well Filter Plates

Introduction

The introduction of 96-well filter plates combined with chromatography media have emerged as an efficient tool for the fractionation of small-volume protein samples. This format can be used to develop protein purification strategies and/or as a platform for moderate- to high-throughput protein isolation. In either case, purified sample can be used for further analysis and/or downstream applications.

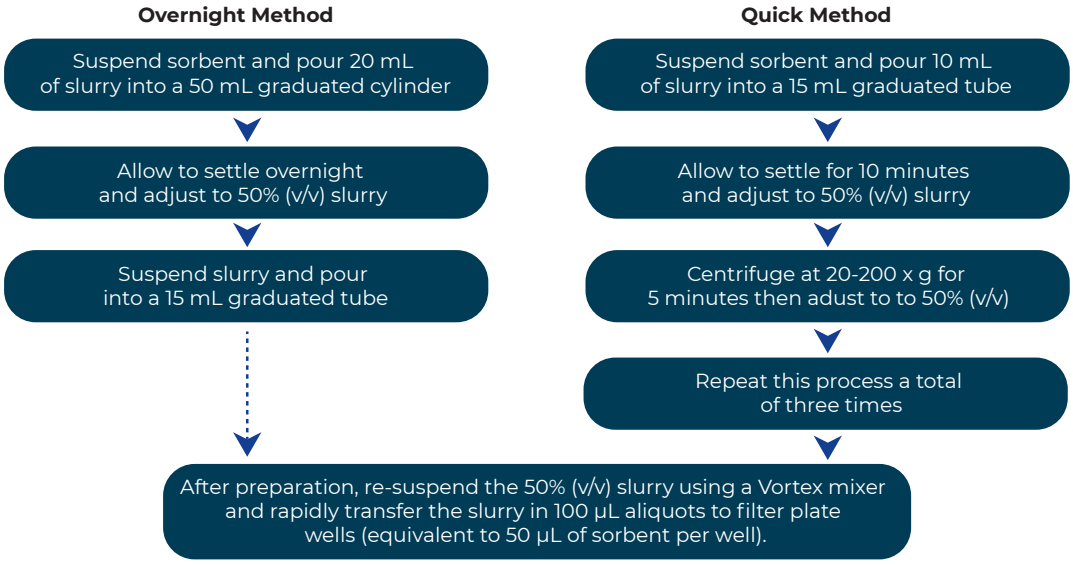
In this paper, development methods for protein purification strategies using small quantities of protein mixtures are reviewed. One method uses AcroPrep™ Advance filter plates with Supor® membrane combined with a mixed-mode chromatography resin to yield complete separation of three proteins in a protein sample. A second method uses AcroPrep Advance filter plates with Mustang® IEX membrane to separate a mixture of three different proteins. In each case, the utilization of a filter plate-based strategy allows for rapid screening of multiple purification schemes. Fractionation and protein purification methods developed within the plate can be used for optimization of larger-scale purifications.

High-Throughput Screening on 96-Well Plates

AcroPrep Advance 96-well filter plates are ideal for analytical applications. By packing chromatography sorbents in the wells of the plates, they can be used for protein or nucleic acid purification. One advantage of using filter plates is their capability to quickly screen various conditions. This allows the analyst to quickly optimize the chromatography resin and purification conditions with minimal sample consumption. The rapid screening ability and process condition optimization achieved with resin-packed filter plates can be transferred and confirmed, or scaled-up. The flexible 96-well plate format can be used with liquid-handling robotic systems or manual multi-channel pipettes. Sample or buffer recovery can be conducted using vacuum aspiration or centrifugation to draw the liquid through the membrane and bead bed into a 96-well receiver plate.

The screening of multiple conditions to optimize the purification step can be performed with an AcroPrep Advance 96-well filter plate. Once the selected sorbent is equilibrated and resuspended as a 50% slurry in the equilibration buffer, the desired amount is dispensed into the wells to a final volume of 50 µL per well. After the slurry has been dispensed into the wells of the plate, the equilibration buffer is then aspirated using a multi-well plate vacuum manifold. Next, a sequence mimicking a chromatographic run is performed on the plates. For each step of the sequence, the corresponding solution is pipetted into the wells. Once the wells are filled, the filter plate is covered with sealing tape and incubated while shaking. After incubation, the liquid is drained from the wells using the vacuum manifold and collected in a 96-well receiver plate. These individual fractions are then analyzed by HPLC, ELISA, or other analytical methods. The analyst can use this data to determine the set of parameters providing the best selectivity for their application – whether protein concentration and recovery or contaminant removal.

Packing of the chromatography sorbent can be achieved in two ways as resin allows, checking resin manufacturing recommendations.



Summary

An AcroPrep Advance filter plate with Supor 0.45 µm or 1.2 µm membrane (depending on the sorbent wet bead size), can be packed with various chromatography resins for small-scale, batch-mode protein purification matrix studies. This technique can be applied using a variety of sample types and optimized with the chromatography resin ideally suited to meet purification requirements. Purification strategies should be developed based on known physical and chemical characteristics of the target molecule, such as net charge, hydrophobicity, and affinity for metals or ligands. Chromatography resins and pH and conductivity variations can then be screened to determine ideal candidates for purification of the target molecules.

Membrane-Based Protein Purification

If resin packing is inappropriate for your application, Ion exchange with AcroPrep Advance 96-well filter plates with Mustang IEX membranes can be considered.

To overcome mass transfer limitations associated with conventional resin-based chromatography, membrane chromatography was developed to obtain better flow distribution, fast flow rates, and increased accessibility for rapid purification of target proteins or removal of contaminants.

These attributes translate into higher throughputs and reduced processing times. Filter plates with two membranes are available with strong IEX ligands: quaternary amine and sulfonic functional groups on Mustang Q (strong anion) and Mustang S (strong cation) membranes respectively. These AcroPrep Advance 96-well filter plates are ideal for small scale, primary protein purification applications.

Results

Performance of Mustang Q membrane is illustrated by separating a three protein mixture using a salt elution strategy. SDS-PAGE analysis of fractions (Figure 3) shows a clear separation of each protein.

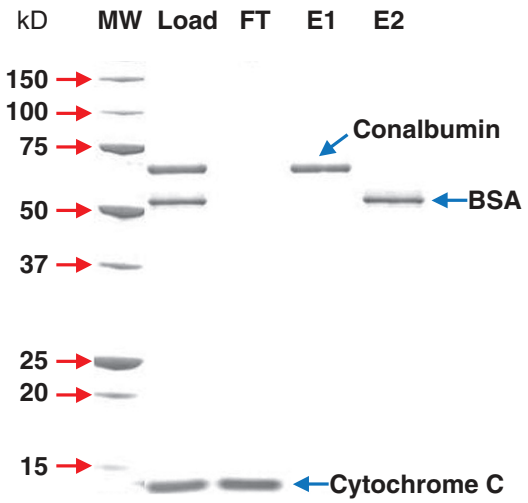
- Cytochrome C (12 kD) does not bind to the Mustang Q membrane at pH 8.8 (Lane FT)
- Conalbumin (78 kD) is eluted with the addition of 0.1 M NaCl (Lane E1)
- Albumin (67 kD) is eluted with 0.4 M NaCl (Lane E2)

Similar levels of protein separation are seen with Mustang S membrane in AcroPrep Advance filter plates, as seen by SDS-PAGE analysis of these fractions (Figure 4).

- All three tested proteins bind to the Mustang S membrane in Na Acetate, pH 4.5
- Trypsinogen (24 kD) elutes first with 0.2 M NaCl (Lane E1)
- Cytochrome C (12 kD) requires higher salt and elutes with 0.5 M NaCl (Lane E2)
- Lysozyme 14.4 kD requires 1.0 M NaCl for elution (Lane E3)

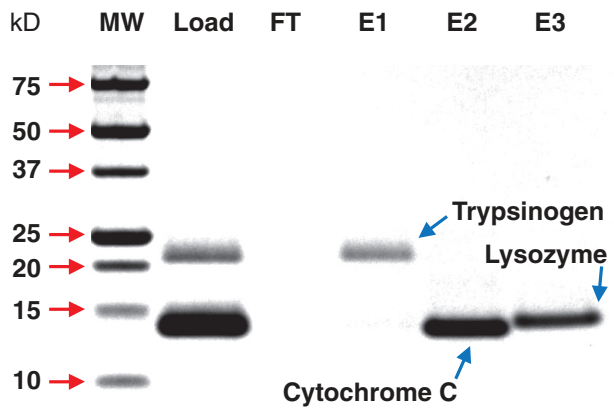
AcroPrep Advance filter plates with Mustang IEX membranes have demonstrated robust charge-based protein separations allowing for rapid protein purification.

Figure 1
Complete Separation of Proteins with AcroPrep Advance Filter Plates with Mustang Q Membrane



1.25-3.75 µg of total protein (not reduced) loaded onto 12% SDS-PAGE. Lanes: MW = molecular weight markers; Load = protein mixture; FT = flow through; E1 and E2 = 0.1 and 0.4 M eluate fractions. GelCode stain used.

Figure 2
Complete Separation of Proteins with Mustang S Membrane



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Discussion

AcroPrep Advance filter plates with Mustang IEX membranes have several performance advantages over resin-based IEX purification. Increased flow rates and better flow distribution allow for rapid binding and elution of biomolecules and wash steps are immediate. Large membrane pores increase accessibility to the IEX chemistries, either for purification or contaminant removal.

In addition, membranes have reduced hold-up compared to the resin. This increases wash efficiency and yields more concentrated elutes. These factors combine to create an efficient platform for protein purification. When reviewing protein purification strategies using IEX chemistries, it is highly recommended that the performance of the membrane chromatography is analyzed.

Conclusion

Two methods for fractionation of protein samples have been demonstrated using AcroPrep Advance filter plates.

The first method combines an AcroPrep Advance filter plate with 1.2 µm Supor membrane with chromatography resin for small scale batch-mode protein separations. Mixed-mode resins, capable of hydrophobic and IEX interactions, are used as a model to separate a three-protein mixture. This technique can be applied using a variety of sample types and optimized with the chromatography resin ideally suited to meet purification requirements. Purification strategies should be developed based on known physical and chemical characteristics of the target molecule such as net charge, hydrophobicity, and affinity for metals or ligands. Ion exchange, mixed-mode, and affinity chromatography resins can then be screened to determine ideal candidates for purification of the target molecules.

The second fractionation method of additional three-protein mixtures was achieved using Mustang Q and S membranes. In both cases, proteins were bound to the membrane and eluted with salt. Each fraction contained one protein that could be used for downstream applications. In contrast to filter plates combined with chromatography resin, AcroPrep Advance filter plates with Mustang membrane are ready-to-use separation devices.

PALL CORPORATION

Corporate Headquarters
Port Washington, NY, USA
+1-800-717-7255 toll free (USA)
+1-516-484-5400 phone

European Headquarters
Fribourg, Switzerland
+41 (0)26 350 53 00 phone

Asia-Pacific Headquarters
Singapore
+65 6389 6500 phone

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Contact us at www.pall.com/contact

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