



## Technical Note

# Optimizing Chromatography Conditions Using AcroPrep™ Advance 96 Well Filter Plates

### Introduction

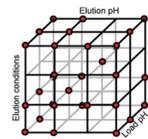
Pall® Laboratory AcroPrep Advance Filter Plates with Supor® membrane (PNs 8129 and 8130) are 96-well filter plates that are suitable for many applications, including chromatography. By packing chromatography sorbents from Pall or other manufacturers in the wells of the plates, they can be used for protein or nucleic acid purification. One advantage of the filter plates is that they can be used to quickly screen various conditions. Thereby allowing the analyst to quickly optimize the chromatography resin and purification conditions, with minimal sample consumption. The rapid screening ability, and optimization of process conditions, achieved with resin packed AcroPrep Advance Filter Plates can be transferred and confirmed, or scaled-up. The flexibility of the 96-well filter plate format allows the AcroPrep Advance Filter Plates to be operated with liquid-handling robotic systems, or manually using multi-channel pipettes. The recovery of either the sample or buffer, can be conducted using vacuum aspiration or centrifugation to draw the liquid through the membrane and bead bed into a 96-well receiver plate.

### High Throughput Screening on 96-Well Plate

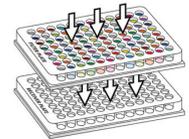
Screening of multiple conditions to optimize the purification step can be performed using an AcroPrep Advance 96-well filter plate. Once the selected sorbent is equilibrated and re-suspended as a 50% slurry in the equilibration buffer, the desired amount is dispensed into the wells to a final volume of 50  $\mu$ L per well. Once the slurry has been dispensed into the wells of the plate, the equilibration buffer then is aspirated using a multi-well plate vacuum manifold. Then 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 AcroPrep Advance is covered “with sealing tape” and incubated while shaking. After incubating, 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 or ELISA or other analytical methods. The analyst can then use this data to determine which set of parameters provide the best selectivity for their application, whether it is protein concentration and recovery or contaminant removal.

#### 1. Design of Experiment (DoE)

- Critical parameters (load, wash, elution conditions)
- Quality attributes (elution yield, HCP, aggregates)

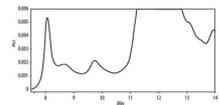


#### 2. Screening on 96-well plate



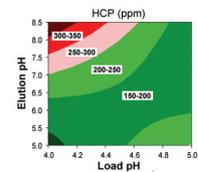
#### 3. Analytical testing

- Aggregate quantification (SEC HPLC)
- HCP quantification (ELISA)
- MAb elution yield (OD<sub>280nm</sub>)



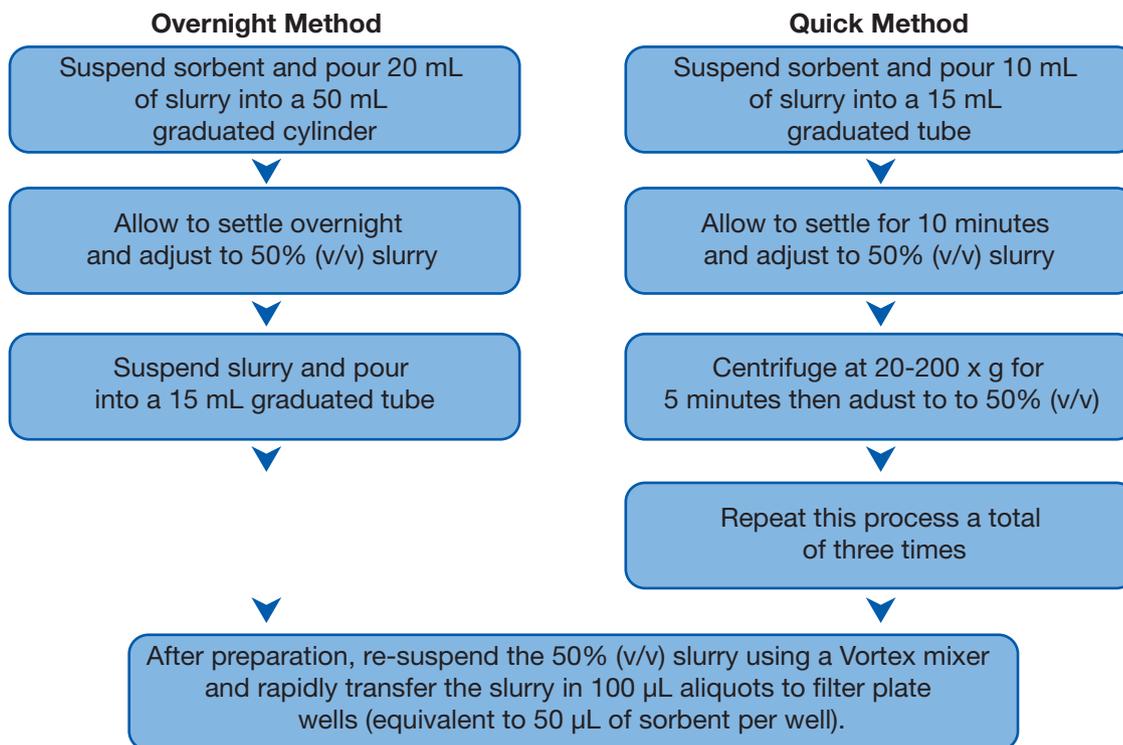
#### 4. Result analysis

- Design space for optimum performances



## Sorbent Packing into Multi-well Filter Plate

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



## Conclusion

An AcroPrep Advance filter plate with Supor membrane, with either 0.45 µm (PN 8129) or 1.2 µm (PN 8130) pore size, 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.



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