Anion Exchange Resins	Cation Exchange Resins
TOYOPEARL DEAE-650C	TOYOPEARL MegaCap II SP-550EC
TOYOPEARL Q-600C AR	TOYOPEARL CM-650C
TOYOPEARL QAE-550C	TOYOPEARL SP-550C
TOYOPEARL SuperQ-650C	TOYOPEARL SP-650C
TOYOPEARL GigaCap DEAE-650M	TOYOPEARL GigaCap CM-650M
TOYOPEARL GigaCap Q-650M	TOYOPEARL GigaCap S-650M
TOYOPEARL DEAE-650M	TOYOPEARL CM-650M
TOYOPEARL SuperQ-650M	TOYOPEARL SP-650M
TOYOPEARL DEAE-650S	TOYOPEARL CM-650S
TOYOPEARL GigaCap Q-650S	TOYOPEARL GigaCap S-650S
TOYOPEARL SuperQ-650S	TOYOPEARL SP-650S
TSKgel DEAE-5PW (30)	TSKgel SP-3PW (30)
TSKgel SuperQ-5PW (30)	TSKgel SP-5PW (30)
TSKgel DEAE-5PW (20)	TSKgel SP-5PW (20)
TSKgel SuperQ-5PW (20)	

The role of Ion Exchange Chromatography in Process Purification

Ion Exchange Chromatography (IEX) plays a major role in the large scale purification of biomolecules. Today, IEX is one of the most commonly used techniques for the purification of proteins, nucleic acids, peptides, and other biomolecules. IEX can be further separated into anion (AEX) and cation (CEX) exchange techniques, both offering high resolution separations with high loading capacities. Ion exchange chromatography is capable of separating species that have minor differences in charges, for example two proteins differing by a single charged amino acid. These attributes make IEX ideally suited to be used at any point in the purification process including capture, intermediate purification, and polishing steps. The scalability of this technique allows it to be used from discovery and analysis through to commercial manufacturing operations.

Ion exchange chromatography functions by separating molecules on the basis of charge differences. Molecules are diverse in their charge properties and interact with charged chromatography media based on differences in their charge density, net charge, and distribution of that charge across the surface of the molecule. Since all molecules with charged groups can be titrated, their net surface charge is largely pH dependent. The net surface charge of proteins, which contain many different amino acids of weakly acidic and basic groups, will change as the environmental pH of the proteins change. IEX chromatography takes advantage of the relationship between net surface charge and pH for each specific protein. In ion exchange chromatography, a reversible interaction between a charged molecule and an oppositely charged ligand are controlled to favor the binding or elution of specific molecules to achieve separation. A protein at a pH above its isoelectric point will bind to a positively charged medium (anion exchanger) and at a pH below its pI, a protein will bind to a negatively charged medium (cation exchanger). The ligand attached to a chromatographic resin determines the charge of an IEX medium, a positively-charged anion or a negatively-charged cation exchanger.

TOYOPEARL Ion Exchange Chromatography Resins

TOYOPEARL IEX resins are functionalized versions of the TOYOPEARL HW size exclusion resins and are therefore based on hydroxylated polymethacrylic polymer beads. Tosoh Bioscience offers four ligands for anion exchange (Q, SuperQ, QAE, and DEAE) and three ligands for cation exchange chromatography (S, SP, and CM). Table 1 lists the properties of these TOYOPEARL IEX resins.

Table 1: Properties of TOYOPEARL ion exchange resins

TOYOPEARL resins	Anion/Cation exchange	Base bead	Pore size	Bead diameter	Ligand type	Ligand pKa	DBC (g/L)	Pressure rating
SuperQ-650C	Anion	HW-65	100 nm	100 µm	Strong Anion	12.2	105 - 155	0.3 MPa
DEAE-650C	Anion	HW-65	100 nm	100 µm	Weak Anion	11.5	25 - 35	0.3 MPa
QAE-550C	Anion	HW-55	50 nm	100 µm	Strong Anion	12.2	60 - 80	0.3 MPa
Q-600C AR	Anion	HW-60	75 nm	100 µm	Strong Anion	12.2	> 120	0.3 MPa
GigaCap Q-650M	Anion	HW-65	100 nm	75 µm	Strong Anion	12.2	105 - 155	0.3 MPa
GigaCap DEAE-650M	Anion	HW-65	100 nm	75 µm	Weak Anion	11.5	> 156	0.3 MPa
SuperQ-650M	Anion	HW-65	100 nm	65 µm	Strong Anion	12.2	105 - 155	0.3 MPa
DEAE-650M	Anion	HW-65	100 nm	65 µm	Weak Anion	11.5	25 - 35	0.3 MPa
SuperQ-650S	Anion	HW-65	100 nm	35 µm	Strong Anion	12.2	105 - 155	0.3 MPa
DEAE-650S	Anion	HW-65	100 nm	35 µm	Weak Anion	11.5	25 - 35	0.3 MPa
GigaCap Q-650S	Anion	HW-65	100 nm	35 µm	Strong Anion	12.2	> 170	0.3 MPa
MegaCap II SP-550EC	Cation	HW-55	50 nm	200 µm	Strong Cation	1.2	60 - 90	0.3 MPa
SP-650C	Cation	HW-65	100 nm	100 µm	Strong Cation	1.2	35 - 55	0.3 MPa
SP-550C	Cation	HW-55	50 nm	100 µm	Strong Cation	1.2	80 - 120	0.3 MPa
CM-650C	Cation	HW-65	100 nm	100 µm	Weak Cation	4.7	25 - 45	0.3 MPa
GigaCap S-650M	Cation	HW-65	100 nm	75 µm	Strong Cation	1.2	80 - 120	0.3 MPa
GigaCap CM-650M	Cation	HW-65	100 nm	75 µm	Weak Cation	3.6	> 110	0.3 MPa
SP-650M	Cation	HW-65	100 nm	65 µm	Strong Cation	1.2	40 - 60	0.3 MPa
CM-650M	Cation	HW-65	100 nm	65 µm	Weak Cation	4.7	30 - 50	0.3 MPa
SP-650S	Cation	HW-65	100 nm	35 µm	Strong Cation	1.2	40 - 60	0.3 MPa
CM-650S	Cation	HW-65	100 nm	35 µm	Weak Cation	3.6	30 - 50	0.3 MPa
GigaCap S-650S	Cation	HW-65	100 nm	35 µm	Strong Cation	1.2	> 150	0.3 MPa

TSKgel Ion Exchange Chromatography Resins

The same SuperQ, DEAE, and SP ligands that are used for the TOYOPEARL resins are also available within the TSKgel IEX resin product line. The TSKgel IEX resins use the same methacrylic polymer chemistry as the TOYOPEARL resins but have a higher degree of crosslinking, making for a more rigid bead. This is necessitated by the higher pressures generated when using smaller particles for chromatography. Greater crosslinking decreases the number of sites available for ligand attachment and thus a TSKgel resin will have a lower dynamic binding capacity than the corresponding TOYOPEARL resin. The polymeric structure of these products also makes them resistant to a wide range of pH conditions and mobile phase ionic strengths. In addition, the hydroxylated surface of the base bead reduces non-specific binding of proteins. Table 2 lists the properties of these TSKgel IEX resins.

The semi-rigid backbone of both TOYOPEARL and TSKgel IEX resins permits high flow rates for maximum throughput and productivity. While TOYOPEARL IEX resins may be operated at pressures up to 0.3 MPa, TSKgel -5PW and -3PW resins may be operated up to 2.0 MPa. Depending on their bead size and the buffer system used, linear velocities of greater than 1,000 cm/hr can be achieved.

Table 3 shows the ligands and particle sizes available for TOYOPEARL and TSKgel IEX resins and is arranged in increasing levels of resolution by bead size (i.e. low, medium, and high resolution). The availability of smaller bead sizes for greater resolution while maintaining the same selectivity is particularly useful in the areas of oligonucleotide and peptide purification.

Table 2: Properties of TSKgel ion exchange resins

TSKgel resins	Anion/Cation exchange	Base bead	Pore size	Bead diameter	Ligand type	Ligand pKa	DBC (g/L)	Pressure rating
DEAE-5PW (20)	Anion	G5000PW	100 nm	20 µm	Weak Anion	11.5	25 -45	2.0 MPa
DEAE-5PW (30)	Anion	G5000PW	100 nm	30 µm	Weak Anion	11.5	20 - 40	2.0 MPa
SuperQ-5PW (20)	Anion	G5000PW	100 nm	20 µm	Strong Anion	12.2	52 - 88	2.0 MPa
SuperQ-5PW (30)	Anion	G5000PW	100 nm	30 µm	Strong Anion	12.2	52 - 88	2.0 MPa
SP-3PW (30)	Cation	G3000PW	25 nm	30 µm	Strong Cation	1.2	> 65	2.0 MPa
SP-5PW (20)	Cation	G5000PW	100 nm	20 µm	Strong Cation	1.2	20 - 40	2.0 MPa
SP-5PW (30)	Cation	G5000PW	100 nm	30 µm	Strong Cation	1.2	20 - 40	2.0 MPa

Table 3: Resolution of TOYOPEARL and TSKgel ion exchange resins

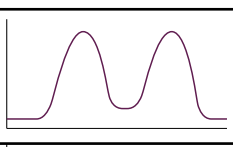
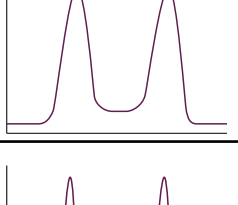
Resolution		Bead diameter (µm)	Pore size (nm)	Resins	
				Anion	Cation
Low		200	50		TOYOPEARL MegaCap II SP-550EC
		100	100 100 50	TOYOPEARL SuperQ-650C TOYOPEARL DEAE-650C TOYOPEARL QAE-550C	TOYOPEARL SP-650C TOYOPEARL CM-650C TOYOPEARL SP-550C
Medium		75	100 100	TOYOPEARL GigaCap Q-650M TOYOPEARL GigaCap DEAE-650M	TOYOPEARL GigaCap S-650M TOYOPEARL GigaCap CM-650M
		65	100 100 75	TOYOPEARL SuperQ-650M TOYOPEARL DEAE-650M TOYOPEARL Q-600C-AR	TOYOPEARL SP-650M TOYOPEARL CM-650M
High		35	100 100 100	TOYOPEARL SuperQ-650S TOYOPEARL DEAE-650S TOYOPEARL GigaCap Q-650S	TOYOPEARL SP-650S TOYOPEARL CM-650S TOYOPEARL GigaCap S-650S
		30	100 100 200	TSKgel SuperQ-5PW (30) TSKgel DEAE-5PW (30)	TSKgel SP-5PW (30) TSKgel SP-3PW (30)
		20	100 100	TSKgel SuperQ-5PW (20) TSKgel DEAE-5PW (20)	TSKgel SP-5PW (20)

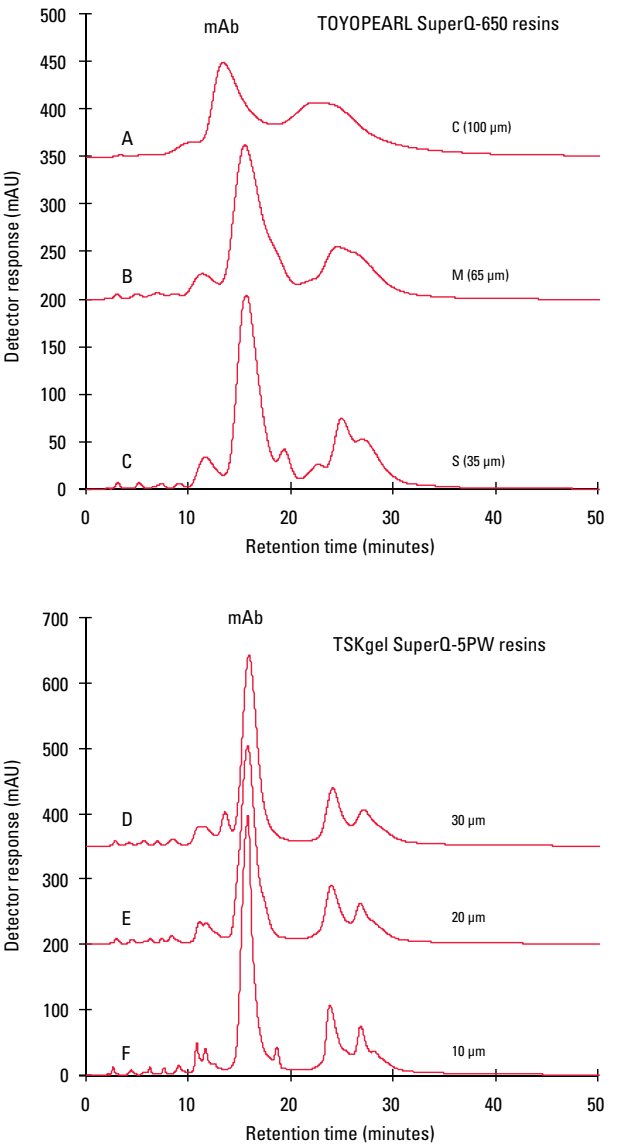
Table 4: DBCs of different chromatography modes

Separation mode	Binding capacity for standard proteins (g/L)	Binding capacity in production processes (g/L)
Ion Exchange	**100 - 200**	**50 - 100**
Hydrophobic Interaction	40 - 60	10 - 30
Affinity (group specific ligands)	40 - 100	20 - 60
Reversed Phase (polymeric media)	60 - 100	30 - 50

Due to the high dynamic binding capacities of ion exchange resins relative to those of the other chromatographic modes (Table 4), IEX is the chromatographic technique selected by many developers for the capture or concentration step.

Because TOYOPEARL and TSKgel IEX resins have the same backbone polymer chemistry, the selectivity for proteins and impurities will be unchanged. Due to this continuity between the TOYOPEARL and TSKgel resins, the chromatographic conditions that work for one particle size will work for all particle sizes with a given ligand functionality. The elution order of the feedstock components will remain the same with increasing resolution as the particle size gets smaller (Figure 1).

Figure 1: Scale up or down using the same ligand

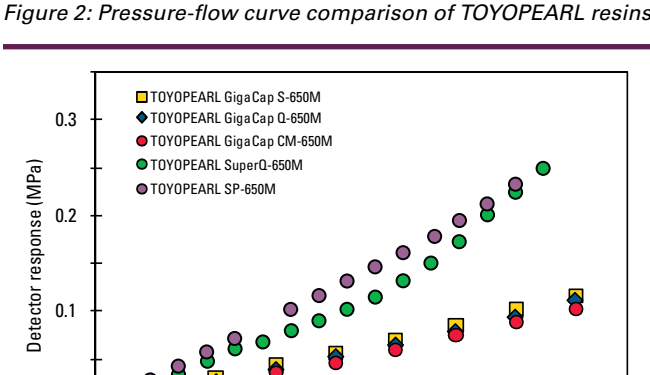


Resins:
A. TOYOPEARL SuperQ-650C, 100 µm
B. TOYOPEARL SuperQ-650M, 65 µm
C. TOYOPEARL SuperQ-650S, 35 µm
D. TSKgel SuperQ-5PW(30), 30 µm
E. TSKgel SuperQ-5PW(20), 20 µm
F. TSKgel SuperQ-5PW, 10 µm

Column size: 7.5 mm ID × 7.5 cm
Mobile phase: Buffer A: 0.02 mol/L Tris-HCl, pH 8.5
Buffer B: 0.5 mol/L NaCl in buffer A
Gradient: 60 min linear gradient from buffer A to buffer B
Flow rate: 136 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Sample: mAb in mouse ascites (dilution, x 5)
Load volume: 100 µL

The TOYOPEARL GigaCap M-grade resins have a particle size of 50-100 µm, which is slightly larger than the normal TOYOPEARL M-grade, 40-90 µm beads. This particle size difference generates a lower back pressure (Figure 2) than the more traditional TOYOPEARL M-grade ion exchange products. The TOYOPEARL GigaCap M-grade resins are high throughput resins that can be used for capture, intermediate, and polishing chromatographic steps.

Figure 2: Pressure-flow curve comparison of TOYOPEARL resins



Resins: **TOYOPEARL GigaCap S-650M**
TOYOPEARL GigaCap Q-650M
TOYOPEARL GigaCap CM-650M
TOYOPEARL SuperQ-650M
TOYOPEARL SP-650M
Column size: 22 mm ID × 20 cm
Mobile phase: distilled H_2O
Detection: pressure (MPa)
Temperature: 25 °C

TOYOPEARL and TSKgel IEX resins are chemically stable from pH 3-13. This allows a constant packing volume over a wide range of salt concentrations and cleaning in place (CIP) with acid or base. Also, these resins can be run at elevated temperatures (4-60 °C) and are autoclavable at 121 °C. Tosoh has focused on improving the alkaline stability of its newer ion exchange resins. Higher capacity resins can bind not only more of the target molecule, but the impurities and isoforms as well. In some cases more rigorous cleaning agents like 0.5 mol/L NaOH and even 1.0 mol/L NaOH are needed to ensure proper resin regeneration. Naturally, the resins need to tolerate these more stringent conditions.

TOYOPEARL IEX resins are available in a broad range of base bead pore sizes (Table 5). Of these, four different mean pore diameters are used: 100 nm, 75 nm, 50 nm, and 20 nm (Table 6). The TSKgel IEX resins have a base bead pore size of 100 nm with the exception of TSKgel SP-3PW, which has a pore size of 25 nm. A bead with a small pore size has theoretically more surface area than the same size bead with a larger pore. Please refer to Table 2 in the SEC section of this catalog (page 5) for the molar mass range of biomolecules covered by each pore size. Figure 3 shows insulin binding capacity on six different pore size beads. As the pore size increases to the point where the insulin has greatest access to the internal surface area, the insulin capacity increases. However, there is a point of diminishing return. Because the absolute surface area decreases as the pores become larger, the insulin capacity decreases accordingly.

Table 5: Methacrylic base beads available for IEC

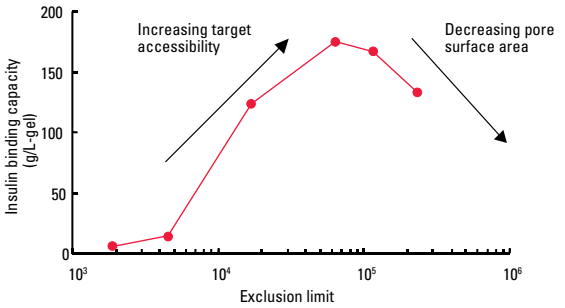
Pore size (nm)	5	12.5	40-50	75	100	>100	>170
Resin							
TOYOPEARL HW-type:	40	50	55	60	65	75	80
TSKgel PW-type:	G1000	G2000	G4000		G5000	G6000	

Increasing pore surface area

Table 6: Mean pore diameters used in TOYOPEARL and TSKgel IEX resins

Base bead	TOYOPEARL HW-65 or TSKgel G5000PW	TOYOPEARL HW-60	TOYOPEARL HW-55	TSKgelG3000PW
Pore diameter	**100 nm**	**75 nm**	**50 nm**	**20 nm**
Resin	TOYOPEARL GigaCap S-650 TOYOPEARL GigaCap CM-650 TOYOPEARL GigaCap Q-650 TOYOPEARL SuperQ-650 TOYOPEARL DEAE-650 TOYOPEARL SP-650 TOYOPEARL CM-650 TSKgel SuperQ-5PW TSKgel SP-5PW TSKgel DEAE-5PW	TOYOPEARL Q-600C AR	TOYOPEARL SP-550 TOYOPEARL MegaCap II SP-550 TOYOPEARL QAE-550	TSKgel SP-3PW

Figure 3: Optimization of insulin binding capacity as a function of pore size of experimental TSKgel SP-type resins



Additional modifications to ligand and bead chemistry resulted in the TOYOPEARL Q-600C AR (alkaline resistant) resin. This is a high capacity, alkaline resistant, Q anion exchange media. TOYOPEARL Q-600C AR resin (using first generation ligand attachment chemistry) was developed by Tosoh for CIP of difficult to remove impurities. This resin has a slightly smaller pore size than TOYOPEARL GigaCap Q-650M resin and has a typical BSA binding capacity of 100 g/L. As shown in Figure 4, after 100 days of exposure to 1.0 mol/L NaOH, the DBC of TOYOPEARL Q-600C AR resin remains unchanged. Figure 5 shows the preservation of selectivity after extensive exposure to caustic.

Figure 4: TOYOPEARL Q-600C AR resin DBC as a function of sodium hydroxide exposure

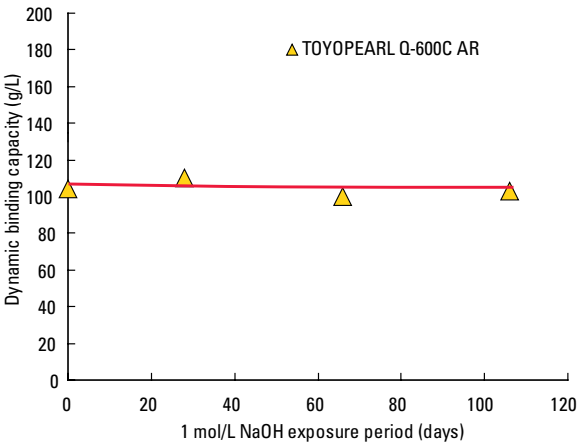
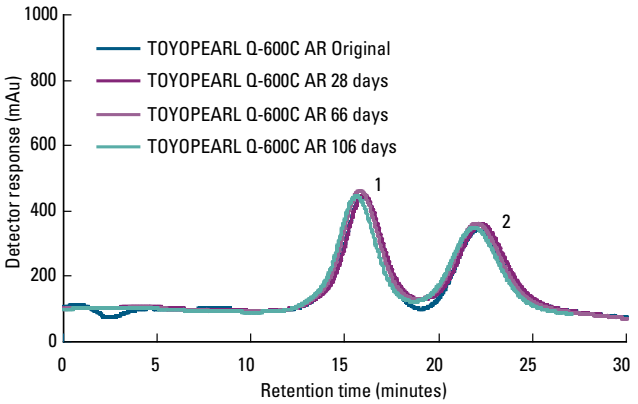


Figure 5: Stability of TOYOPEARL Q-600C AR resin after exposure to 1 mol/L NaOH



Resin:	**TOYOPEARL Q-600C AR**
Column size:	6.0 mm ID × 4 cm
Mobile phase:	Buffer A: 0.05 mol/L Tris-HCl buffer, pH 8.5
	Buffer B: 0.05 mol/L Tris-HCl buffer + 1.0 mol/L NaCl, pH 8.5
Gradient:	60 min linear gradient from buffer A to buffer B
Flow rate:	212 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Samples:	1. ovalbumin
	2. soybean trypsin inhibitor

Following is an explanation of the three ligand attachment chemistries used by Tosoh for TOYOPEARL and TSKgel IEX resins:

Attachment type	TOYOPEARL resins	TSKgel resins
The "traditional" ligand attachment method consists of attaching the ion exchange ligand directly to the resin surface through a proprietary spacer arm.	SP-650 MegaCap II SP-550 EC SP-550 Q-550 DEAE-650 CM-650	SP-3PW SP-5PW DEAE-5PW
The second generation ligand attachment method, for the purpose of increasing protein binding within the accessible surface area, adds a carbon spacer network between the bead surface and the ligand. It is also possible to attach ligand groups along the length of the spacer network, thus improving capacity.	SuperQ-650	SuperQ-5PW
The third generation ligand attachment method improves the accessible location of the ligand groups. This ligand chemistry moves the charged groups to the larger pores where the protein has better access to them. The result of this modification is significantly increased capacity and improved mass transfer. Improved mass transfer also reduces the target molecule elution volume.	GigaCap Q-650 GigaCap CM-650 GigaCap S-650 GigaCap DEAE-650	

Table 7 contains DBC data for five TOYOPEARL resins using three different size proteins. There are three different pore sizes and three different ligand attachment methods represented. TOYOPEARL GigaCap Q-650M resin has the highest capacity for all combinations of pore size and attachment chemistries.

Table 7: DBC varies with protein size

Resin	Pore size (nm)	Binding capacity (g/L-gel)		
		BSA 66 kDA	Human IgG 160 kDA	Thyroglobulin 660 kDA
TOYOPEARL GigaCap Q-650M	100	173	108	71
TOYOPEARL SuperQ-650M	100	145	13	3
TOYOPEARL Q-600C AR	75	108	90	26
TOYOPEARL QAE-550C	50	29	32	6
TOYOPEARL DEAE-650M	100	25	31	3

Column size: 6.0 mm ID × 4 cm
Mobile phase: Buffer A:
BSA 0.05 mol/L Tris-HCl, pH 8.5
Human IgG 0.05 mol/L Tris-HCl, pH 8.7
Thyroglobulin 0.05 mol/L Tris-HCl, pH 8.7 + 0.15 mol/L NaCl
Buffer B: 0.05 mol/L Tris-HCl buffer + 1.0 mol/L NaCl, pH 8.5
Flow rate: 212 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Samples: BSA, human IgG, thyroglobulin, each at 1.0 g/L

The following guidelines may be helpful when selecting a resin that is available in different pore sizes with the same ligand and ligand attachment chemistry:

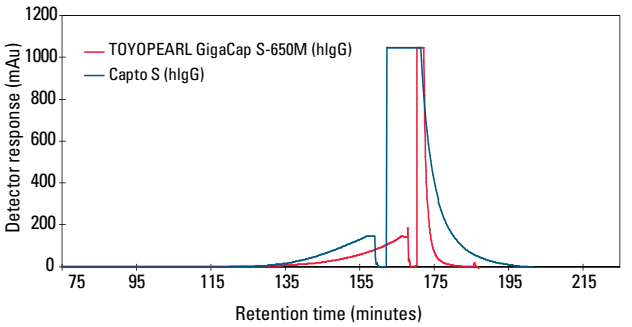
For bind/elute chromatography:	• Select the smallest pore size resin appropriate for the size of the target molecule. • Select a larger particle size for a capture step, a smaller one for intermediate or polishing steps.
For flow through chromatography:	• If the target molecule's size is larger than most components of the feed stream, select a pore size which will tend to exclude it (known as kinetic exclusion, this technique can also be used under binding conditions as the excluded molecule only sees 1% of the resin surface area and the capacity/recovery loss is minimal).
For large molecule impurity clearance:	• Select a pore size which includes the target molecule, but excludes the impurity (see the calibration curves of the TOYOPEARL base beads in the SEC section of the catalog as an aid).

TOYOPEARL GigaCap Resins

TOYOPEARL GigaCap resins have both higher capacity and improved elution kinetics compared to corresponding TOYOPEARL IEX resins. When these parameters are combined, they may significantly reduce elution pool volumes by as much as 75%. The TOYOPEARL GigaCap ligand attachment chemistry results in preferential placement of the functional groups into the larger more protein-accessible pores promoting both higher protein dynamic binding capacities and improved resin binding and desorption.

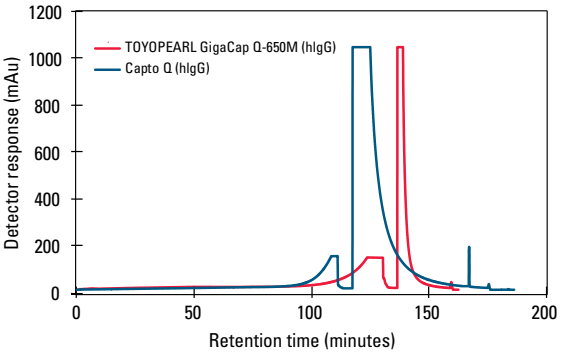
Unmodified TOYOPEARL HW-65 resin is utilized as the base bead for the TOYOPEARL GigaCap M-grade resins. The average particle size of the TOYOPEARL GigaCap M-grade resins, 75 µm, provides for enhanced efficiency and higher resolution than other larger particle size materials, while improved pressure-flow properties are obtained over smaller particle size materials. Figures 6, 7, and 8 show the breakthrough curves for three TOYOPEARL GigaCap M-grade resins. They are compared where possible with the most current equivalent competitive resin. Each trace shows the dynamic binding capacity of the resin up to 10% breakthrough plus the elution profile for the target molecule. **Please note the significant reduction in elution pool volumes of the TOYOPEARL GigaCap resins when compared to other products. The concentration of the eluted peak is proportionally increased as well.**

Figure 6: Elution pool volume comparison of TOYOPEARL GigaCap S-650M vs. Capto™ S resins



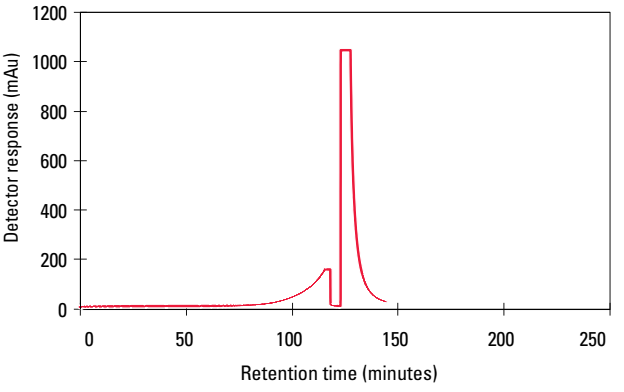
Resins:	**TOYOPEARL GigaCap S-650M**
	Capto S
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 0.1 mol/L acetate buffer, pH 4.7
	Buffer B: 0.1 mol/L acetate buffer, pH 4.7 + 1.0 mol/L NaCl
Flow rate:	212 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Sample:	polyclonal human IgG (1 mg/mL)

Figure 7: Elution pool volume comparison of TOYOPEARL GigaCap Q-650M vs. Capto Q resins



Resins:	**TOYOPEARL GigaCap Q-650M**
	Capto Q
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 15 mmol/LTris-HCl, pH 8.7
	Buffer B: 15 mmol/L Tris-HCl, pH 8.7 + 1.0 mol/L NaCl
Flow rate:	212 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Sample:	polyclonal human IgG (1 g/L)

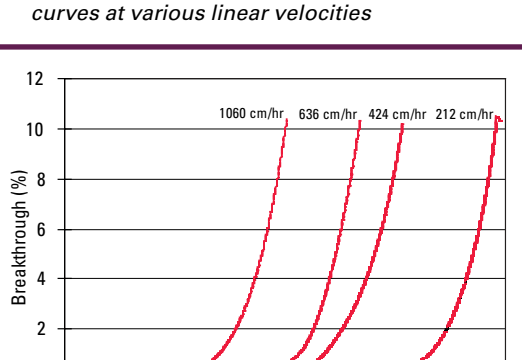
Figure 8: Elution pool volume of TOYOPEARL GigaCap CM-650M resin



Resin:	**TOYOPEARL GigaCap CM-650M**
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 50 mmol/L sodium acetate buffer, pH 4.7
	Buffer B: 50 mmol/L sodium acetate buffer, pH 4.7 + 0.5 mol/L NaCl
Flow rate:	212 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Sample:	polyclonal human IgG (1 mg/mL)

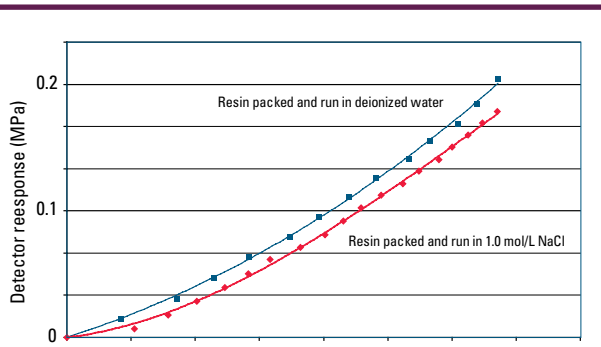
TOYOPEARL GigaCap S-650M resin was specifically developed for the purification of monoclonal antibodies. It has excellent elution kinetics (Figure 6) and maintains reasonably high capacities at higher linear velocities (Figure 9). The slightly larger particle size (50-100 µm) has been optimized to give a unique combination of improved pressure-flow characteristics (Figure 10) with excellent resolution at high loads (Figure 11). In separate studies it was established that DBC values for smaller proteins, such as insulin and lysozyme, were also notably improved with typical values of 133 g/L and 167 g/L, respectively.

Figure 9: TOYOPEARL GigaCap S-650M human IgG breakthrough curves at various linear velocities



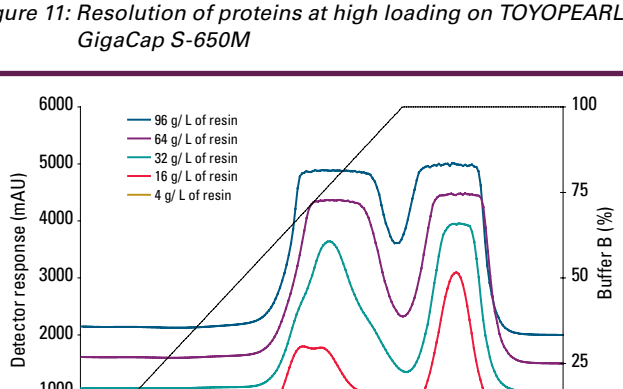
Resins:	**TOYOPEARL GigaCap S-650M**
Column size:	6 mm ID × 4 cm (1.13 mL)
Mobile phase:	0.1 mol/L acetate buffer, pH 4.7
Flow rates:	212, 424, 636, 1060 cm/hr (1.0, 2.0, 3.0, 5.0 mL/min)
Detection:	UV @ 280 nm
Sample:	polyclonal human IgG (1 g/L)

Figure 10: Pressure flow data for TOYOPEARL GigaCap S-650M



TOYOPEARL GigaCap S-650M was packed into a 36 cm ID × 25 cm bed height Eastern Rivers BioStream column to measure the pressure-flow characteristics. The resin had similar profiles when packed and run in both H_2O and 1.0 mol/L NaCl.

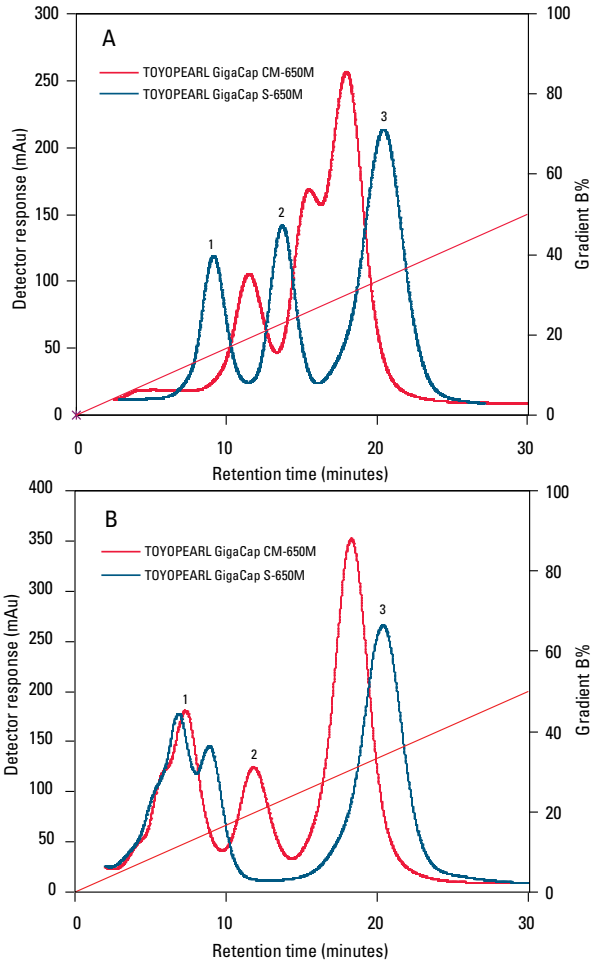
Figure 11: Resolution of proteins at high loading on TOYOPEARL GigaCap S-650M



Resin:	**TOYOPEARL GigaCap S-650M**
Column size:	3 mm ID × 15 cm
Mobile phase:	Buffer A: 20 mmol/L phosphate buffer, pH 6.0 Buffer B: 20 mmol/L phosphate buffer + 500 mmol/L NaCl, pH 6.0
Gradient:	10 CV linear gradient from 0 to 100% B (0-500 mmol/L NaCl)
Flow rate:	300 cm/hr (0.35 mL/min)
Detection:	UV @ 280 nm
Sample:	α-chymotrypsin (2 g/L), lysozyme (2 g/L) (total of 4 g proteins/L)

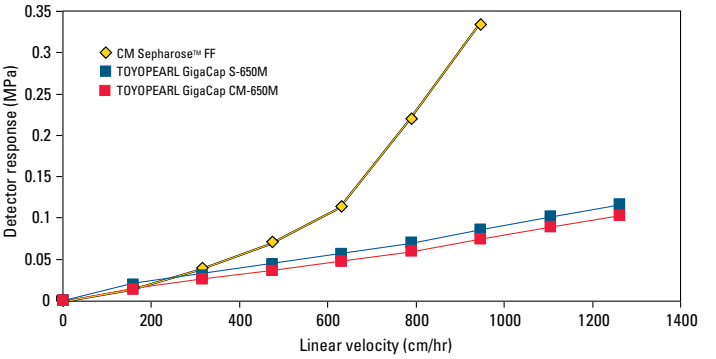
TOYOPEARL GigaCap CM-650M resin was designed for the purification of monoclonal antibodies that require a different chromatographic selectivity than is available with TOYOPEARL GigaCap S-650M resin (Figure 12). Excellent kinetic properties and high capacity are maintained at high linear flow velocities. Since TOYOPEARL GigaCap CM-650M resin is based on the same particle size base beads as the other resins within the TOYOPEARL GigaCap series, very good pressure-flow properties are obtained for this resin as well (Figure 13).

Figure 12: TOYOPEARL GigaCap CM-650M has unique selectivity



Resins:	**TOYOPEARL GigaCap CM-650M** **TOYOPEARL GigaCap S-650M**
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 20 mmol/L phosphate, pH 7.0 Buffer B: 20 mmol/L phosphate + 1.0 mol/L NaCl, pH 7.0
Gradient:	60 min linear gradient from buffer A to buffer B
Flow rate:	212 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Injection vol.:	25 µL
Samples:	(A) 1. ribonuclease A (5.0 g/L) 2. cytochrome C (1.9 g/L) 3. lysozyme (3.8 g/L) (B) 1. trypsinogen (3.8 g/L) 2. ribonuclease A (5.0 g/L) 3. lysozyme (3.8 g/L)

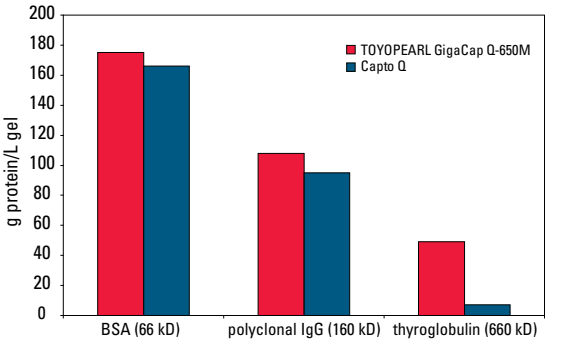
Figure 13: TOYOPEARL GigaCap CM-650M pressure-flow properties



Resin:	**TOYOPEARL GigaCap CM-650M**
Column size:	22 mm ID × 20 cm
Mobile phase:	H_2O
Detection:	pressure (MPa)

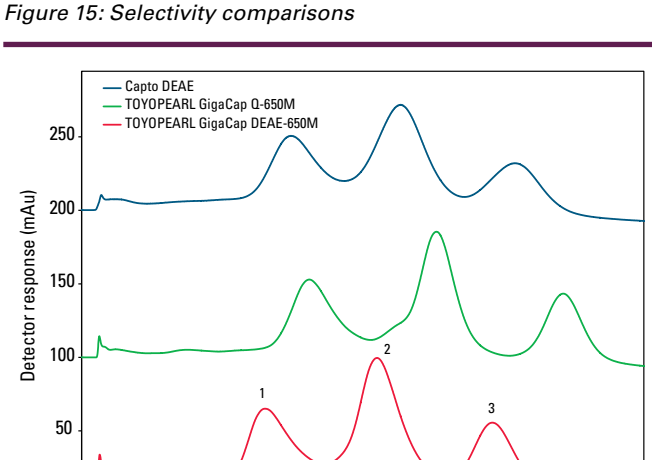
TOYOPEARL GigaCap Q-650M resin was primarily designed for the capture and purification of proteins, although it can also be used for polishing in flow-through chromatography. Of particular note is the excellent capacity of TOYOPEARL GigaCap Q-650M for such large proteins as thyroglobulin when compared to other high capacity resins (Figure 14).

Figure 14: Dynamic binding capacity of proteins with different molar masses @ 212 cm/hr



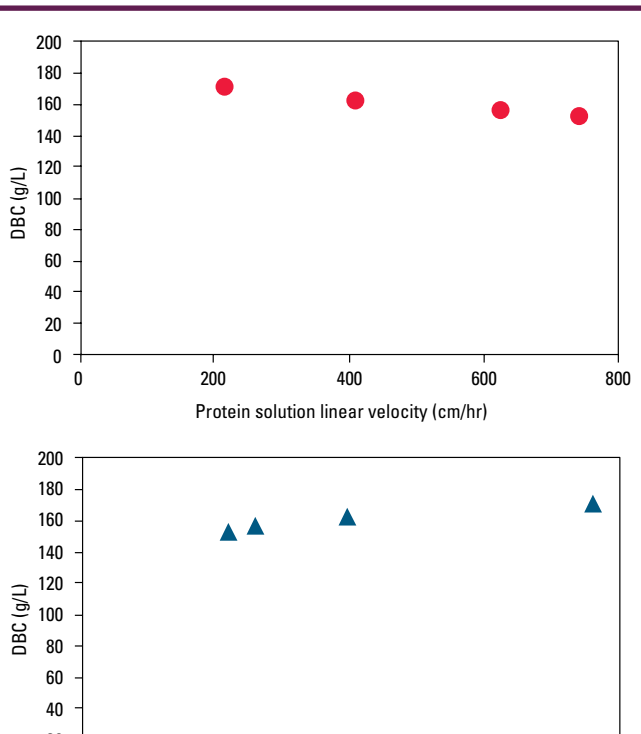
TOYOPEARL GigaCap DEAE-650M resin was designed for the purification proteins that require a different chromatographic selectivity (Figure 15) than is available with TOYOPEARL GigaCap Q-650M resin. As with other TOYOPEARL GigaCap M-grade resins, excellent kinetic properties and high capacity are maintained at high linear flow velocities (Figure 16). Since TOYOPEARL GigaCap DEAE-650M resin is based on the same particle size base beads as the other resins within the TOYOPEARL GigaCap series, very good pressure-flow properties are obtained for this resin as well (Figure 17).

Figure 15: Selectivity comparisons



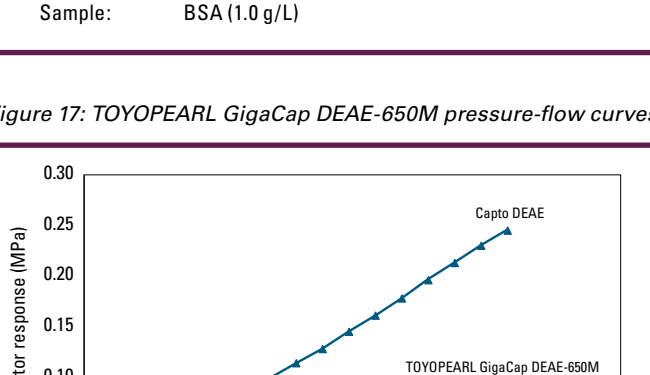
Resins:	**TOYOPEARL GigaCap DEAE-650M** **TOYOPEARL GigaCap Q-650M** Capto DEAE
Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	Buffer A: 50 mmol/L Tris-HCl, pH 8.5 Buffer B: buffer A + 1.0 mol/L NaCl, pH 8.5
Gradient:	120 minutes, 0 - 100% B
Flow rate:	136 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	100 µL
Samples:	1. transferrin, 2.9 g/L 2. ovalbumin, 6.5 g/L 3. trypsin inhibitor, 10.0 g/L
Sample load:	1.94 mg total protein

Figure 16: DBC vs. flow rate and residence time



Resins:	**TOYOPEARL GigaCap DEAE-650M**
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 0.05 mol/L Tris, pH 8.5 Buffer B: mobile phase A + 1.0 mol/L NaCl
Flow rates:	212, 407, 623, and 739 cm/hr (1.0, 1.9, 2.9, 3.5 mL/min)
Detection:	UV @ 280 nm
Sample:	BSA (1.0 g/L)

Figure 17: TOYOPEARL GigaCap DEAE-650M pressure-flow curves



Resin:	**TOYOPEARL GigaCap DEAE-650M**
Column size:	22 mm ID × 20 cm
Mobile phase:	0.1 mol/L NaCl
Detection:	pressure (MPa)

TOYOPEARL GigaCap Q-650 and S-650 resins are also available in a 35 µm S-grade, which is ideal for high resolution applications such as oligonucleotide, peptide, and antibody-drug conjugate purifications. TOYOPEARL GigaCap Q-650S and TOYOPEARL GigaCap S-650S maintain the superior dynamic binding capacities (Tables 8 and 9) and selectivities (Figures 18 and 19) of the M-grade TOYOPEARL GigaCap resins with the benefit of greater resolution due to their smaller bead size. Pressure-flow properties (Figures 20 and 21) are also maintained with the TOYOPEARL GigaCap S-grade resins.

Table 8: Anion exchange resin binding capacity comparisons

Resin	Particle size (µm)	pH stability	Base bead	Ion exchange capacity (meq/L)	Binding capacity (g/L)		DBC recovery (%)	DBC elution volume (CV)
					Static	Dynamic*		
TOYOPEARL GigaCap Q-650S	20 - 50	3 - 13	polymethacrylic	0.20	200	191	99	1.7
TOYOPEARL GigaCap Q-650M	50 - 100	3 - 13	polymethacrylic	0.17	191	172	97	15.8
Capto™ Q ImpRes	36 - 44	2 - 12	agarose	0.12	92	40	100	ND**
Q Sepharose™ HP	24 - 44	2 - 12	agarose	0.15	114	81	99	ND**

*Dynamic binding capacities were determined at 10% breakthrough
**Values not determined

Dynamic Binding Capacity (DBC) Conditions:
Column size: 6 mm ID × 4 cm
Mobile phase: A: 50 mmol/L Tris-HCl buffer, pH 8.5
B: mobile phase A + 0.5 mol/L NaCl
Flow rate: 212 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Sample: 1.0 g/L BSA

Static Binding Capacity (SBC) Conditions:
Adsorption buffer: 50 mmol/L Tris-HCl, pH 8.5
Protein concentration: 10.0 g/L

Table 9: Cation exchange resin binding capacity comparisons

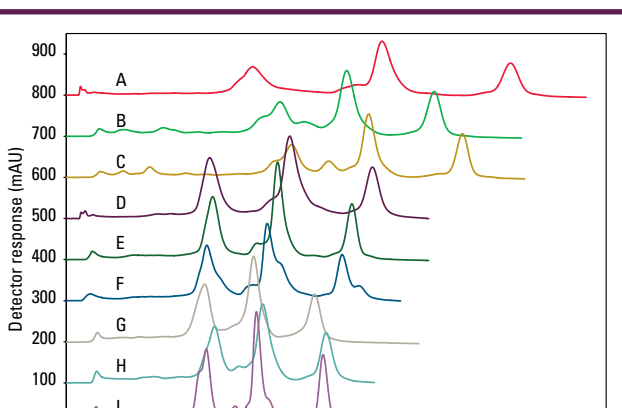
Resin	Particle size (µm)	pH stability	Base bead	Ion exchange capacity (meq/L)	Binding capacity (g/L)		DBC recovery (%)	DBC elution volume (CV)
					Static	Dynamic*		
TOYOPEARL GigaCap S-650S	20 - 50	3 - 13	polymethacrylic	0.24	177	164	99	4.0
TOYOPEARL GigaCap S-650M	50 - 100	3 - 13	polymethacrylic	0.16	156	145	98	13.5
Capto SP ImpRes	36 - 44	2 - 12	agarose	0.12	89	27	100	ND**
SP Sepharose™ HP	24 - 44	2 - 12	agarose	0.15	105	65	100	ND**

*Dynamic binding capacities were determined at 10% breakthrough
**Values not determined

Dynamic Binding Capacity (DBC) Conditions:
Column size: 6 mm ID × 4 cm
Mobile phase: A: 50 mmol/L acetate buffer, pH 4.7
B: mobile phase A + 0.5 mol/L NaCl
Flow rate: 212 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Sample: 1.0 g/L γ-globulin

Static Binding Capacity (SBC) Conditions:
Adsorption buffer: 50 mmol/L acetate buffer, pH 4.7
Sample: 10.0 g/L γ-globulin

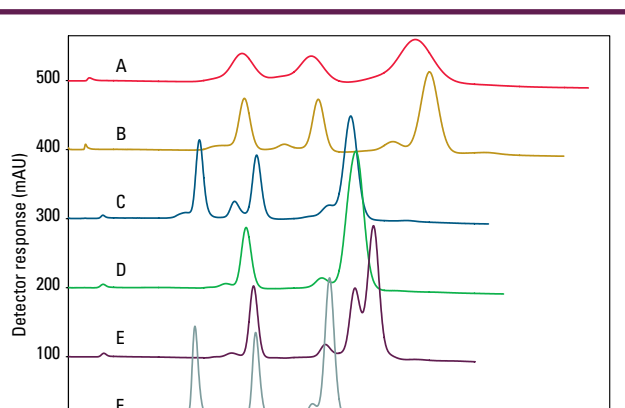
Figure 18: Selectivity comparisons of anion exchange resins



Resins:
A. TOYOPEARL GigaCap Q-650S
B. Capto Q ImpRes
C. Q Sepharose HP
D. TOYOPEARL SuperQ-650S
E. TSKgel SuperQ-5PW (30)
F. TSKgel SuperQ-5PW (20)
G. TOYOPEARL DEAE-650S
H. TSKgel DEAE-5PW (30)
I. TSKgel DEAE-5PW (20)

Column size: 7.5 mm ID × 7.5 cm
Mobile phase: Buffer A: 50 mmol/L Tris-HCl, pH 8.5
Buffer B: buffer A + 1.0 mol/L NaCl
Gradient: 0-100% buffer B (120 min)
Flow rate: 136 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Injection vol.: 100 µL
Samples: transferrin, 2.9 g/L
ovalbumin, 6.5 g/L
trypsin inhibitor, 10.0 g/L

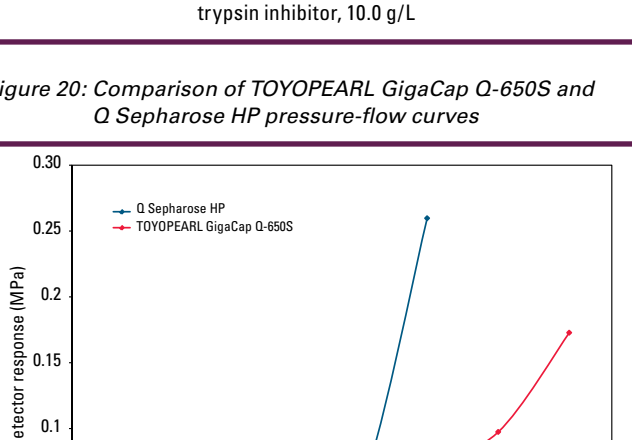
Figure 19: Selectivity comparisons of cation exchange resins



Resins:
A. TOYOPEARL GigaCap S-650M
B. TOYOPEARL GigaCap S-650S
C. TOYOPEARL SP-650S
D. Capto SP ImpRes
E. SP Sepharose HP
F. TSKgel SP-5PW (20)

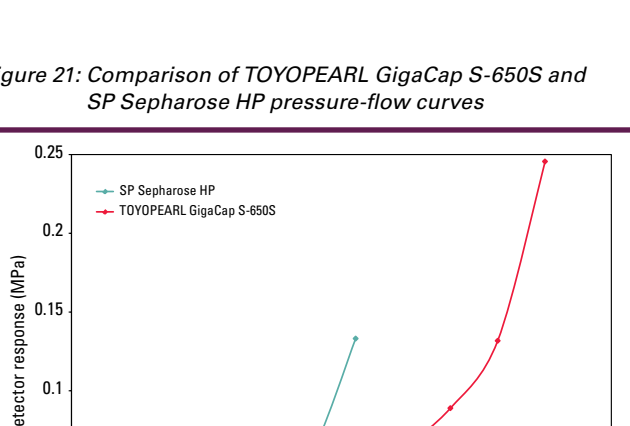
Column size: 7.5 mm ID × 7.5 cm
Mobile phase: Buffer A: 20 mmol/L phosphate, pH 7.0
Buffer B: buffer A + 1.0 mol/L NaCl
Gradient: 0-100% buffer B (60 min)
Flow rate: 136 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Injection vol.: 20 µL
Samples: ribonuclease A, 9.8 g/L
cytochrome C, 3.6 g/L
lysozyme, 6.4 g/L

Figure 20: Comparison of TOYOPEARL GigaCap Q-650S and Q Sepharose HP pressure-flow curves



Resin: **TOYOPEARL GigaCap Q-650S**
Q Sepharose HP
Column size: 22 mm ID × 20 cm
Mobile phase: 0.1 mol/L NaCl
Detection: pressure (MPa)

Figure 21: Comparison of TOYOPEARL GigaCap S-650S and SP Sepharose HP pressure-flow curves



Resin: **TOYOPEARL GigaCap S-650S**
SP Sepharose HP
Column size: 22 mm ID × 20 cm
Mobile phase: 0.1 mol/L NaCl
Detection: pressure (MPa)

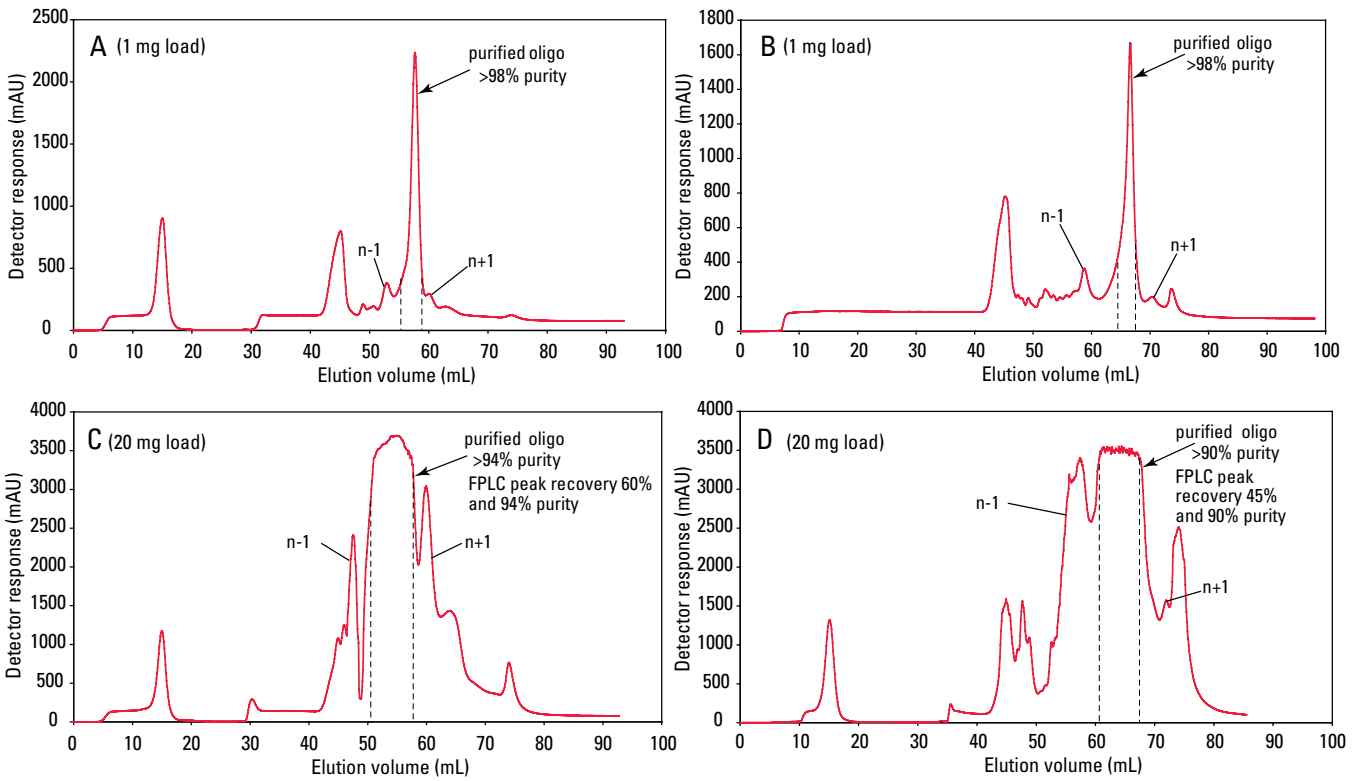
TSKgel SuperQ-5PW Resin

TSKgel SuperQ-5PW resin (offered in 20 and 30 µm particle size) is a strong anion exchange resin used for large and small biomolecules. TSKgel SuperQ-5PW analytical columns have the same backbone chemistry and selectivity as the bulk process scale TSKgel SuperQ-5PW resin, allowing seamless scale-up from analytical to manufacturing. In downstream processing of proteins, TSKgel SuperQ-5PW can be used for intermediate purification and polishing steps.

TSKgel SuperQ-5PW (20) resin is the product of choice for oligonucleotide purification. This resin does an excellent job as a capture resin isolating the full length oligonucleotide from the n-1, n+1, and other impurities generated during synthesis.

Figure 22 shows a comparison of one competitive product, of a smaller particle size, which initially has better resolution than TSKgel SuperQ-5PW (20) resin at 1 g oligonucleotide/L of resin. At 20 g oligonucleotide/L of resin, however, the resolution of peaks on the competitive product deteriorates significantly. The TSKgel SuperQ-5PW (20) resin retains excellent resolution even at this higher oligonucleotide concentration. Under higher loading conditions (Figure 22), the TSKgel SuperQ-type resins maintain their resolution much better than smaller particle, lower capacity resins. The smaller particle products may start out with a slight separation advantage under low oligonucleotide loading conditions, but this vanishes as the feedstock load is increased.

Figure 22: TSKgel SuperQ-5PW (20) resin maintains resolution at high oligonucleotide load



Resins:	**A & C: TSKgel SuperQ-5PW (20)** B & D: SOURCE™ 15Q
Column size:	0.66 cm × 15 cm (5.1 mL)
Mobile phase:	Buffer A: 20 mmol/L Tris-HCl + 10 mmol/L EDTA, pH 9.0 Buffer B: 20 mmol/L Tris-HCl + 10 mmol/L EDTA + 1.0 mol/L NaCl, pH 9.0
Flow rate:	250 cm/hr (1.43 mL/min)
Detection:	UV @ 254 nm
Sample:	DNA based oligonucleotides
Sample load:	A & B: 1 mg/column C & D: 20 mg/column
Separation conditions:	Column was washed with 5CV 100% buffer A followed by 11 mL injection. Column was then washed with 3CV 100% buffer A followed by 6CV of linear gradient 35-53 buffer B. Finally, column was washed with 5CV 100% buffer B.
Fractions:	0.5 mL fractions were taken from peaks of interest and analyzed on a TSKgel DNA-NPR column

Applications for Tosoh Bioscience Ion Exchange Chromatography Resins

Purification of Oligonucleotides

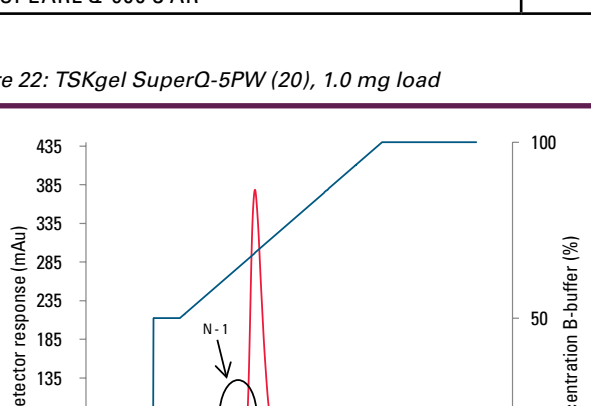
Table 10 shows the different particle sizes that are available in the TSKgel and TOYOPEARL anion exchange resins used for oligonucleotides, and the cation exchange resins used for peptide purifications. The relative binding capacities and predicted resolution of the different particle size resins are depicted by a series of "+" characters. The more "+" characters listed in the table the better one resin is relative to another for that parameter. If a process is developed using one of the resins and more resolution is needed, select an appropriate smaller particle size product. Similarly if more product throughput is needed and resolution is not a critical issue, a larger particle size resin can be selected.

The very high capacity TOYOPEARL GigaCap Q-650 resins (also shown in Table 10) can be used for oligonucleotide purifications, although the selectivity of this resin is somewhat different than the TSKgel and TOYOPEARL SuperQ-type resins. As seen in Figures 22-27, the TOYOPEARL GigaCap Q-650S performs similarly to the TSKgel SuperQ-5PW (20) resin for the purification of oligonucleotides. Table 11 compares the performance of these two resins for purity and recovery of an oligonucleotide from crude feedstock.

Table 10: Oligonucleotide purification products

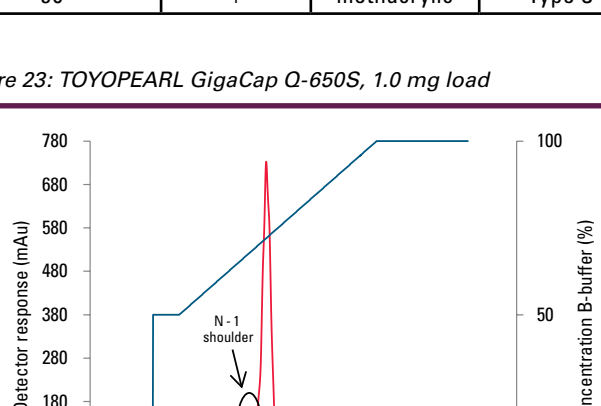
Resin	Bead size (mean µm)	Binding capacity g DNA oligo/L	Resolution	Bead type	Attachment method
TSKgel SuperQ-5PW (20)	20	45	+++++	methacrylic	Type A
TSKgel SuperQ-5PW (30)	30	40	++++	methacrylic	Type A
TOYOPEARL SuperQ-650S	35	54	+++	methacrylic	Type A
TOYOPEARL GigaCap Q-650S	35	40	+++	methacrylic	Type B
TOYOPEARL SuperQ-650M	65	50	++	methacrylic	Type A
TOYOPEARL GigaCap Q-650M	75	55	++	methacrylic	Type B
TOYOPEARL SuperQ-650C	100	50+ (est.)	+	methacrylic	Type A
TOYOPEARL Q-600 C AR	100	50	+	methacrylic	Type C

Figure 22: TSKgel SuperQ-5PW (20), 1.0 mg load



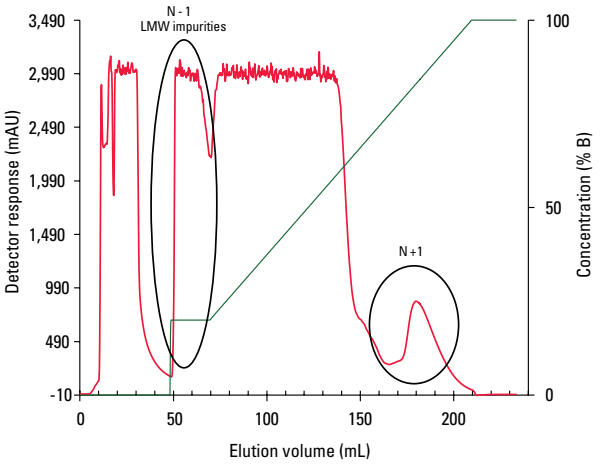
Resin: **TSKgel SuperQ-5PW (20)**
Column size: 6.6 mm ID × 18.5 cm (6.3 mL)
Mobile phase: Buffer A: 20 mmol/L NaOH
Buffer B: 20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient: 50% B (2 CV)
50-100% B (15 CV)
100% B (2 CV)
Flow rate: 200 cm/hr (1.14 mL/min)
Detection: UV @ 254 nm
Sample: crude phosphorothioate deoxyoligonucleotide
Sample load: 1.0 mg

Figure 23: TOYOPEARL GigaCap Q-650S, 1.0 mg load



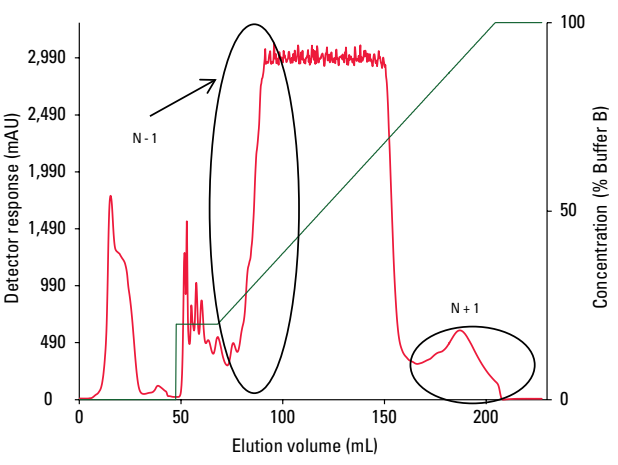
Resin: **TOYOPEARL GigaCap Q-650S**
Column size: 6.6 mm ID × 18.5 cm (6.3 mL)
Mobile phase: Buffer A: 20 mmol/L NaOH
Buffer B: 20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient: 50% B (2 CV)
50-100% B (15 CV)
100% B (2 CV)
Flow rate: 200 cm/hr (1.14 mL/min)
Detection: UV @ 254 nm
Sample: crude phosphorothioate deoxyoligonucleotide
Sample load: 1.0 mg

Figure 24: Purification of oligonucleotide at 80% DBC on TSKgel SuperQ-5PW (20) resin



Resin:	**TSKgel SuperQ-5PW (20)**
Column size:	6.6 mm ID × 18.5 cm (6.3 mL)
Mobile phase:	Buffer A: 20 mmol/L NaOH
	Buffer B: 20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient:	20% B (2 CV)
	20-100% B (20 CV)
	100% B (2 CV)
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 254 nm
Sample:	crude phosphorothioate deoxyoligonucleotide
Sample load:	235 mg

Figure 25: Purification of oligonucleotide at 80% DBC on TOYOPEARL GigaCap Q-650S resin



Resin:	**TOYOPEARL GigaCap Q-650S**
Column size:	6.6 mm ID × 18 cm (6.16 mL)
Mobile phase:	Buffer A: 20 mmol/L NaOH
	Buffer B: buffer A + 3.0 mol/L NaCl
Gradient:	step to 20% B (2 CV)
	20% - 100% B (20 CV)
	100% B (2 CV)
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 254 nm
Sample:	crude phosphorothioate deoxyoligonucleotide
Sample load:	181.4 mg

Figure 26: TSKgel SuperQ-5PW (20) resin: 80% DBC elution peak with fraction purity histogram

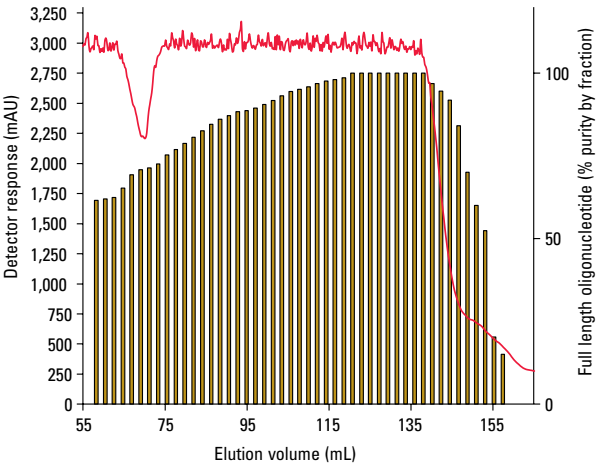


Figure 27: TOYOPEARL GigaCap Q-650S resin: 80% DBC elution peak with fraction purity histogram

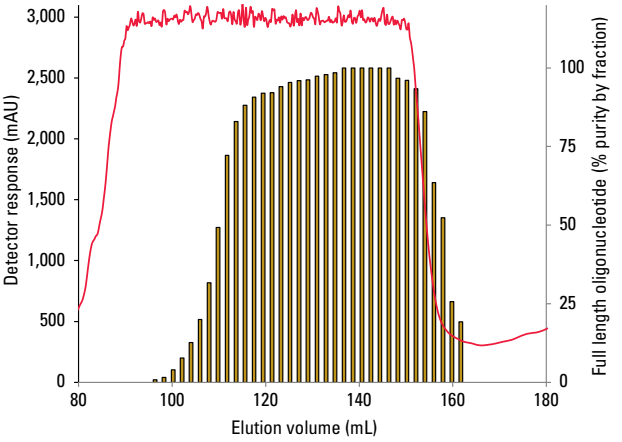


Table 11: Oligonucleotide purity and yield from 80% DBC purifications

Resin	Crude oligo purity	Final oligo purity	% Yield
TSKgel SuperQ-5PW (20)	66.5%	96.4%	72.5%
TOYOPEARL GigaCap Q-650S	66.5%	96.9%	81.3%

Peptide Purifications

Cation exchange chromatography is commonly used for peptide purification. Table 12 shows the same particle size profile availability of TOYOPEARL and TSKgel resins functionalized with the cation exchange SP ligand. Based on the needs for capacity and resolution, an appropriate SP resin should be selected for a particular peptide application.

Table 12: Peptide purification products

Resin	Bead size (mean µm)	Binding capacity	Resolution	Bead type	Attachment method
TSKgel SP-5PW (20)	20	++	+++++	methacrylic	Traditional
TSKgel SP-5PW (30)	30	++	++++	methacrylic	Traditional
TSKgel SP-3PW (30)	30	++	++++	methacrylic	Traditional
TOYOPEARL SP-650S	35	++++	+++	methacrylic	Traditional
TOYOPEARL SP-650M	65	++++	++	methacrylic	Traditional
TOYOPEARL SP-650C	100	++++	+	methacrylic	Traditional
TOYOPEARL GigaCap S-650S	35	+++++	+++	methacrylic	Type B
TOYOPEARL GigaCap S-650M	75	+++++	++	methacrylic	Type B

Insulin Purification

TSKgel SP-3PW (30) resin was developed as a higher resolving and higher capacity resin for insulin purification. Table 13 compares the capacity of this new resin to TSKgel SP-5PW (30) resin and SOURCE 30S resin. The improved resolving power of TSKgel SP-3PW (30) resin is demonstrated in Figure 28.

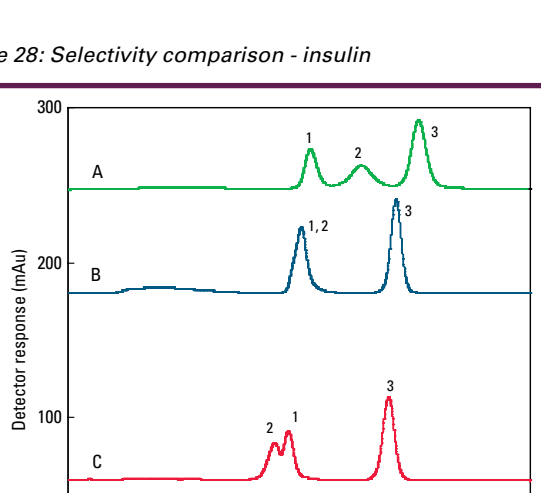
Table 13: Insulin dynamic binding capacity comparison

Resin	TSKgel SP-3PW (30)	TSKgel SP-5PW (30)	SOURCE 30S
Matrix	polymethacrylate	polymethacrylate	polystyrene divinylbenzene
Particle size	30 µm	30 µm	30 µm
Insulin capacity	49 g/L	24 g/L	45 g/L
Pore size	25 nm	100 nm	NR

Dynamic binding capacities were determined at 10% breakthrough

Column size: 4.6 mm ID × 7.5 cm
Mobile phase: gradient elution with 1-propanol by acidic buffer, pH 3.0 containing neutral salt
Flow rate: 270 cm/hr (0.75 mL/min)
Sample: recombinant insulin (7.2 g/L)

Figure 28: Selectivity comparison - insulin

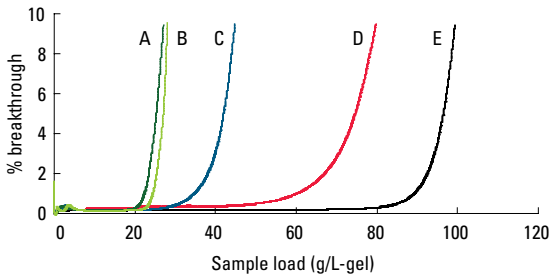


Resins: **A. TSKgel SP-3PW (30)**
B. SOURCE 30S
C. TSKgel SP-5PW (30)
Column size: 7.5 mm ID × 7.5 cm
Mobile phase: Buffer A: 0.02 mol/L sodium citrate buffer, pH 3.2 + ethanol = 8/2 (v/v)
Buffer B: 0.02 mol/L sodium citrate buffer, pH 3.2 + 1.0 mol/L NaCl/ethanol = 8/2 (v/v)
Gradient: 60 min linear gradient from buffer A to buffer B
Flow rate: 136 cm/hr (1.0mL/min)
Detection: UV @ 280 nm
Temperature: ambient
Samples: 1. trypsinogen 2. insulin 3. lysozyme
Sample vol.: 100 µL (0.5 g/L each)

PEGylated Proteins

Ion exchange resins are frequently used for the purification of PEGylated proteins. Figure 29 shows the breakthrough curves of five TOYOPEARL cation exchange resins for mono-PEGylated lysozyme. The selectivities of TOYOPEARL GigaCap CM-650M and TOYOPEARL GigaCap S-650M resins for native lysozyme and its mono-PEGylated counterpart are shown in Figure 30.

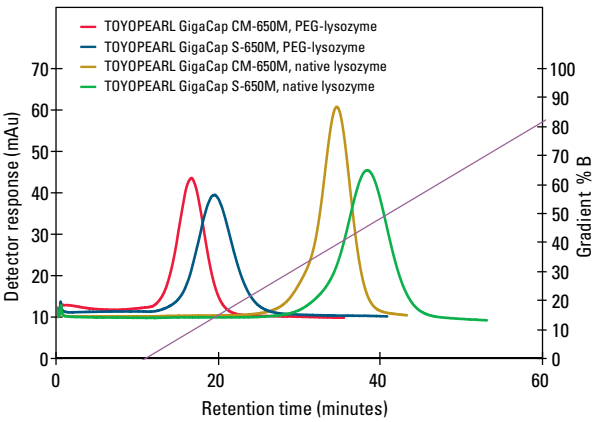
Figure 29: Breakthrough curves of mono-PEGylated lysozyme using TOYOPEARL cation exchange resins



Resins: **A. TOYOPEARL SP-650M**
B. TOYOPEARL CM-650M
C. TOYOPEARL SP-550C
D. TOYOPEARL GigaCap CM-650M
E. TOYOPEARL GigaCap S-650M
Column size: 6 mm ID × 40 mm
Mobile phase: Buffer A: 20 mmol/L phosphate buffer, pH 7.0
Buffer B: 20 mmol/L phosphate buffer, pH 7.0 + 0.5 mol/L NaCl
Flow rate: 212 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Sample: mono-PEGylated lysozyme, 1.0 mg/mL (PEG MW= 5 kDa)

Dynamic binding capacities were determined at 10% breakthrough

Figure 30: Selectivity comparison between native protein and mono-PEGylated protein on TOYOPEARL GigaCap resins



Resins: **TOYOPEARL GigaCap S-650M**
TOYOPEARL GigaCap CM-650M
Column size: 6 mm ID × 4 cm
Mobile phase: Buffer A: 50 mmol/L phosphate buffer, pH 7.0
Buffer B: 50 mmol/L phosphate buffer, pH 7.0 + 0.5 mol/L NaCl

Gradients:

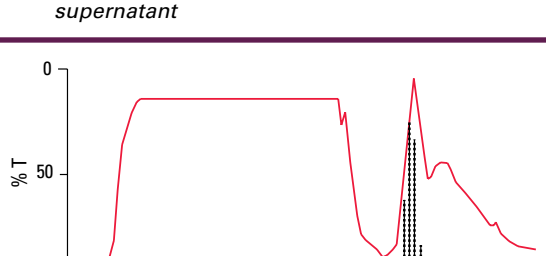
TOYOPEARL GigaCap S-650M	TOYOPEARL GigaCap CM-650M
10 minute 100% buffer A	10 minute 100% buffer A
60 minutes 0%B to 100%B	60 minutes 0%B to 50%B

Flow rate: 212 cm/hr (1 mL/min)
Samples: native lysozyme, 5 g/L
mono-PEGylated lysozyme, 5 g/L (PEG MW= 5 kDa)

Antibody Purification

Klapper *et al.* reported the use of the TOYOPEARL CM-650S for the purification of monoclonal antibodies.[1] Figure 31 shows the elution profile of monoclonal antibody supernatant. Antibody activity is represented in the figure by the black bars.

Figure 31: Separation of monoclonal antibody cell culture supernatant



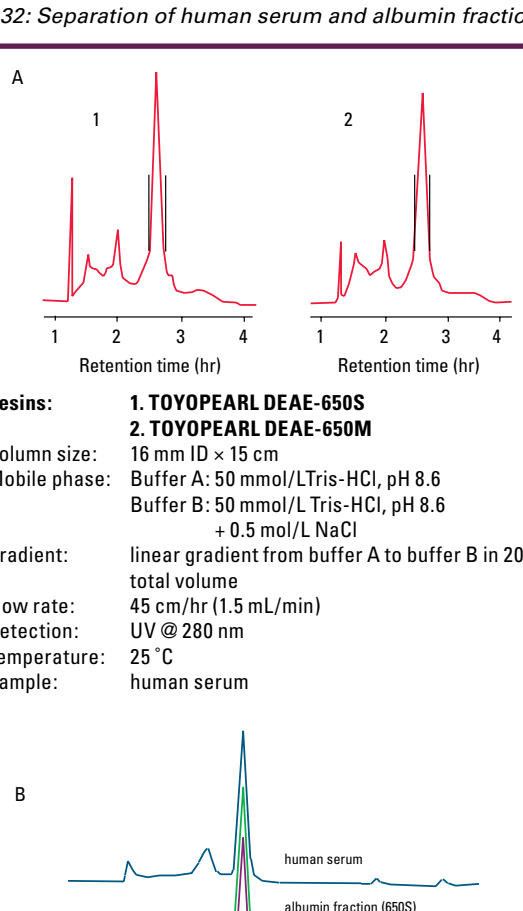
Resin:	**TOYOPEARL CM-650S**
Column size:	16 mm ID × 6 cm
Mobile phase:	Buffer A: 20 mmol/L sodium acetate, pH 5.5 Buffer B: 20 mmol/L sodium acetate, pH 5.5 + 0.5 mol/L NaCl
Gradient:	linear gradient from buffer A to buffer B in 200 mL total volume
Flow rate:	173 cm/hr (5.8 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	100 mL of monoclonal antibody cell culture supernatant

[1]Klapper, D.; Osgood, S.; Esch, R.; Olson, J. Use of new HPLC resins to solve old problems. J. of Liquid Chromatography. 1986, 9, (8), 1613-1633.

Blood Proteins

The separation of human serum on both TOYOPEARL DEAE-650M and TOYOPEARL DEAE-650S is shown in Figure 32. The albumin fractions were collected (between the two vertical lines) and were analyzed via size exclusion chromatography on two TSKgel G3000SW columns in series. As seen in the figure, the albumin fractions contain small amounts of a high formula weight contaminant which is probably α-globulin.[2] Analytical IEX (not shown) demonstrated that the albumin peaks were fairly homogeneous.

Figure 32: Separation of human serum and albumin fractions



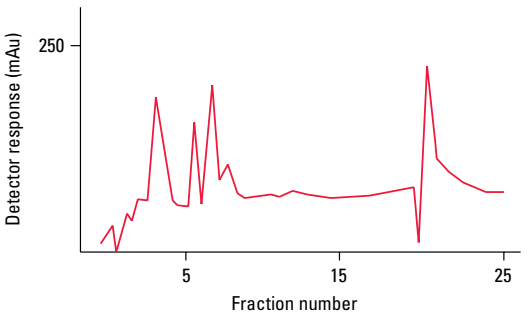
Resins:	**1. TOYOPEARL DEAE-650S** **2. TOYOPEARL DEAE-650M**
Column size:	16 mm ID × 15 cm
Mobile phase:	Buffer A: 50 mmol/LTris-HCl, pH 8.6 Buffer B: 50 mmol/L Tris-HCl, pH 8.6 + 0.5 mol/L NaCl
Gradient:	linear gradient from buffer A to buffer B in 200 mL total volume
Flow rate:	45 cm/hr (1.5 mL/min)
Detection:	UV @ 280 nm
Temperature:	25 °C
Sample:	human serum
Column:	**TSKgel G3000SW, 7.5 mm ID × 30 cm × 2 in series**
Mobile phase:	0.1 mol/L phosphate, pH 6.8 + 0.1 mol/L sodium sulfate
Detection:	UV @ 280 nm
Temperature:	25 °C
Sample:	1. crude human serum 2. albumin fraction from TOYOPEARL DEAE-650S 3. albumin fraction from TOYOPEARL DEAE-650M

[2]Kato, Y.; Nakamura, K.; Hashimoto, T. Characterization of TSK-GEL DEAE-Toyopearl 650 Ion Exchanger. J. Chromatogr. 1982, 245, 193-211.

Tryptic Digests

Tryptic fragments from radiolabeled human immunoglobulin light chain can be separated using anion exchange chromatography on TOYOPEARL DEAE-650S.[1] Figure 33 shows the elution profile of a tryptic digest fraction from an SEC column run on TOYOPEARL DEAE-650S. The recovery of the radiolabeled product was greater than 90%.

Figure 33: Separation of tryptic digest peptide mixture



Resin:	**TOYOPEARL DEAE-650S**
Column size:	6 mm ID × 12 cm
Mobile phase:	pyridine/N-ethyl morpholine
Flow rate:	212 cm/hr (1 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	enzymatic digest of immunoglobulin L chain

[1]Klapper, D.; Osgood, S.; Esch, R.; Olson, J. Use of new HPLC resins to solve old problems. J. of Liquid Chromatography. 1986, 9, (8), 1613-1633.

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

Ordering Information

Anion exchange resins:

Part #	Product description	Container size (mL)	Bead diameter (µm)	Ion Exchange Capacity (eq/L)	Typical BSA capacity (g/L)	
TOYOPEARL and TOYOPEARL GigaCap Anion Exchange Resins						
43271	TOYOPEARL QAE-550C	100	50 - 150	0.28 - 0.38	60 - 80	
14026	TOYOPEARL QAE-550C	250	50 - 150	0.28 - 0.38	60 - 80	
14704	TOYOPEARL QAE-550C	1,000	50 - 150	0.28 - 0.38	60 - 80	
14027	TOYOPEARL QAE-550C	5,000	50 - 150	0.28 - 0.38	60 - 80	
18365	TOYOPEARL QAE-550C	50,000	50 - 150	0.28 - 0.38	60 - 80	
21985	TOYOPEARL Q-600C AR	100	50 - 150	0.15 - 0.20	> 120	
21986	TOYOPEARL Q-600C AR	250	50 - 150	0.15 - 0.20	> 120	
21987	TOYOPEARL Q-600C AR	1,000	50 - 150	0.15 - 0.20	> 120	
21988	TOYOPEARL Q-600C AR	5,000	50 - 150	0.15 - 0.20	> 120	
21989	TOYOPEARL Q-600C AR	50,000	50 - 150	0.15 - 0.20	> 120	
21854	TOYOPEARL GigaCap Q-650M	100	50 - 100	0.20 - 0.30	105 - 155	
21855	TOYOPEARL GigaCap Q-650M	250	50 - 100	0.20 - 0.30	105 - 155	
21856	TOYOPEARL GigaCap Q-650M	1,000	50 - 100	0.20 - 0.30	105 - 155	
21857	TOYOPEARL GigaCap Q-650M	5,000	50 - 100	0.20 - 0.30	105 - 155	
21858	TOYOPEARL GigaCap Q-650M	50,000	50 - 100	0.20 - 0.30	105 - 155	
19823	TOYOPEARL SuperQ-650S	25	20 - 50	0.20 - 0.30	105 - 155	
17223	TOYOPEARL SuperQ-650S	250	20 - 50	0.20 - 0.30	105 - 155	
17224	TOYOPEARL SuperQ-650S	1,000	20 - 50	0.20 - 0.30	105 - 155	
17225	TOYOPEARL SuperQ-650S	5,000	20 - 50	0.20 - 0.30	105 - 155	
19679	TOYOPEARL SuperQ-650S	50,000	20 - 50	0.20 - 0.30	105 - 155	
43205	TOYOPEARL SuperQ-650M	100	40 - 90	0.20 - 0.30	105 - 155	
17227	TOYOPEARL SuperQ-650M	250	40 - 90	0.20 - 0.30	105 - 155	
17228	TOYOPEARL SuperQ-650M	1,000	40 - 90	0.20 - 0.30	105 - 155	
17229	TOYOPEARL SuperQ-650M	5,000	40 - 90	0.20 - 0.30	105 - 155	
21311	TOYOPEARL SuperQ-650M	50,000	40 - 90	0.20 - 0.30	105 - 155	
43275	TOYOPEARL SuperQ-650C	100	50 - 150	0.20 - 0.30	105 - 155	
17231	TOYOPEARL SuperQ-650C	250	50 - 150	0.20 - 0.30	105 - 155	
17232	TOYOPEARL SuperQ-650C	1,000	50 - 150	0.20 - 0.30	105 - 155	
17233	TOYOPEARL SuperQ-650C	5,000	50 - 150	0.20 - 0.30	105 - 155	
19804	TOYOPEARL DEAE-650S	25	20 - 50	0.08 - 0.12	25 - 35	
07472	TOYOPEARL DEAE-650S	250	20 - 50	0.08 - 0.12	25 - 35	
14692	TOYOPEARL DEAE-650S	1,000	20 - 50	0.08 - 0.12	25 - 35	
07973	TOYOPEARL DEAE-650S	5,000	20 - 50	0.08 - 0.12	25 - 35	
21483	TOYOPEARL DEAE-650S	50,000	20 - 50	0.08 - 0.12	25 - 35	

Part #	Product description	Container size (mL)	Bead diameter (µm)	Ion Exchange Capacity (eq/L)	Typical BSA capacity (g/L)	
43201	TOYOPEARL DEAE-650M	100	40 - 90	0.08 - 0.12	25 - 35	
07473	TOYOPEARL DEAE-650M	250	40 - 90	0.08 - 0.12		
14693	TOYOPEARL DEAE-650M	1,000	40 - 90	0.08 - 0.12		
07974	TOYOPEARL DEAE-650M	5,000	40 - 90	0.08 - 0.12		
18367	TOYOPEARL DEAE-650M	50,000	40 - 90	0.08 - 0.12		
07988	TOYOPEARL DEAE-650C	250	50 - 150	0.05 - 0.11	25 - 35	
14694	TOYOPEARL DEAE-650C	1,000	50 - 150	0.05 - 0.11		
07989	TOYOPEARL DEAE-650C	5,000	50 - 150	0.05 - 0.11		
22853	TOYOPEARL DEAE-650C	50,000	50 - 150	0.05 - 0.11		
22865	TOYOPEARL GigaCap DEAE-650M	100	50 - 100	0.15 - 0.25	> 156	
22866	TOYOPEARL GigaCap DEAE-650M	250	50 - 100	0.15 - 0.25		
22867	TOYOPEARL GigaCap DEAE-650M	1,000	50 - 100	0.15 - 0.25		
22868	TOYOPEARL GigaCap DEAE-650M	5,000	50 - 100	0.15 - 0.25		
22869	TOYOPEARL GigaCap DEAE-650M	50,000	50 - 100	0.15 - 0.25		
22881	TOYOPEARL GigaCap Q-650S	25	20 - 50	0.14 - 0.24	>170	
22882	TOYOPEARL GigaCap Q-650S	250	20 - 50	0.14 - 0.24		
22883	TOYOPEARL GigaCap Q-650S	1,000	20 - 50	0.14 - 0.24		
22884	TOYOPEARL GigaCap Q-650S	5,000	20 - 50	0.14 - 0.24		
22885	TOYOPEARL GigaCap Q-650S	50,000	20 - 50	0.14 - 0.24		
TSKgel Anion Exchange Resins						
43383	TSKgel SuperQ-5PW (20)	25	15 - 25	0.12 - 0.18	52 - 88	
18535	TSKgel SuperQ-5PW (20)	250	15 - 25	0.12 - 0.18		
18546	TSKgel SuperQ-5PW (20)	1,000	15 - 25	0.12 - 0.18		
18547	TSKgel SuperQ-5PW (20)	5,000	15 - 25	0.12 - 0.18		
21919	TSKgel SuperQ-5PW (20)	25,000	15 - 25	0.12 - 0.18		
21920	TSKgel SuperQ-5PW (20)	50,000	15 - 25	0.12 - 0.18		
43283	TSKgel SuperQ-5PW (30)	25	20 - 40	0.12 - 0.18	52 - 88	
18536	TSKgel SuperQ-5PW (30)	250	20 - 40	0.12 - 0.18		
18548	TSKgel SuperQ-5PW (30)	1,000	20 - 40	0.12 - 0.18		
18549	TSKgel SuperQ-5PW (30)	5,000	20 - 40	0.12 - 0.18		
43381	TSKgel DEAE-5PW (20)	25	15 - 25	0.05 - 0.11	25 - 45	
14710	TSKgel DEAE-5PW (20)	250	15 - 25	0.05 - 0.11		
14711	TSKgel DEAE-5PW (20)	1,000	15 - 25	0.05 - 0.11		
18436	TSKgel DEAE-5PW (20)	5,000	15 - 25	0.05 - 0.11		
43281	TSKgel DEAE-5PW (30)	25	20 - 40	0.05 - 0.11	20 - 40	
14712	TSKgel DEAE-5PW (30)	250	20 - 40	0.05 - 0.11		
14713	TSKgel DEAE-5PW (30)	1,000	20 - 40	0.05 - 0.11		
18370	TSKgel DEAE-5PW (30)	5,000	20 - 40	0.05 - 0.11		

Cation exchange resins:

Part #	Product description	Container size (mL)	Bead diameter (µm)	Ion Exchange Capacity (eq/L)	Typical BSA capacity (g/L)	
TOYOPEARL and TOYOPEARL GigaCap Cation Exchange Resins						
21833	TOYOPEARL GigaCap S-650M	100	50 - 100	0.14 - 0.18	80 - 120	
21834	TOYOPEARL GigaCap S-650M	250	50 - 100	0.14 - 0.18	80 - 120	
21835	TOYOPEARL GigaCap S-650M	1,000	50 - 100	0.14 - 0.18	80 - 120	
21836	TOYOPEARL GigaCap S-650M	5,000	50 - 100	0.14 - 0.18	80 - 120	
21837	TOYOPEARL GigaCap S-650M	50,000	50 - 100	0.14 - 0.18	80 - 120	
22875	TOYOPEARL GigaCap S-650S	25	20 - 50	0.15 - 0.25	>150 Hu. IgG	
22876	TOYOPEARL GigaCap S-650S	250	20 - 50	0.15 - 0.25	>150 Hu. IgG	
22877	TOYOPEARL GigaCap S-650S	1,000	20 - 50	0.15 - 0.25	>150 Hu. IgG	
22878	TOYOPEARL GigaCap S-650S	5,000	20 - 50	0.15 - 0.25	>150 Hu. IgG	
22879	TOYOPEARL GigaCap S-650S	50,000	20 - 50	0.15 - 0.25	>150 Hu. IgG	
21946	TOYOPEARL GigaCap CM-650M	100	50 - 100	0.18 - 0.28	> 110 γ-globulin	
21947	TOYOPEARL GigaCap CM-650M	250	50 - 100	0.18 - 0.28	> 110 γ-globulin	
21948	TOYOPEARL GigaCap CM-650M	1,000	50 - 100	0.18 - 0.28	> 110 γ-globulin	
21949	TOYOPEARL GigaCap CM-650M	5,000	50 - 100	0.18 - 0.28	> 110 γ-globulin	
21950	TOYOPEARL GigaCap CM-650M	50,000	50 - 100	0.18 - 0.28	> 110 γ-globulin	
43272	TOYOPEARL SP-550C	100	50 - 150	0.14 - 0.18	80 - 120	
14028	TOYOPEARL SP-550C	250	50 - 150	0.14 - 0.18	80 - 120	
14705	TOYOPEARL SP-550C	1,000	50 - 150	0.14 - 0.18	80 - 120	
14029	TOYOPEARL SP-550C	5,000	50 - 150	0.14 - 0.18	80 - 120	
18366	TOYOPEARL SP-550C	50,000	50 - 150	0.14 - 0.18	80 - 120	
19822	TOYOPEARL SP-650S	25	20 - 50	0.13 - 0.17	40 - 60	
08437	TOYOPEARL SP-650S	250	20 - 50	0.13 - 0.17	40 - 60	
14698	TOYOPEARL SP-650S	1,000	20 - 50	0.13 - 0.17	40 - 60	
08438	TOYOPEARL SP-650S	5,000	20 - 50	0.13 - 0.17	40 - 60	
21477	TOYOPEARL SP-650S	50,000	20 - 50	0.13 - 0.17	40 - 60	
43202	TOYOPEARL SP-650M	100	40 - 90	0.13 - 0.17	40 - 60	
07997	TOYOPEARL SP-650M	250	40 - 90	0.13 - 0.17	40 - 60	
14699	TOYOPEARL SP-650M	1,000	40 - 90	0.13 - 0.17	40 - 60	
07998	TOYOPEARL SP-650M	5,000	40 - 90	0.13 - 0.17	40 - 60	
18369	TOYOPEARL SP-650M	50,000	40 - 90	0.13 - 0.17	40 - 60	
07994	TOYOPEARL SP-650C	250	50 - 150	0.12 - 0.18	35 - 55	
14700	TOYOPEARL SP-650C	1,000	50 - 150	0.12 - 0.18	35 - 55	
07995	TOYOPEARL SP-650C	5,000	50 - 150	0.12 - 0.18	35 - 55	
19803	TOYOPEARL CM-650S	25	20 - 50	0.08 - 0.12	30 - 50	
07474	TOYOPEARL CM-650S	250	20 - 50	0.08 - 0.12	30 - 50	

Part #	Product description	Container size (mL)	Bead diameter (µm)	Ion Exchange Capacity (eq/L)	Typical BSA capacity (g/L)	
14695	TOYOPEARL CM-650S	1,000	20 - 50	0.08 - 0.12	30 - 50	
07971	TOYOPEARL CM-650S	5,000	20 - 50	0.08 - 0.12	30 - 50	
43203	TOYOPEARL CM-650M	100	40 - 90	0.08 - 0.12	30 - 50	
07475	TOYOPEARL CM-650M	250	40 - 90	0.08 - 0.12	30 - 50	
14696	TOYOPEARL CM-650M	1,000	40 - 90	0.08 - 0.12	30 - 50	
07972	TOYOPEARL CM-650M	5,000	40 - 90	0.08 - 0.12	30 - 50	
19839	TOYOPEARL CM-650M	50,000	40 - 90	0.08 - 0.12	30 - 50	
07991	TOYOPEARL CM-650C	250	50 - 150	0.05 - 0.11	25 - 45	
14697	TOYOPEARL CM-650C	1,000	50 - 150	0.05 - 0.11	25 - 45	
07992	TOYOPEARL CM-650C	5,000	50 - 150	0.05 - 0.11	25 - 45	
19329	TOYOPEARL CM-650C	50,000	50 - 150	0.05 - 0.11	25 - 45	
21804	TOYOPEARL MegaCap II SP-550EC	100	100 - 300	0.14 - 0.18	60 - 90*	
21805	TOYOPEARL MegaCap II SP-550EC	250	100 - 300	0.14 - 0.18	60 - 90*	
21806	TOYOPEARL MegaCap II SP-550EC	1,000	100 - 300	0.14 - 0.18	60 - 90*	
21807	TOYOPEARL MegaCap II SP-550EC	5,000	100 - 300	0.14 - 0.18	60 - 90*	
21808	TOYOPEARL MegaCap II SP-550EC	50,000	100 - 300	0.14 - 0.18	60 - 90*	
TSKgel Cation Exchange Resins						
43382	TSKgel SP-5PW (20)	25	15 - 25	0.06 - 0.12	20 - 40	
14714	TSKgel SP-5PW (20)	250	15 - 25	0.06 - 0.12	20 - 40	
14715	TSKgel SP-5PW (20)	1,000	15 - 25	0.06 - 0.12	20 - 40	
18435	TSKgel SP-5PW (20)	5,000	15 - 25	0.06 - 0.12	20 - 40	
43282	TSKgel SP-5PW (30)	25	20 - 40	0.06 - 0.12	20 - 40	
14716	TSKgel SP-5PW (30)	250	20 - 40	0.06 - 0.12	20 - 40	
14717	TSKgel SP-5PW (30)	1,000	20 - 40	0.06 - 0.12	20 - 40	
18384	TSKgel SP-5PW (30)	5,000	20 - 40	0.06 - 0.12	20 - 40	
21807	TSKgel SP-5PW (30)	50,000	20 - 40	0.06 - 0.12	20 - 40	
21976	TSKgel SP-3PW (30)	25	20 - 40	0.07 - 0.12	>65 (insulin)	
21977	TSKgel SP-3PW (30)	250	20 - 40	0.07 - 0.12	>65 (insulin)	
21978	TSKgel SP-3PW (30)	1,000	20 - 40	0.07 - 0.12	>65 (insulin)	
21979	TSKgel SP-3PW (30)	5,000	20 - 40	0.07 - 0.12	>65 (insulin)	
21980	TSKgel SP-3PW (30)	50,000	20 - 40	0.07 - 0.12	>65 (insulin)	

* Adsorption capacity for insulin: 90-120 g/L resin