thermoscientific

# Efficient monoclonal antibody aggregate removal by Hydrophobic Interaction Chromatography (HIC)

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## INTRODUCTION

This case study shows the optimization of POROS™ HIC resin-use in high-throughput screening (HTS) and subsequently upscaling in both Bind-Elute (B/E) and Flow-Through (FT) mode. Our study shows that a well-designed process together with a robust resin are key to a successful and efficient Mab polishing process.

## GOAL OF THE STUDY

Design a more efficient, robust and cost-effective polish step utilizing POROS™ HIC resins as an alternative to the mixed-mode step in the original purification process of a clinical Mab containing >10% aggregates.

Salt Tolerability (Data not Shown) → High Throughput Screening → Scale Down Model

## POROS HIC RESINS

POROS Ethyl — Non-POROS HIC resins → POROS Benzyl → POROS Benzyl Ultra

Increasing hydrophobicity

- ✓ Designed for use with lower salt concentrations
- ✓ Differentiated selectivity and ligand chemistry
- ✓ Novel 50 µm base bead
- ✓ Improved recovery, resolution and capacity
- ✓ Superior pressure-flow characteristics
- ✓ Consistent lot-to-lot performance
- ✓ Robust chemical and base stability

## HTS: Resin Selection for Scale-Down

Screening variables used to predict conditions for scale down model: resin type, salt type and salt concentration. Partition selectivity ratio is used to determine to level of separation.

| Resin type | POROS Ethyl | | | | POROS Benzyl | | | | POROS Benzyl ultra | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Salt type | Sodium Chloride | Sodium Acetate | Amm. Sulfate | Sodium Citrate | Sodium Chloride | Sodium Acetate | Amm. Sulfate | Sodium Citrate | Sodium Chloride | Sodium Acetate | Amm. Sulfate | Sodium Citrate |
| | | | | 2.43 | | 1.84 | | | | | | |
| | | | | 3.12 | 2.74 | 2.18 | | | | | | |
| | | | 2.48 | 4.19 | 2.59 | 2.41 | | 2.35 | 2.38 | 2.07 | | |
| | | | | | 2.84 | 2.52 | 2.70 | 2.63 | 2.20 | 2.27 | 2.03 | |
| | | | | | 2.43 | | 2.66 | 3.53 | 2.45 | 3.12 | 2.02 | 2.19 |
| | | | | | | | 2.54 | 3.79 | 2.39 | 3.73 | 2.55 | 2.68 |
| | | | | | | | | | 3.12 | 3.46 | 4.34 | 3.76 |
| | | | | | | | | | 5.36 | 4.23 | 7.17 | 8.16 |

Salt concentration ↑ — Non-Binding Region →

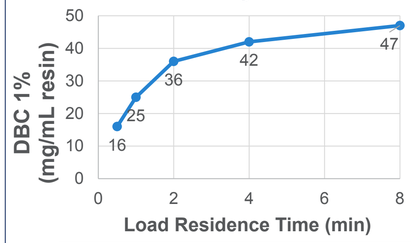
**Screen Constants:**

- pH constant at 7.0
- mAb Load, 66mg/mL resin
- Resin volume 10µL resin

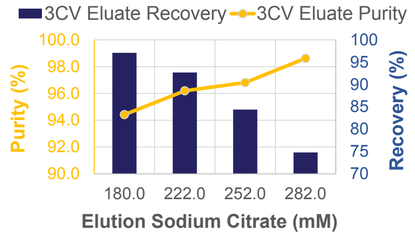
Higher the number = greater separation

- ✓ POROS Benzyl selected for polish in bind/elute
- ✓ POROS Benzyl Ultra selected for polish in flow-through

## POROS Benzyl in Bind/elute mode



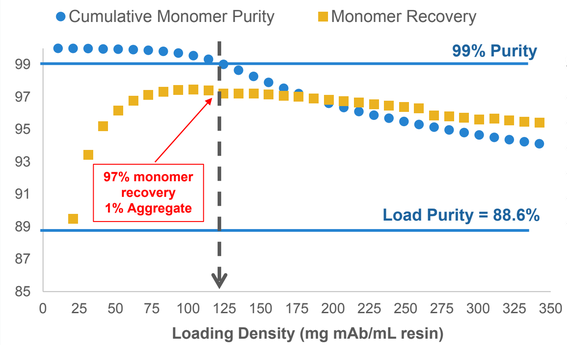

Dynamic Binding Capacity 1% vs. Residence time




Elution Conductivity Optimization Elution 180-282mM Sodium Citrate

- ✓ High DBC at short residence time (2min, 32mg/L resin)
- ✓ High recovery and purity in Elution (265mM Sodium Citrate)

## POROS Benzyl Ultra in Flow-through mode




**Study Format**

- 0.8cmx10cmL (CV 5mL)
- **Load 350 mg/mL resin**
- 2 min residence time
- Load monomer 89%
- 2 mS/cm conductivity
- pH6.8 chosen based on optimized pH of previous AEX polish step

- ✓ 1% Aggregate breakthrough after 125mg/mL resin loading
- ✓ Complete Aggregate clearance achieved with POROS Benzyl Ultra at low salt and flexible pH

## RESULTS SUMMARY AND CONCLUSIONS

| Process Summary | Mixed-Mode (Clinical Process) | POROS Benzyl Bind-Elute Mode | POROS Benzyl Ultra Flow-through Mode |
|---|---|---|---|
| Load Monomer Purity (%) | 90 | 89 | 85.5 |
| Load Density (g/L resin) | 25 | 32 | 100 |
| Monomer Purity Pool (%) | 99 | 99 | >99 |
| Monomer Recovery (%) | 90 | >99 | 98 |
| HCP (ppm) | NA | 120 to 12ppm | 100 to 35 ppm |
| Residence time (min) | 6 | 2 | 1.2 |
| Pool Volume (50-50mAu) | 5CV | 4CV | NA |
| MMV Clearance | NA | 2 | 1 |
| XmuLV Clearance | NA | >5 | >5 |
| **Productivity (g/L/hr)** | **7** | **27** | **89** |

POROS HIC resins drastically improve Mab polish step:

- ✓ Increased load density
- ✓ Improved monomer recovery
- ✓ Shorter residence time
- ✓ 4-12 times higher process step efficiency



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