TOSOH

TOYOPEARL® AF-rProtein A-650F

INTRODUCTION

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 antibodies are captured at near neutral pH and eluted using acidic pH. The rising fermentation titres 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-650F is an affinity resin designed for efficient and robust large-scale purification of monoclonal antibodies. A newly developed recombinant protein A ligand (*E. coli*) was linked to the well proven methacrylic polymer backbone of TOYOPEARL media. The ligand is derived from one of the IgG-binding domains of the *staphylococcus aureus* protein A (*Figure 1*). Its amino acid sequence was optimized in order to increase its stability towards alkaline solutions. Multipoint attachment of the ligand to the TOYOPEARL 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 employed in cleaning-in-place (CIP) procedures.

TOYOPEARL AF-rProtein-650F binds human and mouse immunoglobulin G, as well as immunoglobulin M and Fab fragments.

RECOMBINANT PROTEIN A DERIVED LIGAND

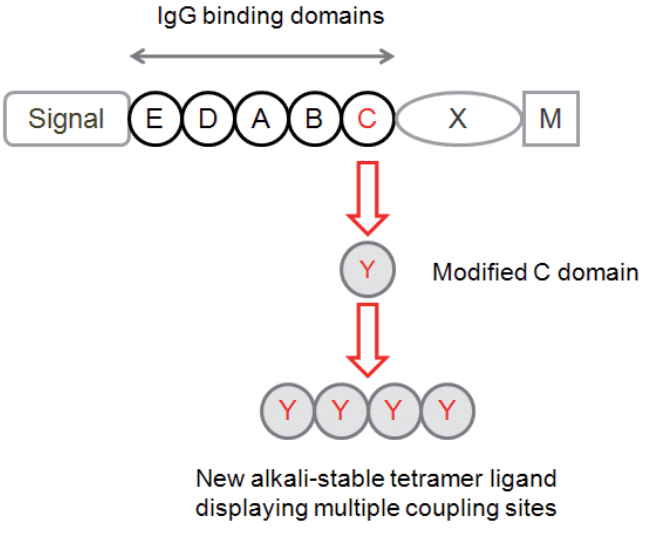

Figure 1

HIGHLIGHTS

- Efficient MAb capturing from high titer feedstocks
- High IgG binding capacity over a wide range of velocities
- High alkaline stability for effective cleaning and sanitization
- Rigid polymethacrylate base matrix with ideal pressure/flow characteristics

FEATURES

HIGH BINDING CAPACITY AT SHORT RESIDENCE TIME

The particle size of 45 µm and the ligand density of TOYOPEARL AF-rProtein A-650F were optimized in order to reach high dynamic binding capacities (DBC) for immunoglobulins. Typical static IgG binding capacity is > 45 mg/mL resin and typical dynamic IgG binding capacity at 10 % breakthrough is > 30 mg/mL resin at 2 minutes residence time (1 mg/mL protein load).

DYNAMIC BINDING CAPACITY

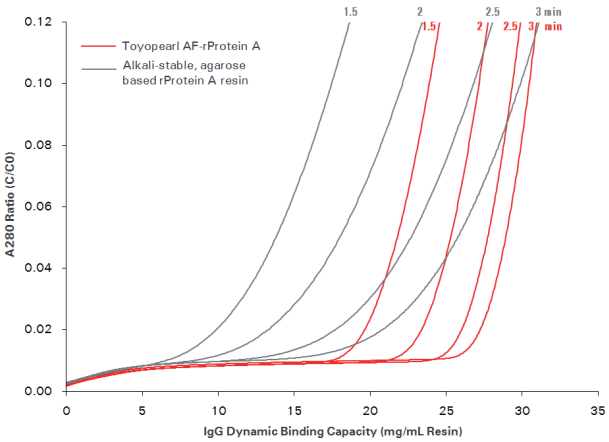

Figure 2

Breakthrough curves for hIgG loading (polyclonal, 10 mg/ml)
Typical DBC at 10% breakthrough:
30,5 mg/mL @ 100 cm/hr (3 min residence time) - 24 mg/mL @ 200 cm/hr (1.5 min residence time)

Column:	5 mm ID x 5 cm L
Mobile phase:	20 mM sodium phosphate buffer pH 7.2 containing 150 mM NaCl
Sample conc.:	10 mg/mL
Residence time:	1.5, 2.0, 2.5, 3.0 min

Fast mass transfer kinetics support high binding capacities even when applying high titer feedstocks at high flow rates. IgG breakthrough curves (*Figure 2*) at various linear velocities demonstrate the high IgG DBC at high velocities and the superior kinetic performance of TOYOPEARL AF-rProtein A-650F.

CIP STUDY WITH 0.1 AND 0.5 M NaOH

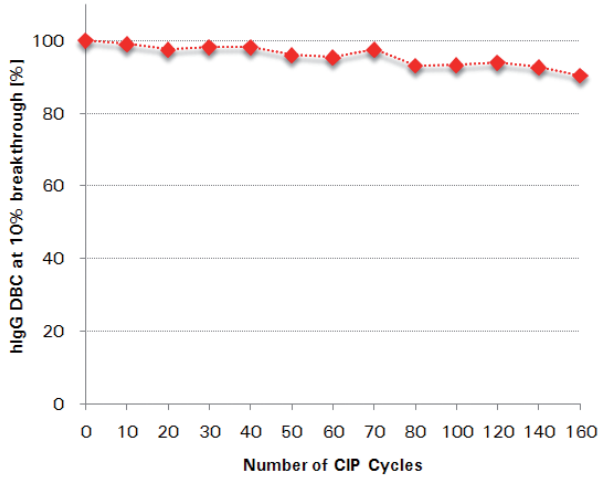

Figure 3A

Cleaning-in-place with 0.1 M NaOH:
Column: 5 mm ID x 5 cm
10 column volumes binding buffer pH 7.4
5 column volumes elution buffer pH 3.0
3 column volumes binding buffer containing 0.1 M NaOH,
16 min contact time
3 column volumes binding buffer pH 7.4

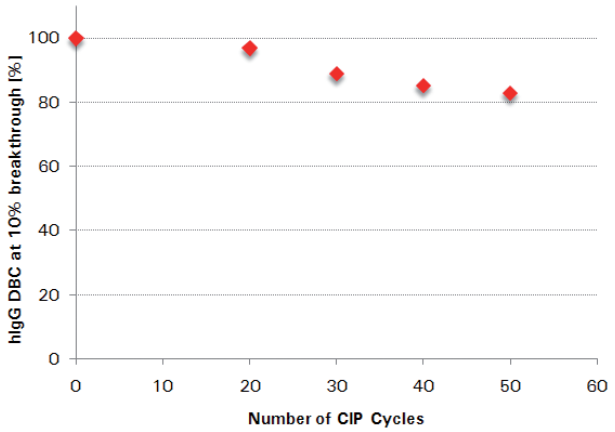

Figure 3B

Cleaning-in-place with 0.5 M NaOH:
Column 4.6 mm ID x 10 cm
10 column volumes binding buffer pH 7.2
5 column volumes elution buffer pH 3.0
3 column volumes binding buffer containing 0.5 M NaOH,
17 min contact time
3 column volumes binding buffer pH 7.2

DBC of hIgG determined at 10% breakthrough

HIGH CIP AND SANITIZATION STABILITY

The structure of the recombinant ligand and its multipoint attachment to the base matrix enhances the stability of TOYOPEARL AF-rProtein A-650F in 0.1 - 0.5 M NaOH. The dynamic binding capacity remains high after repeated CIP cycles. After more than 150 CIP cycles with 0.1 M NaOH at 16 min contact time per cycle more than 90 % of initial dynamic binding capacity was retained (*Figure 3 a*). When performing cleaning-in-place with 0.5 M NaOH about 80 percent of IgG binding capacity remain after 50 cycles with 17 min contact time (*Figure 3b*).

TOYOPEARL AF-rProtein A-650F is also stable in ethanol, 6 M urea, 6 M guanidinium chloride, and 1% phosphoric acid, respectively. Static binding capacity of the resin is not impaired when heated for 30 minutes to temperatures of up to 90°C. It can be stored at room temperature at production site. Recommended conditions for long term storage are a storage solution of 20 % ethanol and temperature of 4 - 8 °C.

PURIFICATION OF HUMANIZED IgG1

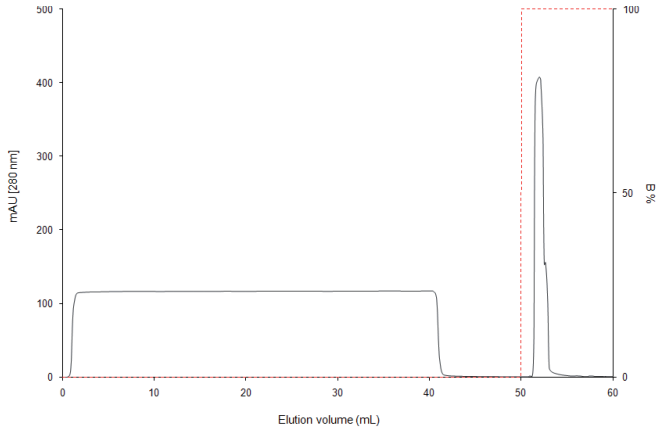

Figure 4

Column:	5 mm ID x 4.9 cm
Flow rate:	0.5 ml/min (150 cm/hr, 2 min residence time)
Sample:	40 ml CHO cell culture lysate containing 0.5 mg /mL humanized IgG1
Binding buffer:	20 mM sodium phosphate, 150 mM NaCl, pH 7.4
Elution buffer:	100 mM glycine-HCl, pH 3.0

HIGH PURITY OF CAPTURED ANTIBODY

Figure 4 shows the purification of a humanized IgG1 from a Chinese Hamster Ovary (CHO) cell lysate. Elisa tests prove that both, remaining host cell proteins and leached protein A ligand amounts, were very low resulting in a very high product purity.

Table 1 shows the removal of host cell protein obtained in a high throughput screening approach to evaluate protein A affinity resins for MAb capture steps. Protein A affinity resins packed in MediaScout® MiniColumns (Repligen GmbH, Weingarten, Germany) were tested by varying the binding buffer pH, the sample load and the residence time. In order to simulate a real feedstock sample a pure monoclonal antibody (IgG1) was spiked into a CHO cell lysate. The purity of the antibody fraction was tested by measuring the amount of CHO host cell proteins

(HCP) and the amount of leached protein A by immunoassays. *Table 1* exemplarily shows the HCP removal when varying the flow rate (residence time) at fixed bed volume and pH of the elution buffer. Fractions purified by TOYOPEARL AF-rProteinA showed a lower amount of remaining host cell proteins under all conditions tested when compared to those purified by a widely used competitive rProtein A resin providing a similar IgG binding capacity. The ligand leaching from TOYOPEARL AF-rProtein A-650F is generally low with typical values of leached protein A ligand between 5 - 25 ppm (ng ligand/mg antibody; determined with Protein A-R28 ELISA kit). Leached recombinant protein A can be removed from the final product through subsequent ion exchange chromatography steps.

Table 2 shows the results of lot-to-lot variation of TOYOPEARL AF-rProtein A-650F with regard to static and dynamic binding capacity and purity.

RIGID POLYMER MATRIX ALLOWS HIGH VELOCITIES

TOYOPEARL AF-rProtein A-650F is based on the well proven polymethacrylate matrix used for all TOYOPEARL resins. The unique pressure flow characteristics allow the use of large production scale columns. *Figure 5* shows the pressure/flow curve for TOYOPEARL AF-rProtein A-650F up to linear velocities of more than 1000 cm/hr.

OPERATION

Typically 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.

PRESSURE/FLOW CURVE

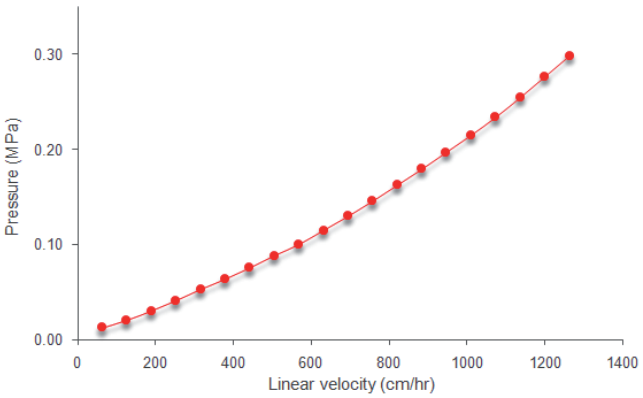

Figure 5

Column: 2.2 mm ID x 20 cm L

For initial scouting of method parameters we recommend using pre-packed SkillPak™ columns or high throughput screening devices.

Suitable load/wash buffers are 20-100 mM sodium phosphate, 150 mM NaCl, pH 7.2 - 7.5 or 100 mM Tris-HCl, 150 mM NaCl, pH 7.2 - 7.5. Washing at reduced pH (e.g. pH 6) might further improve host cell protein reduction. Suitable elution buffers are 100 mM citrate, pH 3 – 4, 100 mM acetate, pH 3 – 4, or 100 mM glycine-HCl, pH 3 - 4. Due to the strong binding of IgGs to the recombinant protein A ligand it might be necessary to modify the elution conditions (lowering pH by 0.1 - 0.3 units) when transferring methods from protein A affinity methods developed on other protein A resins.

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 should be optimized.

HCP REMOVAL

	Bed volume (µl)	Protein load (mg/ml gel)	pH	Flow rate (cm/h)	HCP (ppm)
TOYOPEARL AF-rProtein A	200	5	3.9	100	19.2
Competitive rProtein A resin	200	5	3.9	100	59.7
TOYOPEARL AF-rProtein A	200	5	3.9	250	9.8
Competitive rProtein A resin	200	5	3.9	250	30.5
TOYOPEARL AF-rProtein A	200	25	3.9	250	47.3
Competitive rProtein A resin	200	25	3.9	250	629.6

Table 1

Effect of residence time on the CHO host cell protein (HCP) removal for two alkali-stable rProtein A resins: TOYOPEARL AF-rProtein A-650F and a competitive agarose-based resin. HCP content detected by ELISA (Cygnus Technologies). Data kindly provided by U. Breuninger, University of Applied Science Esslingen.

LOT-TO-LOT VARIATION

	Lot A	Lot B	Lot C
IgG capacitiy (SBC)	51.0 g/L	50.1 g/L	53.1 g/L
IgG capacitiy (DBC)	35.7 g/L	33.6 g/L	35.6 g/L
IgG purity (by SEC)	98.8 %	98.5 %	98.5 %

Table 2

DBC measured at 10 % breakthrough (polyclonal hIgG, 1 mg/ml, 2 min residence time)

For further details of choice and selection of the TOYOPEARL® or TSKgel® resin that best suits your particular separation needs, please contact us:
Tel. +49 (0) 6155 7043700
techsupport.tbg@tosoh.com

Ordering information

TOYOPEARL® AF-rProtein A-650F

Part-No	Description	Resin volume	Pore size	Particle size
22803	TOYOPEARL AF-rProtein A-650F	10 mL	1000 Å	45 µm
22804	TOYOPEARL AF-rProtein A-650F	25 mL	1000 Å	45 µm
22805	TOYOPEARL AF-rProtein A-650F	100 mL	1000 Å	45 µm
22806	TOYOPEARL AF-rProtein A-650F	1 L	1000 Å	45 µm
22807	TOYOPEARL AF-rProtein A-650F	5 L	1000 Å	45 µm
22808	TOYOPEARL AF-rProtein A-650F	50 L	1000 Å	45 µm
22815	Protein A-R28 ELISA Kit	Kit		