

TOYOPEARL® AF-rProtein A HC-650F

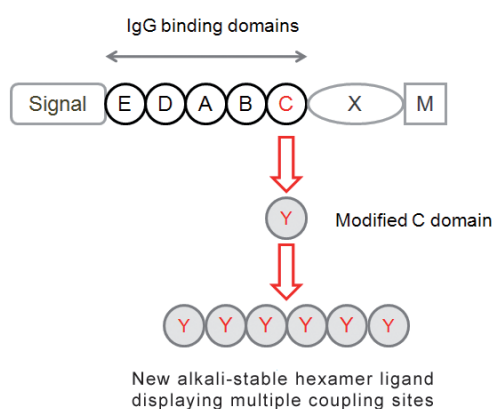
Protein A based affinity chromatography is the industry standard for the capture of monoclonal antibodies (mAbs) in downstream processing. Affinity media offer high selectivity thereby yielding a high purity of the target molecule in one step. The rising fermentation titers in the production of monoclonal antibodies drive the focus on developing resins that increase downstream capacity and efficiency for these processes.

TOYOPEARL AF-rProtein A HC-650F presents the third generation of TOYOPEARL protein A media. It is a rigid, alkaline resistant protein A affinity resin that offers the largest binding capacity for immunoglobulin G of all protein A affinity media currently available on the market. It is based on a recombinant protein A derived ligand expressed in *E. coli*.

The ligand is derived from one of the IgG-binding domains of the *staphylococcus aureus* protein A (*Figure 1*). It was further optimized in order to provide a higher binding capacity and alkali stability.

Multipoint attachment of the ligand to the TOYOPEARL polymer matrix further enhances chemical and thermal stability of the resin. In practice this pays off for a low level of protein A leaching and also for a high resistance to alkaline solutions applied in cleaning-in-place (CIP) procedures.

RECOMBINANT PROTEIN A DERIVED LIGAND



➤ *Figure 1*

HIGHLIGHTS

- Superior dynamic binding capacity for human IgG
- Capacity increases with feedstock titers up to 10 g/L
- Fast binding kinetics and moderate elution conditions
- Optimized alkali-stable recombinant ligand
- Minimized leaching through multi-point attachment

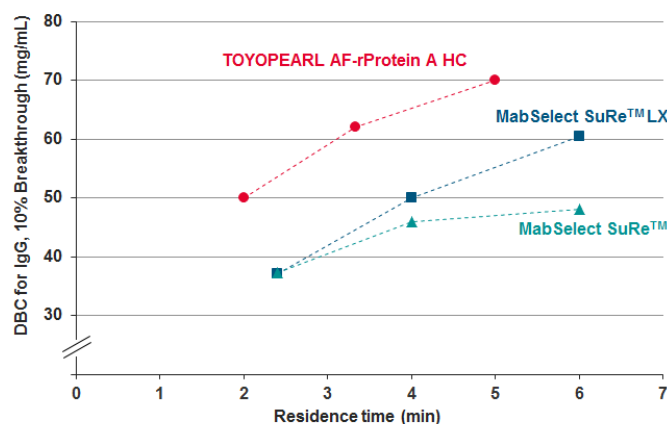
FEATURES

ULTRA-HIGH BINDING CAPACITY

Besides the ligand itself also the base particle and the ligand density were optimized in order to provide extremely high binding capacities for immunoglobulin G. Static IgG binding capacity (SBC) is > 68 mg/mL resin and dynamic binding capacity (DBC) at 10% breakthrough is > 65 mg/mL resin at 5 minutes residence time. *Figure 2* depicts the dynamic binding capacity of TOYOPEARL AF-rProtein A HC-650F at three different linear velocities/residence times compared to the reported DBCs of MabSelect SuRe™ and MabSelect SuRe™ LX. The excellent lot-to-lot reproducibility is depicted in *Figure 3*.

FAST BINDING KINETICS

DBC OF HIGH CAPACITY PROTEIN A MEDIA



➤ *Figure 2*

Column: TOYOPEARL AF-rProtein A HC-650F (5 mm ID x 5 cm)
 Mobile phase: 20 mmol/L sodium phosphate, 150 mmol/L NaCl pH 7.4;
 Residence time: 2, 3.3, 5 min; Detection: UV @ 280 nm;
 Sample: polyclonal human IgG @ 1 mg/mL in mobile phase;
 DBC measured at 10 % breakthrough.
 MabSelect Sure™ and MabSelect Sure™ LX DBC data from GE brochure. MabSelect Sure™ and MabSelect Sure™ LX are registered trademarks of GE Healthcare Bio-Sciences AB, Uppsala, Sweden.

LOT-TO-LOT VARIATION

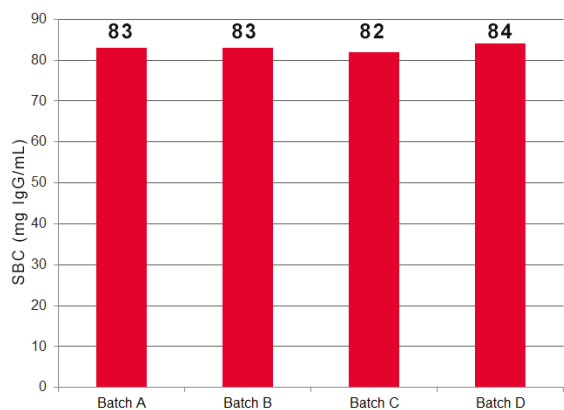


Figure 3

Sample: polyclonal human IgG
 Lot-to-lot variation of static binding capacities for polyclonal human IgG; Buffer: 0.1 mol/L sodium phosphate, pH 7.0;

Fast mass transfer kinetics support high binding capacities even when applying high titer feedstocks at high flow rates. IgG binding capacities measured at various linear velocities and various feedstock concentrations (Figure 4) demonstrate the high IgG DBC at high velocities and the superior kinetic performance of TOYOPEARL AF-rProtein A HC-650F. The DBC increases up to a titer of 10 mg/mL cell culture supernatant. In contrast to other high capacity protein A media TOYOPEARL AF-rProtein A HC-650F reaches more than 70% of the maximum dynamic capacity even at very short residence times. This feature is extremely beneficial when using the resin for mAb capturing in continuous operation on multiple short columns with fast flow.

HIGH CIP AND SANITIZATION STABILITY

DBC AT VARIOUS LOADS AND RESIDENCE TIMES

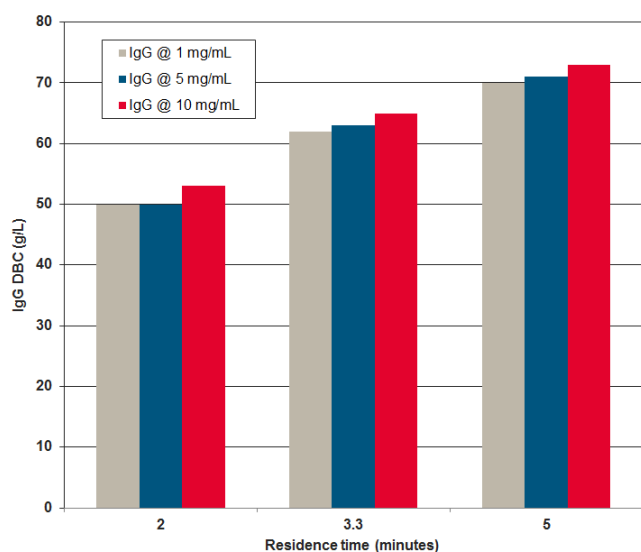


Figure 4

Column: TOYOPEARL AF-rProtein A HC-650F (5 mm ID x 5 cm)
 Mobile phase: 20 mmol/L sodium phosphate, 150 mmol/L NaCl pH 7.4;
 Residence time: 2, 3.3, 5 min; Detection: UV @ 280 nm
 Sample: polyclonal human IgG @ 1, 5, 10 g/L in mobile phase
 DBC measured at 10% breakthrough

The structure of the recombinant ligand and its multipoint attachment to the base matrix enhance the stability of TOYOPEARL AF-rProtein A HC-650F in 0.1 - 0.5 M NaOH. The dynamic binding capacity remains high after repeated CIP cycles. After 200 CIP cycles with 0.1 M NaOH at 15 min contact time per cycle more than 95 percent of initial dynamic binding capacity was retained (Figure 5a). When performing cleaning-in-place with 0.5 M NaOH the resin maintains about 80 percent of initial IgG binding capacity after 40 cycles with 15 min contact time (Figure 5b).

Static binding capacity of the resin is not impaired when stored at room temperature at production site. Recommended conditions for long term storage are a storage solution of 20% ethanol and a temperature of 4 - 8 °C.

LOW LIGAND LEACHING

CIP STUDY WITH 0.1 AND 0.5 M NaOH

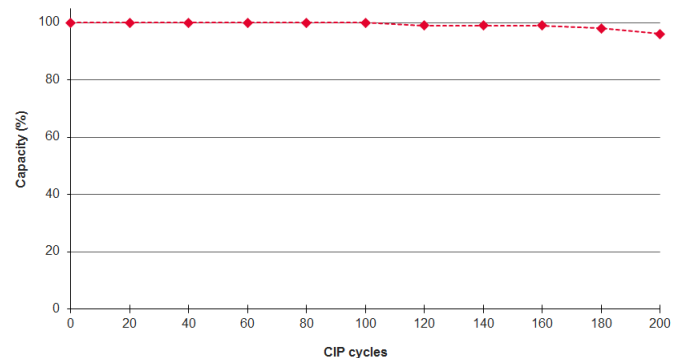


Figure 5a

Column size: 5 mm ID x 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

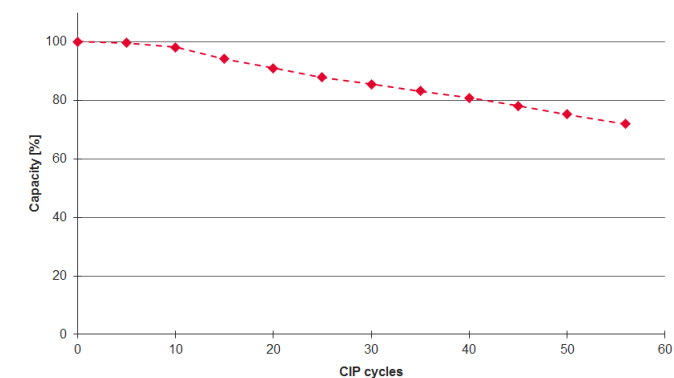


Figure 5b

Column size: 5 mm ID x 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

The binding of the advanced rProtein A ligand to the TOYOPEARL HW-65F base bead via multipoint attachment is not only resulting in high alkaline stability but also the reason for low ligand leakage (*Table 1*).

RIGID POLYMER MATRIX

PROTEIN A LIGAND LEAKAGE

| 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

Table 1

TOYOPEARL AF-rProtein A HC-650F is based on the well-proven polymethacrylate matrix used for all TOYOPEARL resins. *Figure 6* shows the pressure/flow curve for TOYOPEARL AF-rProtein A HC-650F packed in a 20 cm inner diameter column at various bed heights. For bed heights above 15 cm it is recommended to apply a moderate flow. This will avoid conflicts with the maximum system pressure specifications while exploiting the full binding capacity of the resin. For short bed heights the fast adsorption kinetics of the resin allow using higher flow rates/short residence times. This is particularly suitable for continuous chromatography solutions or radial flow columns.

APPLICATION

PRESSURE/FLOW CURVE

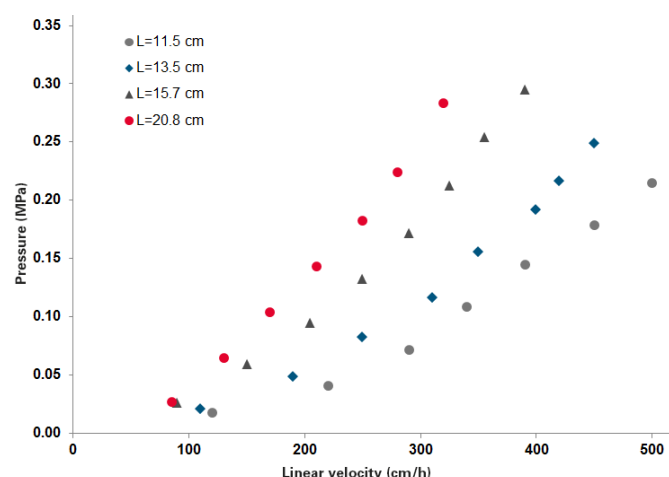


Figure 6

Column size: 20 cm ID; various bed heights; Mobile phase: DI H₂O

DBC FOR A SPECIFIC mAb AT VARIOUS LOADS AND VELOCITIES

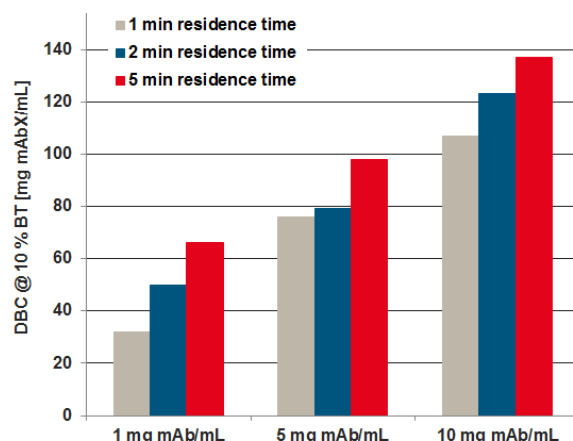


Figure 7

Column: TOYOPEARL AF-rProtein A HC-650F (6.6 mm ID x 2 cm)
 Mobile phase: 100 mmol/L sodium phosphate pH 6.5;
 Residence time: 1, 2, 5 min; Detection: UV @ 280 nm
 Sample: monoclonal antibody mAbX @ 1, 5, 10 g/L in mobile phase
 DBC measured at 10 % breakthrough

Figure 7 shows the results of the capturing of a therapeutic monoclonal IgG1 diluted at different concentrations into CHO cell culture fluid. The binding capacity of the TOYOPEARL AF-rProtein A HC-650F for this specific antibody is increasing dramatically with increasing feed concentrations. Furthermore, when applying a feed concentration of 10 mg mAb/mL an extremely high capacity of more than 100 mg mAb/mL resin was for a wide range of residence times.

OPERATION

Typically antibodies are captured at physiological conditions and eluted using acidic conditions. The clarified feedstock is loaded onto the column at a neutral pH. After sufficient washing with the loading buffer, the antibody is eluted at pH 3-4. However, the physicochemical properties of different mAbs are varying depending on the expression system and antibody subclass. Therefore a generic method needs to be optimized for each individual target in order to establish conditions that will bind the highest amount of the target molecule in the shortest time and elute it with the highest purity. For initial scouting of method parameters we recommend using pre-packed SkillPak™ columns or robotic high throughput screening devices and RoboColumns.

Suitable load/wash buffers are 20-100 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 6.5 - 7.5 or 100 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.2 - 7.5. Washing at reduced pH (e.g. pH 6) might further improve host cell protein reduction. Suitable elution buffer systems are 50-100 mmol/L acetate, citrate or glycine-HCl. The pH shift required for mAb elution depends on a particular mAb and ranges from pH 3.0 to 4.5. For cleaning and sanitization the use of 0.1 to 0.5 molar NaOH is recommended. Depending on the origin and subclass of the antibody, contact time, concentration, and frequency of CIP cycles the conditions should be optimized.

Ordering Information

TOYOPEARL AF-rProtein A HC-650F

| Part-No | Description | Resin volume | Pore size | Particle size |
|------------------|---------------------------------|------------------|-----------|---------------|
| TOYOPEARL | | | | |
| 0023425 | TOYOPEARL AF-rProtein A HC-650F | 10 mL | 100 nm | 45 µm |
| 0023426 | TOYOPEARL AF-rProtein A HC-650F | 25 mL | 100 nm | 45 µm |
| 0023427 | TOYOPEARL AF-rProtein A HC-650F | 100 mL | 100 nm | 45 µm |
| 0023428 | TOYOPEARL AF-rProtein A HC-650F | 1 L | 100 nm | 45 µm |
| 0023429 | TOYOPEARL AF-rProtein A HC-650F | 5 L | 100 nm | 45 µm |
| 0023434 | TOYOPEARL AF-rProtein A HC-650F | 50 L | 100 nm | 45 µm |
| 0022899 | Protein A-R40 Standard | 10 mg/L (0.5 mL) | | |

Process Development Columns

| | | | | |
|---------|---|-------------------|--------|---------------------------------|
| 0045201 | SkillPak AF-rProtein A HC-650F | 1 mL col. | | 7 mm ID x 2.5 cm |
| 0045222 | SkillPak AF-rProtein A HC-650F | 5 x 1 mL col. | | 7 mm ID x 2.5 cm |
| 0045258 | SkillPak AF-rProtein A HC-650F | 5 mL col. | | 8 mm ID x 10 cm |
| 0045228 | SkillPak Antibody AF-rProtein A HC-650F, AF-rProtein L-650F, NH ₂ -750F, Sulfate-650F, GigaCap Q-650M, GigaCap S-650S | 1 mL col. library | | 7 mm ID x 2.5 cm 1 mL x 1 ea |
| 0045229 | SkillPak mAb Platform AF-rProtein A HC-650F, Sulfate-650F, NH ₂ -750F | 1 mL col. library | | 7 mm ID x 2.5 cm 1 mL x 2 ea |
| 0045232 | SkillPak Best in Class AF-rProtein A HC-650F, AF-rProtein L-650F, NH ₂ -750F, Sulfate-650F, Ca ⁺⁺ Pure-HA | 1 mL col. library | | 7 mm ID x 2.5 cm 1 mL x 1 ea |
| 0045263 | SkillPak mAb Platform AF-rProtein A HC-650F, Sulfate-650F, NH ₂ -750F | 5 mL col. library | | 8 mm ID x 10 cm 5 mL x 1 ea |
| 0045266 | SkillPak Best in Class AF-rProtein A HC-650F, AF-rProtein L-650F, NH ₂ -750F, Sulfate-650F, Ca ⁺⁺ Pure-HA | 5mL col. library | | 8 mm ID x 10 cm 5mL x 1 ea |
| 0045063 | RoboColumns AF-rProtein A HC-650F | 200 µL x 8 | 100 nm | 45 µm |
| 0045064 | RoboColumns AF-rProtein A HC-650F | 600 µL x 8 | 100 nm | 45 µm |

Protein A ELISA

Corresponding ELISA kit is supplied by Cygnus Technologies:
https://cygnustechnologies.com/product_detail/tosoh-r40-and-r28-protein-a-mix-n-go-elisa.html