# TOYOPEARL® $NH_2$-750F Flow-through Aggregate Removal from Monoclonal Antibodies

Chromatography Resin
APPLICATION NOTE

## Introduction

Anion exchange chromatography is a widely used technique for protein capture or impurity removal. Typically, anion exchange resins with quaternary amine or DEAE (diethylaminoethyl) ligands are used. However, these more conventional resins have the disadvantage of reduced capacity for proteins in relatively high salt concentrations associated with post-protein A purification of monoclonal antibodies (mAbs) or undiluted biological feedstock. In order to use a DEAE or quaternary amine resin at these process stages, the column load material must be diluted to adjust its conductivity to approximately 5 mS/cm.

TOYOPEARL $NH_2$-750F is a unique salt tolerant anion exchange resin for process scale applications such as aggregate removal and viral clearance. This resin is based on the TOYOPEARL HW-75F size exclusion resin functionalized with primary amine groups. This allows the TOYOPEARL $NH_2$-750F resin to maintain binding capacity at conductivities up to 15 mS/cm when used in bind-and-elute mode.

When used in a flow-through process step, TOYOPEARL $NH_2$-750F can be used at much higher salt concentrations for aggregate removal, allowing for minimal post elution sample adjustment from a capture step prior to loading onto the column. *Table 1* lists the properties of the TOYOPEARL $NH_2$-750F resin.

**Table 1.** Properties of TOYOPEARL $NH_2$-750F

| | **TOYOPEARL $NH_2$-750F** |
|---|---|
| Particle size (µm) | 30-60 |
| Pore size (nm) | > 100 |
| Ion Exchange capacity (eq/L resin) | 0.07 – 0.13 |
| SBC (g/L resin) | ≥ 70 |

TOYOPEARL $NH_2$-750F resin is ideal for the intermediate (post-protein A) purification of mAbs where aggregates and other impurities are removed from the target of interest without having to dilute or buffer exchange the product prior to loading.

The data presented here demonstrate the capabilities of TOYOPEARL $NH_2$-750F to remove dimer and higher order aggregates from the monomer of a protein A purified $IgG_1$ monoclonal antibody when used in a flow-through set-up.

## Experimental Conditions/Results

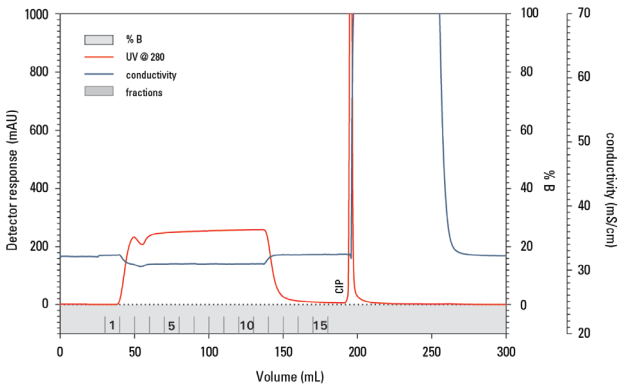
For the dimer/aggregate removal experiment, a 6.6 mm ID × 5.85 cm column was packed with TOYOPEARL $NH_2$-750F resin.

Aggregate removal experiments were conducted in mobile phases of 10 mmol/L Tris-HCl at pH values of 7.0 and 8.0 with different concentrations of NaCl. A 100 mL sample of acid aggregated mAb (1.0 g/L) was loaded onto the column and the unbound mAb was collected in the flow-through. The column was then stripped with 1.0 mol/L NaCl to remove the bound mAb aggregates.

Multiple fractions were taken across the length of the flow-through peaks and were analyzed by size exclusion HPLC using a TSKgel® G3000SW$_{XL}$ column to verify the separation of dimer and aggregates from the mAb monomer.

Flow-through experiments carried out in 10 mmol/L Tris-HCl, 350 mmol/L NaCl, pH 8.0 *(Figure 1)* had an initial aggregate content of 13%. The collected mAb monomer in the flow-through had a final aggregate content of 2% and an overall product yield of 90%.

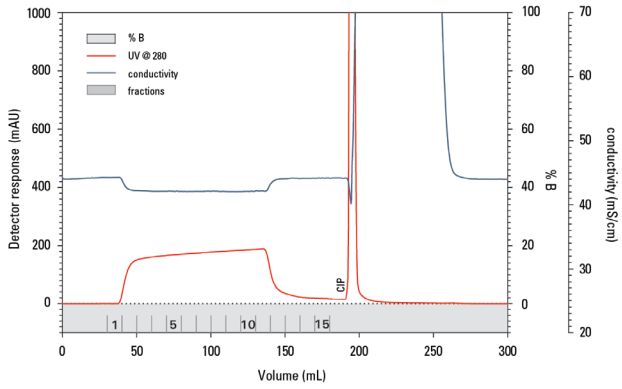
**Figure 1.** 10 mmol/L Tris-HCl, 350 mmol/L NaCl, pH 8.0




| | |
|---|---|
| Resin: | TOYOPEARL $NH_2$-750F |
| Column: | 6.6 mm ID × 5.85 cm (2.0 mL) |
| Mobile phase: | A. 10 mmol/L Tris-HCl, 350 mmol/L NaCl, pH 8.0<br>B. 1.0 mol/L NaCl |
| Gradient: | isocratic |
| Flow rate: | 300 cm/hr |
| Detection: | UV @ 280, conductivity |
| Sample: | acid aggregated mAb (1.0g/L) |
| Load: | 100 mg |

The experiments carried out in 10 mmol/L Tris-HCl, 500 mmol/L NaCl, pH 8.0 *(Figure 2)* had an initial aggregate content of 17%. The collected mAb monomer in the flow-through had a final aggregate content of 3% and an overall product yield of 93%.

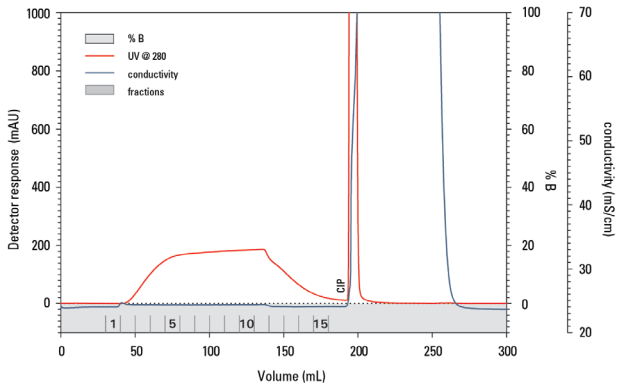
**Figure 2.** 10 mmol/L Tris-HCl, 500 mmol/L NaCl, pH 8.0




| | |
|---|---|
| Resin: | TOYOPEARL $NH_2$-750F |
| Column: | 6.6 mm ID × 5.85 cm (2.0 mL) |
| Mobile phase: | A. 10 mmol/L Tris-HCl, 500 mmol/L NaCl, pH 8.0<br>B. 1.0 mol/L NaCl |
| Gradient: | isocratic |
| Flow rate: | 300 cm/hr |
| Detection: | UV @ 280, conductivity |
| Sample: | acid aggregated mAb (1.0g/L) |
| Load: | 100 mg |

Flow-through experiments carried out in 10 mmol/L Tris-HCl, 250 mmol/L NaCl, pH 7.0 *(Figure 3)* had a much higher initial aggregate content of 28%. The collected mAb monomer in the flow-through had a final aggregate content of 0% and an overall product yield of 75%.

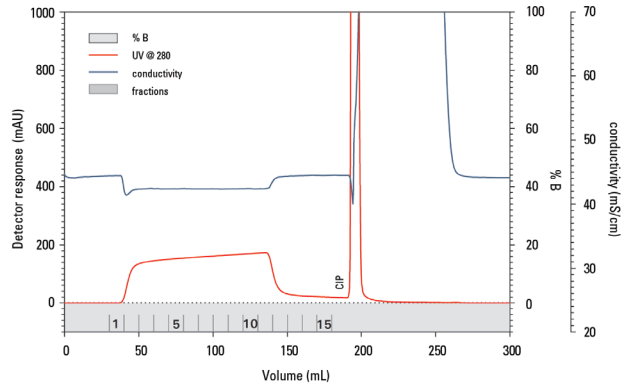
**Figure 3.** 10 mmol/L Tris-HCl, 250 mmol/L NaCl, pH 7.0




| | |
|---|---|
| Resin: | TOYOPEARL $NH_2$-750F |
| Column: | 6.6 mm ID × 5.85 cm (2.0 mL) |
| Mobile phase: | A. 10 mmol/L Tris-HCl, 250 mmol/L NaCl, pH 7.0 |
| | B. 1.0 mol/L NaCl |
| Gradient: | isocratic |
| Flow rate: | 300 cm/hr |
| Detection: | UV @ 280, conductivity |
| Sample: | acid aggregated mAb (1.0g/L) |
| Load: | 100 mg |

The final set of experiments, carried out in 10 mmol/L Tris-HCl, 500 mmol/L NaCl, pH 7.0 *(Figure 4)* had the highest initial aggregate content for all experiments at 32%. The collected mAb monomer in the flow-through had a final aggregate content of 2% and an overall product yield of 75%.

**Figure 4.** 10 mmol/L Tris-HCl, 500 mmol/L NaCl, pH 7.0




| | |
|---|---|
| Resin: | TOYOPEARL $NH_2$-750F |
| Column: | 6.6 mm ID × 5.85 cm (2.0 mL) |
| Mobile phase: | A. 10 mmol/L Tris-HCl, 500 mmol/L NaCl, pH 7.0 |
| | B. 1.0 mol/L NaCl |
| Gradient: | isocratic |
| Flow rate: | 300 cm/hr |
| Detection: | UV @ 280, conductivity |
| Sample: | acid aggregated mAb (1.0g/L) |
| Load: | 100 mg |



The fraction analysis and starting material for the best conditions tested can be seen in *Figure 5*, which demonstrates the complete removal of aggregates from the mAb monomer in the experiments carried out in *Figure 3*. The fraction analysis and starting material for the conditions that had the most residual aggregate can be seen in *Figure 6* from the experiments carried out in *Figure 2*.

**Figure 5.** SEC analysis of aggregated mAb and peak fractions from *Figure 3*

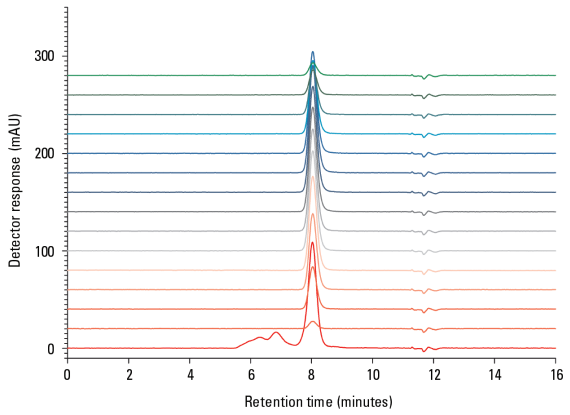
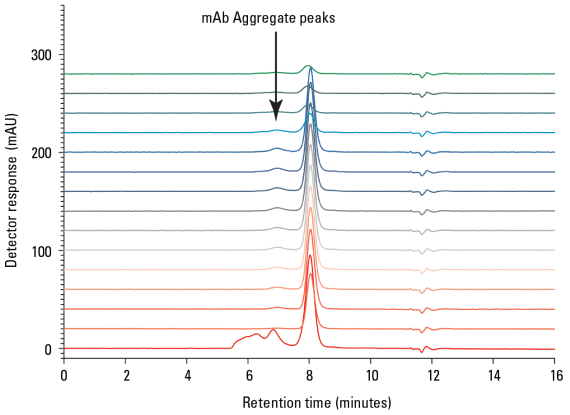



**Figure 6.** SEC analysis of aggregated mAb and peak fractions from *Figure 2*




## Conclusions

TOYOPEARL $NH_2$-750F is an effective anion exchange resin for the removal of dimer and higher order aggregates from mAb monomer in a protein A purified antibody in a flow-through chromatography set-up.

The uniquely high salt tolerance of this resin allows for the retention of mAb aggregates at salt concentrations up to 0.5 mol/L. At these conditions, aggregates can be removed from the mAb monomer without having to dilute or buffer exchange the sample post-protein A capture prior to loading.

Though some aggregate was co-eluted with the monomer peak in a few of these experiments, further method development may further reduce the amount of carryover and increase product yield in the monomer peak.

TOSOH BIOSCIENCE

TOSOH BIOSCIENCE LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Tel: 800-366-4875
email: info.tbl@tosoh.com
www.tosohbioscience.com

**AN91**
**0216**