TOSOH

Scantec Nordic
Analys & Mätteknik
031 336 90 00 • www.scantecnordic.se

TOYOPEARL® AF-rProtein L-650F

Protein L based affinity chromatography is used for the capture of antibodies and antibody fragments that do not bind to Protein A. Unlike Protein A and G, which bind to the Fc region of immunoglobulins (IgGs), Protein L binds through interactions with the variable region of an antibody's kappa light chain. Therefore Protein L binds a wider range of antibody classes than Protein A. Figure 1 shows typical targets, such as antigen binding fragments (Fabs), single-chain variable fragments (scFvs) and domain antibodies (dAbs).

TYPICAL TARGETS FOR PROTEIN L AFFINITY CHROMATOGRAPHY

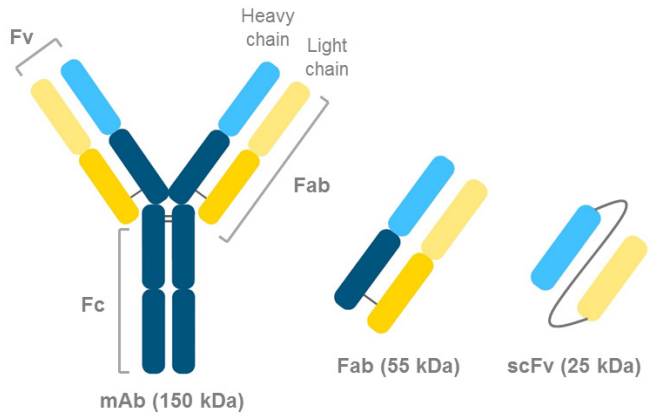

Figure 1

Protein L binds to the variable region of the kappa light chain

TOYOPEARL AF-rProtein L-650F is an affinity chromatography resin that combines a rigid polymer matrix with a recombinant ligand, which is derived from the B4 domain of native Protein L from *Peptostreptococcus magnus* and is expressed in *E.coli* (Figure 2). Code optimization of the domain results in higher binding capacity and an improved stability of the ligand compared to the native molecule.

HIGHLIGHTS

- Capturing of targets that do not bind to Protein A
- Highest binding capacity available on the market
- Binds a broad range of antibody related targets
- Recombinant ligand with high chemical stability
- Improves economics of Protein L unit operations

FEATURES

HIGHEST BINDING CAPACITY

The combination of an optimized recombinant ligand and the proven TOYOPEARL base matrix results in a resin that provides the highest binding capacity available on the market for Fab molecules. Figure 3 shows the binding capacity of TOYOPEARL AF-rProtein L-650F for a Fab fragment at various residence times in comparison to the most popular commercially available agarose based Protein L medium. Due to the excellent mass transfer characteristics of TOYOPEARL AF-rProtein L-650F, especially dynamic binding capacities at 1 to 3 minutes residence time excel capacities obtained with the agarose-based resin.

As the molecular weight of fragments is much smaller compared to full length IgGs, a dynamic binding capacity of about 50 mg/mL for a Fab with a typical molecular weight of 55 kDa equals a DBC of >130 mg/L for a ~150 kDa IgG when considering molar binding capacities.

RECOMBINANT PROTEIN L DERIVED LIGAND

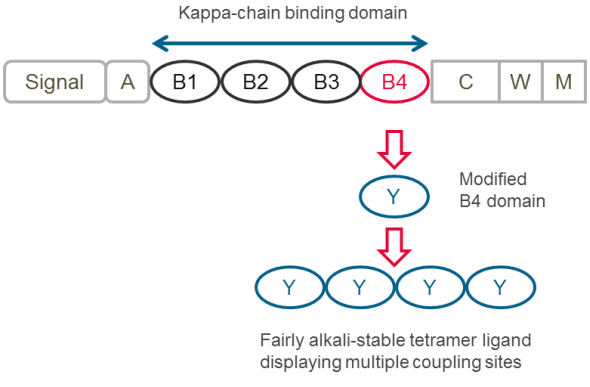

Figure 2

The B4 domain was code optimized and expressed as a tetramer in *E. coli*.

DYNAMIC BINDING CAPACITY OF PROTEIN L MEDIA FOR Fab

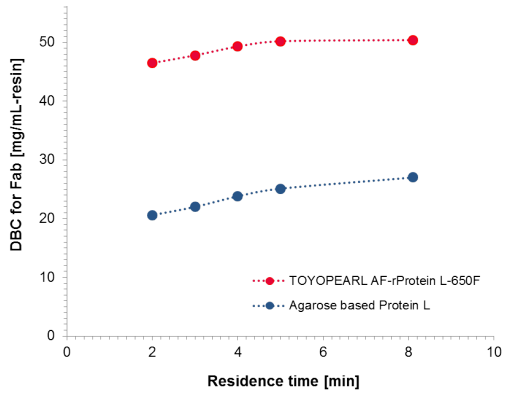

Figure 3

Column: TOYOPEARL AF-rProtein L-650F/competitor resin
4.6 mm ID × 50 mm (0.83 mL)
Detection: UV@280 nm
Sample: 2 g/L human Fab in 0.1 mol/L Na-Phosphate (pH 6.5)
DBC measured at 10 % Breakthrough

IMPROVED PROCESS ECONOMICS

Resin costs represent a considerable part of the overall production costs. The high binding capacity of the new protein L resin can remarkably improve process economics in the production of antibody related recombinant molecules.

RIGID MATRIX ALLOWS HIGH FLOW RATES

TOYOPEARL AF-rProtein L-650F is based on the well proven polymethacrylate matrix used for all TOYOPEARL resins. Figure 4 shows the pressure/flow curve for TOYOPEARL AF-rProtein L-650F packed in a 4.4 cm column with a bed height of 28 cm. Linear velocities up to 600 cm /h can easily be applied to TOYOPEARL Protein L columns.

PRESSURE/FLOW CURVE OF TOYOPEARL AF-rPROTEIN L

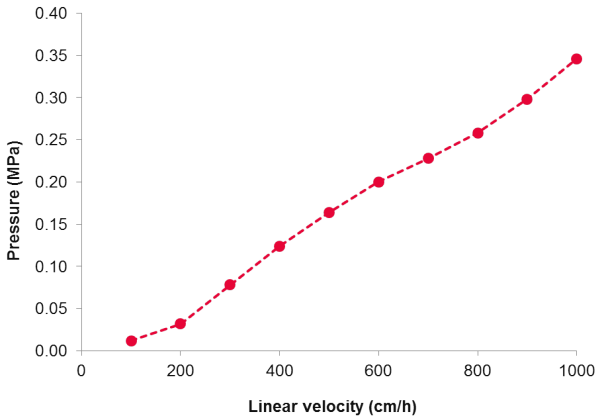

Figure 4

Column size: 4.4 cm ID × 28 cm; Mobile phase: H_2O

ALKALI-STABILITY OF TOYOPEARL PROTEIN L IN 0.1 M NaOH

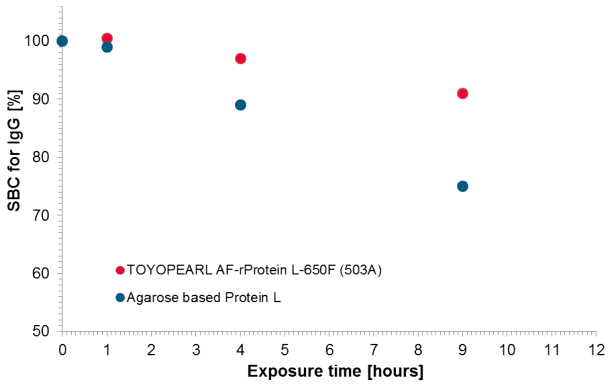

Figure 5

Static binding capacity for IgG in relation to initial binding capacity (100%) after exposure to 0.1M NaOH.

STABILITY TOWARDS CHAOTROPIC AGENTS

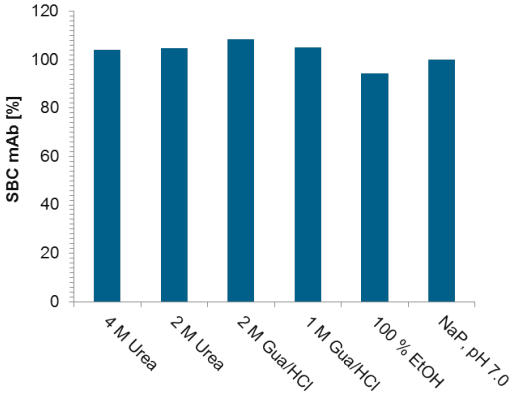

Figure 6

Static binding capacity of TOYOPEARL AF-rProtein L-650F for a mAb determined after 72 hours of exposure to chaotropic agents. Sodium phosphate at pH 7.0 was used as reference (100%).

CHEMICAL STABILITY

The multipoint attachment of the ligand results in a fairly high chemical stability. Figure 5 proves the robustness of the resin towards moderate alkaline solution (0.1 M NaOH) in comparison to a competitor Protein L resin. Figure 6 shows the static binding capacity after storage in several chaotropic agents compared to the initial binding capacity.

SELECTIVITY OF PROTEIN L

Protein L selectively binds to the variable region of kappa light chains of antibodies. It can be used to purify intact immunoglobulins, conventional Fabs as well as single chain variable fragments (scFvs). Protein L also helps to purify those immunoglobulins whose Fc region is not easily accessible for Protein A, such as IgM or IgA.

TOYOPEARL AF-rProtein L has high affinity for mouse and rat antibodies. Table 1 shows the typical Protein L binding affinities to antibody classes and fragments of various species.

PROTEIN L SELECTIVITY

Species	Class	Affinity
GENERAL	Kappa light chain	++
	Lambda light chain	-
	Heavy chain	-
	Fab	++
	ScFv	++
	Dab	++
HUMAN	IgG (1-4)	+
	IgA	+
	IgD	+
	IgE	+
	IgM	+
MOUSE	IgG1	+
	IgG2a	+
	IgG2b	+
	IgA	+
	IgM	+
RAT	IgG1	+
	IgG2a, b, c	+
	IgA	+
HEN*	IgY	+

Table 1

Valid if appropriate kappa light chains are available for binding.
**The results for hen IgY have been obtained through customer collaboration.*

OPERATION

Antibody related targets whether expressed in mammalian cell culture or in microbial systems are typically captured at near neutral pH and eluted using acidic conditions. However, the physicochemical properties of different target molecules are varying depending on the expression system and type of antibody fragment. 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 ToyoScreen or MiniChrom columns or robotic high throughput screening devices with ToyoScreen RoboColumns or Resin Seeker plates.

BINDING

The clarified feedstock is loaded onto the column at a neutral pH. A good starting point is using 100 mmol/L sodium citrate or phosphate buffers at neutral pH. Figure 7 shows the optimization of binding conditions for a F(ab)2 antibody fragment. pH of the loading buffer was varied between 6.0 and 7.5, sodium phosphate concentrations ranged from 50 to 500 mmol/L. Highest binding capacity was achieved at pH 6.5 and 50 mmol/L phosphate.

OPTIMIZATION OF BINDING CONDITIONS

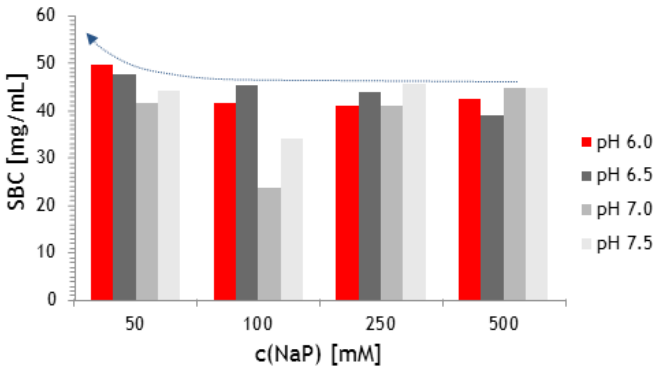

Figure 7

Static binding capacity of TOYOPEARL AF-rProtein L-650F for a F(ab)2 from IgG4 was determined for varying pH and salt concentrations.

WASHING

Up to 10% isopropanol or amino acids such as arginine might be added to the binding buffer to further improve host cell protein reduction.

ELUTION

Suitable elution buffers are 50 to 100 mmol/L sodium citrate, pH 2.0 - 3.0 for fragments and pH 2.5 -3.5 for larger targets such as IgG. Performing elution in a pH linear gradient can help to determine the optimal elution pH.

CLEANING-IN-PLACE AND SANITIZATION

Depending on the origin and type of the target, contact time, concentration, and frequency of CIP cycles, the conditions should be optimized. As a general cleaning-in-place method the column can be flushed with acidic buffer followed by equilibration with 3-5 column volumes of loading buffer. For more rigid cleaning the column can be rinsed with 0.03 to 0.1 M NaOH at short contact times followed by 3-5 column volumes of loading buffer. Although the resin is more alkali stabile than comparable resins on the market, the performance would deteriorate when exposed to alkali solution for long time. Several chaotropic agents can also be applied to remove impurities and improve cleaning (Figure 6).

SUMMARY

TOYOPEARL AF-rProtein L-650F excels all other commercially available Protein L chromatography media with regard to binding capacity and robustness. It is especially suited for the purification of new antibody formats such as antibody fragments, single chain variable fragments and domain antibodies. It can also be used for the capture of immunoglobulin types that cannot be purified with Protein A media. Due to its high binding capacity and improved robustness it can considerably improve process economics of Protein L capturing steps.

Ordering Information

TOYOPEARL AF-rProtein L-650F

Part-No	Description	Resin volume	Pore size	Particle size
TOYOPEARL				
0023486	TOYOPEARL AF-rProtein L-650F	10 mL	100 nm	45 µm
0023487	TOYOPEARL AF-rProtein L-650F	25 mL	100 nm	45 µm
0023488	TOYOPEARL AF-rProtein L-650F	100 mL	100 nm	45 µm
0023489	TOYOPEARL AF-rProtein L-650F	1 L	100 nm	45 µm
0023490	TOYOPEARL AF-rProtein L-650F	5 L	100 nm	45 µm
ToyoScreen				
0023494	ToyoScreen AF-rProtein L-650F	1 mL x 5	100 nm	45 µm
0023495	ToyoScreen AF-rProtein L-650F	5 mL x 1	100 nm	45 µm
0023496	ToyoScreen AF-rProtein L-650F	5 mL x 5	100 nm	45 µm
MiniChrom				
0045162	TOYOPEARL AF-rProtein L-650F	5 mL	100 nm	45 µm
RoboColumns				
0045065	RoboColumn AF-rProtein L-650F	200 µL x 8	100 nm	45 µm
0045066	RoboColumn AF-rProtein L-650F	600 µL x 8	100 nm	45 µm
Resin Seeker Plate				
0045509	Resin Seeker AF-rProtein L-650F	20 µL x 96	100 nm	45 µm

TOSOH BIOSCIENCE I IM LEUSCHNERPARK 4 I 64347 GRIESHEIM I GERMANY I T: +49 (0)6155 7043700 I F: +49 (0)6155 8357900 I SALES-MARKETING.TBG@TOSOH.COM
WWW.TOSOHBIOSCIENCE.DE I WWW.TSKGEL.COM I WWW.TOYOPEARL.COM I WWW.TOYOSCREEN.COM I WWW.ECOSEC.EU