

TOYOPEARL AF-rProtein A-650F

TOYOPEARL AF-rProtein A HC-650F

Protein A Chromatography in Process Purification

Protein A chromatography, the most widely used type of affinity chromatography, relies on the specific and reversible binding of antibodies to an immobilized ligand; in this case protein A. Protein A is a 56 kDa surface protein native to the cell wall of the bacterium *Staphylococcus aureus*. It is composed of five immunoglobulin-binding domains, each of which are able to bind proteins from many mammalian species, most notably Immunoglobulin G (IgG) through the heavy chain within the Fc region. While the native form of protein A was used as the ligand for first generation protein A resins, the recombinant form (rProtein A) produced in *E. coli* is the most prevalent today. Modifications to the protein structure of the ligand, the advent of ligands composed of single domain multimers, and multipoint attachment have given rise to the caustic stable, high capacity and extremely robust protein A resins in use today.

The protein A ligand can either bind directly to the Fc region of an antibody or to an Fc tag that has been fused to the target of interest. Protein A chromatography is a very robust purification procedure and is used as a capture step due to its specificity and, depending on the intended use for the target molecule (antibodies for diagnostic testing), might be the only chromatographic step required to achieve adequate product purity.

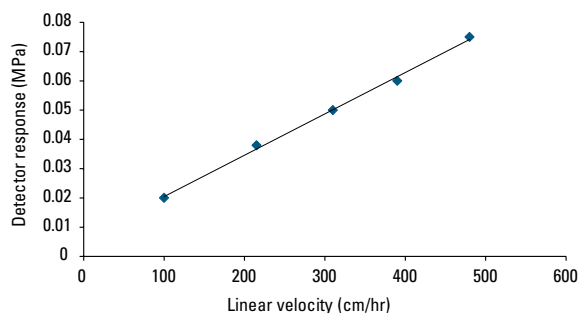
In protein A chromatography, crude feed stock is passed through a column under conditions that promote binding. After loading is complete, the column is washed under conditions that do not interrupt the specific interaction between the target and ligand, but that will disrupt any non-specific interactions between process impurities (host cell proteins, etc.) and the stationary phase. The bound protein is then eluted with mobile phase conditions that disrupt the target/ligand interactions. Elution of the target molecule from protein A resin is most commonly accomplished by lowering the pH of the mobile phase, creating an environment whereby the structure of the target molecule is altered in such a way as to inhibit binding. Low pH elution can have a negative effect on protein stability and it is advised that the eluted protein solution be neutralized to minimize aggregation and denaturation.

TOYOPEARL Protein A Resins

Tosoh Bioscience offers two TOYOPEARL affinity resins with a recombinant protein A ligand. TOYOPEARL AF-rProtein A resins are composed of hydrophilic, dimensionally stable base resins that exhibit excellent pressure-flow characteristics. These resins use the TOYOPEARL HW-65 SEC resin as a base bead. The 100 nm pore diameter of the TOYOPEARL affinity resins can accommodate large globular proteins up to 5×10^6 .

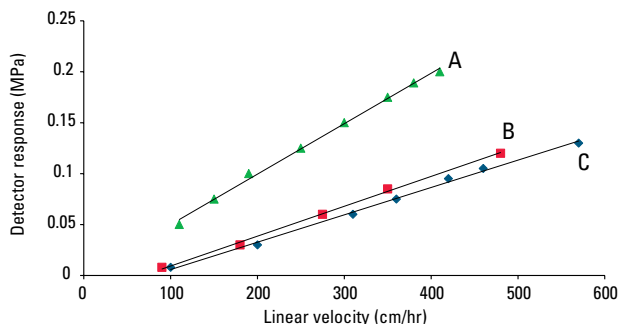
TOYOPEARL AF-rProtein A resins remain dimensionally stable within wide extremes of pH and ionic strength. Moreover, the semi-rigid TOYOPEARL AF-rProtein A particles do not distort under flow rates that generate up to 0.3 MPa pressure. These properties of the resins, combined with the narrow particle size distributions, result in superior pressure-flow characteristics for the packed TOYOPEARL bed. Linear velocities of 300 – 500 cm/hr generate a pressure of between 0.1 and 0.2 MPa in a packed bed (Figures 1A and 1B).

Figure 1A: Linear velocity and pressure curve



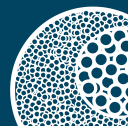
Resin: TOYOPEARL AF-rProtein A -650F
Column and size: Resolute®, 40 cm ID × 8.4 cm
Mobile phase: H₂O
Linear velocity: various
Detection: pressure (MPa)

Figure 1B: Comparison of linear velocity and pressure curves



Resins: A. TOYOPEARL AF-rProtein A -650F, 45 µm, 20 cm ID × 32 cm
 B. TOYOPEARL AF-rProtein A -650F, 45 µm, 20 cm ID × 18 cm
 C. TOYOPEARL Butyl-650M, 65 µm, 20 cm ID × 21 cm
Column: QuikScale®, 20 cm ID
Mobile phase: H₂O
Linear velocity: various
Detection: pressure (MPa)

| TOYOPEARL resin | Functionality | Base bead | Pore size | Bead diameter | Ligand type | Ligand leakage | DBC (g/L) | Pressure rating |
|-----------------------|---------------|-----------|-----------|---------------|-------------|-----------------|--------------|-----------------|
| AF-rProtein A-650F | Protein A | HW-65 | 100 nm | 45 µm | rProtein A | 5 - 25 ng/mg | > 30 @ 3 min | 0.3 MPa |
| AF-rProtein A HC-650F | Protein A | HW-65 | 100 nm | 46 µm | rProtein A | 0.6 - 1.7 ng/mg | > 65 @ 5 min | 0.3 MPa |



TOYOPEARL AF-rProtein A HC-650F resin is the newest affinity resin to be introduced from Tosoh Bioscience. This new, high capacity protein A resin was introduced in the Fall of 2013. An enhanced rProtein A ligand (**Figure 2**) is bound to the TOYOPEARL HW-65F base bead via multipoint attachment resulting in excellent base (**Figure 3**) stability for up to 200 CIP cycle with 0.1 mol/L NaOH. TOYOPEARL AF-rProtein A HC-650F resin maintains 80% of initial dynamic binding capacity after 40 CIP cycles with 0.5 mol/L NaOH (**Figure 4**). TOYOPEARL AF-rProtein A HC-650F resin exhibits dynamic binding capacities of greater than 65 g/L at residence times of 5 minutes and greater than 50 g/L at 2 minutes residence time with feed stock concentrations from 1.0 g/L to 10.0 g/L (**Figure 5**).

Figure 2: Ligand structure of TOYOPEARL AF-rProtein A HC-650F resin

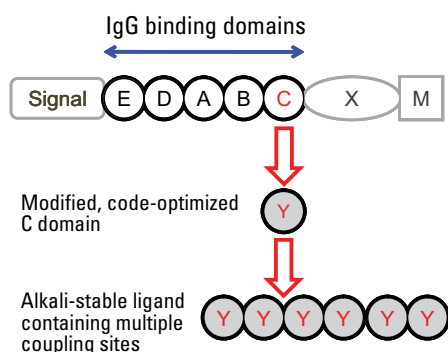
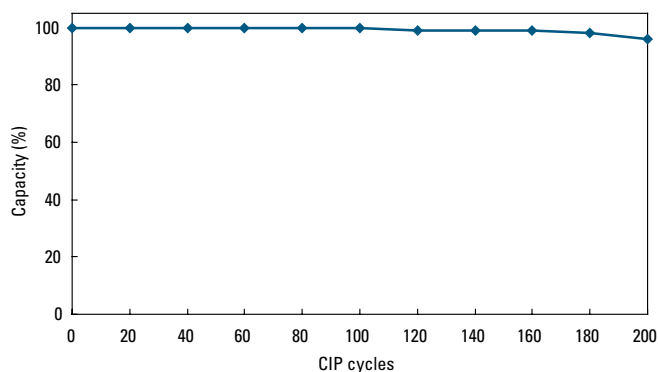
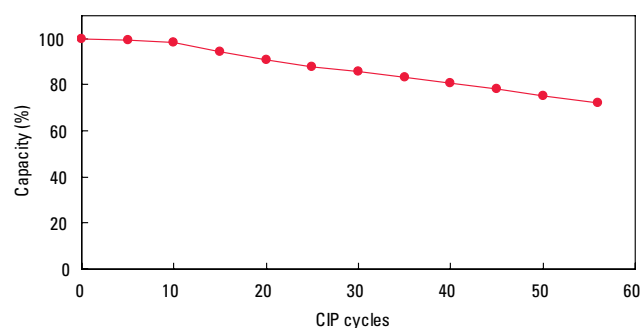


Figure 3: Base stability of TOYOPEARL AF-rProtein A HC-650F



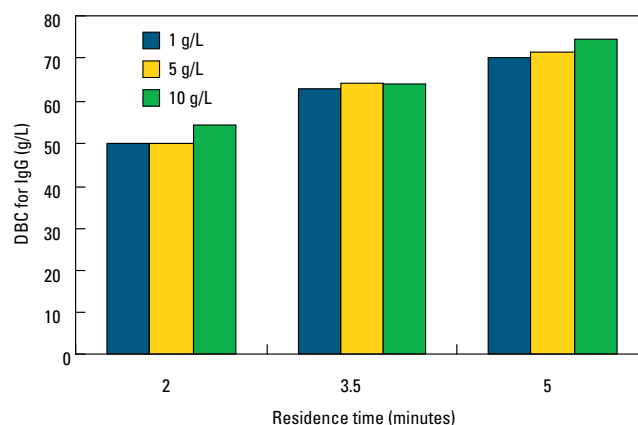
Column size: 5 mm ID × 5 cm
 Wash procedure: A: 20 mmol/L Na₂HPO₄, 0.15 mol/L NaCl, pH 7.4 (10 CV)
 B: 0.1 mol/L citrate, pH 3.0 (5 CV)
 C: 20 mmol/L Na₂HPO₄, 0.15 mol/L NaCl, pH 7.4 (7 CV)
 D: 0.1 mol/L NaOH (3 CV – 15 min contact time)
 E: 20 mmol/L Na₂HPO₄, 0.15 mol/L NaCl, pH 7.4 (5 CV)
 Capacity: DBC was determined at 10% breakthrough after every 20 cycles

Figure 4: DBC of TOYOPEARL AF-rProtein A HC-650F resin after CIP with 0.5 mol/L NaOH



Column size: 5 mm ID × 5 cm
 Wash procedure: A: 20 mmol/L Na₂HPO₄, 0.15 mol/L NaCl, pH 7.4 (10 CV)
 B: 0.1 mol/L citrate, pH 3.0 (5 CV)
 C: 20 mmol/L Na₂HPO₄, 0.15 mol/L NaCl, pH 7.4 (7 CV)
 D: 0.5 mol/L NaOH (3 CV – 15 min contact time)
 E: 20 mmol/L Na₂HPO₄, 0.15 mol/L NaCl, pH 7.4 (5 CV)
 Capacity: DBC was determined at 10% breakthrough after every 5 cycles

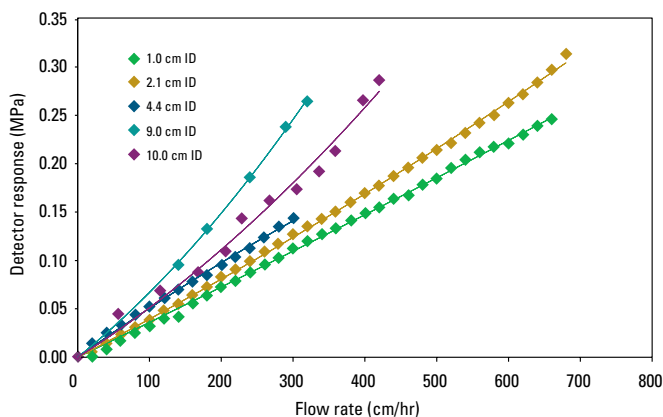
Figure 5: DBC of of TOYOPEARL AF-rProtein A HC-650F



Resin: TOYOPEARL AF-rProtein A HC-650F
 Column size: 5 mm ID × 5 cm
 Mobile phase: 0.02 mol/L sodium phosphate, 0.15 mol/L NaCl, pH 7.4
 Residence time: 2, 3.5, 5 min
 Detection: UV @ 280 nm (10% breakthrough)
 Sample: human IgG @ 1, 5, 10 g/L in mobile phase

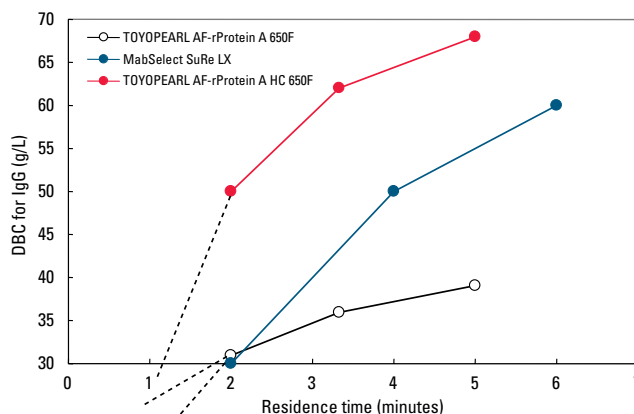
Achievement of high linear velocities at relatively low pressure enables high throughput at production scale using equipment with moderate pressure limitations (Figure 6). Improved mass transfer characteristics allow it to maintain a larger percent of its capacity at lower residence times (Figure 7) relative to agarose base stable resins. Typical leakage for this rProtein A ligand is 0.6 -1.7 ng rProtein A / mg eluted antibody by ELISA testing (Table 1). Though TOYOPEARL AF-rProtein A-650F resin is shipped in 20% ethanol, it can be stored in 2% benzyl alcohol if necessary.

Figure 6: Comparison of linear velocity and pressure curves



Column sizes: 1.0 cm ID, 2.1 cm ID, 4.4 cm ID, 9.0 cm ID, 10.0 cm ID
20 cm normalized bed height
Mobile phase: DI H₂O
Detection: pressure (MPa)

Figure 7: Comparison of residence time and capacity



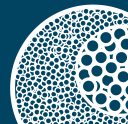
Resins: TOYOPEARL AF-rProtein A HC-650F
TOYOPEARL AF-rProtein A-650F
MabSelect SuRe™ LX
Column size: 5 mm ID x 5 cm
Mobile phase: 0.02 mol/L sodium phosphate, 0.15 mol/L NaCl, pH 7.4
Residence time*: 2, 3.5, 5 min
Detection: UV @ 280 nm
Sample: human IgG @ 1 g/L in mobile phase

*MabSelect SuRe DBC data taken from product brochure (2, 4, and 6 minute residence times).

DBC was calculated at 10% breakthrough

Table 1: Ligand leakage before and after CIP

| Amount of ligand leakage (ppm) | Before CIP | | After 200 CIP cycles | |
|--|------------------|----------------------|----------------------|----------------------|
| | Elution Buffer | | Elution Buffer | |
| | citrate (pH 3.0) | glycine-HCl (pH 3.0) | citrate (pH 3.0) | glycine-HCl (pH 3.0) |
| | 1.7 | 1.6 | 0.6 | 0.5 |
| Amount of ligand leakage was determined with TOYOPEARL AF-rProtein A HC-650F ELISA | | | | |
| ppm = ng/mg IgG | | | | |



TOYOPEARL AF-rProtein A-650F resin is an affinity resin for monoclonal antibody purification. The recombinant ligand (Figure 8) is expressed in *E. coli* and is free of animal derived products. The ligand is bound to the TOYOPEARL HW-65F base bead via multipoint attachment resulting in excellent base (Figure 9 and 10) and thermal stability (Figure 11). TOYOPEARL AF-rProtein A-650F resin exhibits dynamic binding capacities of greater than 30 g/L at residence times of 3 minutes and greater.

Figure 8: Ligand structure of TOYOPEARL AF-rProtein A-650F resin

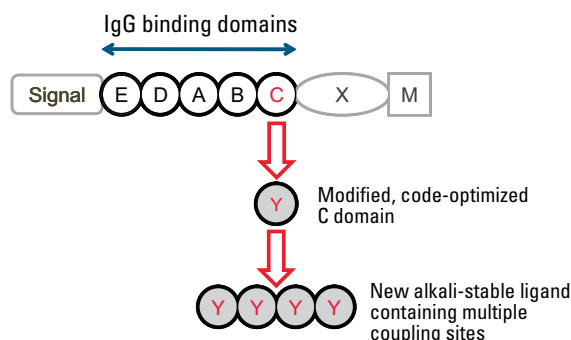
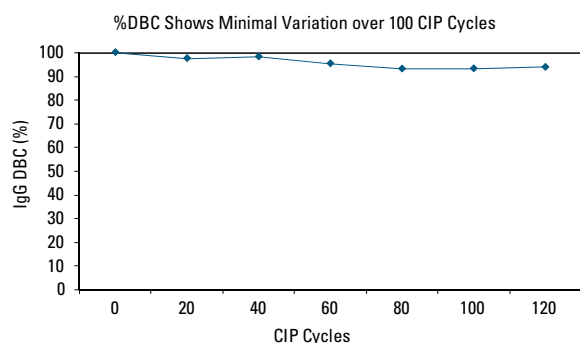


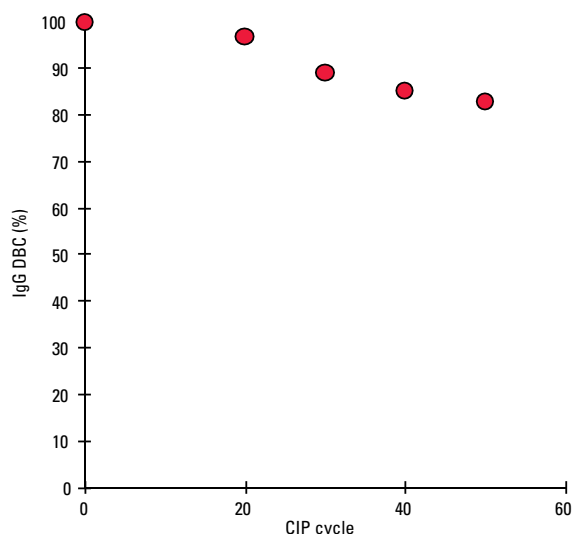
Figure 9: CIP stability to 0.1 mol/L NaOH



Resin: TOYOPEARL AF-rProtein A-650F
Column size: 4.6 mm ID × 10 cm
Mobile phase: Buffer A: 0.15 mol/L NaCl in 0.02 mol/L sodium phosphate buffer, pH 7.2
 Buffer B: 0.10 mol/L citrate buffer, pH 3.0
Cycle volumes: 5 column volumes binding buffer
 10 column volumes elution buffer
 3 column volumes 0.1 mol/L NaOH, (16 min contact time)
 5 column volumes H₂O

DBC was calculated at 10% breakthrough

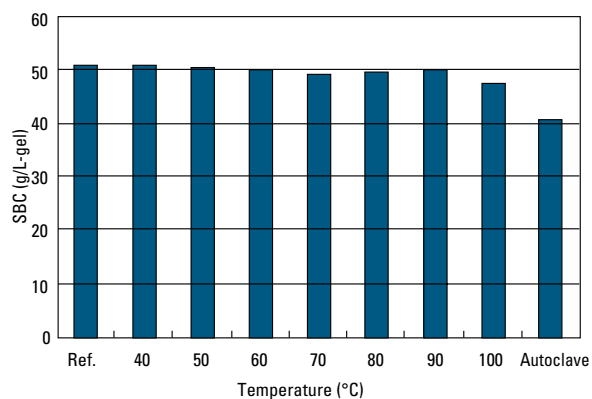
Figure 10: CIP stability to 0.5 mol/L NaOH



Resin: TOYOPEARL AF-rProtein A-650F
Column size: 5.0 mm ID × 5 cm
Mobile phase: Buffer A: 0.15 mol/L NaCl in 0.02 mol/L sodium phosphate buffer, pH 7.2
 Buffer B: 0.10 mol/L citrate buffer, pH 3.0
Cycle volumes: 5 column volumes binding buffer
 10 column volumes elution buffer
 8 column volumes 0.5 mol/L NaOH, (16 min contact time)
 5 column volumes H₂O

DBC was calculated at 10% breakthrough

Figure 11: Temperature stability



Resin: TOYOPEARL AF-rProtein A-650F
Mobile phase: deionized H₂O
Autoclave settings: 120 °C, 1.2 atm, 15 min
Heating time: 30 min

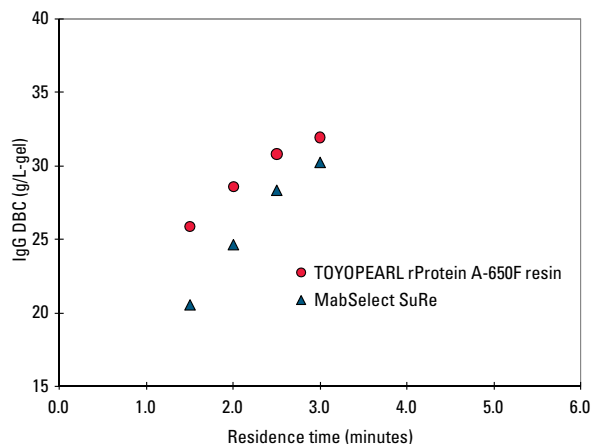
TOYOPEARL AF-rProtein is stable at 35 °C for least 3 years (data not shown)

Improved mass transfer characteristics allow TOYOPEARL AF-rProtein A-650F to maintain a larger percent of its capacity at lower residence times (Figure 12) relative to agarose base stable resins. Typical leakage for this rProtein A ligand is 5-25 ng rProtein A /mg eluted antibody by ELISA testing.

Achievement of high linear velocities at relatively low pressure enables high throughput at production scale using equipment with moderate pressure limitations. Sanitization or cleaning may be conducted with up to 0.5 mol/L NaOH or 0.5 mol/L HCl depending upon the ligand.

An important aspect of the use of a Protein A resin in the capture step is its ability to remove host cell protein (HCP) from the feedstock. TOYOPEARL AF-rProtein A-650F addresses this key area as well (Table 2).

Figure 12: DBC at various residence times

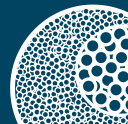


Resins: TOYOPEARL AF-rProtein A-650F
MabSelect SuRe
Column size: 5 mm ID x 5 cm (1 mL)
Mobile phase: 0.02 mol/L sodium phosphate buffer, pH 7.2 + 0.15 mol/L NaCl
Residence time: 1.5, 2.0, 2.5, 3.0 min

Table 2: TOYOPEARL AF-rProtein A-650F resin vs. MabSelect SuRe resin

| Resin | Protein load (mg/mL gel) | pH | Flow (cm/hr) | BV (µL) | Buffer | CHO (ng/mL) |
|------------------------------|--------------------------|-----|--------------|---------|-----------|-------------|
| Toyopearl AF-rProtein A-650F | 5 | 3,9 | 250 | 200 | Tris | 9,76 |
| MabSelect SuRe | 5 | 3,9 | 250 | 200 | Phosphate | 30,52 |
| Toyopearl AF-rProtein A-650F | 45 | 3,4 | 100 | 200 | Tris | 0,67 |
| MabSelect SuRe | 45 | 3,4 | 100 | 200 | Phosphate | 36,52 |
| Toyopearl AF-rProtein A-650F | 25 | 3,9 | 250 | 200 | Tris | 47,26 |
| MabSelect SuRe | 25 | 3,9 | 250 | 200 | Phosphate | >310 |
| Toyopearl AF-rProtein A-650F | 5 | 3,9 | 100 | 200 | Tris | 19,16 |
| MabSelect SuRe | 5 | 3,9 | 100 | 200 | Phosphate | 81,32 |

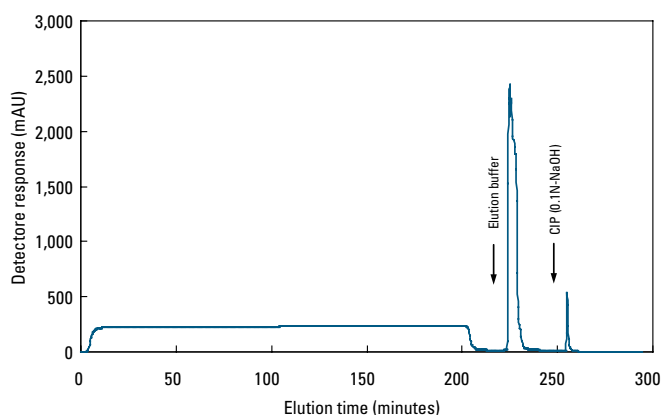
Data kindly provided by U. Breuninger, University of Applied Science Esslingen. Both resins were packed in Media Scout® Columns, Atoll GmbH, Weingarten.



Purification of Monoclonal Antibodies

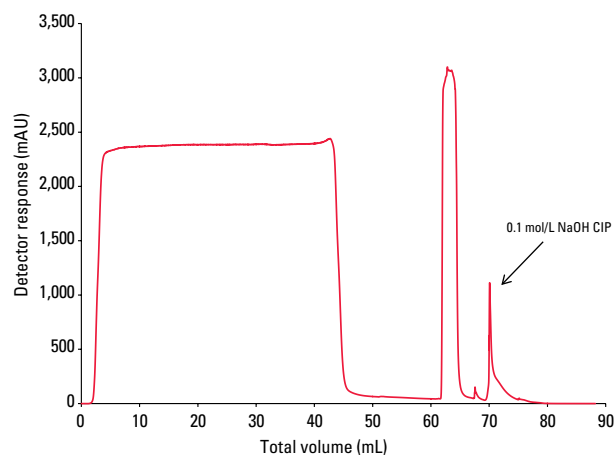
TOYOPEARL AF-rProtein A HC-650F was used for the purification of a monoclonal antibody from CHO cell culture supernatant with a concentration of 1.0 g/L (Figure 13) at 5 minutes residence time in a 5 cm bed height column. As can be seen from the chromatogram, tailing is minimal on the elution peak and the eluted mAb is > 95% pure by SEC. A second purification using a 9.7 cm bed height with a feedstock concentration of 2.9 g/L and 5 minute residence time can be seen in Figure 14.

Figure 13: Purification of monoclonal antibody



Resin: TOYOPEARL Protein A
Column size: 5 mm ID × 5.0 cm
Mobile phase: Buffer A: 20 mmol/L sodium phosphate containing 0.15 mol/L NaCl, pH 7.4
 Buffer B: 0.1 mol/L citrate, pH 3.0
Flow rate: 61 cm/hr (0.2 mL/min)
Residence time: 5 min
Sample: 40 mL of CHO cell culture, containing 1.0 g/L humanized IgG₁

Figure 14: Second purification of monoclonal antibody



Resin: TOYOPEARL AF-rProtein A HC-650F
Column size: 5.0 mm ID × 9.7 cm (1.90 mL)
Mobile phase: Buffer A: 20 mmol/L Na₂HPO₄, 150 mmol/L NaCl, pH 7.39
 Buffer B: 100 mmol/L Na citrate, pH 3.0
Flow rate: 116 cm/hr (0.38 mL/min)
Residence time: 5 min
Detection: UV @ 280 nm
Sample: Crude feedstock (mAb)

A selection of screening tools are available for TOYOPEARL Protein A resins. See the Process Development Products section of this Product Guide for details.

Ordering Information

TOYOPEARL Protein A resins:

| Part # | Product description | Container size (mL) | |
|--------|---|---------------------|--|
| 22803 | TOYOPEARL AF-rProtein A-650F | 10 | |
| 22804 | TOYOPEARL AF-rProtein A-650F | 25 | |
| 22805 | TOYOPEARL AF-rProtein A-650F | 100 | |
| 22806 | TOYOPEARL AF-rProtein A-650F | 1,000 | |
| 22807 | TOYOPEARL AF-rProtein A-650F | 5,000 | |
| 22808 | TOYOPEARL AF-rProtein A-650F | 50,000 | |
| 22815 | TOYOPEARL AF-rProtein A-650F ELISA* | | |
| 23425 | TOYOPEARL AF-rProtein A HC-650F | 10 | |
| 23426 | TOYOPEARL AF-rProtein A HC-650F | 25 | |
| 23427 | TOYOPEARL AF-rProtein A HC-650F | 100 | |
| 23428 | TOYOPEARL AF-rProtein A HC-650F | 1,000 | |
| 23429 | TOYOPEARL AF-rProtein A HC-650F | 5,000 | |
| 23434 | TOYOPEARL AF-rProtein A HC-650F | 50,000 | |
| 23433 | TOYOPEARL AF-rProtein A HC-650F ELISA** | | |

* This kit is specifically prepared for TOYOPEARL AF-rProtein A-650F resin. Test kits for other commercially available Protein A products may not work properly for TOYOPEARL AF-rProtein A-650F resin.

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