Resin Type	Process Media
Protein A	TOYOPEARL AF-rProtein A-650F TOYOPEARL AF-rProtein A HC-650F
Protein L	TOYOPEARL AF-rProtein L-650F
Activated Resins	TOYOPEARL AF-Epoxy-650 TOYOPEARL AF-Tresyl-650
Reactive Resins	TOYOPEARL AF-Carboxy-650 TOYOPEARL AF-Amino-650 TOYOPEARL AF-Formyl-650
Ready-to-Use Resins with Group Specific Ligands	TOYOPEARL AF-Chelate-650 TOYOPEARL AF-Red-650

The Role of Affinity Chromatography in Process Purification

Affinity chromatography (AFC) is distinctive as a chromatographic technique as it is the only mode that enables the purification of a biomolecule on the basis of functionality or unique chemical structure. Affinity chromatography works on the basis of a specific, reversible, interaction between a target molecule and a specific ligand coupled to a base chromatography bead. Affinity chromatography is inherently a high resolution media because it is highly selective for the molecule of interest, and usually high capacity as well. Because affinity resins are highly specific, post-purification product titer increases of more than 1000× are not unheard of. Purifications that would otherwise be difficult or even impractical using other modes can often be easily accomplished through the use of affinity chromatography.

TOYOPEARL Affinity Resins

There are many custom designed affinity ligands available to the chromatographer. TOYOPEARL affinity chromatography resins are functionalized with chemically active groups or with group-specific ligands. TOYOPEARL affinity resins can be used in combinatorial chemistry or for solid phase synthesis of biomolecules such as peptides, proteins, antibodies, and oligonucleotides because of their excellent stability in a variety of organic solvents and under extreme pHs.

TOYOPEARL affinity 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.

Tosoh Bioscience offers a spectrum of carefully selected TOYOPEARL affinity resins with activated or reactive groups which can be used to covalently attach almost any custom ligand. The structures of TOYOPEARL resins with activated and reactive ligands are shown in Figure 1. Resins with activated functional groups are ready to directly couple a protein or other ligand. The coupled ligand must preserve its specific binding affinity for the target molecule and the binding between the ligand and target molecule must be reversible to allow the target to be eluted without substantial alterations. Resins with reactive groups require carbodiimide coupling or reductive amination to achieve a stable covalent linkage.

In general, TOYOPEARL AF-Tresyl-650M and TOYOPEARL AF-Formyl-650M resins are recommended for coupling proteins, while TOYOPEARL AF-Epoxy-650M resin is suited for coupling lower molecular weight ligands. TOYOPEARL AF-Amino-650M and TOYOPEARL AF-Carboxy-650M resins may be used for both.

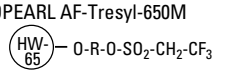
The structure of TOYOPEARL resins with group-specific ligands are shown in Figure 2. Note that due to the proprietary bonding chemistry of TOYOPEARL AF rProtein A-650F, TOYOPEARL AF-rProtein A HC-650F and TOYOPEARL AF rProtein L-650F resins, only ligand structures are shown for these resins (located in their respective sections).

TOYOPEARL AF-Red-650ML is functionalized with Procion Red HE-3B (also known as Reactive Red 120). This resin is useful for the purification of nucleotide-dependent enzymes, lipoproteins, plasminogen, peptides, hormones and cytotoxins. TOYOPEARL AF-Chelate-650M resin carries a chelating ligand, iminodiacetic acid (IDA), that can form stable chelate complexes with selected metal ions such as Cu2+, Ni2+, Zn2+ and Co2+. The resultant resin can be used for immobilized metal affinity chromatography (IMAC). It binds to histidine rich/histidine tagged proteins and to cysteine containing proteins.

Figure 1: Activated and reactive TOYOPEARL affinity resins

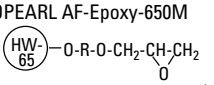
Activated TOYOPEARL affinity resins

TOYOPEARL AF-Tresyl-650M



Ligand Density: 80 µmol/g (dry)

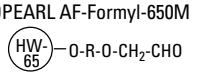
TOYOPEARL AF-Epoxy-650M



Ligand Density: 800 µmol/g (dry)

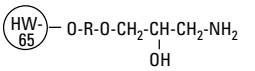
Reactive TOYOPEARL affinity resins

TOYOPEARL AF-Formyl-650M



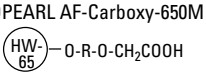
Ligand Density: 60 µeq/mL

TOYOPEARL AF-Amino-650M



Ligand Density: 100 µmol/mL

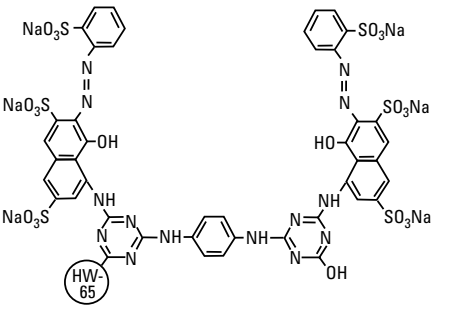
TOYOPEARL AF-Carboxy-650M



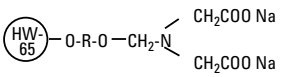
Ligand Density: 100 eq/mL

Figure 2: Group-specific TOYOPEARL affinity resins

TOYOPEARL AF-Red



TOYOPEARL AF-Chelate



Ligand Density: 20 µmol/mL

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 (Table 1). 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 HC-650F is a high capacity protein A resin for monoclonal antibody purification. An enhanced rProtein A ligand (Figure 3) is bound to the TOYOPEARL HW-65F base bead via multipoint attachment resulting in excellent base (Figure 4) 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 5). 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 6).

Figure 3: Ligand structure of TOYOPEARL AF-rProtein A HC-650F resin

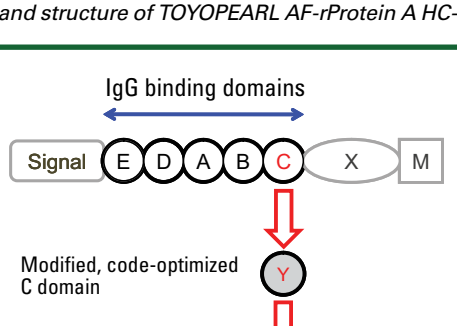
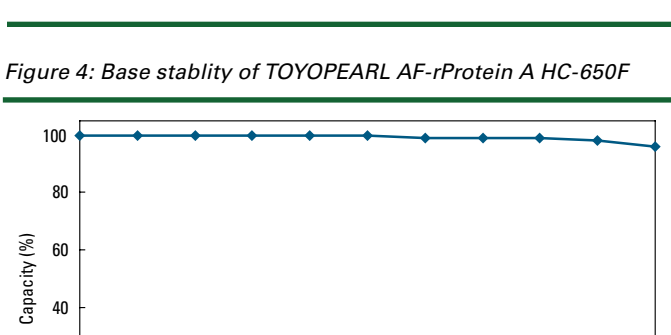


Figure 4: Base stablity of TOYOPEARL AF-rProtein A HC-650F

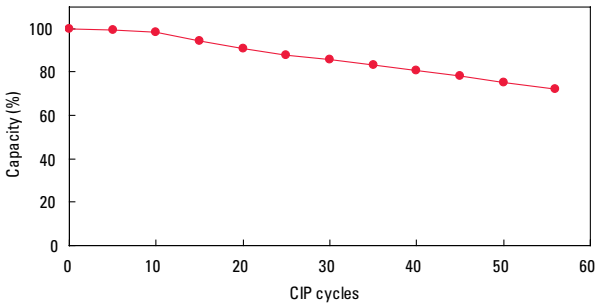


Column size: 5 mm ID × 5 cm
Wash procedure: A: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (10 CV)
B: 0.1 mol/L citrate, pH 3.0 (5CV)
C: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (7 CV)
D: 0.1 mol/L NaOH (3 CV – 15 min contact time)
E: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (5 CV)
Capacity: DBC was determined at 10% breakthrough after every 20 cycles

Table 1: Properties of TOYOPEARL Protein A resins

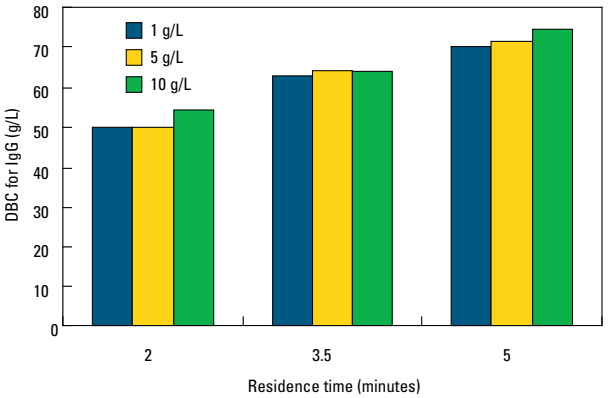
TOYOPEARL resin	Functionality	Base bead	Pore size	Bead diameter	Ligand type	Ligand leakage	DBC (g/L)	Pressure rating
AF-rProtein A-650F	Protein A	HW-65	100 nm	45 µm	rProtein A	5 - 25 ng/mg	> 30 @ 3 min	0.3 MPa
AF-rProtein A HC-650F	Protein A	HW-65	100 nm	45 µm	rProtein A	0.6 - 1.7 ng/mg	> 65 @ 5 min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

Figure 5: 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 Na_2HPO_4, 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 Na_2HPO_4, 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 Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (5 CV)
Capacity:	DBC was determined at 10% breakthrough after every 5 cycles

Figure 6: 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

The selected recombinant Protein A ligand used in the TOYOPEARL AF-rProtein A HC-650F resin has an affinity for a broad range of antibody subclasses, as demonstrated in Table 2.

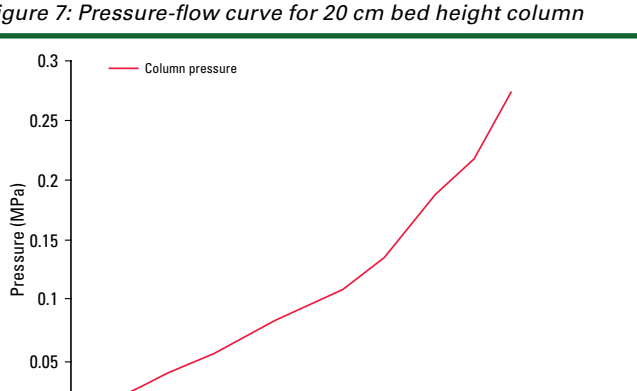
Note that this selected recombinant protein A ligand has very high affinity for mAbs from mouse, goat, rat and hybridoma cell lines.

Table 2: TOYOPEARL AF-rProtein A HC-650F ligand with a broad affinity range for mAb subclasses

Species	Subclass	rProtein A ligand (TOYOPEARL AF-rProtein A HC-650F)	Native Protein A
Human	IgG_1	+++++	++++
	IgG_2	+++++	++++
	IgG_3	-	-
	IgG_4	+++++	++++
Mouse	IgG_1	++++	+
	IgG_{2a}	+++++	++++
	IgG_{2b}	+++++	+++
	IgG_3	++++	++
Rat	IgG_1	++++	-
	IgG_{2a}	-	-
	IgG_{2b}	+++	-
	IgG_{2c}	++++	-
Goat	IgG_s	++++	-
Chicken	IgY	-	-
Rabbit	IgG	+++++	++++

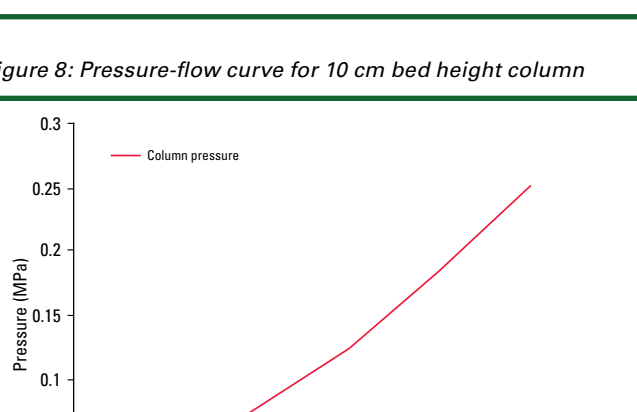
Achievement of high linear velocities at relatively low pressure enables high throughput at production scale using equipment with moderate pressure limitations (Figures 7 and 8).

Figure 7: Pressure-flow curve for 20 cm bed height column



Resin:	**TOYOPEARL AF-rProtein A HC-650F**
Column type:	Merck Superformance
Column size:	10 cm ID × 20 cm (1,570 mL)
Mobile phase:	DI water
Flow rate:	various

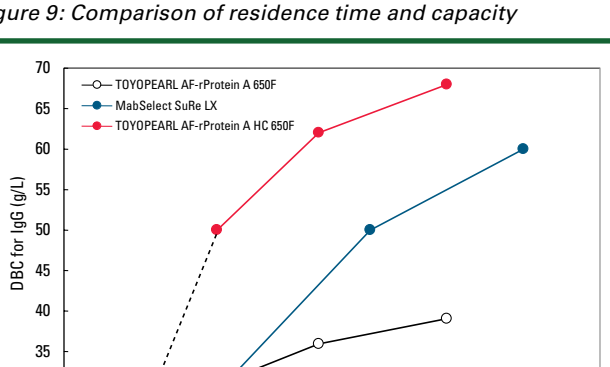
Figure 8: Pressure-flow curve for 10 cm bed height column



Resin:	**TOYOPEARL AF-rProtein A HC-650F**
Column type:	GE Healthcare AxiChrom™
Column size:	20 cm ID × 10 cm (3,140 mL)
Mobile phase:	DI water
Flow rate:	various

Improved mass transfer characteristics allow it to maintain a larger percent of its capacity at lower residence times (Figure 9) 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 3).

Figure 9: 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 ×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

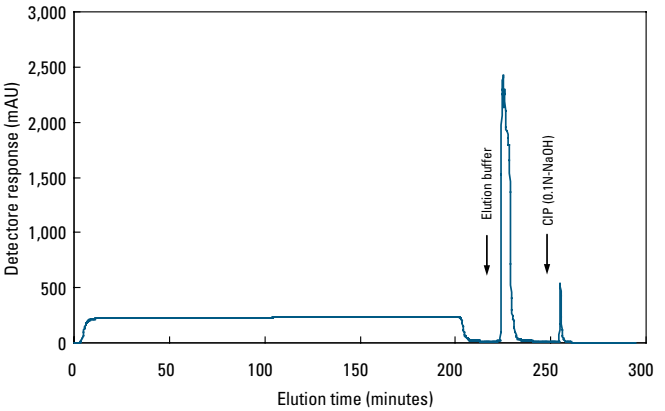
Table 3: Ligand leakage before and after CIP

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				

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 10) 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 series of purification was performed to study the effects of resin loading.

Figure 10: Purification of monoclonal antibody



Resin:	**TOYOPEARL AF-rProtein A HC-650F**
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 gL humanized IgG_1

A 5 mm ID column with a 9.7 cm bed height was loaded with consecutively larger quantities of feedstock so that loads of 35 g/L, 50 g/L, and 65 g/L were achieved. Table 4 shows the load, yield and purity for each of the purifications performed.

Table 4: mAb purity and yield of varying loads of feedstock

Load	% Monomer	% Recovery
35 g/L	96.1	87.2
50 g/L	96.8	86.5
65 g/L	96.1	89.5

DOE Characterization of mAb Capture Step

A four factor, central composite, experimental design was developed to compare the performance of TOYOPEARL AF-rProtein A-650F, TOYOPEARL AF-rProtein A HC-650F and MabSelect SuRe LX resins in terms of product recovery, aggregates, leached protein A ligand, and host cell protein removal. Factors included in the experimental design are elution pH, resin load, feedstock titer, and initial HCP concentration. Figure 11 shows the design space parameters for the experiments carried out with the protein A resins.

Purifications were carried out using the Tecan Freedom EVO® robotic liquid handling instrument according to the experimental design protocol generated by the Design-Expert® DOE software. Experiments were carried out with both citrate and acetate as the elution buffer for a total of 60 experiments performed per resin.

The feed stock material and eluted mAb was analyzed for host cell protein content using a Cygnus Technologies third generation CHO HCP ELISA kit. Figure 12 shows the host cell protein removal for each experiment conveyed in terms of log reduction of HCP from the feed stock material while Figure 13 shows the effects of feedstock titer on the amount of HCP eluted from the TOYOPEARL AF-rProtein A HC-650F resin.

Figure 11: Design space parameters

Variables: feedstock titer
resin load
HCP spiking
pH

Center point values

Four factor, central composite experimental design

Factor	Variable	Min. value	Max. value	-1 Actual	+1 Actual	Mean value
A	Elution pH	2.25	4.25	2.75	3.75	3.25
B	Resin load (g/L)	10.0	50.0	20.0	40.0	30.0
C	Feedstock titer (g/L)	0.25	9.25	2.5	7.00	4.75
D	HCP Spike %	5.0	25.0	10.0	20.0	15.0
	HCP concentration (µg/mL)	100	500	200	400	300

Variable: elution buffer (citrate or acetate)

Figure 12: HCP removal for all resins evaluated

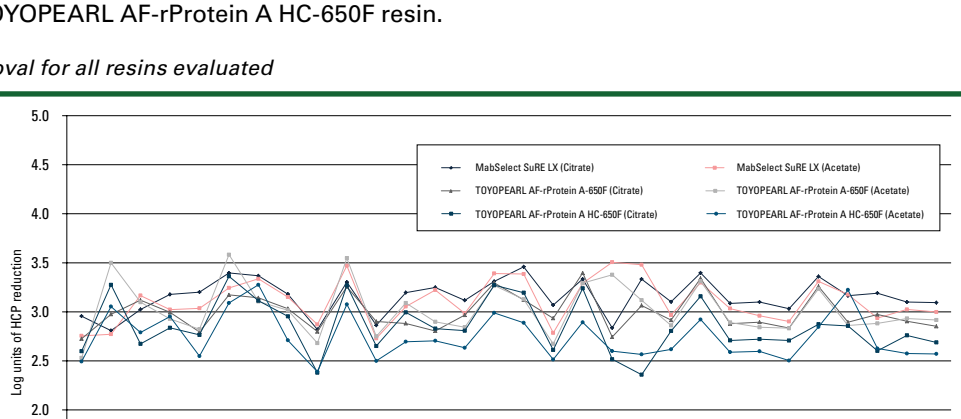


Figure 13: Effect of feedstock titer on HCP concentration in column elution

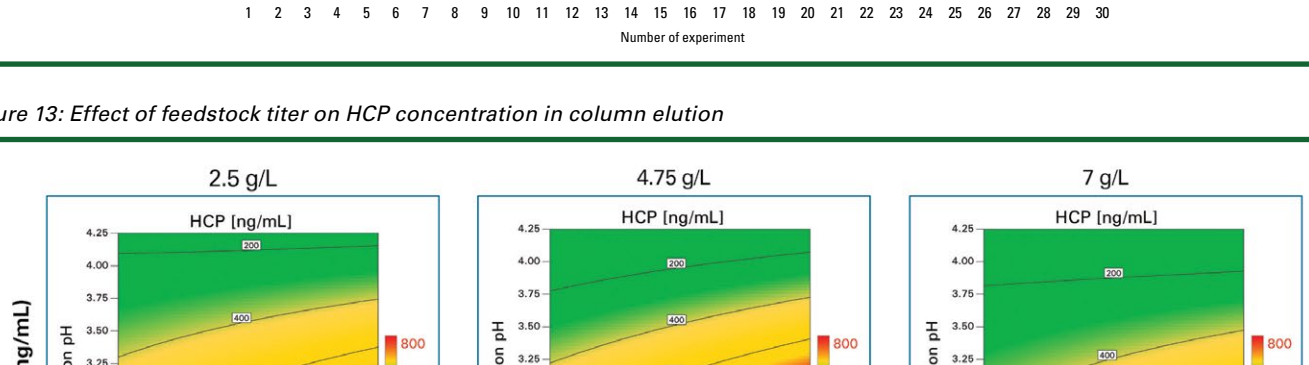


Figure 14 shows the results of the DOE experiments for ligand leakage (ng/mL) for all three resins using both citrate and acetate as an elution buffer. Acceptable levels of ligand leakage were seen for all resins tested; however, the TOYOPEARL AF-rProtein A HC-650F showed levels of leakage an order of magnitude lower than that seen with the MabSelect SuRe LX.

Figure 14: Ligand leaching from Protein A resins tested

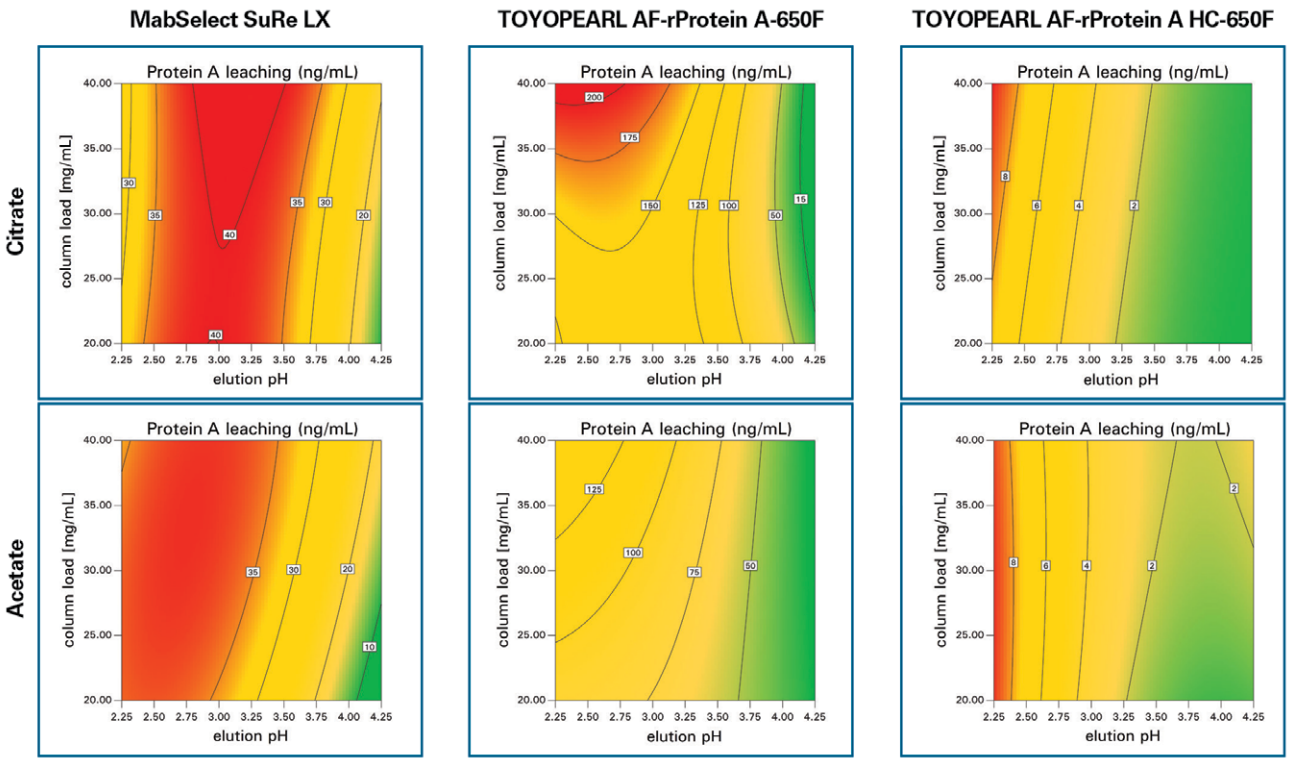
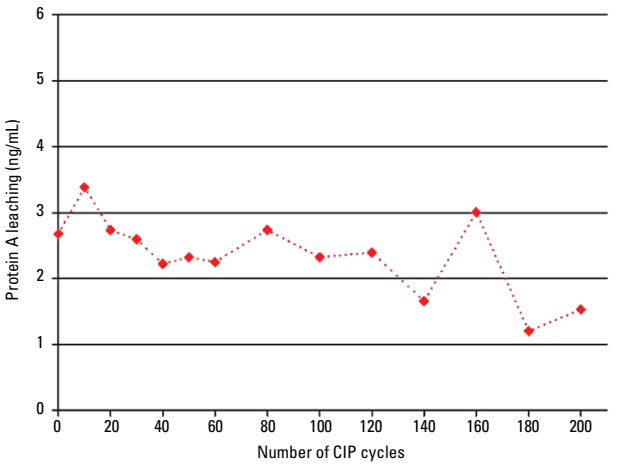


Figure 15 shows the amount of ligand eluted from the TOYOPEARL AF-rProtein A HC-650F resin over 200 cycles using 0.2 mol/L NaOH to clean in place (CIP) between each cycle. As the number of CIP cycles increased, the amount of ligand present in the eluted product decreased. This indicates that the TOYOPEARL AF-rProtein A HC-650F resin has a very stable ligand attachment and meets the performance expectations required in the biopharmaceutical industry for ligand leaching.

Figure 15: TOYOPEARL AF-rProtein A HC-650F ligand stability, 0.2 mol/L NaOH CIP



TOYOPEARL AF-rProtein A-650F resin is an affinity resin for monoclonal antibody purification. The recombinant ligand (Figure 16) 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 17 and 18) and thermal stability (Figure 19). 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 16: Ligand structure of TOYOPEARL AF-rProtein A-650F resin

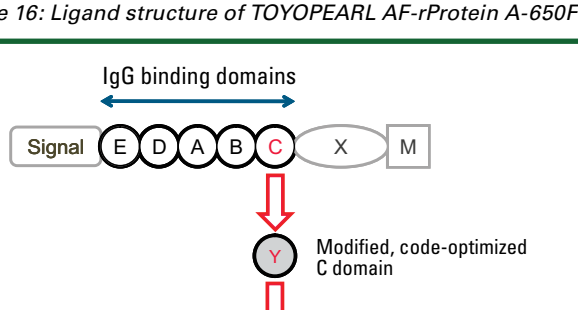
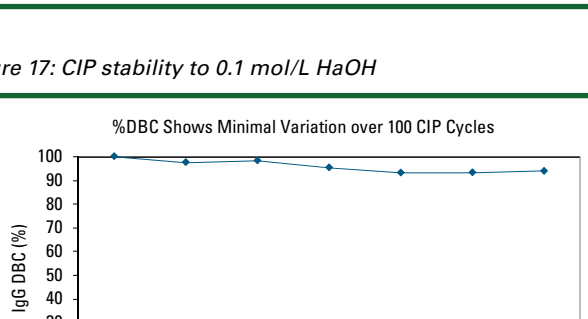


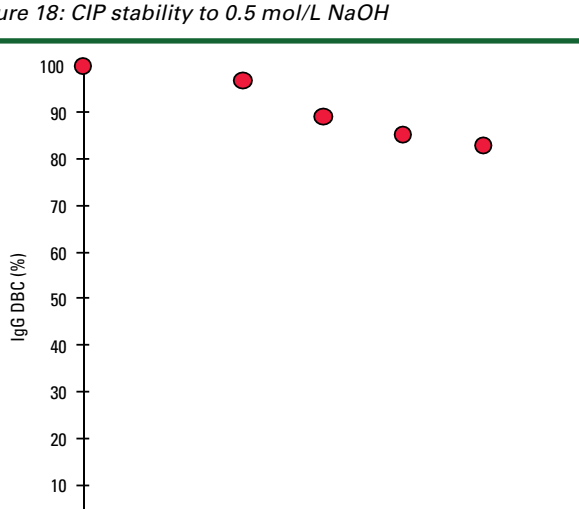
Figure 17: CIP stability to 0.1 mol/L HaOH



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 H_2O

DBC was calculated at 10% breakthrough

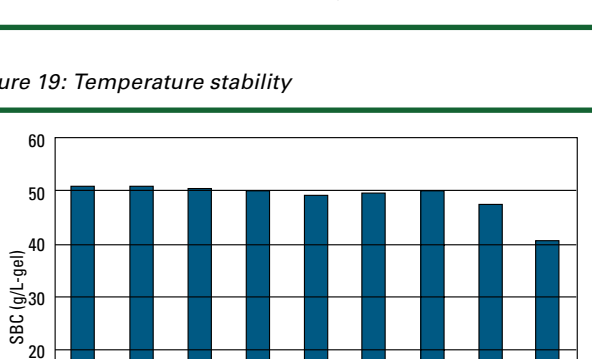
Figure 18: CIP stability to 0.5 mol/L NaOH



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 H_2O

DBC was calculated at 10% breakthrough

Figure 19: Temperature stability

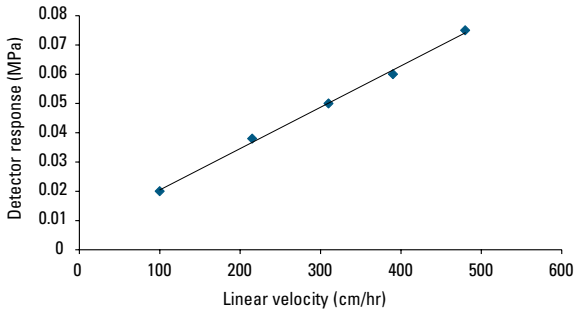


Resin:	**TOYOPEARL AF-rProtein A-650F**
Mobile phase:	deionized H_2O
Autoclave settings:	120 °C, 1.2 atm, 15 min
Heating time:	30 min

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

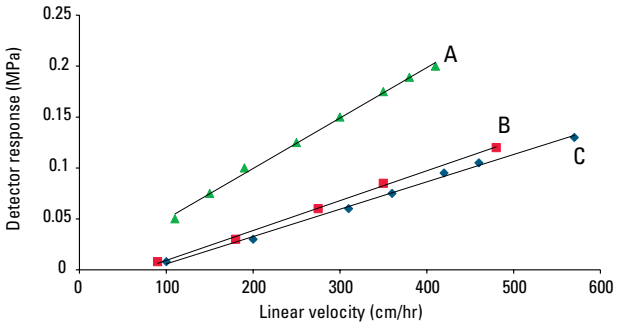
TOYOPEARL AF-rProtein A resins remain dimensionally stable within wide extremes of pH and ionic strength. Moreover, the semi-rigid TOYOPEARL AF-rProtein A particles do not distort under flow rates that generate up to 0.3 MPa pressure. These properties of the resins, combined with the narrow particle 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 20 and 21).

Figure 20: Linear velocity and pressure curve



Resin:	**TOYOPEARL AF-rProtein A -650F**
Column and size:	Resolute®, 40 cm ID × 8.4 cm
Mobile phase:	H_2O
Linear velocity:	various
Detection:	pressure (MPa)

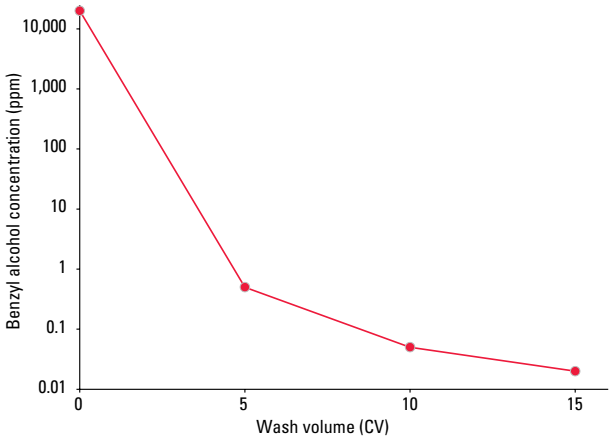
Figure 21: Comparison of linear velocity and pressure curves



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:	H_2O
Linear velocity:	various
Detection:	pressure (MPa)

A 2% solution of benzyl alcohol in water has been identified as a suitable alternative to 20% ethanol as a preservative in resin storage solutions. A sample of TOYOPEARL AF-rProtein A-650F resin was prepared by adding 100 mL of aqueous 2% benzyl alcohol to 100 mL of suction filtered resin. The TOYOPEARL AF-rProtein A-650F was packed in a 1.6 cm ID × 10 cm column and washed with DI water at a flow rate of 800 cm/hr. A sample of the effluent was taken after 5, 10, and 15 column volumes and analyzed for benzyl alcohol concentration (Figure 22). As demonstrated in the figure, a 2% benzyl alcohol solution can be effectively removed from the TOYOPEARL AF-rProtein A-650F resin by thorough washing with DI water.

Figure 22: Concentration of benzyl alcohol in TOYOPEARL AF-rProtein A-650F supernatant (packed column)



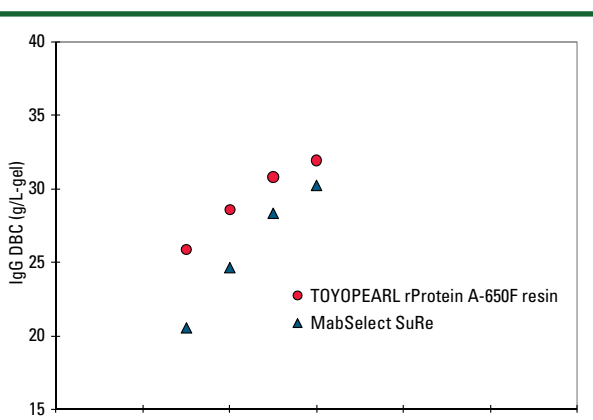
Resin:	**TOYOPEARL AF-rProtein A-650F**
Column size:	1.6 cm ID × 10 cm (20.1 mL)
Mobile phase:	DI H_2O
Flow rate:	800 cm/hr (26.8 mL/min)
Residence time:	45 sec

Improved mass transfer characteristics allow TOYOPEARL AF-rProtein A-650F to maintain a larger percent of its capacity at lower residence times (Figure 23) 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 5).

Figure 23: DBC at various residence times



Resins:	**TOYOPEARL AF-rProtein A-650F**
	MabSelect SuRe
Column size:	5 mm ID × 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 5: TOYOPEARL AF-rProtein A-650F resin vs. MabSelect SuRe resin

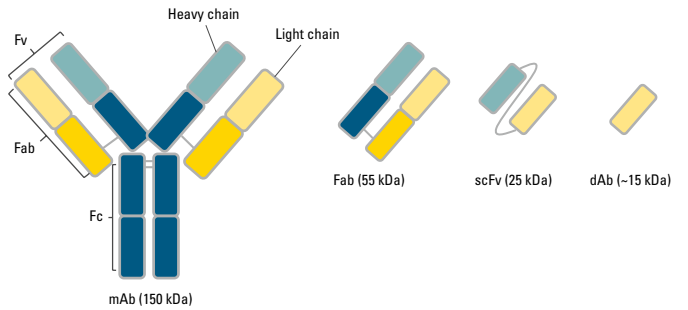
Resin	Protein load (mg/mL gel)	pH	Flow (cm/hr)	BV (µL)	Buffer	CHO (ng/mL)
Toyopearl AF-rProtein A-650F MabSelect SuRe	5 5	3,9 3,9	250 250	200 200	Tris Phosphate	9,76 30,52
Toyopearl AF-rProtein A-650F MabSelect SuRe	45 45	3,4 3,4	100 100	200 200	Tris Phosphate	0,67 36,52
Toyopearl AF-rProtein A-650F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

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

Protein L Chromatography in Process Purification

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 such as IgG, IgM, IgA, IgE, and IgD. Figure 24 shows typical protein L binding regions, such as antigen binding fragments (Fabs), single-chain variable fragments (scFvs) and domain antibodies (dAbs).

Figure 24: Protein L binds to the variable region of the kappa light chain



TOYOPEARL AF-rProtein L-650F Resin

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 25). Code optimization of the domain results in higher binding capacity and an improved stability of the ligand compared to the native molecule. The key characteristics of TOYOPEARL AF-rProtein L-650F resin are listed in Table 6.

Figure 25: The modified recombinant Protein L ligand used in TOYOPEARL AF rProtein L-650F resin

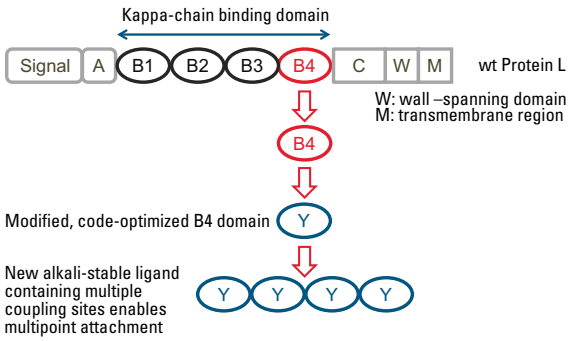


Table 6: Properties of TOYOPEARL AF-rProtein L-650F resin

Resin matrix	Polymer
Particle size (mean)	45 µm
Pore size (mean)	100 nm
Ligand	Recombinant Protein L (*E. Coli*)
DBC at 4 min retention time	≥ 38 g human Fab/L resin
SBC	>64 g human IgG/L resin
Pressure rating	0.2 MPa
pH stability	2-13
Shipping buffer	20% ethanol
Storage	20% ethanol, 2-8 °C

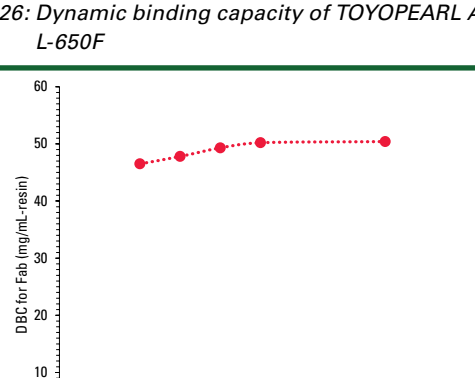
The selected recombinant Protein L ligand used in the TOYOPEARL AF-rProtein L-650F resin has an affinity for a broad range of antibody subclasses, as demonstrated in Table 7.

Table 7: TOYOPEARL AF-rProtein L-650F ligand with a broad affinity range for mAb subclasses

Species	Subclass	Affinity
General	Kappa light chain	++
	Lambda light chain	-
	Heavy chain	-
	Fab	++
	ScFv	++
	Dab	++
Human	$IgG_{(1-4)}$	+
	IgA	+
	IgD	+
	IgE	+
	IgM	+
Mouse	IgG_1	+
	IgG_{2a}	+
	IgG_{2b}	+
	IgA	+
	IgM	+
Rat	IgG_1	+
	$IgG_{2a,b,c}$	+
	IgA	+
Hen	IgM	+
	IgY	+

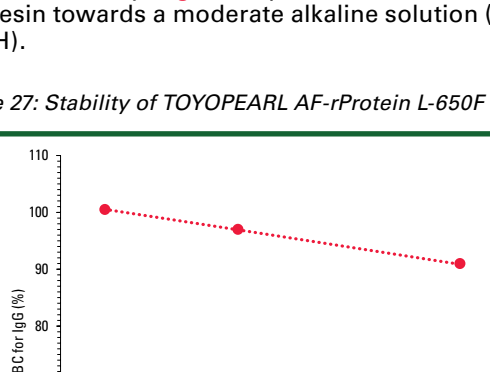
The combination of an optimized recombinant ligand and the proven TOYOPEARL base matrix results in a resin that provides the highest binding capacity available on the market for Fab molecules. Figure 26 shows the excellent binding capacity of TOYOPEARL AF-rProtein L-650F for a Fab fragment at various residence times in comparison to an agarose based protein L medium. The binding capacity of the TOYOPEARL AF-rProtein L-650F resin is 50 mg/mL for a Fab with a typical molecular weight of 55 kDa, which equates to a dynamic binding capacity (DBC) of >130 mg/L for a ~150 kDa IgG when considering molar binding capacities.

Figure 26: Dynamic binding capacity of TOYOPEARL AF-rProtein L-650F



The multipoint attachment of the modified, code-optimized B4 domain of the recombinant protein L used in the TOYOPEARL AF rProtein L-650F resin results in a high chemical stability. Figure 27 proves the robustness of this resin towards a moderate alkaline solution (0.1 mol/L NaOH).

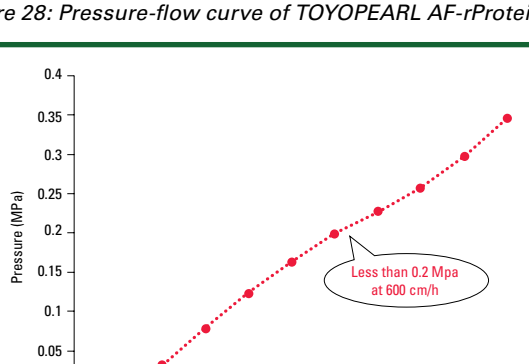
Figure 27: Stability of TOYOPEARL AF-rProtein L-650F



Resin costs represent a considerable part of overall production costs. The high binding capacity and great alkaline resistance of the TOYOPEARL AF-rProtein L-650F resin can remarkably improve process economics in the production of antibody related recombinant molecules.

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

Figure 28: Pressure-flow curve of TOYOPEARL AF-rProtein L-650F

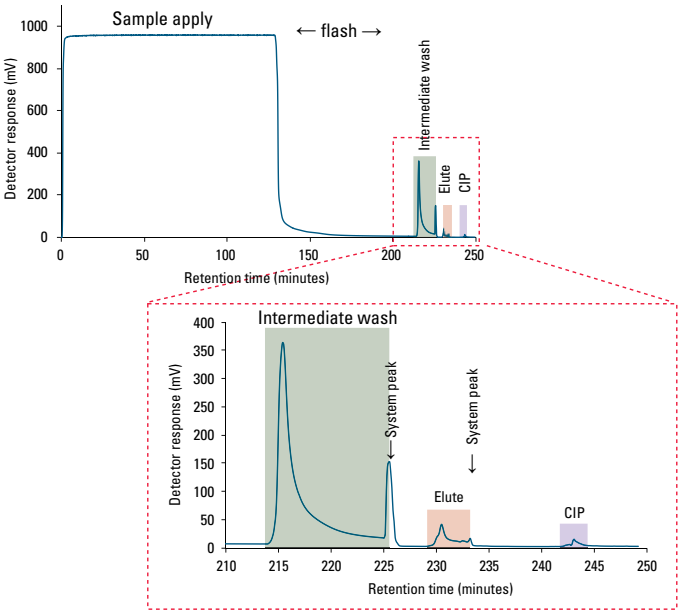


The protein L ligand is immobilized to the highly cross bead matrix via a multi-point coupling that also gives the TOYOPEARL AF rProtein L-650F resin a low ligand leakage. The analysis of the protein L ligand leakage is determined by using a commercially available ELISA-protein L ligand leakage kit in the presence of Fab. Typical values found in the Fab-containing eluates from purification of E. coli homogenate feed showed ligand leakage below the quantitation limit (protein L level of <1.4 ppm of purified Fab).

Purification and Analysis of scFv Fragment of hIgG$_1$

scFv fragments were expressed in a mammalian cell line. After harvesting, the sample was spun and filtered. Approximately 2 mg of total protein (including scFv fragments) was loaded onto a TOYOPEARL AF-rProtein L-650F column (0.5 mL volume). The approximate residence time was 1.4 minutes. A step gradient protocol was used. The intermediate wash peak, system peak, eluted peak, and CIP peak were collected for further analysis as shown in Figure 29 (zoom in view). The bound sample was eluted with 0.1 mol/L Na-citrate, pH 2.3.

Figure 29: Purification of scFv fragments using TOYOPEARL AF-rProtein L-650F



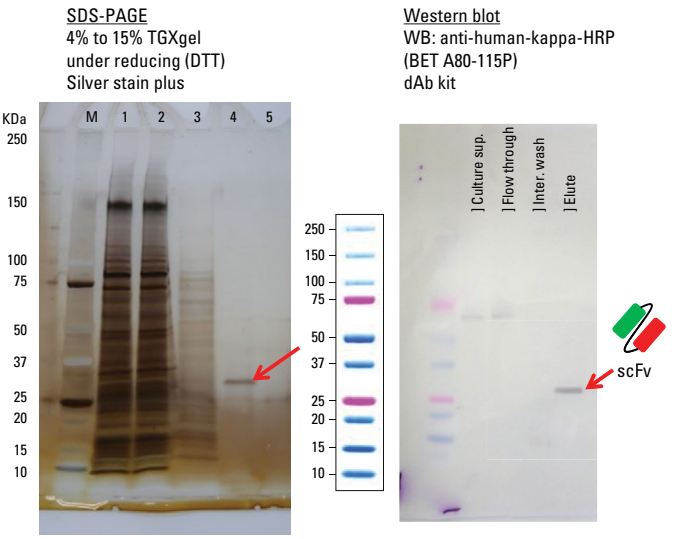
Resin:	**TOYOPEARL AF-rProtein L-650F**
Column size:	5 mm ID × 2.5 cm (0.5 mL)
Equib & flash wash:	0.1 mol/L Na-citrate, pH 6.5
Intermediate wash:	0.1 mol/L Na-citrate, pH 6.5 + 1 mol/L Arg-HCl, 10% iPrOH
Elute:	0.1 mol/L Citrate, pH 2.3
CIP:	0.05 mol/L NaOH
Flow rate:	0.35 mL/min (residence time = 1.4 min)
Detection:	UV @ 280 nm
Sample:	hu-scFv1 CHO culture sup. 40 mL

Figure 30, left panel, shows the results of silver stain from the collected fractions after the sample containing scFv fusion protein was injected onto a TOYOPEARL AF-rProtein L-650F column. 10 µL from each fraction was loaded onto the 4-15% TGXgel under a reduced condition with DTT. The gel was stained with silver stain plus kit. Data from the silver stain gel shows that there is only a single band from the eluted peak (Figure 30, left panel, lane 4) with a molecular weight of approximately 26 kDa. This indicates that only the sample containing a molecule of about 26 kDa is captured by the resin. The data suggests that this is the scFv.

Figure 30, right panel, shows Western blot data using anti-human-kappa-HRP from a dAb kit to determine whether the eluted peak of 26 kDa is the scFv. The result from the Western blot analysis reconfirmed that the anti-human-kapp-HRP interacts with this single 26 kDa band.

Based on the data from the silver stained SDS-PAGE and the Western blot, this 26 kDa molecule is confirmed to be the scFv fusion protein. The estimated yield of the scFv fusion protein was >98%.

Figure 30: Purification of scFv fragments using TOYOPEARL AF-rProtein L-650F

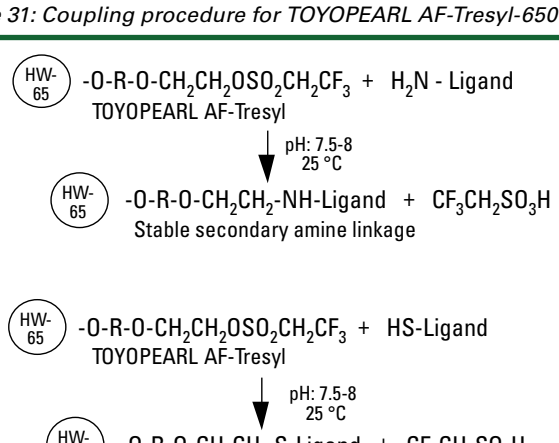


M prestained size marker (Bio-Rad 1610374)
1 Applied sample (hu_scFv_1)
2 Flow through
3 Intermediate wash (Arg+iPrOH)
4 Elute (pH 2.3) 1114series
5 CIP (50 mmol/L NaOH)

Activated resins – ready for direct ligand attachment

TOYOPEARL AF-Tresyl-650M activated resin is highly reactive toward amine and thiol groups. It is provided in dry form, ready for reaction in buffered solutions containing the ligand to be coupled. Coupling is accomplished in a neutral to slightly alkaline (pH 7 - 8) solution (Figure 31).

Figure 31: Coupling procedure for TOYOPEARL AF-Tresyl-650M



Under such conditions even proteins of limited stability may be successfully coupled. Coupling leads to the formation of a highly stable secondary amine or thio-ether linkage. The optimized tresyl density (ca. 20 µmol/mL hydrated resin) is sufficient to provide substantial protein binding while avoiding excessive multi-point attachment and consequent impairment of ligand affinity and activity. Representative data are presented in Table 8.

TOYOPEARL AF-Epoxy-650M activated resin, also packaged in dry form, has a high density of epoxy-functionality (ca. 800 µmol/mL). Under appropriate reaction conditions, this may be used to immobilize proteins or low molecular weight ligands. It is particularly useful when high densities of low molecular weight ligands must be attached (Figure 32). Glutathione and glycine have, for example, been coupled at densities greater than 100 µmol/mL hydrated resin. TOYOPEARL AF-Epoxy-650M resin is a highly versatile starting material for conversion to other chemically active functional groups required in special applications. This resin may be readily activated to hydrazide-bearing materials. This is particularly useful for immobilization of carbohydrates or glycoproteins.

Figure 32: Coupling procedure for TOYOPEARL AF-Epoxy-650M

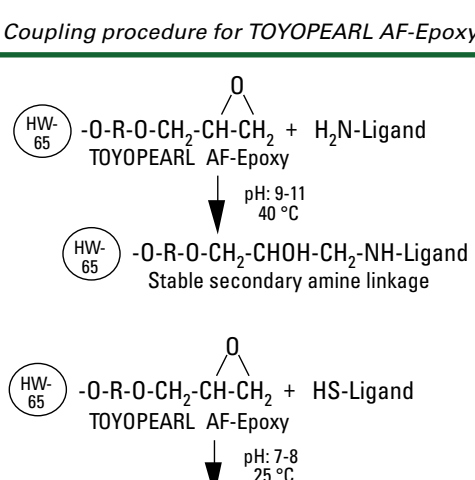


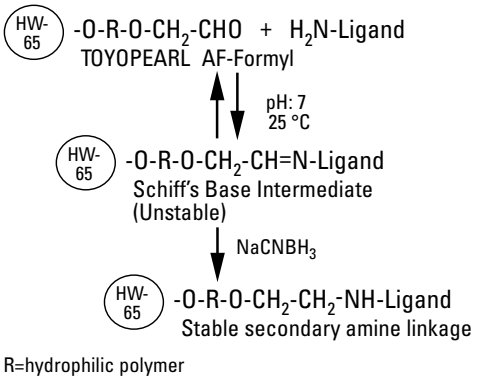
Table 8: Representative coupling densities for activated and reactive TOYOPEARL media

TOYOPEARL resin *Protein coupled (g/L resin)*	**AF-Tresyl-650M**	**AF-Formyl-650M**	**AF-Amino-650M**	**AF-Carboxy-650M**
soybean trypsin inhibitor	16	3.5	5.8	15
protein A	1.9	—	—	—
concanavalin A	13	—	—	—
α1-antitrypsin	12.3	—	—	—
α-chymotrypsin	12.5	—	—	—
myoglobin	12.4	—	—	—
ovalbumin	—	2.5	6.7	0.8
bovine serum albumin	12.4	14	19.2	3.3
human IgG	10.0	15	6.7	11.7
cytochrome	—	5.8	3.3	7.5
lysozyme	60	20	5.8	17.5
coupling agent	*not required*	*$NaCNBH_3$*	*$NaCNBH_3$ or carbodiimide*	*carbodiimide*
optimal pH	7.0 - 9.0	6.9 - 9.0	4.5 - 6.0	4.5 - 6.0

Reactive resins - require activation for ligand attachment

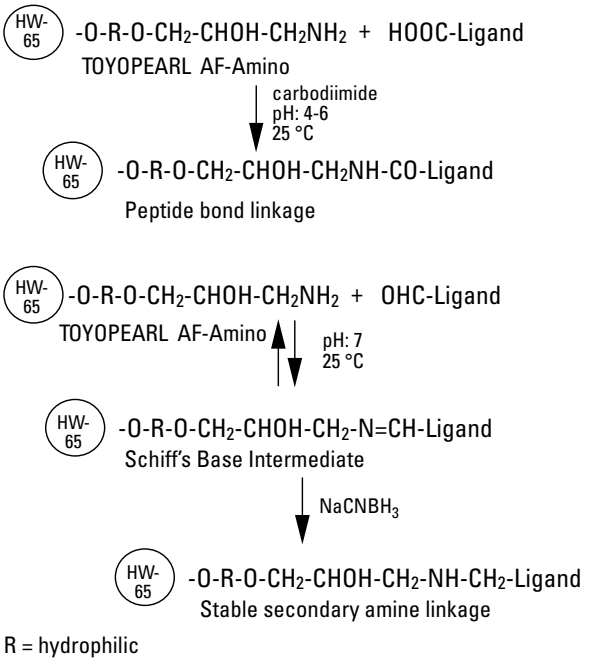
Ligands may be coupled to TOYOPEARL AF-Formyl-650M (aldehyde-bearing) resin under mild conditions exclusively using primary amines. The ligand is bound to the resin by a stable secondary amine linkage (Figure 33). A wide variety of industrial enzymes have been immobilized on aldehyde-bearing supports. Typically, these supports have been synthesized by industrial users by partial oxidation of polysaccharide supports (e.g. cellulose and agarose) or partial hydrolysis of polyacetals. In contrast, TOYOPEARL AF-Formyl-650M resin is a ready-to-use aldehyde support formulated from a dimensionally stable, macroporous matrix. Consistent aldehyde content and physical properties are ensured from batch to batch.

Figure 33: Coupling procedure for TOYOPEARL AF-Formyl-650M



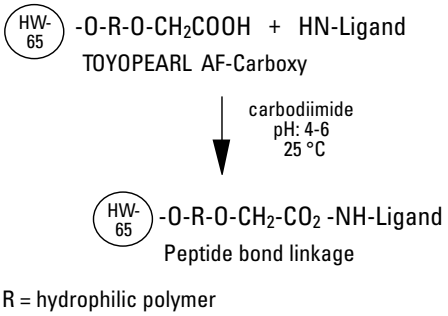
TOYOPEARL AF-Amino-650M resin may be used to couple ligands using their carboxyl groups through peptide bond formation or aldehyde groups by reductive amination as shown in Figure 34. Aldehyde groups may be present in a carbohydrate or glycoprotein ligand or may be introduced into the ligand by mild, periodate oxidation. The optimized functional group density of TOYOPEARL AF-Amino-650M (100 µmol/mL) is ideal for coupling of either proteins or low molecular weight ligands. For example, lactose was coupled by reductive alkylation to yield a ligand density of ca. 30 µmol/mL resin.

Figure 34: Coupling procedure for TOYOPEARL AF-Amino-650M



TOYOPEARL AF-Carboxy-650M resin provides another useful, though milder, approach for coupling to amino groups of proteins or low molecular weight ligands. The carbodiimide mediated coupling reaction produces an amide bond between ligand and support (Figure 35).

Figure 35: Coupling procedure for TOYOPEARL AF-Carboxy-650M



Resins with group specific ligands

TOYOPEARL AF-Chelate-650M resin is derivatized with iminodiacetic acid (IDA) at a concentration of ca. 20 µmol/mL. In typical applications, selected metal ions, most often Cu^{2+}, Ni^{2+}, Zn^{2}+ and Co^{2+}, are bound to the support by stable chelation. The resultant metal ion-bearing resin binds to histidine and free cysteine containing sequences of a peptide or protein. Immobilized metal ion affinity chromatography (IMAC) has been used for purification of recombinant human growth factor, tissue plasminogen activator, glycophorins, and whole cells.

TOYOPEARL AF-Red-650ML resins are functionalized with Procion Red HE-3B (also known as Reactive Red 120). This resin is useful for the purification of nucleotide-dependent enzymes, lipoproteins, plasminogen, peptides, hormones and cytotoxins. TOYOPEARL AF-Red-650ML resin is useful for the purification of nucleotide-dependent enzymes, albumin, cell growth factors, interferons, transferases, cyclases, and polymerases. Typical binding capacities are shown in Table 9.

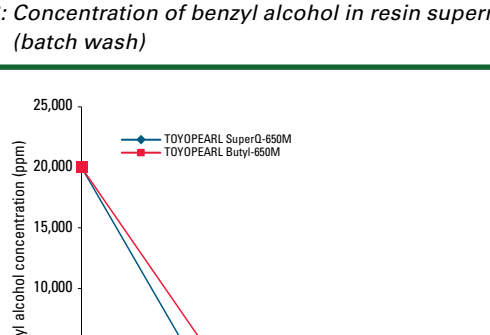
Table 9: Representative binding capacities for TOYOPEARL AF-Red-650ML

Protein (g/L)	TOYOPEARL AF-Red-650ML
human serum albumin	3.5 ± 1
lactate dehydrogenase	11

A 2% solution of benzyl alcohol in water has been identified as a suitable alternative to 20% ethanol as a preservative in resin storage solutions. Samples of TOYOPEARL SuperQ-650M and Butyl-650M resin (which serve as a representative sample of all TOYOPEARL resins, including the TOYOPEARL affinity resins) were prepared by adding 100 mL of aqueous 2% benzyl alcohol to 100 mL of suction filtered resin. A 100 mL aliquot of DI water was added to the filtered resin and stirred to make a slurry. This resin/ DI water slurry was allowed to stand for 5 minutes and was then suction filtered to remove the supernatant. This procedure was repeated 14 more times, for a total of 15 washes.

Samples of the filtered supernatant from the TOYOPEARL SuperQ-650M and Butyl-650M resin were taken after the 5th, 10th, and 15th washes and analyzed for benzyl alcohol concentration (Figure 36). As demonstrated in the figure, a 2% benzyl alcohol solution can be effectively removed from the TOYOPEARL SuperQ-650M and Butyl-650M resin by thorough washing with DI water.

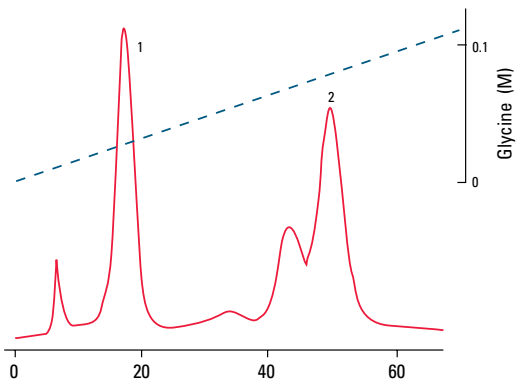
Figure 36: Concentration of benzyl alcohol in resin supernatant (batch wash)



Separation of Two Proteins

Metal ion affinity chromatography is often used for the purification of histidine-rich or histidine-tagged proteins. For example, in the separation of two proteins, zinc ions were immobilized to the resin and salt was used in the eluent to suppress the ionic interactions between the sample and the carboxyl groups of the AF-Chelate-650M resin (Figure 37). These conditions favor chelation of the proteins by the resin-bound metal ions over potential ion exchange interactions. Typical elution gradients use imidazole (1 mmol/L to 20 mmol/L), glycine (0 to 0.2 mol/L), or a pH gradient (8.0 to 4.0).

Figure 37: Immobilized metal ion affinity chromatography with TOYOPEARL AF-Chelate-650M

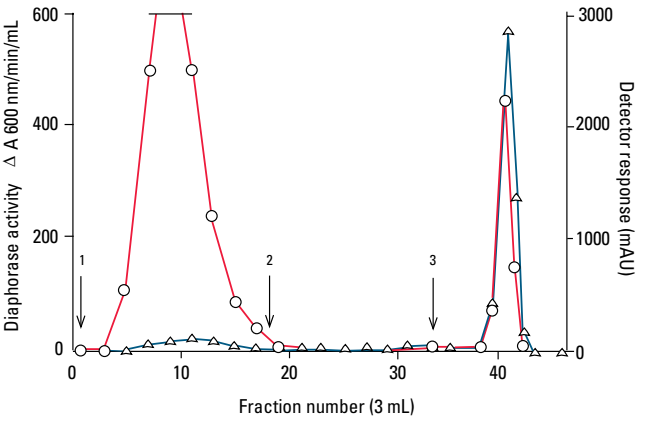


Resin:	**TOYOPEARL AF-Chelate-650M**
Column size:	8 mm ID × 7.5 cm
Metal ion:	Zn^{+2}
Mobile phase:	Buffer A: 20 mmol/L Tris-HCl, 0.5 mol/L NaCl, pH 8.0 Buffer B: buffer A + 0.2 mol/L glycine
Gradient:	0 - 100% B (120 min)
Flow rate:	60 cm/hr (0.5 mL/min)
Detection:	UV @ 280 nm
Samples:	1. ribonuclease A. 250 µg 2. transferrin, 250 µg

Purification of Ferredoxin-NADP Reductase

Synechococcus ferredoxin (Fd) was coupled to TOYOPEARL AF-Tresyl using a 0.1 mol/L $NaHCO_3$, pH 8, coupling buffer. The resulting *Synechococcus* Fd-TOYOPEARL was used to purify ferredoxin-NADP reductase, as shown in Figure 38[1]. The TOYOPEAERL AF-Tresyl was preferred by the authors over agarose-based affinity resins due to the superior flow properties of the TOYOPEARL resin.

Figure 38: Affinity chromatography of spinach FNR on a Synechococcus Fd-TOYOPEARL column



Resin:	***Synechococcus* Fd-TOYOPEARL**
Column size:	22 mm ID × 10 cm
Mobile phase:	1. Load with crude spinach FNR in 20 mmol/L Tris-HCl, pH 7.5 2. Wash with 20 mmol/L Tris-HCl, pH 7.5 3. Elute with 20 mmol/L Tris-HCl, pH 7.5 with 0.25 mol/L NaCl
Flow rate:	16 cm/hr (1 mL/min)
Detection:	UV @ 275 nm, specific activity
Sample:	spinach FNR

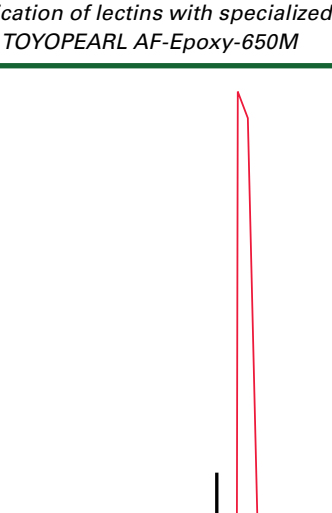
[1]Sakihama, N.; Nagai, K.; Ohmori, H.; Tomizawa, H.; Tsujita, M.; Shin, M. Immobilized ferredoxins for affinity chromatography of ferredoxin-dependent enzymes. *J. Chroma. A.* **1992**, *597*, 147-153.

Purification of Lectins

The high density of epoxy functionality is especially useful for generating specialized affinity supports with low molar mass ligands. For example, 150 mg N-acetylgalactosamine (GalNAc) was couple to 1.0 g of hydrated resin by reaction in 3 mL of 0.1 mol/L sodium hydroxide at 45 °C for 16 hours with gentle agitation[2]. The product was washed with distilled water, 1 mol/L sodium chloride, and distilled water. Residual epoxy groups were blocked by treatment with 1 mol/L ethanolamine (25 °C, 12 hours).

The TOYOPEARL AF-GalNAc resin was used to purify a lection from *Grifola frondosa* (GFL), an edible mushroom (Figure 39). A two-step affinity chromatography scheme yielded 3.2 mg of FGL with 86% of the initial activity found in 2.34 g of crude protein from an ammonium sulfate precipitation.

Figure 39: Purification of lectins with specialized supports prepared from TOYOPEARL AF-Epoxy-650M



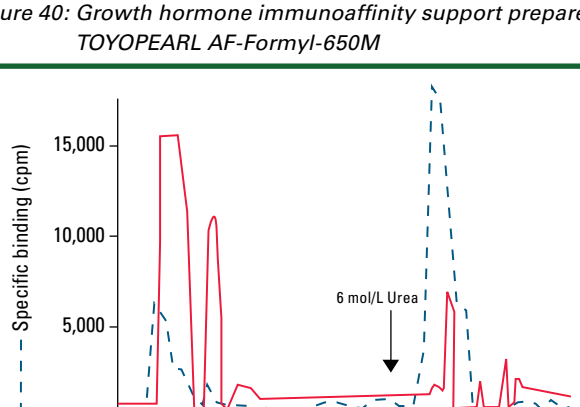
Resin:	**GalNAc-Epoxy TOYOPEARL**
Column size:	10 mm ID × 5 cm
Mobile phase:	Buffer A: 10 mmol/L phosphate-buffered saline, pH 7.4
	Buffer B: buffer A + 0.1mol/L lactose
Gradient:	equilibrate/load/wash 100% buffer A isocratic elution 100% buffer B
Detection:	UV @ 275 nm
Sample:	4.0 mg impure *Grifola frondosa* lectin

[2]Kawagishi, H.; Nomura, A.; Mizuno, T.; Kimura, A.; Chiba. S. Isolation and characterization of a lectin from Grifola frondosa fruiting bodies. Biochimica et Biophysica Acta (BBA) - General Subjects. **1990**, *1034*, (3), 247-252.

Purification of GH Receptor Protein

As shown in Figure 40, growth hormone (GH) was coupled to TOYOPEARL AF-Formyl-650M, and then was used to purify GH receptor protein[3]. A size exclusion column (TSKgel G3000SW) was directly connected to the affinity column. This approach eliminated the urea that co-eluted with the GH receptor from the affinity column, and enabled high receptor activity as denaturation was minimized. This one-step procedure provided a 1,000-fold purification, yielding 50 mg of GH receptor.

Figure 40: Growth hormone immunoaffinity support prepared with TOYOPEARL AF-Formyl-650M



Resin:	**GH-Formyl TOYOPEARL**
Column size:	4.6 mm ID × 15 cm in series with TSKgel 3000SW, 7.6 mm ID × 60 cm
Mobile phase:	Buffer A: 50 mmol/L Tris-HCl, 20 mmol/L NaCl, 10 mmol/L $MgCl_2$, 0.3 mmol/L phenylmethylsulfonyl fluoride GH receptor protein, 0.1% Triton™ X-100, pH 7.4
	Buffer B: buffer A + 6 mol/L urea
Gradient:	isocractic elution 100% buffer B
Flow rate:	36 cm/hr for 60 min, then 220 cm/hr
Detection:	UV @ 280 nm, specific binding assay
Sample:	16 mg growth hormone (GH) receptor protein in 6 mL Triton X-100

[3]Yagi, S.; Izawa, K.; Nakagawa, T.; Tanaka, H.; Yoshitake, A.; Mohri, Z. Efficient high performance liquid chromatographic system for protein purification. *J. Chroma. A.* **1989**, *493*, (1), 27-33.

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

Ordering Information

TOYOPEARL Affinity resins:

Part #	Product description	Container size (mL)
	TOYOPEARL Protein A Resins	
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
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

Part #	Product description	Container size (mL)
	TOYOPEARL Protein L Resin	
23486	TOYOPEARL AF-rProtein L-650F	10
23487	TOYOPEARL AF-rProtein L-650F	25
23488	TOYOPEARL AF-rProtein L-650F	100
23489	TOYOPEARL AF-rProtein L-650F	1,000
23490	TOYOPEARL AF-rProtein L-650F	5,000

Part #	Product description	Container size (mL)	Typical ligand density	Typical capacity (g/L)
	TOYOPEARL Affinity Resins with Group Specific Ligands			
08651	TOYOPEARL AF-Red-650ML	25	7 µmol/mL	2.5 - 4.5 (HSA)
19801	TOYOPEARL AF-Red-650ML	100	7 µmol/mL	2.5 - 4.5 (HSA)
42102	TOYOPEARL AF-Red-650ML	1,000	7 µmol/mL	2.5 - 4.5 (HSA)
14475	TOYOPEARL AF-Chelate-650M	25	25 - 45 µeq/mL	≥ 60 (lysozyme)
19800	TOYOPEARL AF-Chelate-650M	100	25 - 45 µeq/mL	≥ 60 (lysozyme)
14907	TOYOPEARL AF-Chelate-650M	1,000	25 - 45 µeq/mL	≥ 60 (lysozyme)
14908	TOYOPEARL AF-Chelate-650M	5,000	25 - 45 µeq/mL	≥ 60 (lysozyme)

HSA = Human Serum Albumin

Part #	Product description	Container size (mL)	Typical ligand density	Typical capacity (g/L)
TOYOPEARL Reactive Affinity Resins				
43411	TOYOPEARL AF-Amino-650M	10	70 - 130 µeq/mL	
08002	TOYOPEARL AF-Amino-650M	25	70 - 130 µeq/mL	
08039	TOYOPEARL AF-Amino-650M	100	70 - 130 µeq/mL	
18074	TOYOPEARL AF-Amino-650M	1,000	70 - 130 µeq/mL	
18316	TOYOPEARL AF-Amino-650M	5,000	70 - 130 µeq/mL	
43412	TOYOPEARL AF-Carboxy-650M	10	80 - 120 µeq/mL	
08006	TOYOPEARL AF-Carboxy-650M	25	80 - 120 µeq/mL	
08041	TOYOPEARL AF-Carboxy-650M	100	80 - 120 µeq/mL	
18827	TOYOPEARL AF-Carboxy-650M	1,000	80 - 120 µeq/mL	
18828	TOYOPEARL AF-Carboxy-650M	5,000	80 - 120 µeq/mL	
43413	TOYOPEARL AF-Formyl-650M	10	40 - 70 µeq/mL	
08004	TOYOPEARL AF-Formyl-650M	25	40 - 70 µeq/mL	
08040	TOYOPEARL AF-Formyl-650M	100	40 - 70 µeq/mL	
17396	TOYOPEARL AF-Formyl-650M	1,000	40 - 70 µeq/mL	
17397	TOYOPEARL AF-Formyl-650M	5,000	40 - 70 µeq/mL	

Part #	Product description	Container size (g)	Typical ligand density	Adsorption capacity (mg/g)
TOYOPEARL Activated Affinity Resins				
43402	TOYOPEARL AF-Epoxy-650M*	5	600 - 1,000 µeq/g	> 60**
08000	TOYOPEARL AF-Epoxy-650M*	10	600 - 1,000 µeq/g	> 60**
08038	TOYOPEARL AF-Epoxy-650M*	100	600 - 1,000 µeq/g	> 60**
14471	TOYOPEARL AF-Tresyl-650M*	5	80 µmol/mL	≥ 60**
14472	TOYOPEARL AF-Tresyl-650M*	100	80 µmol/mL	≥ 60**
14905	TOYOPEARL AF-Tresyl-650M*	200	80 µmol/mL	≥ 60**
18371	TOYOPEARL AF-Tresyl-650M*	5,000	80 µmol/mL	≥ 60**

**Shipped dry. 1 g yields approximately 3.5 mL of hydrated resin*
***Measured as amount of test protein coupled per gram of dry gel.*


TOYOPEARL®
AF-rProtein L-650
Net Vol. 100mL
Part No. 23488
Lot No. 60PPGMB501F
FOR IN VITRO USE ONLY
TOYOPEARL®
AF-rProtein L-650F
25mL
23487
65ETS99R
TOSOH
TOYOPEARL®
AF-rProtein A-650F
25mL
22804
65ETS99R
TOSOH