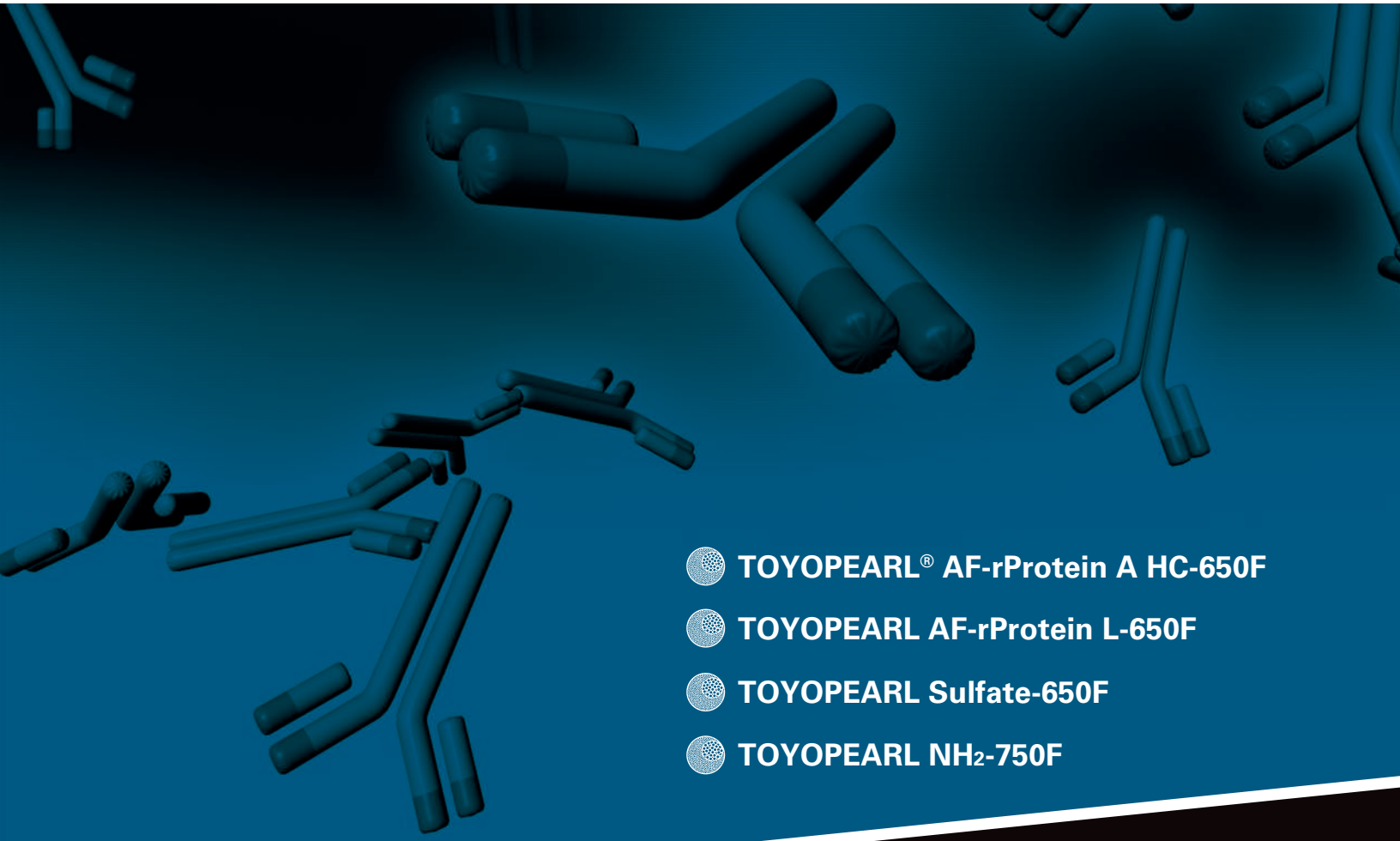
TOSOH

Best-In-Class Bioprocessing Resins



- TOYOPEARL® AF-rProtein A HC-650F
- TOYOPEARL AF-rProtein L-650F
- TOYOPEARL Sulfate-650F
- TOYOPEARL NH_2-750F

Experts in Chromatography

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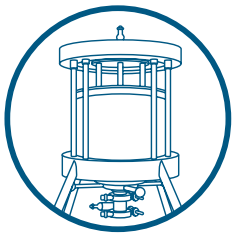
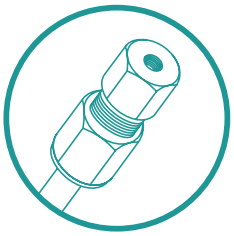
Solutions for Your Purification and Separation Tasks from Lab to Production

Tosoh Bioscience is a leading supplier of chromatographic media, columns and gel permeation chromatography (GPC) instruments with over 500 specialty products to meet your analysis and purification needs.



TOYOPEARL, TSKgel® & Ca++Pure-HA™ Resins

TOYOPEARL and TSKgel chromatography resins are specifically designed for the purification of biomolecules. Ca++Pure-HA is a hydroxyapatite resin and has unique separation properties for biomolecules. These resins show excellent physical strength and ideal flow characteristics for industrial downstream processing.



TSKgel U/HPLC Columns

Extensively used in laboratories all over the world, our TSKgel columns are designed for researchers seeking the highest level of performance. Covering the total range of U/HPLC, these columns offer high resolution, excellent reproducibility and long column life. Scaling up from analytical to preparative columns is made simple and easy.

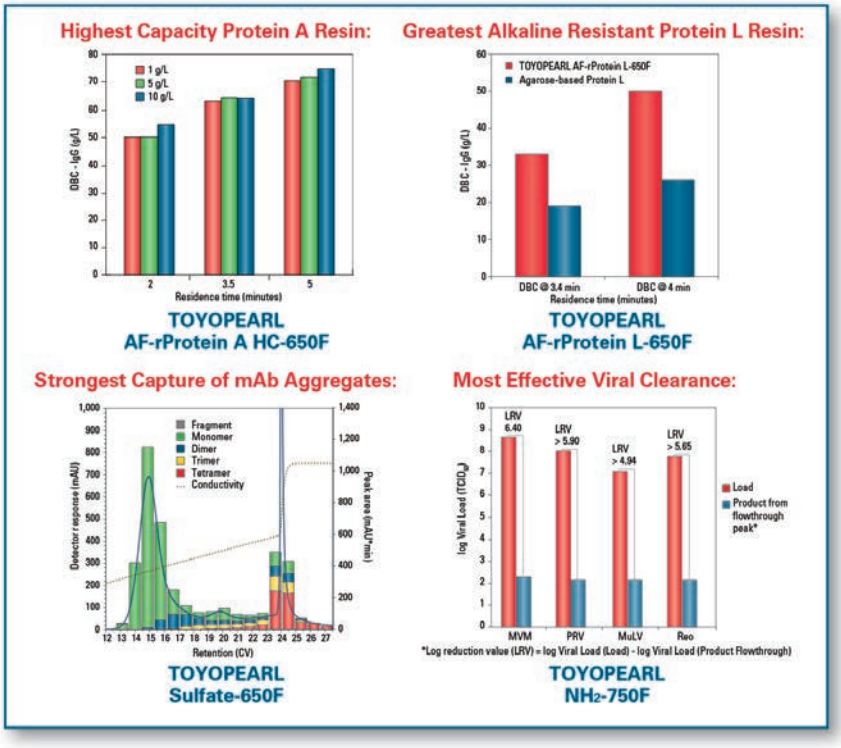
EcoSEC® GPC Systems

The EcoSEC series of fully automated liquid chromatography systems for gel permeation chromatography is designed for robust polymer analysis. Both solutions, for ambient and for high temperature GPC, combine dual pump solvent systems, sophisticated heating and a highly efficient detection system to deliver the highest reproducibility.

In this brochure, four best-in-class TOYOPEARL resins are introduced.

These resins are designed for mAb purification with exceptionally high capacities and excellent impurity removal properties.

These features provide process developers with greater flexibility of operational parameters, leading to higher productivity.



TOYOPEARL AF-rProtein A HC-650F

The Best-In-Class Resin: an economical protein A resin with the highest dynamic binding capacity

Protein A chromatography is the most widely used type of affinity chromatography. Its high specificity for the Fc region of antibodies is the primary reason for its dominance as the affinity ligand of choice in antibody purification applications. Increasing usage of monoclonal antibodies (mAbs) as biological drugs in management of chronic diseases has driven the increased demand of protein A resins.

With pressures mounting to reduce production costs at many companies, and protein A being the most expensive resin used in mAb purification, the use of a high capacity protein A resin can significantly impact the overall cost of doing business. To reduce the production cost at many companies, scientists are often looking for high binding capacity protein A resins with high recovery at various flow rates.

TOYOPEARL AF-rProtein A HC-650F is a hydroxylated methacrylic polymer high capacity protein A resin for monoclonal antibody purification. Table 1 lists the properties and dynamic binding capacities of this resin.

TOYOPEARL AF-rProtein A HC-650F resin offers:

- **High binding capacity** – up to 70 g/L, the highest in its class
- **Reduced operating cost** – use of shorter columns requires less resin
- **Superior recovery** – up to 90% at 65 g/L load
- **High alkaline resistance** – ensures long operational lifetime and improves process economics
- **Excellent pressure-flow stability** – low pressure drop allows high flexibility of operational parameters
- **Lowest ligand leaching** – allows long term usage of resin before its effective operational life is complete
- **High affinity to more subclasses of mAbs** – the best-in-class and most cost-effective resin for capturing mAbs from hybridoma, mouse and CHO cell lines

Table 1. Characteristics of TOYOPEARL AF-rProtein A HC-650F resin

Particle size	45 µm
Pore diameter	100 nm
DBC (5 min)	70 g/L
DBC (2 min)	50 g/L
Caustic stability	> 200 CIP cycles (0.1 mol/L NaOH)
Max. pressure	0.3 MPa

High dynamic binding capacity

The results of a dynamic binding capacity (DBC) comparison study between TOYOPEARL AF-rProtein A HC-650F and another commercially available protein A resin is detailed in Table 2. The data shows that TOYOPEARL AF-rProtein A HC-650F has much higher DBC. Indeed, at 2 minutes residence time, this resin is able to absorb as much as 50 g IgG/L resin and at 5 minutes residence time, TOYOPEARL AF-rProtein A HC-650F resin's dynamic binding capacity reaches 70 g/L. This is almost 20% higher than the dynamic binding capacity of a competitive resin.

Table 2. Dynamic binding capacity of TOYOPEARL AF-rProtein A HC-650F resin and competitor

Product name	Supplier	Bead diameter	DBC-2 min (g/L)	DBC-5 min (g/L)
TOYOPEARL AF-rProtein A HC-650F	Tosoh Bioscience	45 µm	50	70
Competitive Product	Competitor X	85 µm	30	58

Reduced operating cost

With its high dynamic binding capacity, TOYOPEARL AF-rProtein A HC-650F resin does not have to be packed in the typical column bed height range of 20 cm. Use of a shorter column requires less resin. As can be seen from the below comparisons in Table 3, making use of a high capacity protein A resin in your purification process is an excellent way to save on production costs.

Table 3. Production cost comparisons using TOYOPEARL AF-rProtein A HC-650F resin and competitor

Resin	Packed bed height (cm)	Column diameter (cm)	Column volume (L)	Resin compression	Resin volume to pack column	Resin cost per liter	Packed column cost	DBC at residence time (g/L)	Column capacity 80% DBC (g)	Residence time (min)
TOYOPEARL AF-rProtein A HC-650F	15	36	15	1.25	20	$12,000	$240,000	60	732	3
Resin X	21	40	26	1.16	31	$12,000	$372,000	35	739	3

Superior recovery

TOYOPEARL AF-rProtein A HC-650F is capable of delivering high purity monoclonal antibodies with excellent recovery at loading levels approaching the resin capacity without any increase in aggregate levels (Table 4). This level of performance will allow chromatographers to fully utilize the increased capacity of this resin without compromising quality while at the same time increasing the productivity of the antibody capture step.

Table 4. Load and recovery levels of TOYOPEARL AF-rProtein A HC-650F resin

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

High alkaline resistance

TOYOPEARL AF-rProtein A HC-650F resin was subjected to 300 purification cycles. Clean-in-place (CIP) was done with 0.2 mol/L NaOH following each cycle, with a contact time of 15 minutes. As can be seen from Figure 1, the capacity, normalized to 100% prior to the first CIP cycle, remains virtually unchanged for approximately 200 cycles. This data indicates that the TOYOPEARL AF-rProtein A HC-650F resin is exceptionally stable in NaOH, ensuring a long operational lifetime, thereby improving process economics.

Excellent pressure-flow stability

TOYOPEARL AF-rProtein A HC-650F resin is based on a hydrophilic, dimensionally stable base resin, TOYOPEARL HW-65, that exhibits excellent pressure-flow characteristics (see Figure 2). The 100 nm pore diameter of the TOYOPEARL affinity resins can accommodate large globular proteins up to 5×10^6. The semi-rigid TOYOPEARL AF-rProtein A HC-650F beads do not distort under flow rates that generate up to 0.3 MPa pressure.

Figure 1. Stability of TOYOPEARL AF-rProtein A HC-650F resin

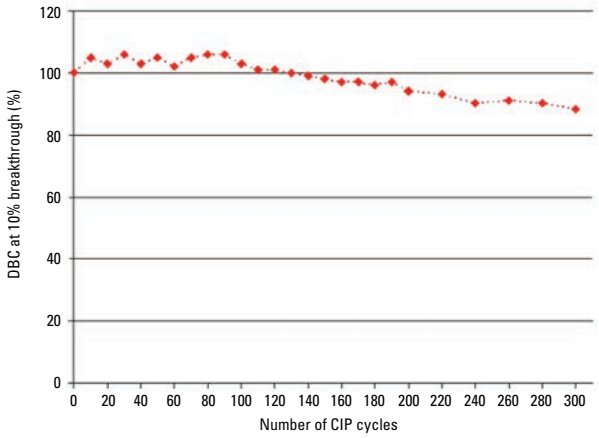
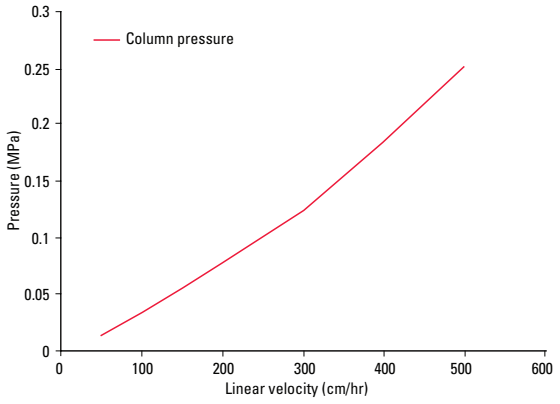


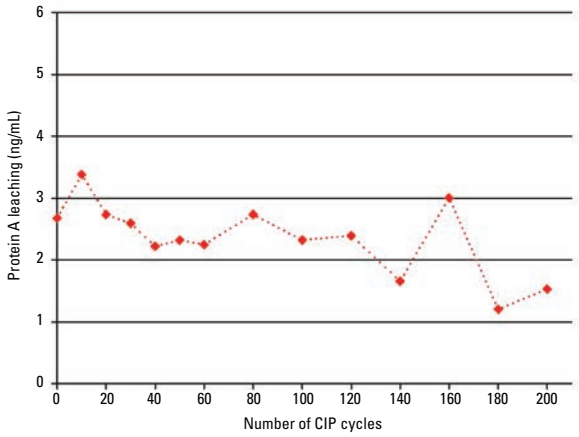
Figure 2. Pressure-flow of TOYOPEARL AF-rProtein A HC-650F resin



Lowest ligand leaching

Figure 3 below 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. This also demonstrates the potential for long term use of TOYOPEARL AF-rProtein A HC-650F resin before its effective operational life is complete.

Figure 3. Low ligand leaching of TOYOPEARL AF-rProtein A HC-650F resin



High affinity to more subclasses of mAbs

Because the recombinant protein A ligand of the TOYOPEARL AF-rProtein A HC-650F resin is a code-modified hexamer of the C domain, this resin has an affinity for various antibodies that the native protein A and some other recombinant protein A ligands do not possess. For example, it has high affinity for different subclasses of antibodies from rat and goat which native protein A does not have any affinity for, as demonstrated in Table 5.

Table 5. 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 Ligand
Hybridoma	IgG	+++++	++
Mouse	IgG_1 IgG_{2a} IgG_{2b} IgG_3	++++ +++++ +++++ ++++	+ ++++ +++ ++
Human (CHO)	IgG_1 IgG_2 IgG_3 IgG_4	+++++ +++++ - +++++	++++ ++++ - ++++
Rat	IgG_1 IgG_{2a} IgG_{2b} IgG_{2c}	++++ - +++ ++++	- - - -
Goat	IgG_s	++++	-
Rabbit	IgG	+++++	++++

Ordering Information

Part#	Description
23425	TOYOPEARL AF-rProtein A HC-650F, 10 mL, 45 µm
23426	TOYOPEARL AF-rProtein A HC-650F, 25 mL, 45 µm
23427	TOYOPEARL AF-rProtein A HC-650F,100 mL, 45 µm
23428	TOYOPEARL AF-rProtein A HC-650F, 1 L, 45 µm
23429	TOYOPEARL AF-rProtein A HC-650F, 5 L, 45 µm
45201	SkillPak™ TOYOPEARL AF-rProtein A HC-650F 1 mL column
45222	SkillPak TOYOPEARL AF-rProtein A HC-650F 1 mL columns (qty. 5)
45258	SkillPak TOYOPEARL AF-rProtein A HC-650F 5 mL column
OC41MDAFPA-650F	HC Protein A 20 µL 96-well Plate Kit
45063	ToyoScreen RoboColumn AF-rProtein A HC-650F, 8 × 200 µL
45064	ToyoScreen RoboColumn AF-rProtein A HC-650F, 8 × 600 µL

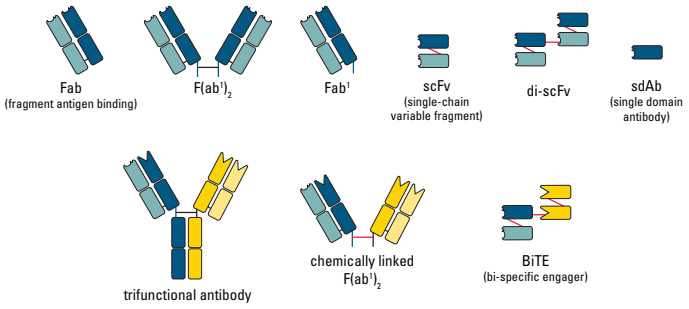
Interested in trying more than one of our best-in-class resins?
See the last page of this brochure for ordering information for SkillPak Column Libraries.

TOYOPEARL AF-rProtein L-650F

The Best-In-Class Resin: an alkaline resistant protein L resin with the highest binding capacity for non-Fc mAb fragments

Antibody fragments such as single domain antibodies (dAbs), antigen binding fragment (Fabs) and single chain variable fragments (scFv) have been found to be better suited to certain therapeutic conditions than full size antibodies. Scientists are able to conjugate these fragments to create more powerful antibodies, known as bispecific monoclonal antibodies (BsMAb or BsAb), as shown in Figure 4. Based on their size, these antibodies can effectively reach their targets and have the ability to target more than one antigen. Therefore, they have the widest applications in cancer immunotherapy and drug delivery currently.

Figure 4. Types of bispecific antibodies (bottom row): trifunctional antibody, chemically linked Fab and bi-specific T-cell engager. Blue and yellow parts are from separate monoclonal antibodies



However, purification of these fragments presents some challenges. Protein A affinity chromatography resin is the gold standard for capturing full size monoclonal antibodies which contain the Fc region. This region has high affinity to protein A. But protein A resin fails to capture these dAbs, Fabs and scFv fragments because these fragments lack the Fc region of an antibody. Therefore, protein L resin is used to purify these fragments because protein L binds antibodies through light chain interactions where dAbs, Fabs and scFv are located. Since no part of the heavy chain, such as Fc, is involved in the binding interaction to protein L, this resin binds a wider range of antibody classes than Protein A or G. Protein L resin binds to representatives of all antibody classes, including IgG, IgM, IgA, IgE, and IgD, and binds to the variable region of the kappa light chain without interfering with the antigen binding site.

TOYOPEARL AF-rProtein L-650F resin combines the rigid TOYOPEARL polymer matrix with an engineered recombinant Protein L ligand. The primary characteristics of this resin are shown in Table 6.

Table 6. Characteristics of TOYOPEARL AF-rProtein L-650F resin

Pore size (mean):	100 nm
Particle size (mean):	45 µm (F-grade)
Pressure rating:	0.3 MPa
Shipping buffer:	20% ethanol
pH stability:	2-13

TOYOPEARL AF-rProtein L-650F resin overcomes the deficiencies in other protein L resins that are currently available, namely alkaline instability of the ligand and low binding capacity. Resin costs represent a considerable part of the overall production costs. The high binding capacity, excellent recovery 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-r Protein L-650F resin offers:

- **High alkaline stability** – stable in a high concentration (0.1 mol/L) of NaOH
- **High dynamic binding capacity** – up to 50 g/L of Fab
- **Broad affinity to a wide range of mAbs** – binds all Kappa light chain antibody fragments (Fabs, scFvs, dAbs) including IgM and IgA
- **Excellent pressure-flow stability** – low pressure drop allows high flexibility of operational parameters
- **High sample purity** – estimated yield of scFv and Fab is >98%

High alkaline stability

The multipoint attachment of the protein L ligand of TOYOPEARL AF-rProtein L-650F resin, as shown in the structure below (Figure 5), results in a high chemical stability. Figure 6 proves the robustness of the TOYOPEARL AF-rProtein L-650F resin towards a moderate alkaline solution (0.1 mol/L NaOH) in comparison to a competitive protein L resin.

Figure 5. Structure of TOYOPEARL AF-rProtein L-650F resin

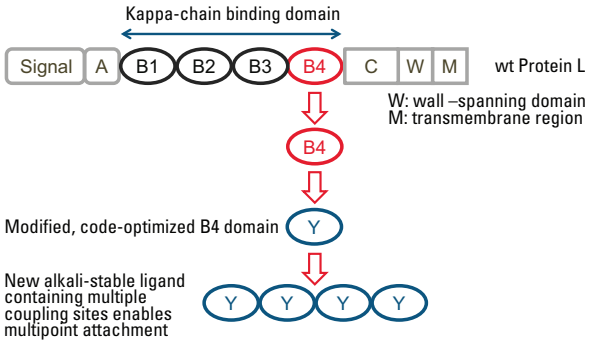
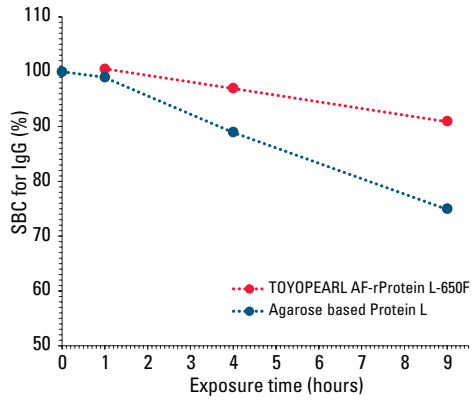


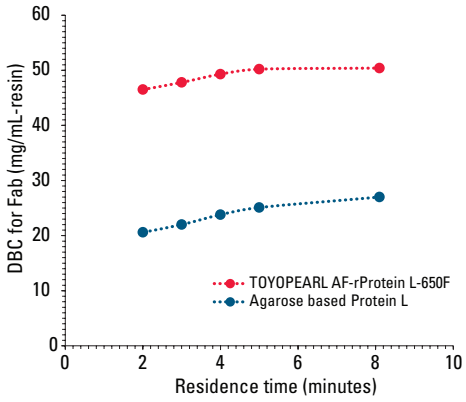
Figure 6. Stability of TOYOPEARL AF-rProtein L-650F resin



High dynamic 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 7 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 of >130mg/L for a ~150 kDa IgG when considering molar binding capacities.

Figure 7. Dynamic binding capacity for Fab



Broad affinity to a wide range of mAbs

The selected recombinant protein L ligand also has an affinity for a broad range of antibody subclasses as demonstrated in Table 7.

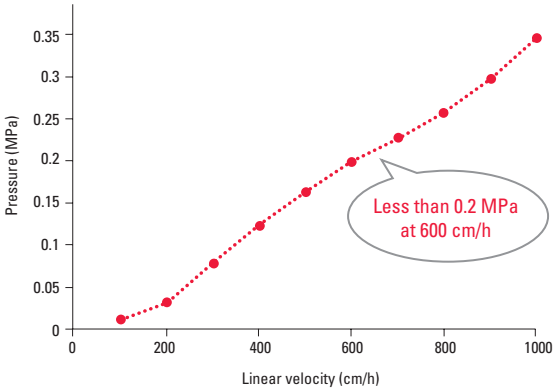
Table 7. TOYOPEARL AF-rProtein L-650F ligand with a broad affinity range for mAb subclasses

Species	Class	TOYOPEARL AF-rProtein L-650F Affinity
General	Kappa light chain	++
	Fab	++
	ScFv	++
	Dab	++
Human	$IgG_{(1-4)}$	+
	IgA	+
	IgD	+
	IgE	+
	IgM	+
Mouse	IgG_1	+
	IgG_2a	+
	IgG_2b	+
	IgA	+
	IgM	+
Rat	IgG	+
	$IgG_{2a,b,c}$	+
	IgA	+
Hen	IgM	+
	IgY	+

Excellent pressure-flow stability

TOYOPEARL AF-rProtein L-650F is based on the well proven polymethacrylate matrix used for all TOYOPEARL resins. Figure 8 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.

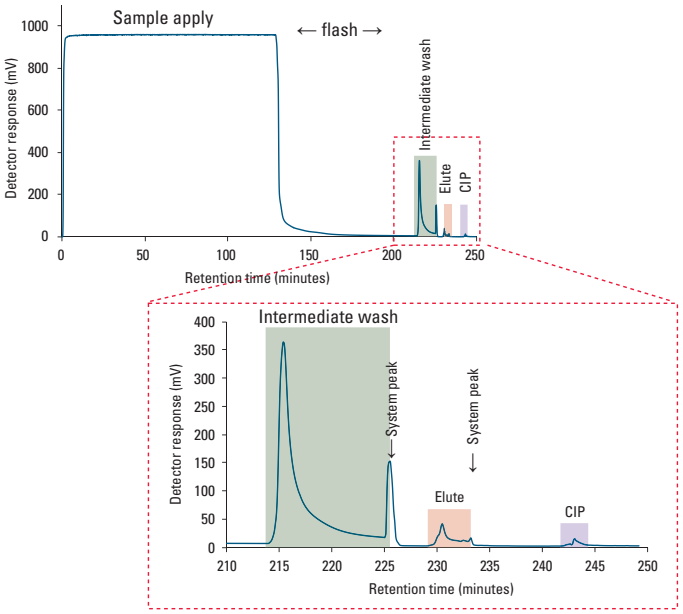
Figure 8. Pressure-flow of TOYOPEARL AF-rProtein L-650F resin



High sample purity

scFv fragments were expressed in a mammalian cell line. After harvesting, the sample was spun and filtered. The sample was then loaded onto a TOYOPEARL AF-rProtein L-650F column. 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 9.

Figure 9. Capture of scFv fragments using TOYOPEARL AF-rProtein L-650F column

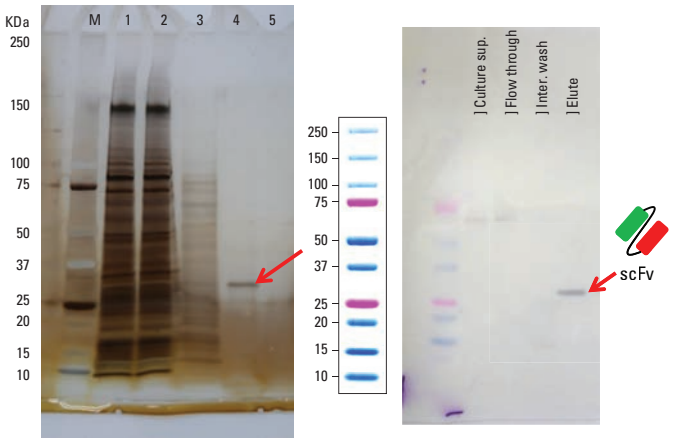


Resin:	TOYOPEARL AF-rProtein L-650F
Column size:	5 mm ID × 2.5 cm (0.5 mL)
Equib. & flash wash:	0.1 mol/L Na-citrate, pH 6.5
Intermediate wash:	0.1 mol/L Na-citrate, pH 6.5 + 1 mol/L Arg-HCl, 10% iPrOH
Elute:	0.1 mol/L Citrate, pH 2.3
CIP:	0.05 mol/L NaOH
Flow rate:	0.35 mL/min (residence time = 1.4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

The collected fractions were loaded onto a 4-15% TGXgel under a reduced condition with DTT. The gel was stained with silver stain plus kit. Data from the gel shows that there is only a single band from the eluted peak (Figure 10, panel A, 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 10, panel B, shows the 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-kappa-HRP interacts with this single 26 kDa band (see Figure 10, panel B). 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 estimated >98%.

Figure 10. : Identification of molecular weight and purity of scFv fragments using TOYOPEARL AF-rProtein L-650F resin



M prestained size marker (Bio-Rad 1610374)
1 Applied sample (hu_scFv_1)
2 Flowthrough
3 Intermediate wash (Arg+iPrOH)
4 Elute (pH 2.3) 1114series
5 CIP (50 mmol/L NaO_4H)

Ordering Information

Part#	Description
23486	TOYOPEARL AF-rProtein L-650F, 10 mL, 45 µm
23487	TOYOPEARL AF-rProtein L-650F, 25 mL, 45 µm
23488	TOYOPEARL AF-rProtein L-650F, 100 mL, 45 µm
23489	TOYOPEARL AF-rProtein L-650F, 1 L, 45 µm
23490	TOYOPEARL AF-rProtein L-650F, 5 L, 45 µm
45200	SkillPak TOYOPEARL AF-rProtein L-650F 1 mL column
45221	SkillPak TOYOPEARL AF-rProtein L-650F 1 mL columns (qty. 5)
45257	SkillPak TOYOPEARL AF-rProtein L-650F 5 mL column
OC41MDAFPL-650F	Protein L Plate 20 µL 96-well Kit
45065	ToyoScreen RoboColumn AF-rProtein L-650F, 8 × 200 µL
45066	ToyoScreen RoboColumn AF-rProtein L-650F, 8 × 600 µL

Interested in trying more than one of our best-in-class resins?
See the last page of this brochure for ordering information for SkillPak Column Libraries.

TOYOPEARL Sulfate-650F

The Best-In-Class Resin: a highly effective cation exchange resin for the capture and removal of mAb aggregates

Ion exchange chromatography is often used as an intermediate purification step in monoclonal antibody purification for the removal of protein aggregates, host cell proteins (HCP) and leached protein A ligand. Industry trends are focusing on the development of continuous downstream processing. Typically scientists in biopharmaceutical settings use cation exchange (CEX) and anion exchange (AEX) chromatography steps in series to further polish a purified mAb after the protein A purification step.

TOYOPEARL Sulfate-650F resin is a novel strong cation exchange resin with the following benefits: strong capture of mAb aggregates, high salt-tolerance, wide working pH range, and high dynamic binding capacity. The 100 nm pore size of this resin, along with proprietary bonding technology, makes TOYOPEARL Sulfate-650F ideal for applications performed in physiological conditions or for post-protein A removal of aggregates in a single step process. Its particle characteristics are shown in Table 8.

Table 8. Characteristics of TOYOPEARL Sulfate-650F resin

Pore size (mean):	100 nm
Particle size (mean):	45 µm (F-grade)
Pressure rating:	0.3 MPa
Shipping buffer:	20% ethanol
pH stability:	2-13

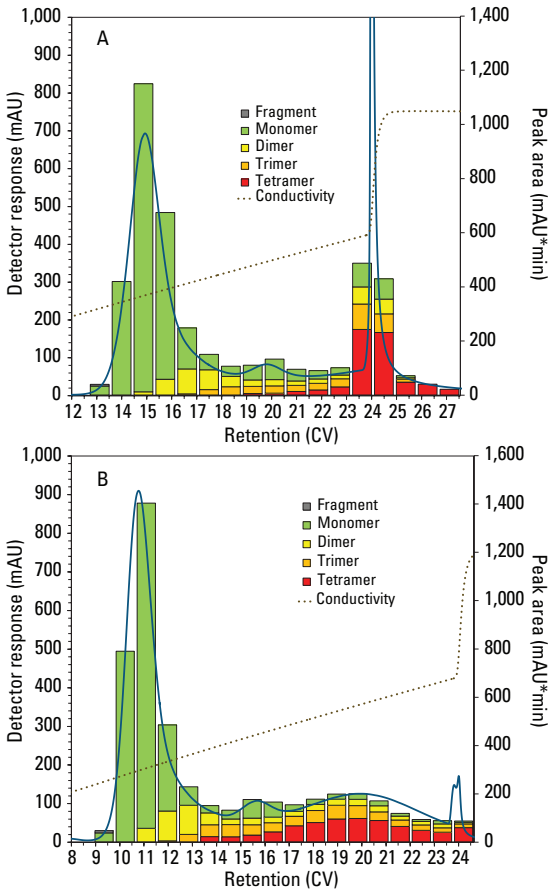
TOYOPEARL Sulfate-650F resin offers:

- **Effective removal of aggregates from IgG** - ≥10 fold can be achieved
- **Wide pH working range** – works well with pH 5.0 to 6.0 without losing its binding capacity for IgG
- **High dynamic binding capacity** – >120 g/L of IgG
- **High salt concentration tolerance** – Samples containing ≥150 mmol/L can be loaded on the resin
- **Excellent pressure-flow stability** – >600 cm/hr at 0.2 MPa
- **Durability at high pH** – no sign of losing DBC when resin exposed to 0.5 mol/L NaOH >60 days

Effective removal of aggregates from IgG

TOYOPEARL Sulfate-650F resin is effective at removing aggregates from IgG, as demonstrated in Figure 11. A protein A-purified IgG sample was loaded onto a TOYOPEARL Sulfate-650F column, fractions were collected using an ÄKTA® and further analyzed using a TSKgel G3000SWXL HPLC column. The comparison between TOYOPEARL Sulfate-650F resin and a competitor SO_3^- resin shows that TOYOPEARL Sulfate-650F resin provides stronger binding of mAb aggregates, resulting in the high resolution separation of monomer and aggregates.

Figure 11. High resolution separation of IgG monomer and aggregates using TOYOPEARL Sulfate-650F resin



Resins:	**A: TOYOPEARL Sulfate-650F** **B: Competitor SO_3^-**
Column size:	6.6 mm × 20 cm (6.8 mL)
Mobile phase:	A: 0.1 mol/L sodium acetate, pH 5.0 B: mobile phase A + 1 mol/L NaCl
Gradient:	0 – 50% B, 20 CV 100% B, 5 CV
Flow rate:	300 cm/hr (4 min residence time)
Detection:	UV @ 280 nm (mAU), conductivity (mS/cm)
Temperature:	ambient
Injection vol.:	6.8 mL (10 mg/mL-resin load ratio)
Sample:	IgG, protein A-purified, aggregated, 10 g/L
Instrument	ÄKTA avant 25

The monomer peak was fractioned and analyzed using SEC analysis of the eluate pool at 260 mmol/L NaCl, 9 column volumes. The peaks from the SEC column were analyzed for the total amount of high molecular weight, HCP and protein A ligand content. Table 9 shows that after passing through the TOYOPEARL Sulfate-650F resin, the collected IgG peak has significantly reduced amounts of HMW, HCP and protein A ligand. This suggests that TOYOPEARL Sulfate-650F resin can effectively remove and reduce the impurities of IgG.

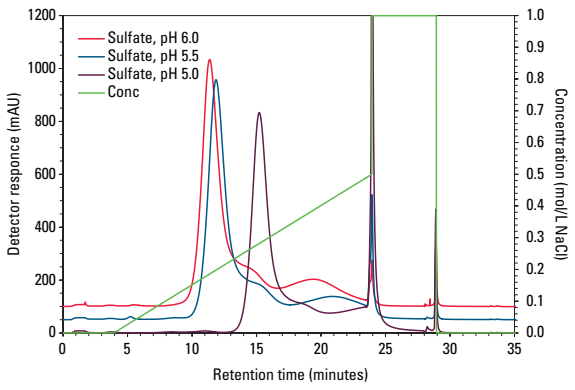
Table 9. The reduction of impurities from IgG sample, post-protein A, after passing through TOYOPEARL Sulfate-650F resin

Impurity	ProA eluate	Sulfate eluate
Dimer (%)	3.9	2.4
HMW (%)	0.54	0.07
HCP (ppm)	1260	134
ProA (ppm)	1.2	0.040

Wide pH working range

The strong cation characteristics of the sulfate group and the proprietary bonding technology of TOYOPEARL Sulfate-650F allows this resin to have a wide working pH range while still maintaining its elution profiles for IgG, as shown in Figure 12. The retention time is shifted but the selectivity remains unchanged. This benefit allows users the flexibility to select a pH that is more suitable to their sample.

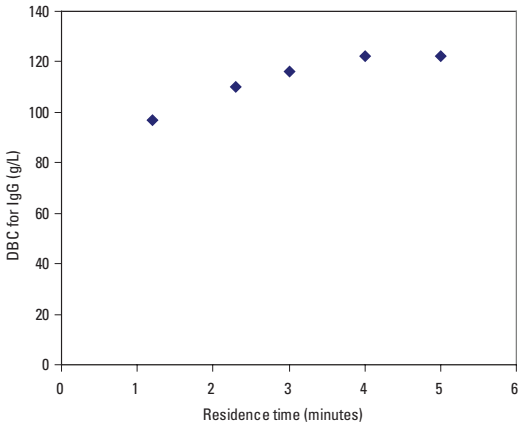
Figure 12. Wide working range of pH



High dynamic binding capacity

TOYOPEARL Sulfate-650F offers high dynamic binding capacities for IgG. These capacities can be obtainable even at higher flow rates, as shown in Figure 13.

Figure 13. Dynamic binding capacity of TOYOPEARL Sulfate-650F resin at various flow rates

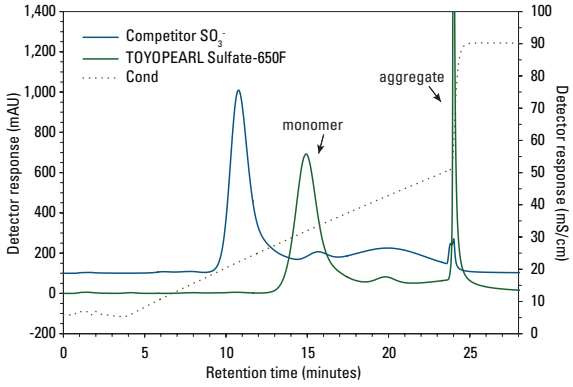


Resin:	TOYOPEARL Sulfate-650F
Column size:	6.0 mm ID × 4 cm
Mobile phase:	0.15 mol/L NaCl + 0.054 mol/L acetate buffer, pH 4.7
Residence time:	1.2, 2.3, 3.0, 4.0 or 5.0 min
Sample:	polyclonal human IgG, 1.0 g/L

High salt concentration tolerance

The increased salt tolerance of the TOYOPEARL Sulfate-650F resin as compared to another cation exchange resin can be seen in Figure 14. The mAb peak begins to elute from the TOYOPEARL Sulfate-650F column at a concentration of approximately 0.3 mol/L NaCl compared to 0.15 mol/L for the other anion exchange resin.

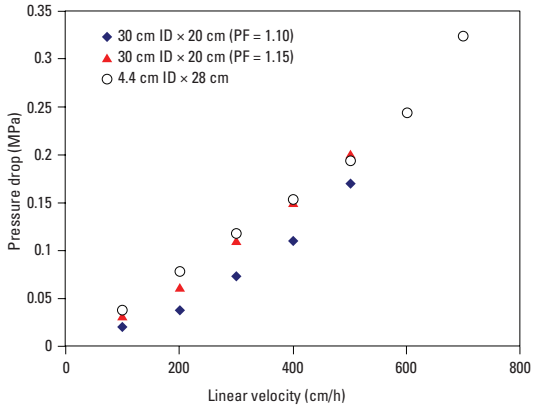
Figure 14. Salt tolerance comparison



Excellent pressure-flow stability

Figure 15 demonstrates the excellent pressure-flow rate properties of the TOYOPEARL Sulfate-650F resin. A flow rate of >600 cm/hr on a large process column is easily achieved at a pressure drop of only 0.2 MPa.

Figure 15. Pressure-flow rate curve on large process column (30 cm ID × 20 cm bed height)

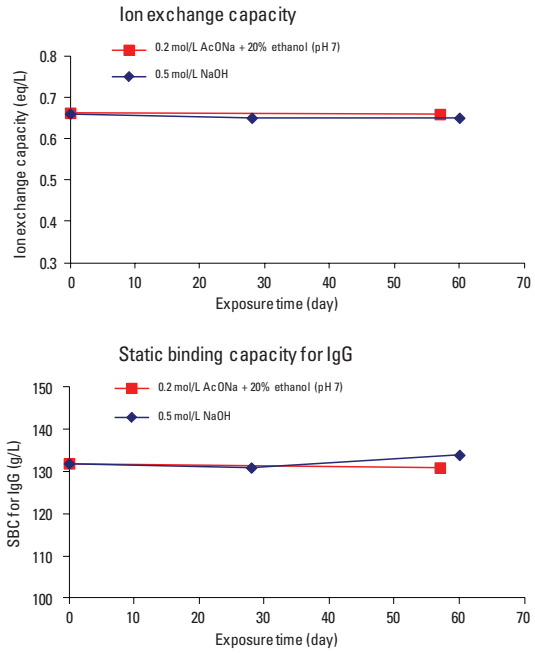


Column system: AxiChrom® 30 cm ID × 20 cm, QuickScale 4.4 cm ID × 28 cm
Packing Factor (PF): 1.10 or 1.15
Packing: Slurry 50% in 0.2 mol/L NaCl
Eluent: water

Durability at high pH

TOYOPEARL Sulfate-650F resin is stable in 0.5 mol/L NaOH (Figure 16). It can be stored in this solution for up to 8 weeks without loss in its binding capacity.

Figure 16. Alkaline stability of TOYOPEARL Sulfate-650F



Ordering Information

Part#	Description
23467	TOYOPEARL Sulfate-650F, 100 mL, 45 µm
23468	TOYOPEARL Sulfate-650F, 250 mL, 45 µm
23469	TOYOPEARL Sulfate-650F, 1 L, 45 µm
23470	TOYOPEARL Sulfate-650F, 5 L, 45 µm
23471	TOYOPEARL Sulfate-650F, 50L, 45 µm
45205	SkillPak TOYOPEARL Sulfate-650F 1 mL columns (qty. 5)
45241	SkillPak TOYOPEARL Sulfate-650F 5 mL column
OC41MDSLFT-650F	Sulfate-650F 20 µL 96-well Plate Kit
45027	ToyoScreen RoboColumn Sulfate-650F, 8 × 200 µL
45028	ToyoScreen RoboColumn Sulfate-650F, 8 × 600 µL

Interested in trying more than one of our best-in-class resins?
See the last page of this brochure for ordering information for SkillPak Column Libraries.

TOYOPEARL NH_2-750F

The Best-In-Class Resin: a salt tolerant anion exchange resin for efficient endotoxin and viral removal

Typically anion exchange resins with quaternary amine or DEAE ligands are used for the removal of impurities from mAbs. However, these conventional resins have the disadvantage of reduced capacity for samples in relatively high salt concentrations, such as collected fractions from post-protein A purification of monoclonal antibodies (mAbs) or undiluted biological feedstock. In order to use a DEAE or quaternary amine resin at these process stages, the column load material must be diluted to adjust its conductivity to approximately >5 mS/cm.

TOYOPEARL NH_2-750F resin is a salt tolerant anion exchange resin for process scale applications. This resin is ideal for the intermediate purification of mAbs and other proteins where aggregates and other negatively charged impurities, such as DNA, endotoxins and viruses, are removed from the target of interest within a *single* step without having to dilute or buffer exchange the product prior to loading. This resin is based on the TOYOPEARL HW-75F size exclusion resin functionalized with primary amine groups. This allows the TOYOPEARL NH_2-750F resin to maintain its capacity at conductivities up to 15 mS/cm. Its particle characteristics are shown in Table 10.

Table 10. Properties of TOYOPEARL NH_2-750F

Particle size	30-60 µm
Pore diameter	>100
Ion exchange capacity (eq/L resin)	0.07- 0.13
SBC (g/L resin)	≥ 70

TOYOPEARL NH_2-750F resin offers:

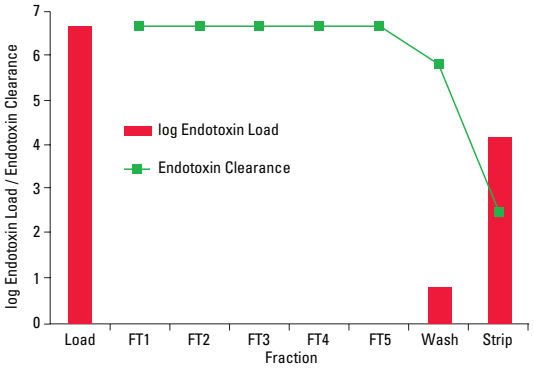
- **Effective endotoxin and viral removal** – in flowthrough chromatography mode; a clearance of >4 logs can be achieved
- **Removal of mAb aggregates** – in bind-and-elute and flowthrough chromatography mode
- **High salt tolerance** – samples containing ≥150 mmol/L NaCl can be loaded on the resin
- **Excellent pressure-flow characteristics** – resin can tolerate >600 cm/hr
- **Alkaline stability** – resin can be exposed to 0.5-1.0 mol/L NaOH

Effective endotoxin removal

TOYOPEARL NH_2-750F is a very effective anion exchange resin for the removal of endotoxin in a flowthrough chromatography mode. To demonstrate this, a solution of *E. coli* lipopolysaccharide was prepared in water, giving a starting endotoxin concentration of 89,000 EU/mL with a total load of 4,450,000 EU (89,000 EU/ mL × 50 mL). The column was then loaded with spiked equilibration buffer and 2 CV (10 mL) flowthrough fractions were collected. Fractions were also collected for both wash and strip steps.

As can be seen in Figure 17, a graphical representation of the log endotoxin clearance for each step in the process, the endotoxin concentration of the flowthrough fractions was less than the limit of detection for an LAL assay (0.1 EU/mL); therefore, the minimum log reduction value for each flowthrough fraction was 6.7. Although there was some minor breakthrough of endotoxin during the wash phase (the log reduction value for this fraction was 5.82), this represents a breakthrough of less than 0.0002% of endotoxin from the original load material.

Figure 17. Endotoxin clearance using TOYOPEARL NH_2-750F



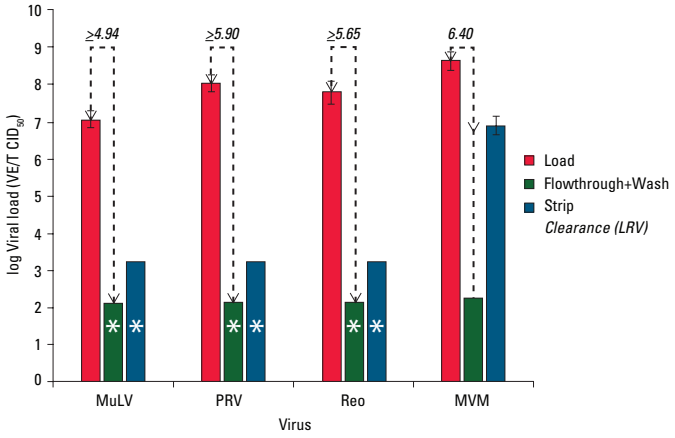
Resin:	TOYOPEARL NH_2-750F
Column:	MiniChrom, 8.0 mm ID × 10.0 cm (5.0 mL)
Mobile phase:	A: 20 mmol/L Tris base, pH 7.4
	B: 20 mmol/L Tris base, 1 mol/L sodium chloride, pH 7.4
Flow rate:	150 cm/hr (1.25 mL/min, 4 min residence time)
Detection:	LAL endotoxin assay
Temperature:	ambient
Injection vol.:	5 × 2 CV (10 mL) injections
	10 CV (50 mL) total
Sample:	89,000 EU/mL LPS in equilibration buffer
Instrument:	ÄKTA Explorer 100

Effective viral removal

Two chromatography steps in the purification of a monoclonal antibody for viral clearance were evaluated using four model viruses. Studies were performed as spike/chase experiments, where a known quantity of virus is added to unprocessed material and remaining virus is quantitated following processing.

Protein A-purified mAb was spiked with 1% (Reo, MVM) or 5% (MuLV, PRV) (v/v) and was then passed through TOYOPEARL NH_2-750F resin. As shown in Figure 18, TOYOPEARL NH_2-750F resin effectively removed all viruses with a clearance of >4 logs.

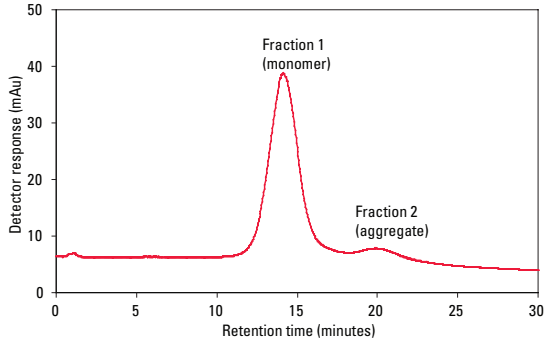
Figure 18. Viral clearance results from flowthrough mode using TOYOPEARL NH_2-750F resin



Removal of mAb aggregates

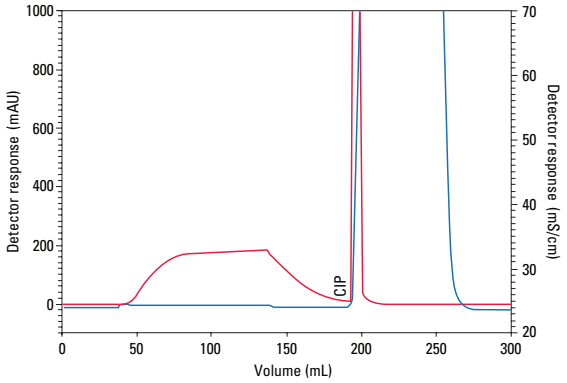
TOYOPEARL NH_2-750F is effective at removing aggregates from mAbs, in both bind-and-elute mode as well as in flowthrough, as demonstrated in Figures 19a and 19b. SEC analysis of the peaks (data not shown) shows that high molecular weight aggregates are completely removed from the main mAb peak.

Figure 19a. Removal of aggregates from IgG_1 monomer on TOYOPEARL NH_2-750F resin



Resin:	TOYOPEARL NH_2-750F
Column size:	5 mm ID × 5 cm
Mobile phase:	A: 20 mmol/L Tris-HCl, pH 8.0; B: mobile phase A + 1.0 mol/L NaCl
Gradient:	0 -100% B (60 min)
Flow rate:	300 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	mAb (IgG_1), 0.5 g/L

Figure 19b. Flowthrough removal of aggregates from mAb monomer on TOYOPEARL NH_2-750F resin

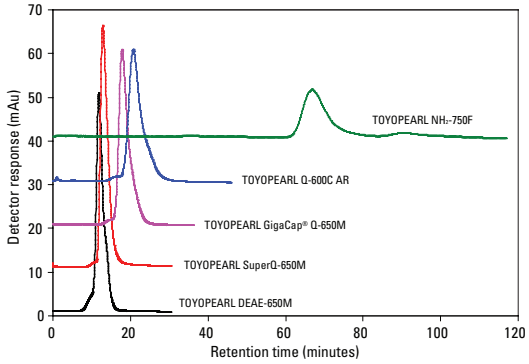


Resin:	TOYOPEARL NH_2-750F
Column size:	6.6 mm ID × 6 cm
Mobile phase:	A: 10 mmol/L Tris-HCl, 250 mmol/L NaCl, pH 7.0; B: 1.0 mol/L NaOH (CIP)
Gradient:	isocratic
Flow rate:	300 cm/hr (1.71 mL/min)
Detection:	UV @ 280 nm, conductivity
Temperature:	ambient
Sample:	mAb (IgG1), 1.0 g/L

High salt tolerance

Increased salt tolerance of TOYOPEARL NH_2-750F as compared to other TOYOPEARL anion exchange resins can be seen in Figure 20. The BSA peak begins to elute from the TOYOPEARL NH_2-750F column at a concentration of approximately 1.0 mol/L NaCl compared to 0.14 – 0.40 mol/L for the other anion exchange resins.

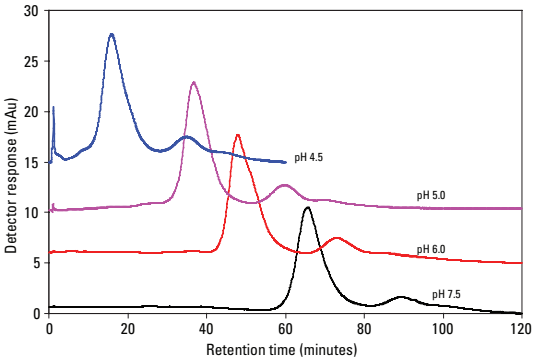
Figure 20. Comparison of anion exchange resins for salt tolerance



Resins:	various
Column size:	5 mm ID × 5 cm
Mobile phase:	A: 20 mmol/L Tris-HCl, pH 8.0 B: mobile phase A + 2.0 mol/L NaCl
Gradient:	0 -100% B (120 min)
Flow rate:	300 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	BSA (pI 4.7 – 4.9), 1.0 g/L

TOYOPEARL NH_2-750F resin also shows that it can withstand pH changes without greatly modifying its selectivity, as demonstrated in Figure 21. This allows for a large design space in which to develop a separation protocol.

Figure 21. Selectivity of TOYOPEARL NH_2-750F resin when pH buffer is changed

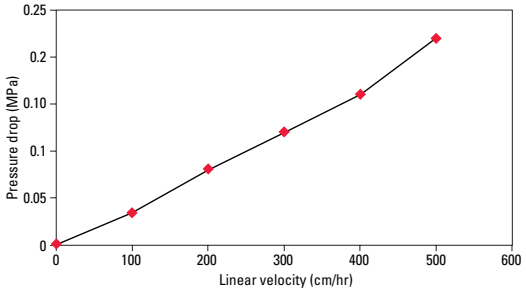


Resins:	TOYOPEARL NH_2-750F
Column size:	5 mm ID × 5 cm
Mobile phase:	A: 20 mmol/L N-methyl piperazine, pH 4.5 and 5.0 20 mmol/L Bis-Tris, pH 6.0 20 mmol/L Tris-HCl, pH 7.5 B: mobile phase A + 2.0 mol/L NaCl
Gradient:	0 -100% B (120 min)
Flow rate:	300 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	BSA (pI 4.7 – 4.9), 1.0 g/L

Excellent pressure-flow characteristics

TOYOPEARL NH_2-750F resin is based on the well proven polymethacrylate matrix used for all TOYOPEARL resins. Figure 22 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 columns packed with TOYOPEARL NH_2-750F resin.

Figure 22. TOYOPEARL NH_2-750F resin; flow rate vs. pressure

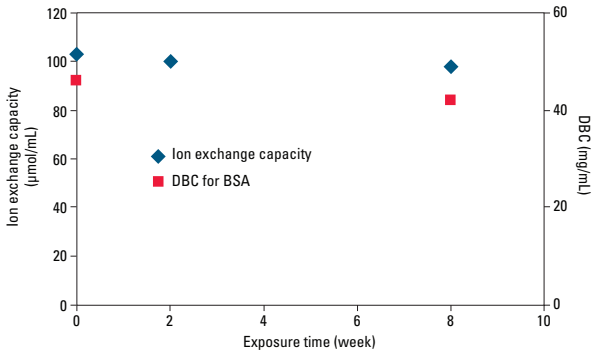


Resin:	TOYOPEARL NH_2-750F
Column size:	4.4 cm ID × 29 cm
Mobile phase:	0.1 mol/L NaCl
Flow rate:	multiple

Alkaline stability

TOYOPEARL NH_2-750F is alkaline stable in 0.5 mol/L NaOH and can be stored in this solution for up to 8 weeks with little appreciable loss of capacity (Figure 23).

Figure 23. Alkaline stability of TOYOPEARL NH_2-750F resin



Ordering Information

Part#	Description
23438	TOYOPEARL NH_2-750F, 100 mL, 45 µm
23439	TOYOPEARL NH_2-750F, 250 mL, 45 µm
23440	TOYOPEARL NH_2-750F, 1 L, 45 µm
23441	TOYOPEARL NH_2-750F, 5 L, 45 µm
23442	TOYOPEARL NH_2-750F, 50 L, 45 µm
45209	SkillPak TOYOPEARL NH_2-750F 1 mL columns (qty. 5)
45245	SkillPak TOYOPEARL NH_2-750F 5 mL column
OC41MDNH2-750F	NH_2-750F 20 µL 96-well Plate Kit
45021	ToyoScreen RoboColumn NH_2-750F, 8 × 200 µL
45022	ToyoScreen RoboColumn NH_2-750F, 8 × 600 µL

Interested in trying more than one of our best-in-class resins?
See the last page of this brochure for ordering information for SkillPak Column Libraries.

Ordering Information: SkillPak Column Libraries

Part#	Description
1 mL Columns	
45229	SkillPak mAb Platform 1 mL column library, 1 mL × 2 ea (TOYOPEARL AF-rProtein A HC-650F, Sulfate-650F, NH_2-750F)
45230	SkillPak Salt Tolerant 1 mL column library, 1 mL × 3 ea (TOYOPEARL Sulfate-650F, NH_2-750F)
45232	SkillPak Best-in-Class 1 mL column library, 1 mL × 1 ea (Ca^{++}Pure-HA, TOYOPEARL AF-rProtein A HC-650F, AF-rProtein L-650F, NH_2-750F, Sulfate-650F)
5 mL Columns	
45245	SkillPak mAb Platform 5 mL column library, 5 mL × 1 ea (TOYOPEARL AF-rProtein A HC-650F, Sulfate-650F, NH_2-750F)
45264	SkillPak Salt Tolerant 5 mL column library, 5 mL × 1 ea (TOYOPEARL Sulfate-650F, NH_2-750F)
45266	SkillPak Best-in-Class 5 mL column library, 5 mL × 1 ea (Ca^{++}Pure-HA, TOYOPEARL AF-rProtein A HC-650F, AF-rProtein L-650F, NH_2-750F, Sulfate-650F)

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