TOSOH

TOYOPEARL, TSKgel & Ca^{++}Pure-HA Bulk Resin

Experts in Chromatography

2019 Product Guide

TOYOPEARL® Bulk Resin • TSKgel® Bulk Resin • Ca^{++}Pure-HA® Resin • TSKgel U/HPLC Columns • EcoSEC® GPC Systems

TOSOH BIOSCIENCE

What's in a Name?

Would a resin by any other name purify as well? Tosoh Bioscience has the most comprehensive selection of process media resins, with a variety of pore and particle size combinations for several modes of chromatography. When it comes to naming our resins, we've got it down to a science (literally). Here's how you can identify the right resin for your purification process:

1. Resin Type:

Tosoh Bioscience offers 3 base beads for our resin products: TOYOPEARL, TSKgel, and Ca^{++}Pure-HA.

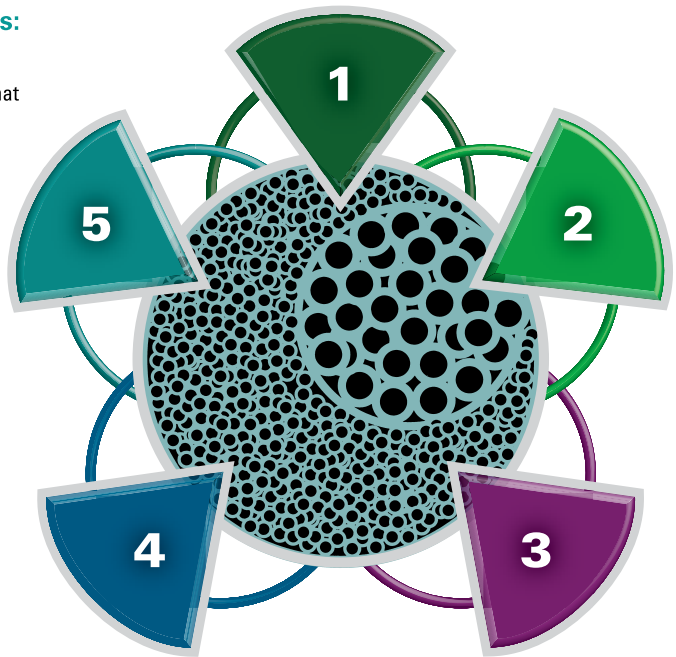
TOYOPEARL and TSKgel products are hydroxylated methacrylic polymer resins and are offered in many different pore sizes and particle diameters. The key differences between the two types are particle size availability, degree of crosslinking, dynamic binding capacity, and operating pressures. Since similarly functionalized TOYOPEARL HW and TSKgel PW resins have the same backbone polymer chemistry, the selectivity remains the same as you scale up or down.

Ca^{++}Pure-HA is a hydroxyapatite resin (a form of calcium phosphate) and has unique separation properties for biomolecules. Unlike TOYOPEARL and TSKgel resins, Ca^{++}Pure-HA is both the ligand and the base bead. Its highly selective nature often separates proteins otherwise shown to be homogeneous by other chromatographic techniques.

5. Additional abbreviations:

Some of our products have additional features or need clarification about what type of product they are. We use the following abbreviations to highlight these features:

HC = High Capacity
AR = Alkaline Resistant
MX = Mixed-Mode
AF = Affinity



2. Ligand:

TOYOPEARL or TSKgel resins are available in the following modes of chromatography functionalized with these ligands:

HIC:	*Ether, PPG, Phenyl, Butyl, Hexyl*
Anion Exchange:	*DEAE, QAE, Q, NH_2*
Cation Exchange:	*CM, SP, SO_3, Sulfate*
Affinity:	*Amino, Carboxy, Iminodiacetic acid, Epoxy, Formyl, Reactive Red, Tresyl, r-Protein A, r-Protein L*
Mixed-Mode:	*Tryptophan*
Hydroxyapatite:	*Calcium phosphate*

4. Particle Size:

Particle size is typically denoted in the product name as letters or numbers denoting the grade, except in Ca^{++}Pure-HA products.

Particle Size of TOYOPEARL and TSKgel Resins (µm)				
Grade	TOYOPEARL	TOYOPEARL GigaCap	TSKgel	Ca^{++}Pure-HA
EC	200			
C	100 (SEC resins are 75)			
M	65 (MX-Trp is 75)	75		
F	45			
S-	35 (SEC resins are 30)	35		
n/a				39
(30)			30	
(20)			20	

3. Pore Size: *(applies to TOYOPEARL and TSKgel resins only)*

TOYOPEARL and TSKgel Resin Number Key		
TOYOPEARL 550 resins	HW-55 base resin	50 nm pore size
TOYOPEARL 600 resins	HW-60 base resin	75 nm pore size
TOYOPEARL 650 resins	HW-65 base resin	100 nm pore size
TOYOPEARL 750 resins	HW-75 base resin	> 100 nm pore size
TSKgel 3PW resin	PW-3000 base resin	25 nm pore size
TSKgel 5PW reisn	PW-5000 base resin	100 nm pore size

For more detailed information, email us to request your Tosoh Bioscience Resin Selection Guide: info.tbl@tosoh.com

Contents

Get Started

Additional resources are available for helping you implement TOYOPEARL, TSKgel and Ca^{++}Pure-HA resins into your process:

Web
Visit tosohbioscience.com for videos, product information and ordering.

Email
Our technical service staff is ready to answer questions:
techservice.tbl@tosoh.com

In Person
A technical seminar can be arranged on-site or via the web.
Request via seminars@tosoh.com


TOYOPEARL®
HW-55F
Net Vol. 500 mL
Part No. 07457
Lot No.
FOR IN VITRO USE ONLY
TOSOH
TOYOPEARL®
HW-65F
Net Vol. 500 mL
Part No. 07465
Lot No.
FOR IN VITRO USE ONLY
TOSOH
TOYOPEARL®
HW-75F
Net Vol. 500 mL
Part No. 07469
Lot No.
FOR IN VITRO USE ONLY
TOSOH

TOYOPEARL HW-40

TOYOPEARL HW-50

TOYOPEARL HW-55

TOYOPEARL HW-65

TOYOPEARL HW-75

The Role of Size Exclusion Chromatography in Process Purification

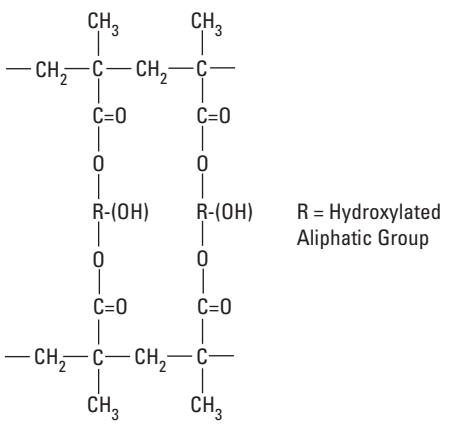
Size exclusion chromatography (SEC), also known as gel filtration chromatography, separates molecules in an aqueous mobile phase according to their hydrodynamic radius in solution as they pass through a porous structure. Molecules with a diameter greater than the largest pores within the resin are unable to enter the particle. Because they are excluded from the pores they travel quickly through the column and elute first. Smaller molecules, which are able to access pores within the resin particles, permeate a larger accessible volume within the column and are eluted later, in order of decreasing molar mass.

Because SEC has no adsorption capacity and its separation mechanism dilutes the sample during elution, it is not normally used in the capture or intermediate steps of manufacturing processes. It is most often used as a final polishing step where a target protein is being separated from its aggregates or other significantly different molar mass impurities. Another related application would be the desalting of the purified target protein in lieu of a more traditional diafiltration step.

TOYOPEARL HW SEC Resins

Tosoh Bioscience offers a number of TOYOPEARL HW resins for size exclusion chromatography (H = *hydrophilic,* W = *water-compatible)*. TOYOPEARL HW size exclusion resins are hydroxylated polymethacrylic polymer beads (Figure 1). Surface hydroxyl groups render these resins very hydrophilic, therefore minimal non-specific adsorption occurs, making the TOYOPEARL HW resins useful for protein separations. The semi-rigid polymeric nature and the narrow particle size distribution of these resins give them better pressure-flow properties than softer materials such as agarose. In addition, good mechanical stability of the TOYOPEARL HW resins produces excellent flow characteristics in large industrial size columns (up to 0.3 MPa).

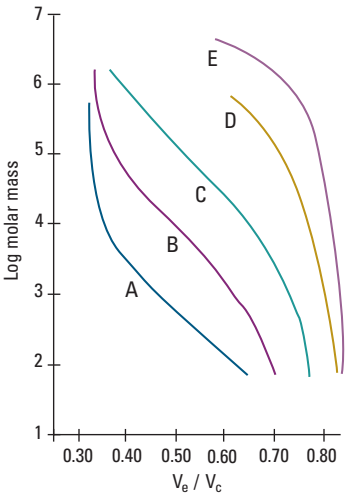
Figure 1: Resin Chemistry of TOYOPEARL HW resins (Hydroxylated Acrylic)



TOYOPEARL HW resins are chemically stable from pH 2-14. 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.

Commercial TOYOPEARL HW size exclusion resins are available in five pore sizes covering five different fractionation ranges, though there is some overlap among the listed ranges. The choice of TOYOPEARL HW resin depends on the molar mass of the feedstock components. Tables 1 and 2 show this information for proteins, dextrans and PEG (polyethylene glycol) polymers. The TOYOPEARL HW resin molar mass ranges span peptide and protein sizes between 100 to 5 x 10^7 daltons. Each TOYOPEARL HW resin exhibits a typical calibration curve and exclusion limit for globular proteins (Figure 2).

Figure 2: Calibration curves for globular proteins on TOYOPEARL resins

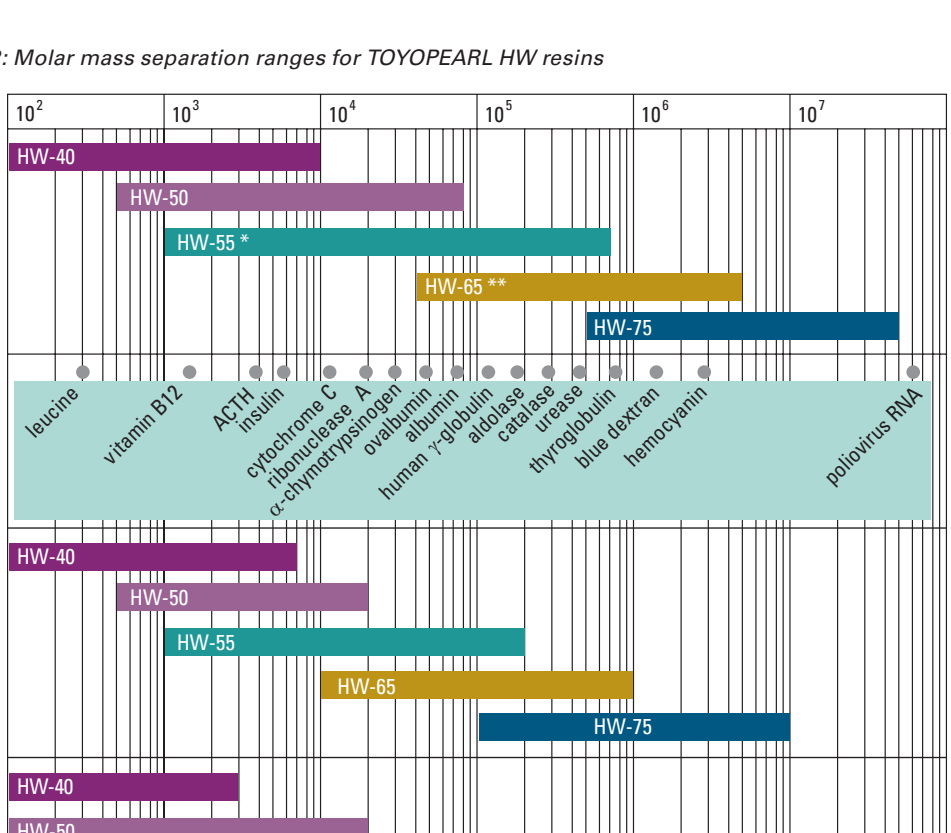


Resins:	**A. TOYOPEARL HW-40** **B. TOYOPEARL HW-50** **C. TOYOPEARL HW-55** **D. TOYOPEARL HW-65** **E. TOYOPEARL HW-75**
Column size:	22 mm ID × 30 cm
Mobile phase:	0.06 mol/L phosphate buffer, pH 7.0, + 0.06 mol/L KCl
Detection:	UV @ 280 nm
Temperature:	ambient
Samples:	protein standards
Legend:	V_e = elution volume V_c = column volume

Table 1: Properties and molar mass separation ranges of TOYOPEARL HW resins

			Molar mass of sample (Da)		
TOYOPEARL resin	**Particle size (µm)**	**Pore size (nm)**	**Polyethylene glycols and oxides**	**Dextrans**	**Globular proteins**
HW-40S HW-40F HW-40C	20 - 40 30 - 60 50 - 100	5	100 - 3,000	100 - 7,000	100 - 1×10^4
HW-50S HW-50F	20 - 40 30 - 60	12.5	100 - 1.8×10^4	500 - 2×10^4	500 - 8×10^4
HW-55S HW-55F	20 - 40 30 - 60	50	100 - 1.5×10^5	1,000 - 2×10^5	1,000 - 7×10^5
HW-65S HW-65F	20 - 40 30 - 60	100	500 - 1×10^6	1×10^4 - 1×10^6	4×10^4 - 5×10^6
HW-75S HW-75F	20 - 40 30 - 60	>100	4,000 - 5×10^6	1×10^5 - 1×10^7	5×10^5 - 5×10^7

Table 2: Molar mass separation ranges for TOYOPEARL HW resins



Globular proteins

* TOYOPEARL HW-55 is base material for some IEC and HIC products

** TOYOPEARL HW-65 is base material for most IEC, HIC, and AFC products

Calibration molecules

Dextrans

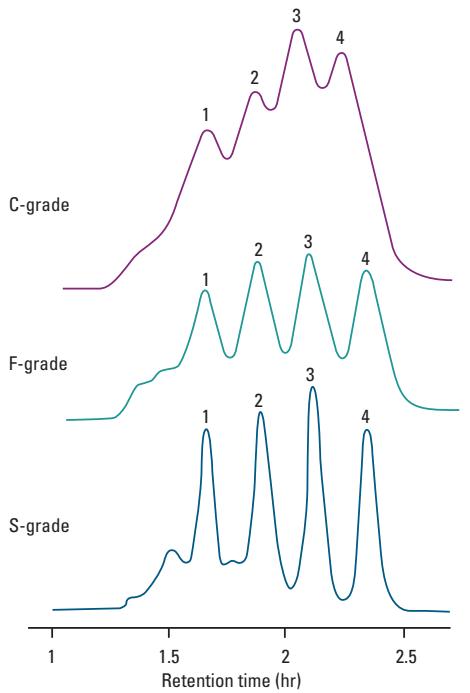
Polyethylene glycols

Resolution increases with decreasing particle size (Figure 3). Resin particle size is proportional to HETP and inversely proportional to the column efficiency and resolution of two peaks. Most TOYOPEARL HW resins are available in three particle size ranges:

- S-grade = 20 - 40 µm (Superfine)
- F-grade = 30 - 60 µm (Fine)
- C-grade = 50 - 100 µm (Coarse)

When the highest resolution is needed, the smaller S and F grade beads are preferred for process SEC. For desalting, where the resin is used in a filtration mode to remove the target from a buffer, the C grade is primarily employed because of its better flow dynamics at lower operating pressures. TOYOPEARL HW-40 is manufactured in an "EC-grade" (extra coarse) with a 100 - 300 µm bead.

Figure 3: Comparison of resolution on different particle sizes of TOYOPEARL HW-55 resin



Resin:	**TOYOPEARL HW-55**
Column size:	26 mm ID × 70 cm
Mobile phase:	33.3 mmol/L phosphate buffer, pH 7.0, 0.2 mol/L NaCl
Flow rate:	20 cm/hr (1.77 mL/min)
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	1 mL
Samples:	1. thyroglobulin (0.3%) 2. γ-globulin (0.3%) 3. β-lactoglobulin (0.3%) 4. cytochrome C (0.1%)

General properties of TOYOPEARL HW resins in aqueous eluents are detailed in Table 3. TOYOPEARL HW resins can be used in organic solvents or mixtures of organic solvents and water. Bed volumes may swell or shrink relative to water depending on the solvent as shown in Tables 4 and 5. DMSO can be used for SEC of oligosaccharides and polyethylene glycols. The compatibility of DMF with TOYOPEARL also permits SEC separation of hydrophobic substances such as polystyrenes.

Table 3: Properties of TOYOPEARL HW resins in aqueous eluents

High mechanical stability	All TOYOPEARL resins can be operated at pressures up to 3 bar without deformation.
Minimum change in gel bed volume	Changes in the column bed volume under operational salt conditions are negligible. TOYOPEARL does not shrink or swell even in high concentrations of strong denaturing agents such as urea or guanidine hydrochloride.
Chemical stability	TOYOPEARL is stable from pH 2-13 and can tolerate levels outside of that range (pH 0-14) for short periods of time. Biomolecules which are only soluble at extreme pH values can be readily separated.
Sharp chromatographic peaks	TOYOPEARL's narrow particle size distribution (min. 80% – within declared limits) results in better peak shapes and higher elution target concentrations than other SEC materials.
Temperature stability	TOYOPEARL HW SEC resins are thermally stable and do not degrade or denature even in boiling water. TOYOPEARL resins can be sterilized by autoclaving at 121 °C.
Microorganism resistance	TOYOPEARL is an organosynthetic material and is resistant to degradation by microorganisms.
Suitability for enzyme immobilization	TOYOPEARL resins contain numerous hydroxyl groups on the external and internal bead surfaces. These, in combination with the chemical stability of the polymer, make the resin well suited for the covalent bonding of enzymes or other ligands. Please see the affinity chromatography section for more information.

Table 4: Swelling properties in various solvents

Mobile phase	TOYOPEARL resin				
	HW-40	HW-50	HW-55	HW-65	HW-75
Water	100	100	100	100	100
0.2 mol/L KCl	100	100	100	100	100
MeOH	100	100	100	100	105
EtOH	100	100	100	100	110
DMF	110	110	105	105	120
Acetone	80	80	85	90	110
Toluene	65	70	70	75	90

Table 5: Additional swelling data for TOYOPEARL HW-40 resin

TOYOPEARL resin	Mobile phase				
	DMSO	Ethyl Acetate	Benzene	$CHCl_3$	$CHCl_3$/ MeOH (1:1)
HW-40	140	80	70	105	120

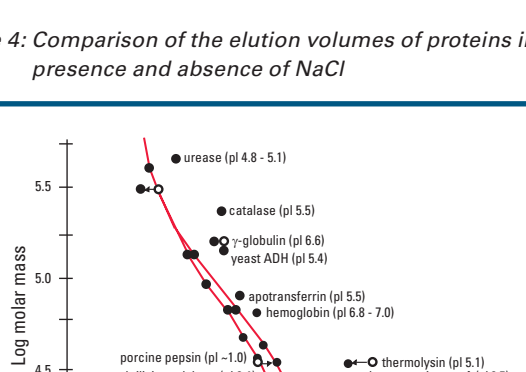
Mobile phase components, such as salts, can affect SEC separations. The presence or absence of sodium chloride influences the elution volume of proteins. This is demonstrated in Figure 4, in which a mixture of various proteins was separated on a column packed with TOYOPEARL HW-55F resin. Salt concentrations can change the hydrodynamic radius of proteins and either increase or decrease their molar mass as a function of salt strength.

TOYOPEARL HW resins are commonly used in size exclusion chromatography and desalting applications though they can be used for other important functions, such as:

- Removal of surfactants such as Triton® X-100 from biological solutions by an adsorption mechanism
- Use in hydrophobic interaction chromatography (HIC) for the separation of very hydrophobic molecules
- Use in HIC separations as a guard column for hydrophobic impurities
- Possible use as a stationary phase for either normal or reversed phase separations depending on solvent system selected

All of the physical and chemical properties discussed for the TOYOPEARL HW SEC resins make them an excellent choice for use as the base beads for the ion exchange, hydrophobic interaction, mixed-mode, and affinity chromatographic resins discussed in the later sections of this catalog.

Figure 4: Comparison of the elution volumes of proteins in presence and absence of NaCl

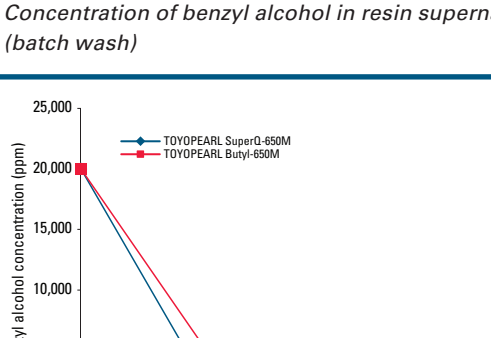


Resin:	**TOYOPEARL HW-55F**
Column size:	22 mm ID × 50 cm
Mobile phase:	25 mmol/L Tris-HCl with (•) or without (•) 0.5 mol/L NaCl, pH 7.5
Flow rate:	16 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm, 420 nm for heme proteins, 200 nm for proteins without aromatic amino acids
Temperature:	5-10 °C

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

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

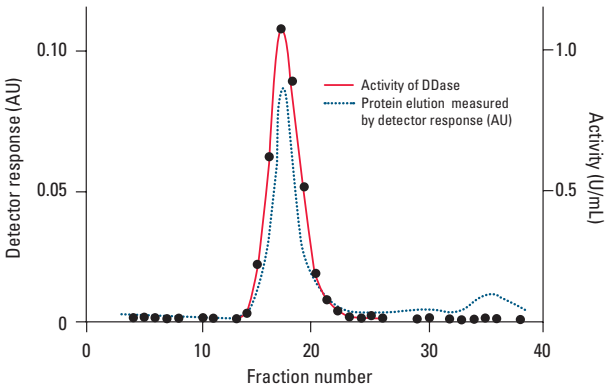
Figure 5: Concentration of benzyl alcohol in resin supernatant (batch wash)



Polishing Step for Enzyme Purification

TOYOPEARL HW SEC resins are an excellent choice when looking for a polishing step for enzyme purification. Dextrin dextranase, a 300 kDa enzyme, was purified from *Acetobacter capsulatus* using a two-step process consisting of TOYOPEARL Phenyl-650M and TOYOPEARL HW-65S. The elution profile of Dextrin dextranase is shown in (Figure 6). Due to the hydrophobic nature of the enzyme, it aggregates in 100% aqueous mobile phases, thus it was necessary to add 40% ethylene glycol to the mobile phase.

Figure 6: Elution profile of protein and activity on TOYOPEARL HW-65S

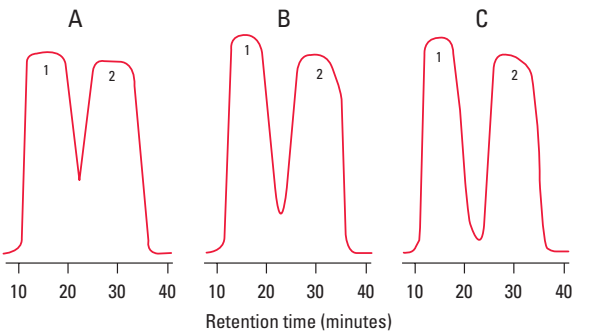


Resin:	**TOYOPEARL HW-65S**
Column size:	16 mm ID × 80 cm
Mobile phase:	20 mol/L acetate buffer with 40% ethylene glycol, pH 4.8
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	concentrated active fractions from TOYOPEARL Phenyl-650 column

Desalting Step for Proteins

Though SEC is typically used as the polishing step in a purification process, it can also be used as an ideal desalting step for proteins that may be sensitive to membrane concentration and diafiltration steps. TOYOPEARL HW-40F allows for high total protein and activity recovery, allowing the operator to use it as a desalting resin. Figure 7 demonstrates the effect of the ionic strength of a volatile salt on the desalting of bovine serum albumin (BSA) from sodium acetate. It is important to note that the loading volumes for a desalting application are much higher than for regular SEC purifications. As much as 25% of the bed volume can be loaded for desalting steps, compared with 1% to 5% of the bed volume for normal SEC purifications.

Figure 7: Desalting of bovine serum albumin



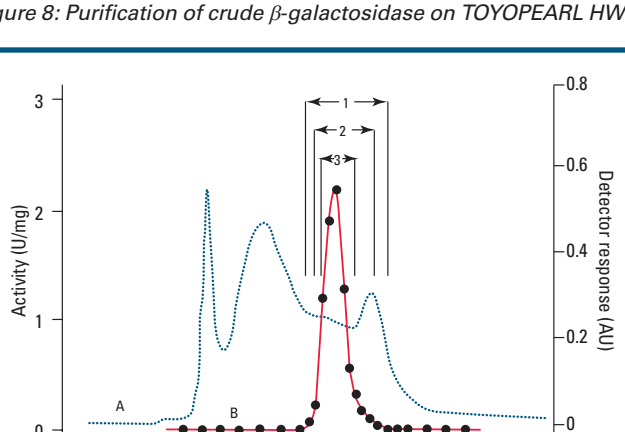
Resin:	**TOYOPEARL HW-40F**
Column size:	7.5 mm ID× 30 cm
Mobile phase:	A. distilled water B. 10 mmol/L ammonium formate C. 50 mmol/L ammonium formate
Flow rate:	48 cm/hr (0.4 mL/min)
Detection:	UV @ 220 nm
Temperature:	25 °C
Samples:	2.5 mL of: 1. bovine serum albumin (0.1%) 2. sodium acetate (10%)

Recovery of Activity

Recovery of activity is a very important consideration when purifying an enzyme. As shown in Figure 8, crude β-galactosidase has been purified using TOYOPEARL HW-55F with excellent recovery yields (Table 6).

The 1, 2, and 3 brackets are representative of the pooling of fractions and what the yield and recoveries would be and are listed in Table 6. The widest pool sample corresponds to the greatest recovery.

Figure 8: Purification of crude β-galactosidase on TOYOPEARL HW-55F



Resin:	**TOYOPEARL HW-55F**
Column size:	25 mm ID × 55 cm × 2
Mobile phase:	0.2 mol/L phosphate, pH 6.7
Flow rate:	12 cm/hr (1.0 mL/min)
Detection:	A. UV @ 280 nm B. enzymatic activity
Temperature:	22 °C
Sample:	3 mL containing 45 mg of crude β-galactosidase

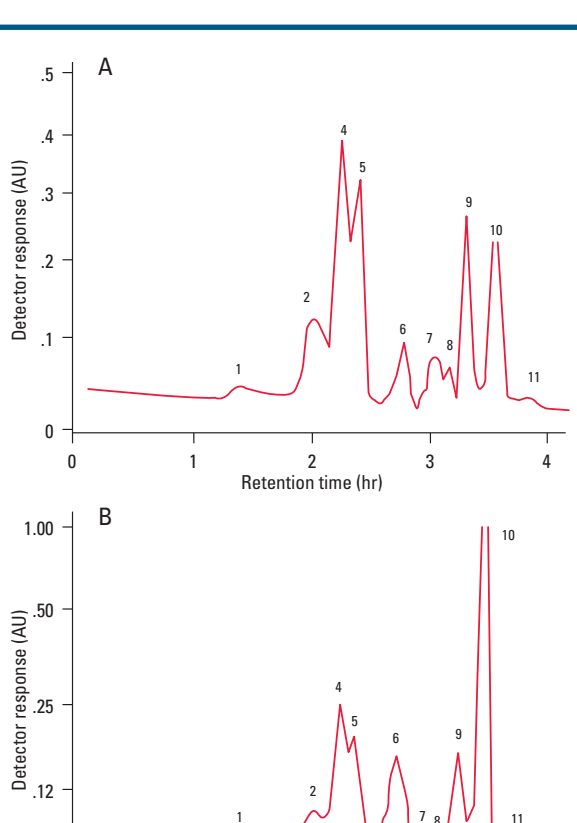
Table 6: Purification of crude β-galactosidase on TOYOPEARL HW-55F

Fraction	Yield (%)	Specific activity (units/mg)	Degree of purification
Original sample		0.95	
1	94	2.8	2.9 ×
2	93	3.7	3.9 ×
3	83	6.4	6.7 ×

Antibody Separation

Antibodies have been separated from bovine colostral whey and human serum using TOYOPEARL HW-55F resin. Figure 9 shows the separation of colostral whey on TOYOPEARL HW-55F after centrifugation. Peak #2 is IgG_1, and the chromatogram shows both the 254 and 280 nm absorbance profiles.

Figure 9: Elution profiles of colostral whey

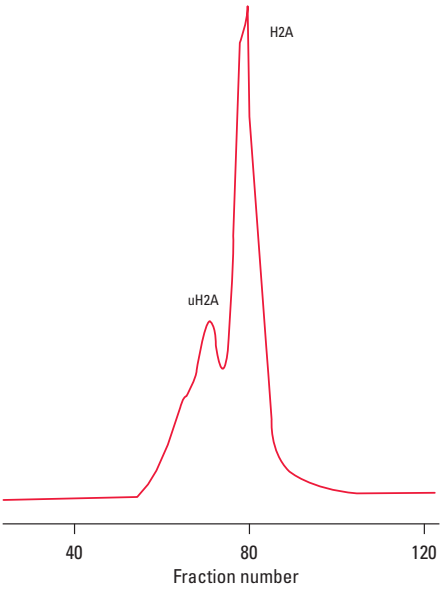


Resin:	**TOYOPEARL HW-55F**
Column size:	17 mm ID × 64 cm
Mobile phase:	25 mmol/L Tris-HCl with 0.3 mol/L NaCl, pH 7.5
Flow rate:	18 cm/hr (0.7 mL/min)
Detection:	A. UV @ 280 nm B. UV @ 254 nm
Temperature:	23 °C
Samples:	1. unknown 2. IgG_1 3. serum albumin 4. β-lactoglobulin (dimer) 5. α-lactalbumin 6 - 12. unknown

Isolation Based on Polypeptide Difference

TOYOPEARL HW-50S can help to isolate the ubiquitin-histone conjugate uH2A from the unicellular protozoan *Tetrahymena pyriformis*. Figure 10 shows the separation of uH2A from the histone, H2A. The sole difference between these two components is a small polypeptide, ubiquitin (approximately 8,500 Da).

Figure 10: Isolation of a complex protein conjugate on TOYOPEARL HW-50S

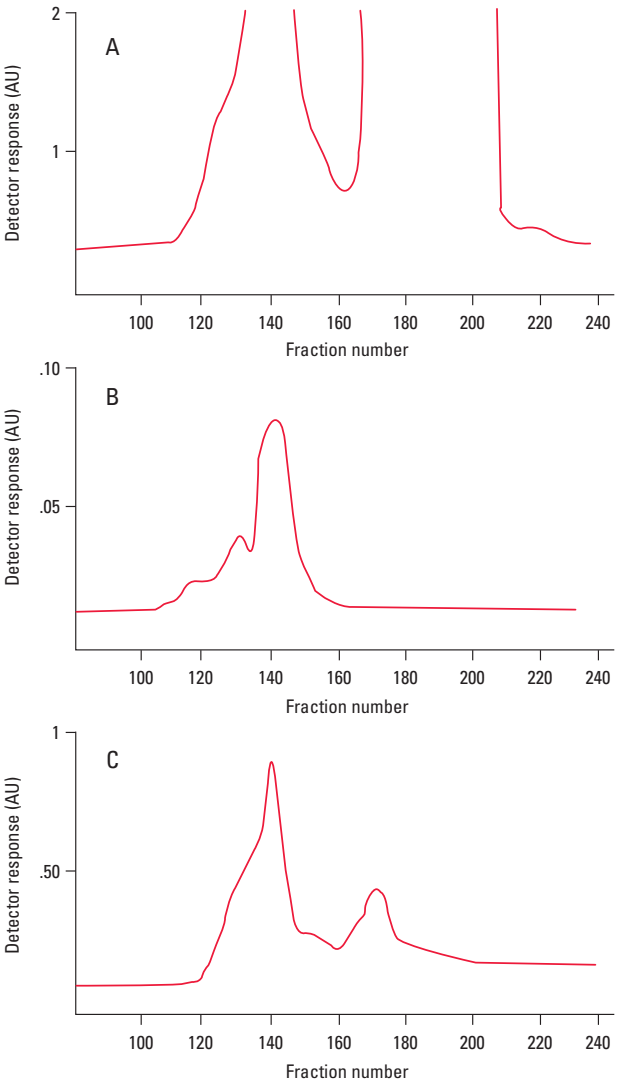


Resin:	**TOYOPEARL HW-50S**
Column size:	22 mm ID × 83 cm
Mobile phase:	10 mmol/L HCl
Flow rate:	1.6 cm/hr (0.1 mL/min)
Detection:	UV @ 230 nm
Sample:	fraction of crude *Tetrahymena* H2A containing the ubiquitin-histone conjugate uH2A

Amylose Isolation

TOYOPEARL HW-75F resins, with pores larger than 100 nm, has been used in place of ultra-centrifugation steps for purification of plasmid DNA. Ultra-centrifugation is a time-consuming process and requires expensive chemicals, such as cesium chloride. TOYOPEARL HW-75F resin provides superior separation performance for plasmid DNA, and also provides high yields. Figure 11 shows the separation of crude pBR322 DNA from contaminating RNA species using TOYOPEARL HW-75F.

Figure 11: Separation of pBR322 DNA



Resin:	**TOYOPEARL HW-75F**
Column size:	16 mm ID × 130 cm
Mobile phase:	10 mmol/L Tris-HCl, 1 mmol/L EDTA, 0.2 mol/L NaCl, pH 8
Flow rate:	7 cm/hr (0.23 mL/min)
Detection:	UV @ 260 nm
Temperature:	ambient
Samples:	A. crude plasmid DNA extract B. DNA from A C. DNA from hydroxyapatite chromatography

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

Ordering Information

TOYOPEARL SEC resins:

Conditions: Exclusion limits are +/- 30% and are determined using PEG, PEO, or dextran standards, as appropriate.

Part #	Product description	Container size (mL)	Bead diameter (µm)	Exclusion limit (Da)
19809	TOYOPEARL HW-40S	150	20-40	3×10^3
07451	TOYOPEARL HW-40S	250	20-40	3×10^3
07447	TOYOPEARL HW-40S	500	20-40	3×10^3
14681	TOYOPEARL HW-40S	1,000	20-40	3×10^3
07967	TOYOPEARL HW-40S	5,000	20-40	3×10^3
19808	TOYOPEARL HW-40F	150	30-60	3×10^3
07448	TOYOPEARL HW-40F	500	30-60	3×10^3
14682	TOYOPEARL HW-40F	1,000	30-60	3×10^3
07968	TOYOPEARL HW-40F	5,000	30-60	3×10^3
19807	TOYOPEARL HW-40C	150	50-100	3×10^3
07449	TOYOPEARL HW-40C	500	50-100	3×10^3
14683	TOYOPEARL HW-40C	1,000	50-100	3×10^3
07969	TOYOPEARL HW-40C	5,000	50-100	3×10^3
21484	TOYOPEARL HW-40C	50,000	50-100	3×10^3
07450	TOYOPEARL HW-40EC	500	100-300	3×10^3
07970	TOYOPEARL HW-40EC	5,000	100-300	3×10^3
19811	TOYOPEARL HW-50S	150	20-40	1.8×10^4
07455	TOYOPEARL HW-50S	250	20-40	1.8×10^4
07452	TOYOPEARL HW-50S	500	20-40	1.8×10^4
14684	TOYOPEARL HW-50S	1,000	20-40	1.8×10^4
08059	TOYOPEARL HW-50S	5,000	20-40	1.8×10^4
19810	TOYOPEARL HW-50F	150	30-60	1.8×10^4
07453	TOYOPEARL HW-50F	500	30-60	1.8×10^4
14685	TOYOPEARL HW-50F	1,000	30-60	1.8×10^4
08060	TOYOPEARL HW-50F	5,000	30-60	1.8×10^4
18368	TOYOPEARL HW-50F	50,000	30-60	1.8×10^4
19813	TOYOPEARL HW-55S	150	20-40	1.5×10^5
07459	TOYOPEARL HW-55S	250	20-40	1.5×10^5
07456	TOYOPEARL HW-55S	500	20-40	1.5×10^5
14686	TOYOPEARL HW-55S	1,000	20-40	1.5×10^5
08062	TOYOPEARL HW-55S	5,000	20-40	1.5×10^5

Part #	Product description	Container size (mL)	Bead diameter (µm)	Exclusion limit (Da)
19812	TOYOPEARL HW-55F	150	30-60	1.5×10^5
07457	TOYOPEARL HW-55F	500	30-60	1.5×10^5
14687	TOYOPEARL HW-55F	1,000	30-60	1.5×10^5
08063	TOYOPEARL HW-55F	5,000	30-60	1.5×10^5
21918	TOYOPEARL HW-55F	50,000	30-60	1.5×10^5
19815	TOYOPEARL HW-65S	150	20-40	1×10^6
07467	TOYOPEARL HW-65S	250	20-40	1×10^6
07464	TOYOPEARL HW-65S	500	20-40	1×10^6
14688	TOYOPEARL HW-65S	1,000	20-40	1×10^6
08068	TOYOPEARL HW-65S	5,000	20-40	1×10^6
18377	TOYOPEARL HW-65S	50,000	20-40	1×10^6
19814	TOYOPEARL HW-65F	150	30-60	1×10^6
07465	TOYOPEARL HW-65F	500	30-60	1×10^6
14689	TOYOPEARL HW-65F	1,000	30-60	1×10^6
08069	TOYOPEARL HW-65F	5,000	30-60	1×10^6
21852	TOYOPEARL HW-65F	50,000	30-60	1×10^6
21481	TOYOPEARL HW-65C	150	50-100	1×10^6
07466	TOYOPEARL HW-65C	500	50-100	1×10^6
14690	TOYOPEARL HW-65C	1,000	50-100	1×10^6
08070	TOYOPEARL HW-65C	5,000	50-100	1×10^6
21482	TOYOPEARL HW-65C	50,000	50-100	1×10^6
07471	TOYOPEARL HW-75S	250	20-40	8.25×10^6
07468	TOYOPEARL HW-75S	500	20-40	8.25×10^6
08071	TOYOPEARL HW-75S	5,000	20-40	8.25×10^6
19816	TOYOPEARL HW-75F	150	30-60	8.25×10^6
07469	TOYOPEARL HW-75F	500	30-60	8.25×10^6
14691	TOYOPEARL HW-75F	1,000	30-60	8.25×10^6
08072	TOYOPEARL HW-75F	5,000	30-60	8.25×10^6

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 NH_2-750F	TOYOPEARL Sulfate-650F
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 five ligands for anion exchange (Q, SuperQ, QAE, DEAE, and NH_2) and four ligands for cation exchange chromatography (S, SP, Sulfate, 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 pKa	DBC (g/L)	Pressure rating
NH_2-750F	Weak Anion	HW-75	> 100 nm	45 µm	8.5	≥ 70	0.3 MPa
SuperQ-650C	Strong Anion	HW-65	100 nm	100 µm	12.2	105 - 155	0.3 MPa
DEAE-650C	Weak Anion	HW-65	100 nm	100 µm	11.5	25 - 35	0.3 MPa
QAE-550C	Strong Anion	HW-55	50 nm	100 µm	12.2	60 - 80	0.3 MPa
Q-600C AR	Strong Anion	HW-60	75 nm	100 µm	12.2	> 120	0.3 MPa
GigaCap Q-650M	Strong Anion	HW-65	100 nm	75 µm	12.2	≥ 162	0.3 MPa
GigaCap DEAE-650M	Weak Anion	HW-65	100 nm	75 µm	11.5	> 156	0.3 MPa
SuperQ-650M	Strong Anion	HW-65	100 nm	65 µm	12.2	105 - 155	0.3 MPa
DEAE-650M	Weak Anion	HW-65	100 nm	65 µm	11.5	25 - 35	0.3 MPa
SuperQ-650S	Strong Anion	HW-65	100 nm	35 µm	12.2	105 - 155	0.3 MPa
DEAE-650S	Weak Anion	HW-65	100 nm	35 µm	11.5	25 - 35	0.3 MPa
GigaCap Q-650S	Strong Anion	HW-65	100 nm	35 µm	12.2	> 170	0.3 MPa
Sulfate-650F	Strong Cation	HW-65	100 nm	45 µm	N/A	≥ 114	0.3 MPa
MegaCap II SP-550EC	Strong Cation	HW-55	50 nm	200 µm	1.2	100 - 155	0.3 MPa
SP-650C	Strong Cation	HW-65	100 nm	100 µm	1.2	35 - 55	0.3 MPa
SP-550C	Strong Cation	HW-55	50 nm	100 µm	1.2	80 - 120	0.3 MPa
CM-650C	Weak Cation	HW-65	100 nm	100 µm	4.7	25 - 45	0.3 MPa
GigaCap S-650M	Strong Cation	HW-65	100 nm	75 µm	1.2	136 - 176	0.3 MPa
GigaCap CM-650M	Weak Cation	HW-65	100 nm	75 µm	3.6	> 110	0.3 MPa
SP-650M	Strong Cation	HW-65	100 nm	65 µm	1.2	40 - 60	0.3 MPa
CM-650M	Weak Cation	HW-65	100 nm	65 µm	4.7	30 - 50	0.3 MPa
SP-650S	Strong Cation	HW-65	100 nm	35 µm	1.2	40 - 60	0.3 MPa
CM-650S	Weak Cation	HW-65	100 nm	35 µm	3.6	30 - 50	0.3 MPa
GigaCap S-650S	Strong Cation	HW-65	100 nm	35 µm	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 pKa	DBC (g/L)	Pressure rating
DEAE-5PW (20)	Weak Anion	G5000PW	100 nm	20 µm	11.5	25 -45	2.0 MPa
DEAE-5PW (30)	Weak Anion	G5000PW	100 nm	30 µm	11.5	20 - 40	2.0 MPa
SuperQ-5PW (20)	Strong Anion	G5000PW	100 nm	20 µm	12.2	52 - 88	2.0 MPa
SuperQ-5PW (30)	Strong Anion	G5000PW	100 nm	30 µm	12.2	52 - 88	2.0 MPa
SP-3PW (30)	Strong Cation	G3000PW	25 nm	30 µm	1.2	> 65	2.0 MPa
SP-5PW (20)	Strong Cation	G5000PW	100 nm	20 µm	1.2	20 - 40	2.0 MPa
SP-5PW (30)	Strong Cation	G5000PW	100 nm	30 µm	1.2	20 - 40	2.0 MPa

Table 3: Resolution of TOYOPEARL and TSKgel ion exchange resins

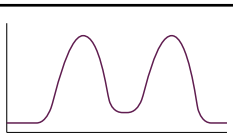
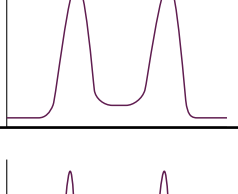
Resolution		Bead diameter (µm)	Pore size (nm)	Resins	
				Anion	Cation
Low		200	50		TOYOPEARL MegaCap II SP-550EC
		100	100 100 50	TOYOPEARL SuperQ-650C TOYOPEARL DEAE-650C TOYOPEARL QAE-550C	TOYOPEARL SP-650C TOYOPEARL CM-650C TOYOPEARL SP-550C
Medium		75	100 100	TOYOPEARL GigaCap Q-650M TOYOPEARL GigaCap 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		45	100	TOYOPEARL NH_2-750F	TOYOPEARL Sulfate-650F
		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)

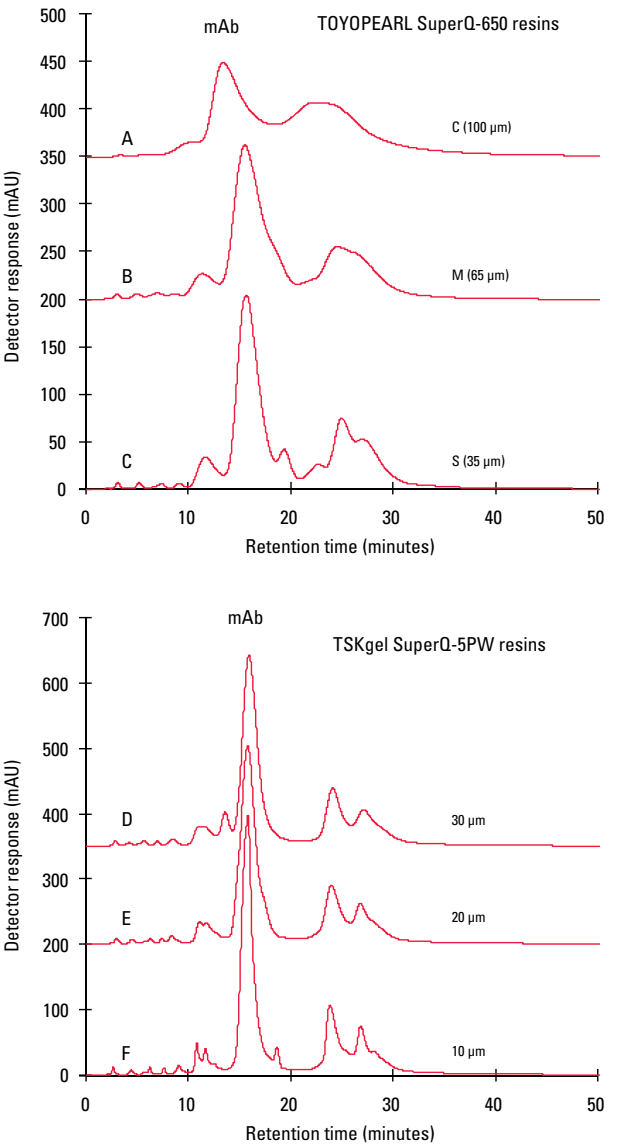
Table 4: DBCs of different chromatography modes

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

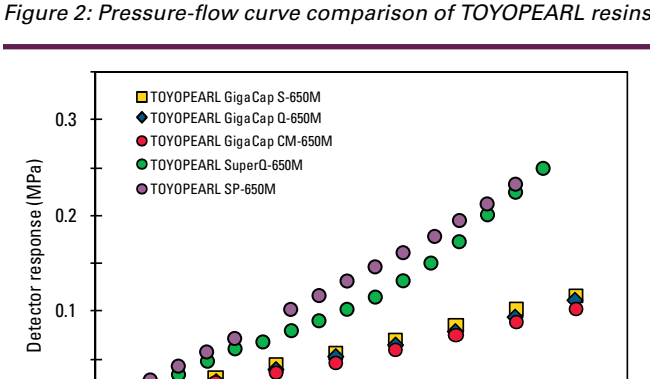


Resins:
A. TOYOPEARL SuperQ-650C, 100 µm
B. TOYOPEARL SuperQ-650M, 65 µm
C. TOYOPEARL SuperQ-650S, 35 µm
D. TSKgel SuperQ-5PW(30), 30 µm
E. TSKgel SuperQ-5PW(20), 20 µm
F. TSKgel SuperQ-5PW, 10 µm

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

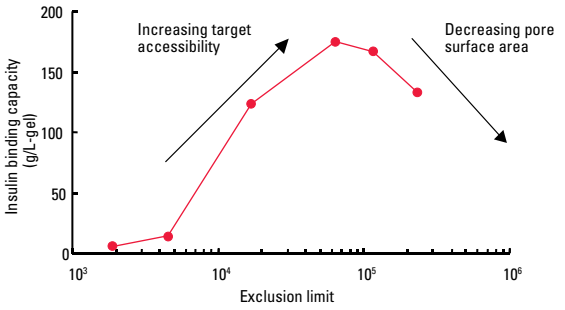
Pore size (nm)	5	12.5	40-50	75	100	>100	>170
Resin							
TOYOPEARL HW-type:	40	50	55	60	65	75	80
TSKgel PW-type:	G1000	G2000	G4000		G5000	G6000	

Increasing pore surface area

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

Base bead	TOYOPEARL HW-75	TOYOPEARL HW-65 or TSKgel G5000PW	TOYOPEARL HW-60	TOYOPEARL HW-55	TSKgelG3000PW
Pore diameter	**>100 nm**	**100 nm**	**75 nm**	**50 nm**	**25 nm**
Resin	TOYOPEARL NH_2-750	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 Sulfate-650	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

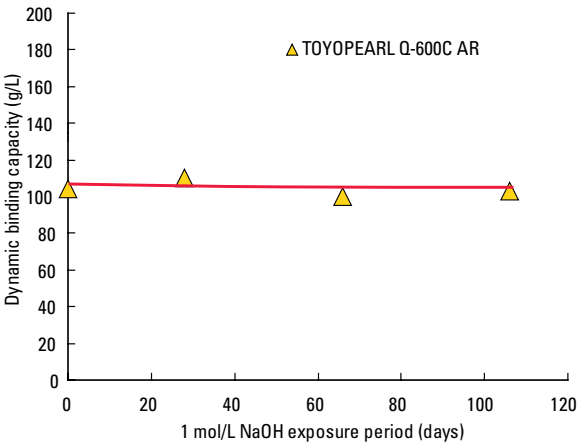
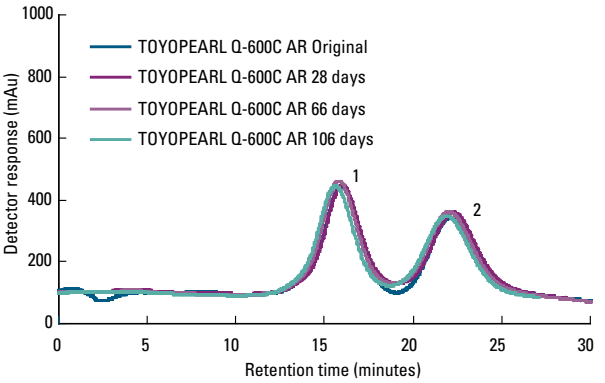


Figure 5: Stability of TOYOPEARL Q-600C AR resin after exposure to 1 mol/L NaOH

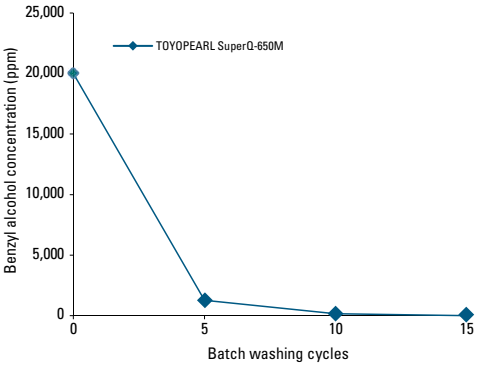


Resin:	**TOYOPEARL Q-600C AR**
Column size:	6.0 mm ID × 4 cm
Mobile phase:	Buffer A: 0.05 mol/L Tris-HCl buffer, pH 8.5 Buffer B: 0.05 mol/L Tris-HCl buffer + 1.0 mol/L NaCl, pH 8.5
Gradient:	60 min linear gradient from buffer A to buffer B
Flow rate:	212 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Samples: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

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

Samples of the filtered supernatant from the TOYOPEARL SuperQ-650M resin was taken after the 5th, 10th, and 15th washes and analyzed for benzyl alcohol concentration (Figure 6). As demonstrated in the figure, a 2% benzyl alcohol solution can be effectively removed from the TOYOPEARL SuperQ-650M resin by thorough washing with DI water.

Figure 6: Concentration of benzyl alcohol in resin supernatant (batch wash)



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 NH_2-750F Sulfate-650F	

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 Sulfate-650F Resin

TOYOPEARL Sulfate-650F resin is a novel strong cation exchange resin that exhibits high salt tolerance. This resin offers the strongest capture of monoclonal antibody (mAb) aggregates over a wide pH range without losing its binding capacity for mAb. With the use of optimized binding conditions, a dynamic binding capacity of ≥114 g/L of mAb can be easily achieved with TOYOPEARL Sulfate-650F resin. This high dynamic binding capacity translates into lower operating costs per gram of antibody produced.

A TOYOPEARL HW-65F polymeric bead has been functionalized with a sulfate (SO_4^-) group. The 100 nm pore size of this resin, along with proprietary bonding technology, makes TOYOPEARL Sulfate-650F resin ideal for applications performed in physiological conditions or for post-protein A removal of aggregates. The 45 µm particle size is stable up to 0.3 MPa.

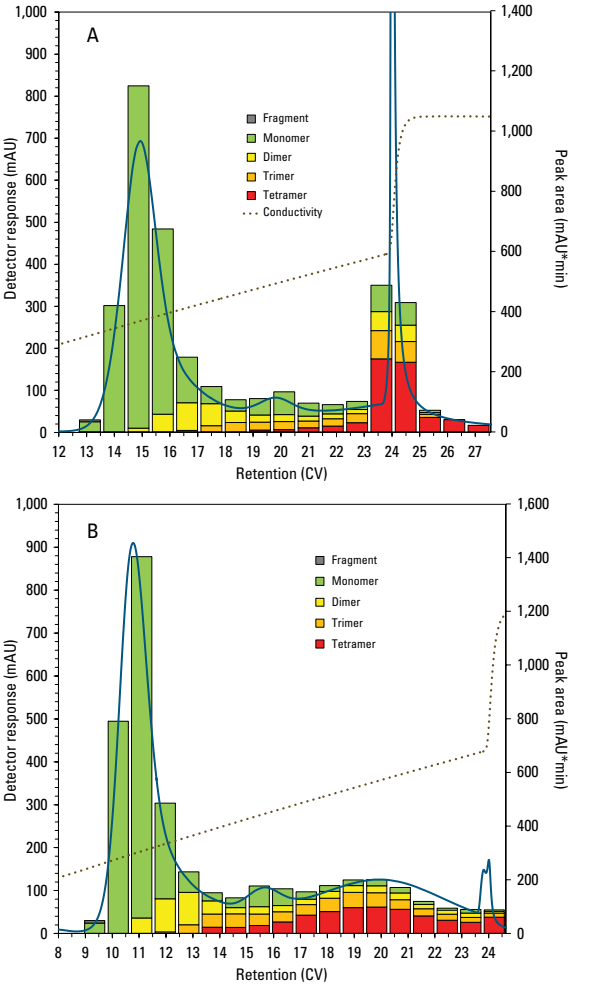
TOYOPEARL Sulfate-650F resin offers:

- **Effective removal of aggregates from IgG**
- **Wide pH working range –** works well with pH 5.0 to 6.0 without losing its binding capacity for IgG
- **High dynamic binding capacity**
- **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 7. 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 7: 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 8 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.

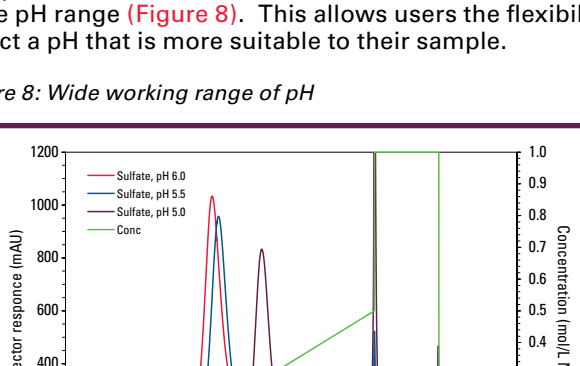
Table 8: The reduction of impurities from IgG sample, post-protein A, after passing through TOYOPEARL Sulfate-650F resin

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.TOYOPEARL Sulfate-650F resin can be used within a wide pH range (Figure 8). This allows users the flexibility to select a pH that is more suitable to their sample.

Figure 8: Wide working range of pH

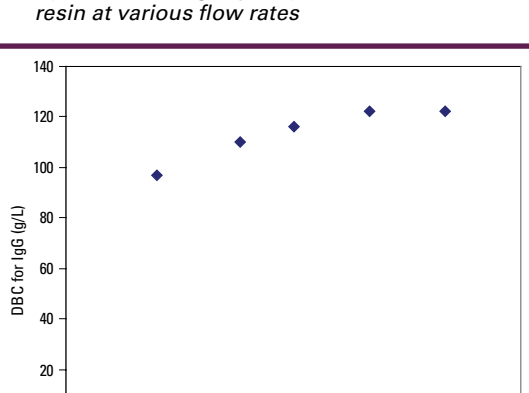


Resin:	**TOYOPEARL Sulfate-650F**
Column:	6.6 mm × 20 cm (6.8 mL)
Mobile phase:	A: 0.1 mol/L sodium acetate, pH 5.0 and 5.5, or MES, pH 6.0 B: mobile phase A + 1 mol/L NaCl
Gradient:	0 - 50% B, 20 CV 100% B, 5 CV
Flow rate:	300 cm/hr (4 min residence time)
Detection:	UV @ 280 nm (mAU), conductivity (mS/cm)
Temperature:	ambient
Injection vol.:	6.8 mL (10 mg/mL-resin load ratio)
Sample:	10 g/L IgG (protein A-purified, followed by aggregate induction)
Instrument:	ÄKTA avant 25

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 9.

Figure 9: Dynamic binding capacity of TOYOPEARL Sulfate-650F resin at various flow rates

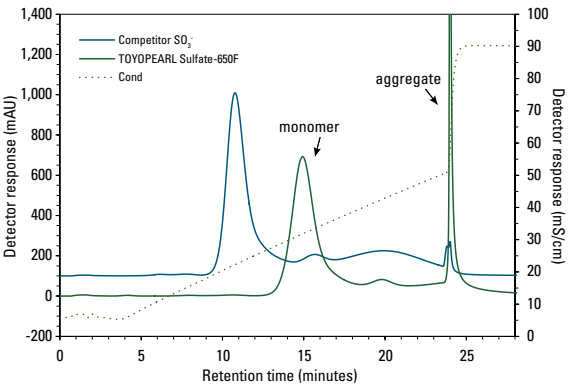


Resin:	**TOYOPEARL Sulfate-650F**
Column size:	6.0 mm ID × 4 cm
Mobile phase:	0.15 mol/L NaCl + 0.054 mol/L acetate buffer, pH 4.7
Residence time:	1.2, 2.3, 3.0, 4.0 or 5.0 min
Sample:	polyclonal human IgG, 1.0 g/L

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 10. 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 10: Salt tolerance comparison

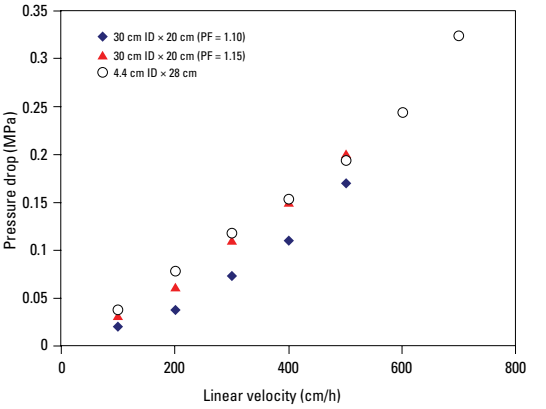


Resins:	**TOYOPEARL Sulfate-650F**
	Competitor SO_3^-
Column:	6.6 mm × 20 cm (6.8 mL)
Mobile phase:	A: 0.1 mol/L sodium acetate, pH 5.0
	B: mobile phase A + 1 mol/L NaCl
Gradient:	0 - 50% B, 20 CV
	100% B, 5 CV
Flow rate:	300 cm/hr (4 min residence time)
Detection:	UV @ 280 nm (mAU), conductivity (mS/cm)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

Excellent Pressure-Flow Stability

Figure 11 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 11: Pressure-flow rate curve on large process column (30 cm ID × 20 cm bed height)

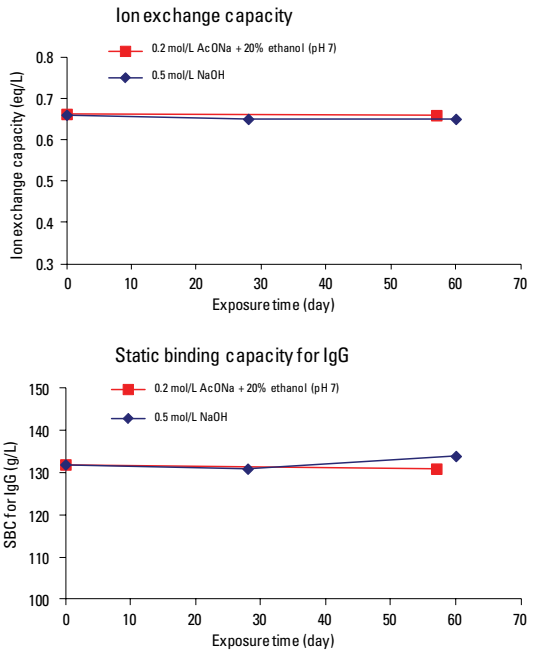


Column system:	**AxiChrom® 30 cm ID × 20 cm, QuickScale 4.4 cm ID × 28 cm Packing Factor (PF): 1.10 or 1.15**
Packing:	Slurry 50% in 0.2 mol/L NaCl
Eluent:	water

Durability at High pH

TOYOPEARL Sulfate-650F resin is stable in 0.5 mol/L NaOH (Figure 12). It can be stored in this solution for up to 8 weeks without loss in its binding capacity..

Figure 12: Alkaline stability of TOYOPEARL Sulfate-650F



TOYOPEARL NH_2-750F Resin

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.

TOYOPEARL NH_2-750F resin offers:

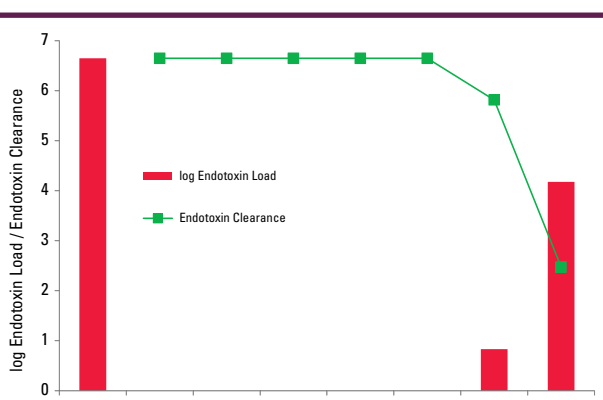
- **Effective endotoxin and viral removal –** in flowthrough chromatography mode; a clearance of >4 logs can be achieved
- **Removal of mAb aggregates –** in bind-and-elute and flowthrough chromatography mode
- **High salt tolerance –** samples containing ≥150 mmol/L NaCl can be loaded on the resin
- **Excellent pressure-flow characteristics –** resin can tolerate >600 cm/hr
- **Alkaline stability –** resin can be exposed to 0.5 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 13, 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 13: Endotoxin clearance using TOYOPEARL NH_2-750F



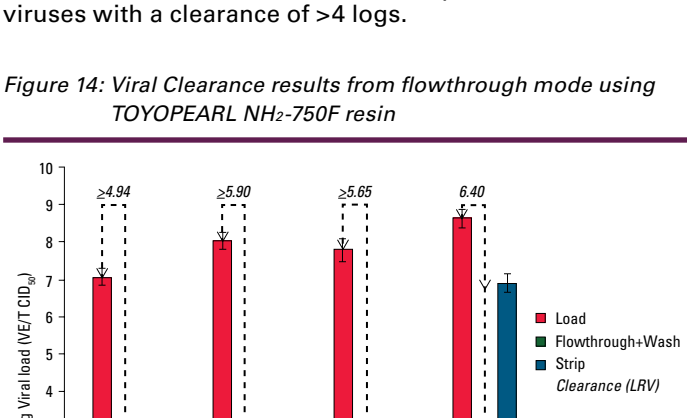
Resin:	**TOYOPEARL NH_2-750F**
Column size:	MiniChrom, 8.0 mm ID × 10.0 cm (5 mL)
Mobile phase:	A: 20 mmol/L Tris base, pH 7.4
	B: 20 mmol/L Tris base, 1 mol/L NaCl, pH 7.4
Flow rate:	150 cm/hr (1.25 mL/min, 4 min residence time)
Detection:	LAL endotoxin assay
Temperature:	ambient
Sample:	89,000 EU/ml 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 14, TOYOPEARL NH_2-750F resin effectively removed all viruses with a clearance of >4 logs.

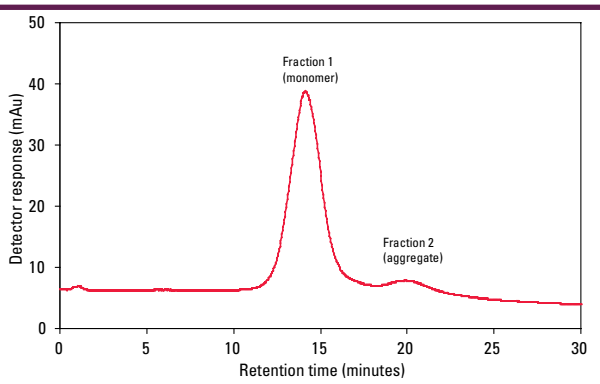
Figure 14: Viral Clearance results from flowthrough mode using TOYOPEARL NH_2-750F resin



Removal of mAb Aggregates

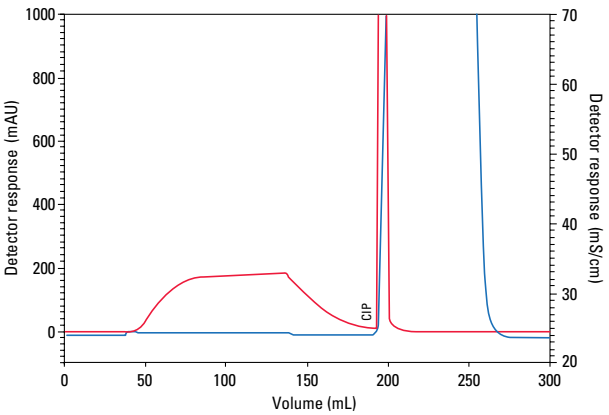
TOYOPEARL NH_2-750F is effective at removing aggregates from mAbs, in both bind-and-elute mode as well as in flow-through, as demonstrated in Figures 15a and 15b. SEC analysis of the peaks (data not shown) shows that high molecular weight aggregates are completely removed from the main mAb peak.

Figure 15a: Removal of aggregates from IgG_1 monomer on TOYOPEARL NH_2-750F



Resin:	**TOYOPEARL NH_2-750F**
Column size:	5 cm 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 15b: Flow-through removal of aggregates from mAb monomer on TOYOPEARL NH_2-750F

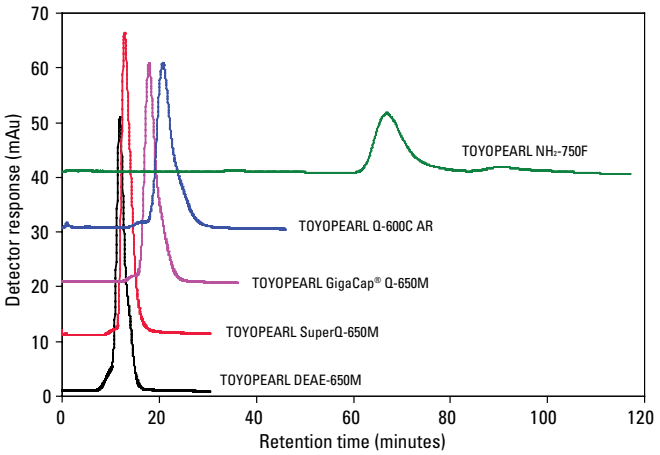


Resin:	**TOYOPEARL NH_2-750F**
Column size:	6.6 mm I.D. × 6 cm
Mobile phase:	A: 10 mmol/L Tris-HCl, 250 mmol/L NaCl, pH 7.0 B: 1.0 mol/L NaOH (CIP)
Gradient:	isocratic
Flow rate:	300 cm/hr (1.71 mL/min)
Detection:	UV@ 280 nm, conductivity
Temperature:	ambient
Sample:	mAb (IgG_1), 1.0 g/L

High Salt Tolerance

Increased salt tolerance of TOYOPEARL NH_2-750F as compared to other TOYOPEARL anion exchange resins can be seen in Figure 16. 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.

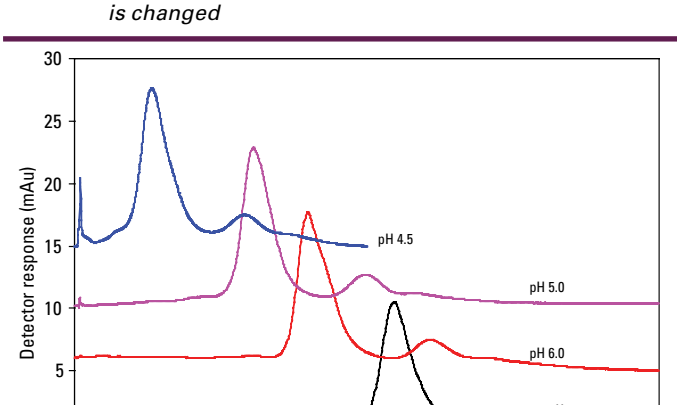
Figure 16: Comparison of anion exchange resins for salt tolerance



Resins:	**various**
Column size:	5 cm 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 (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 17. This allows for a large design space in which to develop a separation protocol.

Figure 17: Selectivity of TOYOPEARL NH_2-750F resin when pH buffer is changed

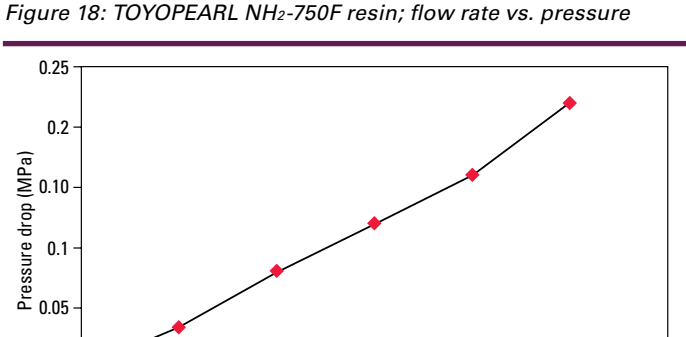


Resin:	**TOYOPEARL NH_2-750F**
Column size:	5 mm ID × 5 cm
Mobile phase:	A: 20 mmol/L N-methyl piperazine, pH 4.5 and 5.0 20 mmol/L Bis-Tris, pH 6.0 20 mmol/L Tris-HCl, pH 7.5 B: mobile phase A + 2.0 mol/L NaCl
Gradient:	0 - 100% B (120 min)
Flow rate:	300 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Temperature:	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 18 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 18: TOYOPEARL NH_2-750F resin; flow rate vs. pressure

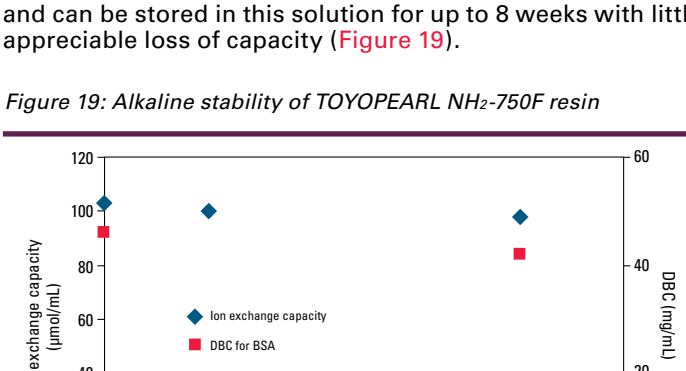


Resin:	**TOYOPEARL NH_2-750F**
Column size:	4.4 cm ID × 29 cm
Mobile phase:	0.1 mol/L NaCl
Flow rate:	multiple

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 19).

Figure 19: Alkaline stability of TOYOPEARL NH_2-750F resin

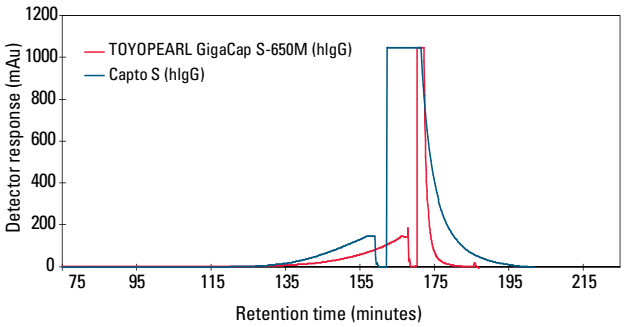


TOYOPEARL GigaCap Resins

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.

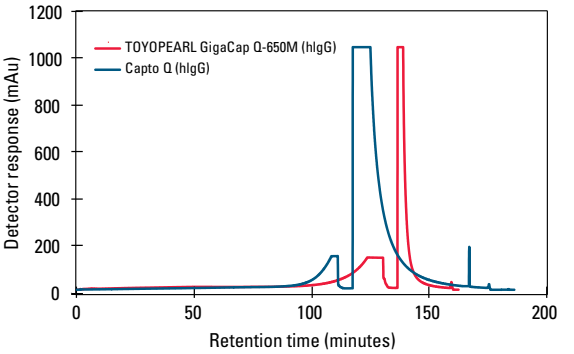
Unmodified TOYOPEARL HW-65 resin is utilized as the base bead for the TOYOPEARL GigaCap M-grade resins. The average particle size of the TOYOPEARL GigaCap M-grade resins, 75 µm, provides for enhanced efficiency and higher resolution than other larger particle size materials, while improved pressure-flow properties are obtained over smaller particle size materials. Figures 20, 21, and 22 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 20: Elution pool volume comparison of TOYOPEARL GigaCap S-650M vs. Capto™ S resins



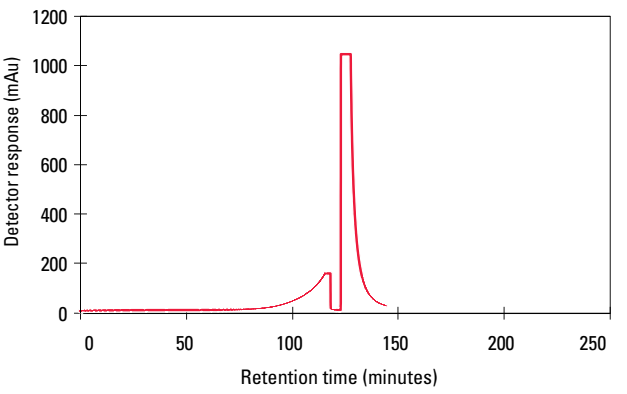
Resins:	**TOYOPEARL GigaCap S-650M**
	Capto S
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 0.1 mol/L acetate buffer, pH 4.7
	Buffer B: 0.1 mol/L acetate buffer, pH 4.7 + 1.0 mol/L NaCl
Flow rate:	212 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Sample:	polyclonal human IgG (1 mg/mL)

Figure 21: 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)

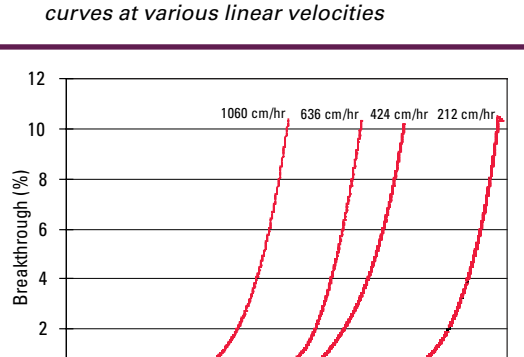
Figure 22: Elution pool volume of TOYOPEARL GigaCap CM-650M resin



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)

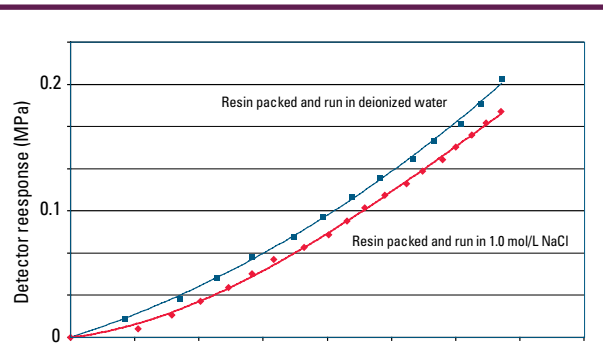
TOYOPEARL GigaCap S-650M resin was specifically developed for the purification of monoclonal antibodies. It has excellent elution kinetics (Figure 20) and maintains reasonably high capacities at higher linear velocities (Figure 23). The slightly larger particle size (50-100 µm) has been optimized to give a unique combination of improved pressure-flow characteristics (Figure 24) with excellent resolution at high loads (Figure 25). 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 23: TOYOPEARL GigaCap S-650M human IgG breakthrough curves at various linear velocities



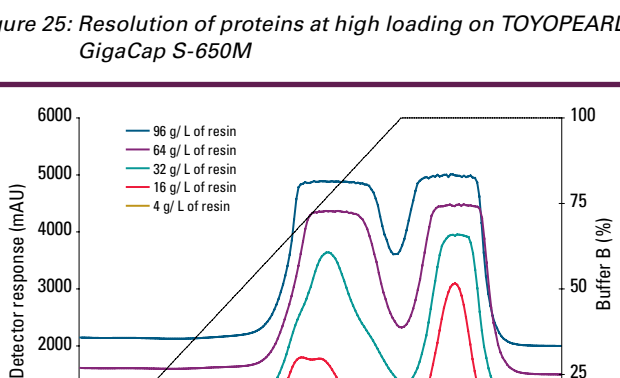
Resins:	**TOYOPEARL GigaCap S-650M**
Column size:	6 mm ID × 4 cm (1.13 mL)
Mobile phase:	0.1 mol/L acetate buffer, pH 4.7
Flow rates:	212, 424, 636, 1060 cm/hr (1.0, 2.0, 3.0, 5.0 mL/min)
Detection:	UV @ 280 nm
Sample:	polyclonal human IgG (1 g/L)

Figure 24: 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.

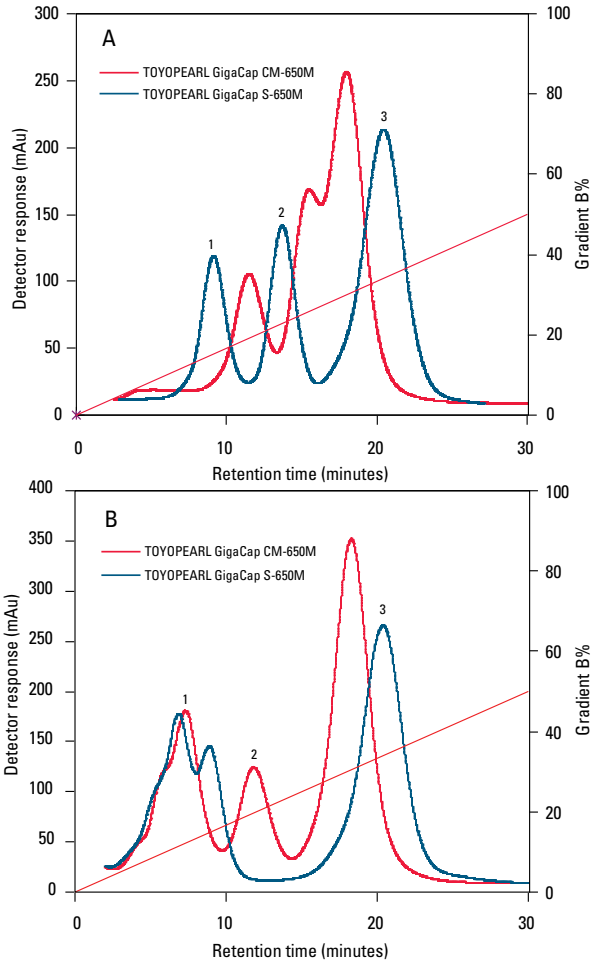
Figure 25: Resolution of proteins at high loading on TOYOPEARL GigaCap S-650M



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)

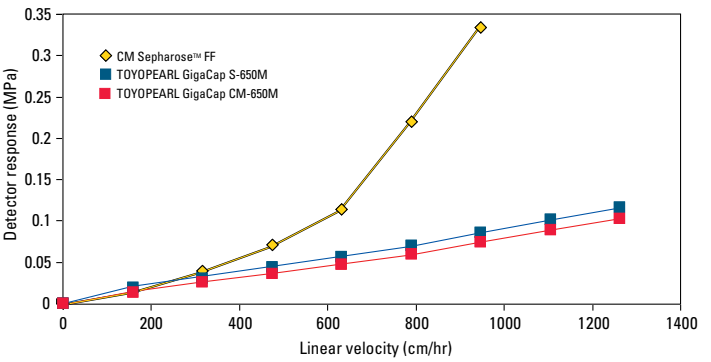
TOYOPEARL GigaCap CM-650M resin was designed for the purification of monoclonal antibodies that require a different chromatographic selectivity than is available with TOYOPEARL GigaCap S-650M resin (Figure 26). Excellent kinetic properties and high capacity are maintained at high linear flow velocities. 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 27).

Figure 26: 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)

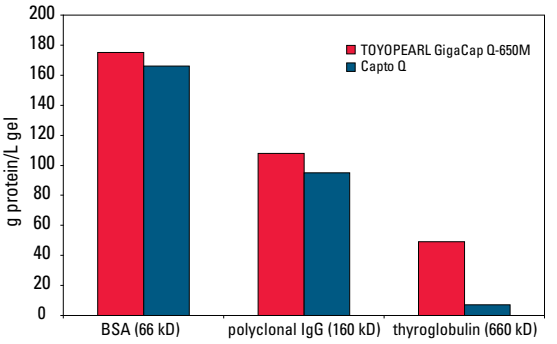
Figure 27: TOYOPEARL GigaCap CM-650M pressure-flow properties



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

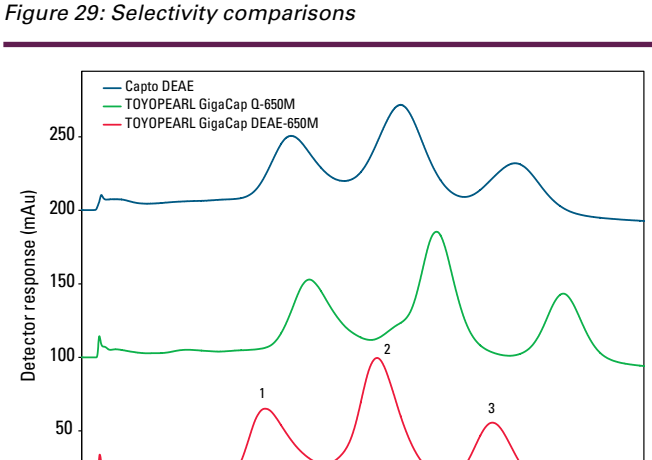
TOYOPEARL GigaCap Q-650M resin was primarily designed for the capture and purification of proteins, although it can also be used for polishing in flow-through chromatography. Of particular note is the excellent capacity of TOYOPEARL GigaCap Q-650M for such large proteins as thyroglobulin when compared to other high capacity resins (Figure 28).

Figure 28: Dynamic binding capacity of proteins with different molar masses @ 212 cm/hr



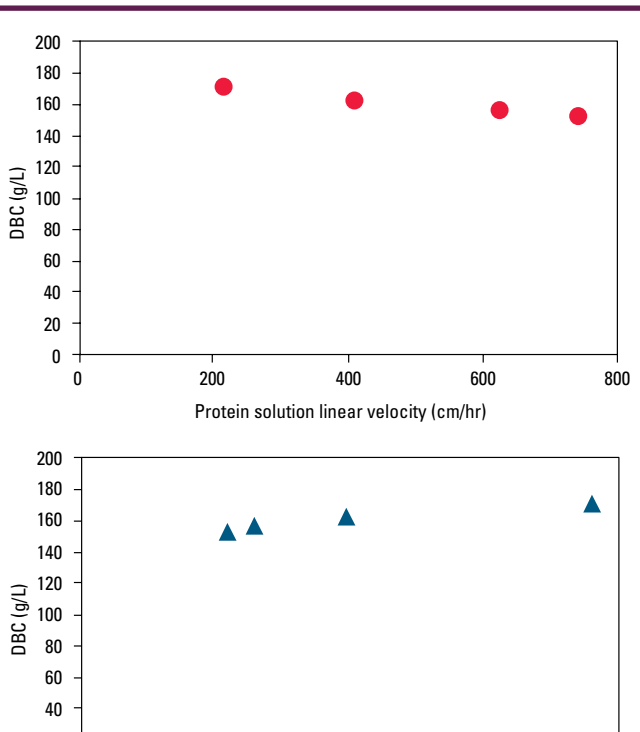
TOYOPEARL GigaCap DEAE-650M resin was designed for the purification proteins that require a different chromatographic selectivity (Figure 29) than is available with TOYOPEARL GigaCap Q-650M resin. As with other TOYOPEARL GigaCap M-grade resins, excellent kinetic properties and high capacity are maintained at high linear flow velocities (Figure 30). 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 31).

Figure 29: Selectivity comparisons



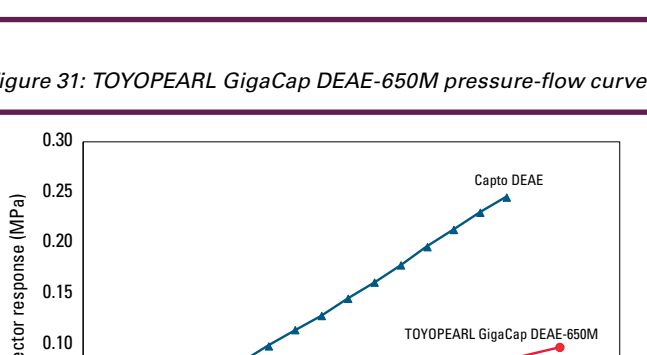
Resins:	**TOYOPEARL GigaCap DEAE-650M**
	TOYOPEARL GigaCap Q-650M
	Capto DEAE
Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	Buffer A: 50 mmol/L Tris-HCl, pH 8.5
	Buffer B: buffer A + 1.0 mol/L NaCl, pH 8.5
Gradient:	120 minutes, 0 - 100% B
Flow rate:	136 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Temperature:	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 30: 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 31: 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 9 and 10) and selectivities (Figures 32 and 33) of the M-grade TOYOPEARL GigaCap resins with the benefit of greater resolution due to their smaller bead size. Pressure-flow properties (Figures 34 and 35) are also maintained with the TOYOPEARL GigaCap S-grade resins.

Table 9: 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 10: Cation exchange resin binding capacity comparisons

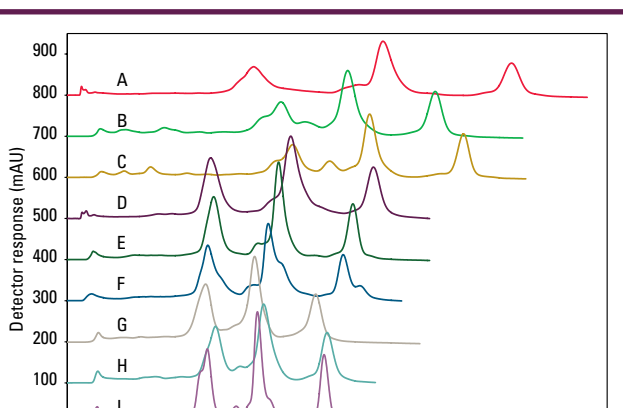
Resin	Particle size (µm)	pH stability	Base bead	Ion exchange capacity (meq/L)	Binding capacity (g/L)		DBC recovery (%)	DBC elution volume (CV)
					Static	Dynamic*		
TOYOPEARL GigaCap S-650S	20 - 50	3 - 13	polymethacrylic	0.24	177	164	99	4.0
TOYOPEARL GigaCap S-650M	50 - 100	3 - 13	polymethacrylic	0.16	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

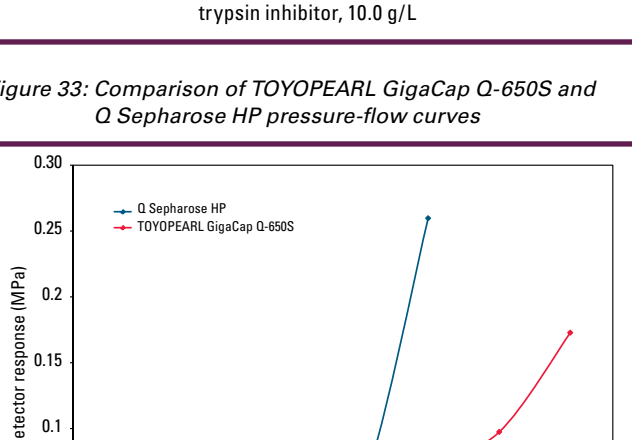
Static Binding Capacity (SBC) Conditions:
Adsorption buffer: 50 mmol/L acetate buffer, pH 4.7
Sample: 10.0 g/L γ-globulin

Figure 32: Selectivity comparisons of anion exchange resins



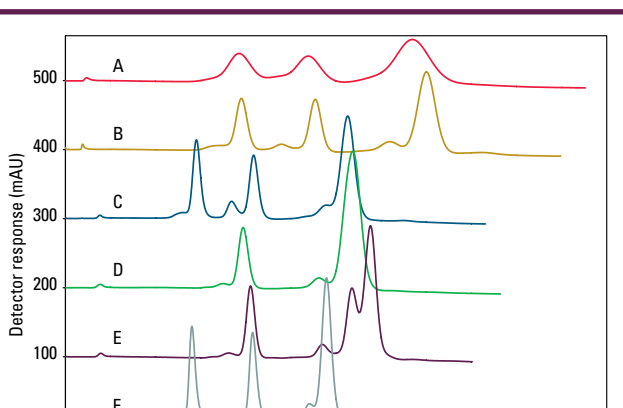
Resins:	**A. TOYOPEARL GigaCap Q-650S**
	B. Capto Q ImpRes
	C. Q Sepharose HP
	D. TOYOPEARL SuperQ-650S
	E. TSKgel SuperQ-5PW (30)
	F. TSKgel SuperQ-5PW (20)
	G. TOYOPEARL DEAE-650S
	H. TSKgel DEAE-5PW (30)
	I. TSKgel DEAE-5PW (20)
Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	Buffer A: 50 mmol/L Tris-HCl, 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 33: Comparison of TOYOPEARL GigaCap Q-650S and Q Sepharose HP pressure-flow curves



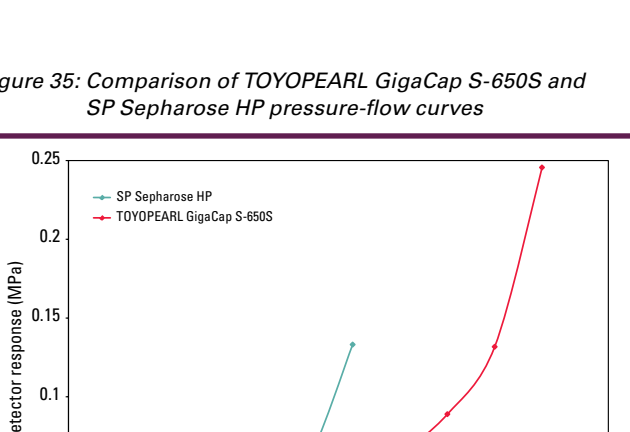
Resin:	**TOYOPEARL GigaCap Q-650S**
	Q Sepharose HP
Column size:	22 mm ID × 20 cm
Mobile phase:	0.1 mol/L NaCl
Detection:	pressure (MPa)

Figure 34: 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 35: 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)

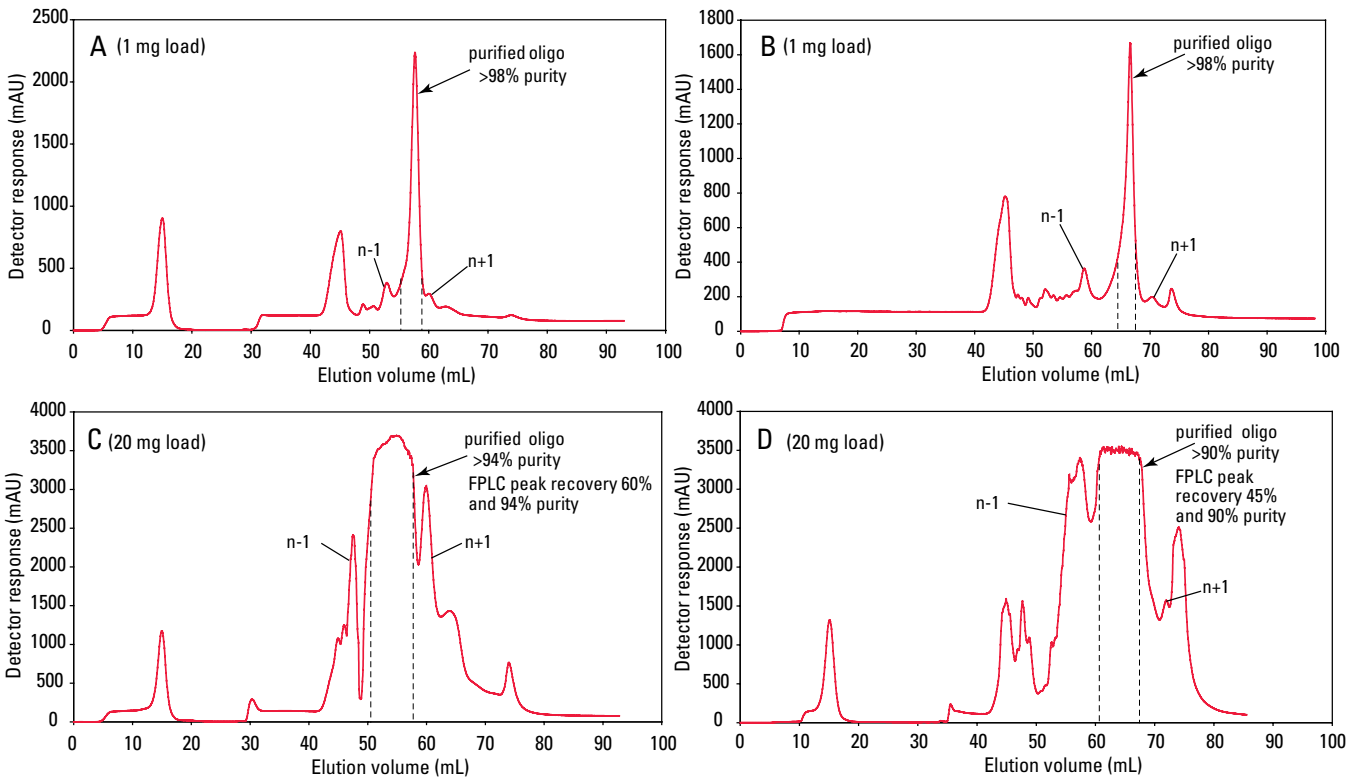
TSKgel SuperQ-5PW Resin

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 36 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 36), 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 36: 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

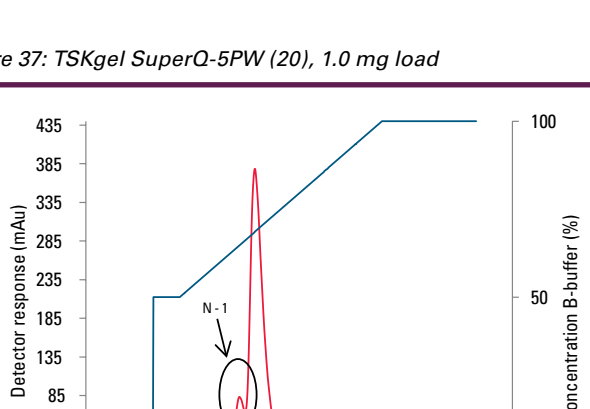
Table 11 shows the different particle sizes that are available in the TSKgel and TOYOPEARL anion exchange resins used for oligonucleotides, and the cation exchange resins used for peptide purifications. The relative binding capacities and predicted resolution of the different particle size resins are 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 11) 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 37-42, the TOYOPEARL GigaCap Q-650S performs similarly to the TSKgel SuperQ-5PW (20) resin for the purification of oligonucleotides. Table 12 compares the performance of these two resins for purity and recovery of an oligonucleotide from crude feedstock.

Table 11: Oligonucleotide purification products

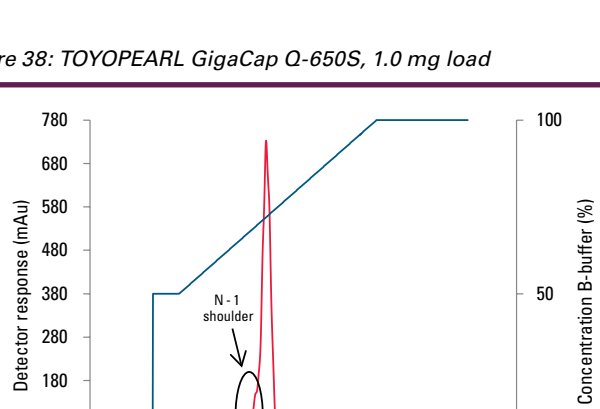
Resin	Bead size (mean µm)	Binding capacity g DNA oligo/L	Resolution	Bead type	Attachment method
TSKgel SuperQ-5PW (20)	20	45	+++++	methacrylic	Type A
TSKgel SuperQ-5PW (30)	30	40	++++	methacrylic	Type A
TOYOPEARL SuperQ-650S	35	54	+++	methacrylic	Type A
TOYOPEARL GigaCap Q-650S	35	40	+++	methacrylic	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 37: TSKgel SuperQ-5PW (20), 1.0 mg load



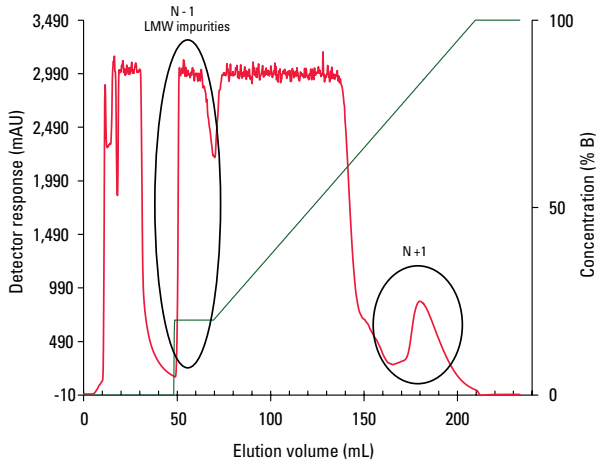
Resin: **TSKgel SuperQ-5PW (20)**
Column size: 6.6 mm ID × 18.5 cm (6.3 mL)
Mobile phase: Buffer A: 20 mmol/L NaOH
Buffer B: 20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient: 50% B (2 CV)
50-100% B (15 CV)
100% B (2 CV)
Flow rate: 200 cm/hr (1.14 mL/min)
Detection: UV @ 254 nm
Sample: crude phosphorothioate deoxyoligonucleotide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

Figure 38: TOYOPEARL GigaCap Q-650S, 1.0 mg load



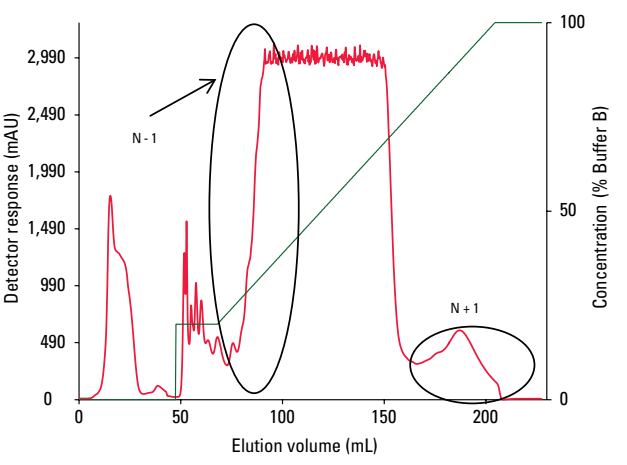
Resin: **TOYOPEARL GigaCap Q-650S**
Column size: 6.6 mm ID × 18.5 cm (6.3 mL)
Mobile phase: Buffer A: 20 mmol/L NaOH
Buffer B: 20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient: 50% B (2 CV)
50-100% B (15 CV)
100% B (2 CV)
Flow rate: 200 cm/hr (1.14 mL/min)
Detection: UV @ 254 nm
Sample: crude phosphorothioate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

Figure 39: 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 40: 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 41: TSKgel SuperQ-5PW (20) resin: 80% DBC elution peak with fraction purity histogram

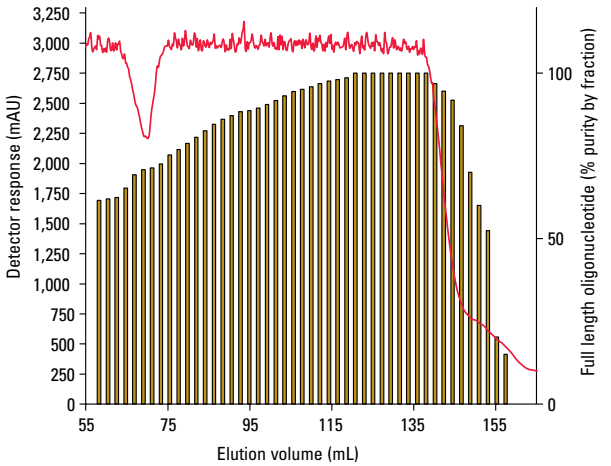


Figure 42: TOYOPEARL GigaCap Q-650S resin: 80% DBC elution peak with fraction purity histogram

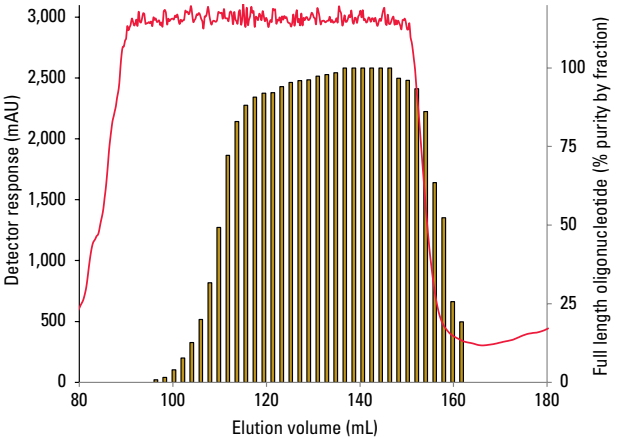


Table 12: Oligonucleotide purity and yield from 80% DBC purifications

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 13 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 13: 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 14 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 43.

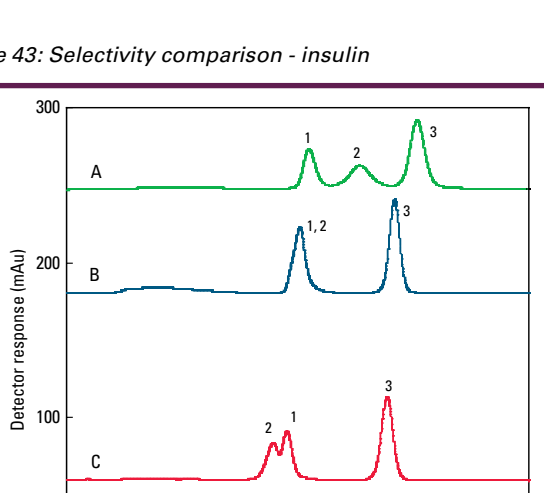
Table 14: Insulin dynamic binding capacity comparison

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 43: Selectivity comparison - insulin

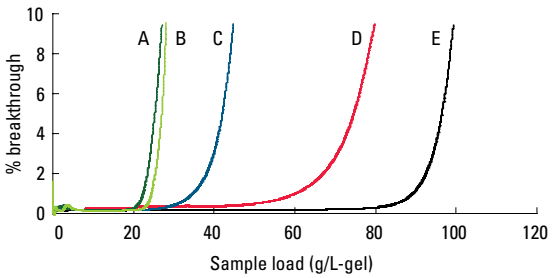


Resins: **A. TSKgel SP-3PW (30)**
B. SOURCE 30S
C. TSKgel SP-5PW (30)
Column size: 7.5 mm ID × 7.5 cm
Mobile phase: Buffer A: 0.02 mol/L sodium citrate buffer, pH 3.2 + ethanol = 8/2 (v/v)
Buffer B: 0.02 mol/L sodium citrate buffer, pH 3.2 + 1.0 mol/L NaCl/ethanol = 8/2 (v/v)
Gradient: 60 min linear 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 44 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 45.

Figure 44: Breakthrough curves of mono-PEGylated lysozyme using TOYOPEARL cation exchange resins

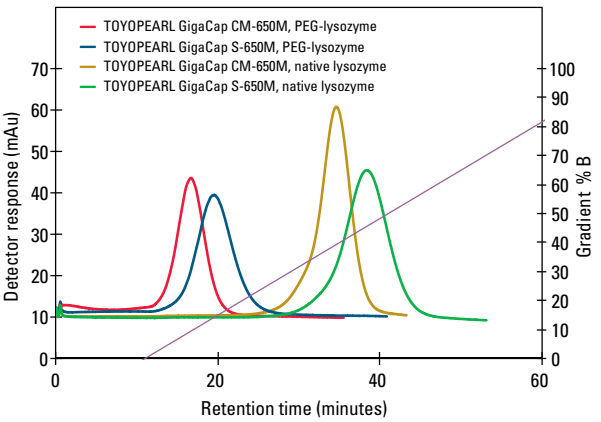


Resins: **A. TOYOPEARL SP-650M**
B. TOYOPEARL CM-650M
C. TOYOPEARL SP-550C
D. TOYOPEARL GigaCap CM-650M
E. TOYOPEARL GigaCap S-650M

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 45: 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 46 shows the elution profile of monoclonal antibody supernatant. Antibody activity is represented in the figure by the black bars.

Figure 46: Separation of monoclonal antibody cell culture supernatant

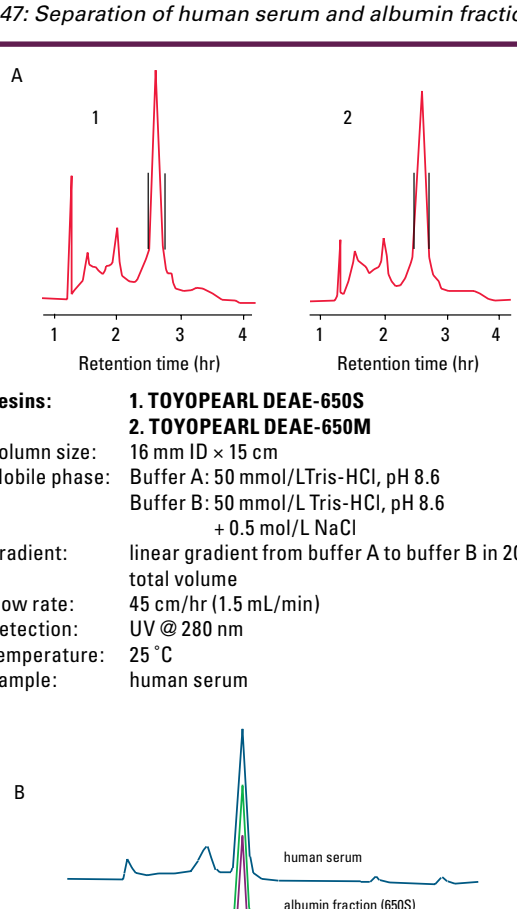
Resin:	**TOYOPEARL CM-650S**
Column size:	16 mm ID × 6 cm
Mobile phase:	Buffer A: 20 mmol/L sodium acetate, pH 5.5 Buffer B: 20 mmol/L sodium acetate, pH 5.5 + 0.5 mol/L NaCl
Gradient:	linear gradient from buffer A to buffer B in 200 mL total volume
Flow rate:	173 cm/hr (5.8 mL/min)
Detection:	UV @ 280 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 47. 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 47: 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

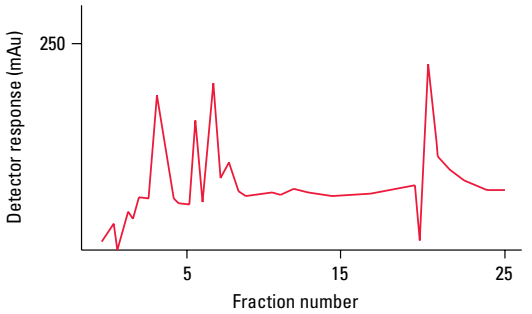
Column:	**TSKgel G3000SW, 7.5 mm ID × 30 cm × 2 in series**
Mobile phase:	0.1 mol/L phosphate, pH 6.8 + 0.1 mol/L sodium sulfate
Detection:	UV @ 280 nm
Temperature:	25 ˚C
Sample:	1. crude human serum 2. albumin fraction from TOYOPEARL DEAE-650S 3. albumin fraction from TOYOPEARL DEAE-650M

[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 48 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 48: 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					
23438	TOYOPEARL NH_2-750F	100	30 - 60	0.07 - 0.13	>70 Hu. IgG
23439	TOYOPEARL NH_2-750F	250	30 - 60	0.07 - 0.13	>70 Hu. IgG
23440	TOYOPEARL NH_2-750F	1,000	30 - 60	0.07 - 0.13	>70 Hu. IgG
23441	TOYOPEARL NH_2-750F	5,000	30 - 60	0.07 - 0.13	>70 Hu. IgG
23442	TOYOPEARL NH_2-750F	50,000	30 - 60	0.07 - 0.13	>70 Hu. IgG
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	0.14 - 0.23	> 120
21986	TOYOPEARL Q-600C AR	250	50 - 150	0.14 - 0.23	> 120
21987	TOYOPEARL Q-600C AR	1,000	50 - 150	0.14 - 0.23	> 120
21988	TOYOPEARL Q-600C AR	5,000	50 - 150	0.14 - 0.23	> 120
21989	TOYOPEARL Q-600C AR	50,000	50 - 150	0.14 - 0.23	> 120
21854	TOYOPEARL GigaCap Q-650M	100	50 - 100	0.10 - 0.20	≥ 162
21855	TOYOPEARL GigaCap Q-650M	250	50 - 100	0.10 - 0.20	≥ 162
21856	TOYOPEARL GigaCap Q-650M	1,000	50 - 100	0.10 - 0.20	≥ 162
21857	TOYOPEARL GigaCap Q-650M	5,000	50 - 100	0.10 - 0.20	≥ 162
21858	TOYOPEARL GigaCap Q-650M	50,000	50 - 100	0.10 - 0.20	≥ 162
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

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

Part #	Product description	Container size (mL)	Bead diameter (µm)	Ion Exchange Capacity (eq/L)	Typical BSA* capacity (g/L)
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	20 - 40
14713	TSKgel DEAE-5PW (30)	1,000	20 - 40	0.05 - 0.11	20 - 40
18370	TSKgel DEAE-5PW (30)	5,000	20 - 40	0.05 - 0.11	20 - 40

⁺ Typical BSA capacity (g/L) unless otherwise noted

Cation exchange resins:

Part #	Product description	Container size (mL)	Bead diameter (µm)	Ion Exchange Capacity (eq/L)	Typical Lysozyme⁺ capacity (g/L)
TOYOPEARL and TOYOPEARL GigaCap Cation Exchange Resins					
23467	TOYOPEARL Sulfate-650F	100	50 - 100	≥ 0.53	≥ 114 Hu. γ-globulin
23468	TOYOPEARL Sulfate-650F	250	50 - 100	≥ 0.53	≥ 114 Hu. γ-globulin
23469	TOYOPEARL Sulfate-650F	1,000	50 - 100	≥ 0.53	≥ 114 Hu. γ-globulin
23470	TOYOPEARL Sulfate-650F	5,000	50 - 100	≥ 0.53	≥ 114 Hu. γ-globulin
23471	TOYOPEARL Sulfate-650F	50,000	50 - 100	≥ 0.53	≥ 114 Hu. γ-globulin
21833	TOYOPEARL GigaCap S-650M	100	50 - 100	0.10 - 0.20	136 - 176 Hu. γ-globulin
21834	TOYOPEARL GigaCap S-650M	250	50 - 100	0.10 - 0.20	136 - 176 Hu. γ-globulin
21835	TOYOPEARL GigaCap S-650M	1,000	50 - 100	0.10 - 0.20	136 - 176 Hu. γ-globulin
21836	TOYOPEARL GigaCap S-650M	5,000	50 - 100	0.10 - 0.20	136 - 176 Hu. γ-globulin
21837	TOYOPEARL GigaCap S-650M	50,000	50 - 100	0.10 - 0.20	136 - 176 Hu. γ-globulin
22875	TOYOPEARL GigaCap S-650S	25	20 - 50	0.15 - 0.25	>150 Hu. γ-globulin
22876	TOYOPEARL GigaCap S-650S	250	20 - 50	0.15 - 0.25	>150 Hu. γ-globulin
22877	TOYOPEARL GigaCap S-650S	1,000	20 - 50	0.15 - 0.25	>150 Hu. γ-globulin
22878	TOYOPEARL GigaCap S-650S	5,000	20 - 50	0.15 - 0.25	>150 Hu. γ-globulin
22879	TOYOPEARL GigaCap S-650S	50,000	20 - 50	0.15 - 0.25	>150 Hu. γ-globulin
21946	TOYOPEARL GigaCap CM-650M	100	50 - 100	0.17 - 0.28	> 110 Hu. γ-globulin
21947	TOYOPEARL GigaCap CM-650M	250	50 - 100	0.17 - 0.28	> 110 Hu. γ-globulin
21948	TOYOPEARL GigaCap CM-650M	1,000	50 - 100	0.17 - 0.28	> 110 Hu. γ-globulin
21949	TOYOPEARL GigaCap CM-650M	5,000	50 - 100	0.17 - 0.28	> 110 Hu. γ-globulin
21950	TOYOPEARL GigaCap CM-650M	50,000	50 - 100	0.17 - 0.28	> 110 Hu. 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

Part #	Product description	Container size (mL)	Bead diameter (µm)	Ion Exchange Capacity (eq/L)	Typical Lysozyme+ capacity (g/L)
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
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	0.10 - 0.20	100 - 155 insulin
21805	TOYOPEARL MegaCap II SP-550EC	250	100 - 300	0.10 - 0.20	100 - 155 insulin
21806	TOYOPEARL MegaCap II SP-550EC	1,000	100 - 300	0.10 - 0.20	100 - 155 insulin
21807	TOYOPEARL MegaCap II SP-550EC	5,000	100 - 300	0.10 - 0.20	100 - 155 insulin
21808	TOYOPEARL MegaCap II SP-550EC	50,000	100 - 300	0.10 - 0.20	100 - 155 insulin
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
21976	TSKgel SP-3PW (30)	25	20 - 40	0.07 - 0.22	>65 insulin
21977	TSKgel SP-3PW (30)	250	20 - 40	0.07 - 0.22	>65 insulin
21978	TSKgel SP-3PW (30)	1,000	20 - 40	0.07 - 0.22	>65 insulin
21979	TSKgel SP-3PW (30)	5,000	20 - 40	0.07 - 0.22	>65 insulin
21980	TSKgel SP-3PW (30)	50,000	20 - 40	0.07 - 0.22	>65 insulin

+ Typical Lysozyme capacity (g/L) unless otherwise noted

TOYOPEARL Butyl-600M		
TOYOPEARL Butyl-650C	TOYOPEARL Butyl-650M	TOYOPEARL Butyl-650S
TOYOPEARL Ether-650M	TOYOPEARL Ether-650S	
TSKgel Ether-5PW (20)	TSKgel Ether-5PW (30)	
TOYOPEARL Hexyl-650C		
TOYOPEARL Phenyl-600M		
TOYOPEARL Phenyl-650C	TOYOPEARL Phenyl-650M	TOYOPEARL Phenyl-650S
TSKgel Phenyl-5PW (20)	TSKgel Phenyl-5PW (30)	
TOYOPEARL PPG-600M		
TOYOPEARL SuperButyl-550C		

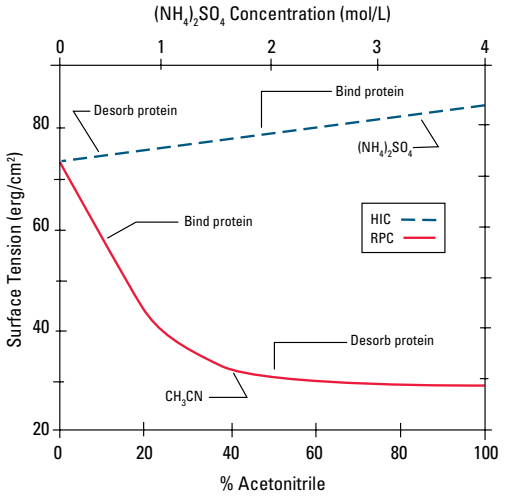
The Role of Hydrophobic Interaction Chromatography in Process Purification

Hydrophobic interaction chromatography (HIC) is a powerful tool for the process purification of biomolecules. The technique utilizes the accessible hydrophobic regions located on protein surfaces and their interactions with a weakly hydrophobic stationary phase. HIC is an excellent complement to ion exchange and size exclusion chromatography particularly when protein isoforms exist or when feedstock impurities are of similar isoelectric point or molar mass. The selectivity differences exploited by HIC can also be used after affinity separations in which closely related proteins with similar recognition sites are not distinguishable by the affinity ligand.

Proteins and other molecules with hydrophobic surfaces are attracted to the hydrophobic ligands of HIC resins. Proteins are bound to the resin by employing an aqueous high salt mobile phase. The salt conditions contribute to a lyotropic effect which allows the proteins to bind to the lower surface coverage of a hydrophobic ligand. Proteins are eluted by the simple technique of decreasing the salt concentration. Most therapeutic targets are eluted in a low salt or a no salt buffer.

During elution, the energy of interaction for a HIC step is less than that of a reversed phase chromatography (RPC) step. One means of gauging the relative binding energy between the two techniques is to measure the surface tension of the two sets of binding and elution conditions. Figure 1 provides a comparison of the surface tension generated by HIC and RPC elution systems.[1] Since HIC separates under milder eluting conditions, biological activity is typically retained.

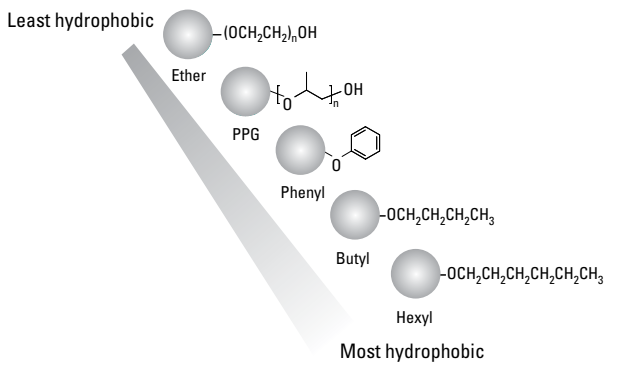
Figure 1: The surface tension of aqueous solutions used in HIC and RPC



Mode	Gradient (Typical)	Δ Surface Tension (erg/cm²)
HIC	1.8 to 0 mol/L $(NH_4)_2SO_4$/ aqueous buffer	4
RPC	10 to 50% ACN/ 0.1%TFA	23

[1]C. Horvath et. al., Separation Processes in Biotechnology, Volume 9; Asenjo, J. ed.; Marcel Dekker, Inc.: New York, 1990, p 447.

Figure 2: Available HIC ligands



TOYOPEARL Hydrophobic Interaction Chromatography Resins

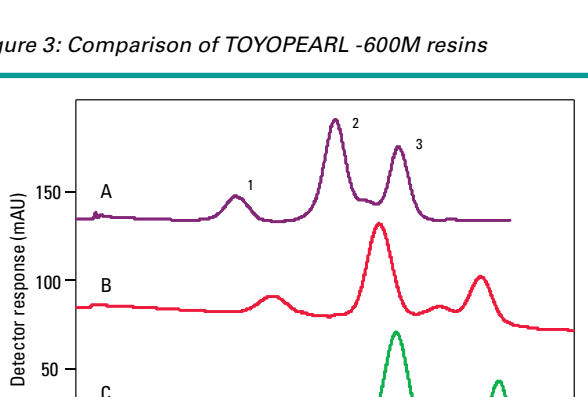
TOYOPEARL HIC resins are functionalized versions of the TOYOPEARL HW size exclusion resins and are therefore based on hydroxylated polymethacrylic polymer beads. Tosoh Bioscience offers five HIC ligands featuring different degrees of hydrophobicity and selectivity. Table 1 lists the properties of these TOYOPEARL HIC resins. The hydrophobicity of TOYOPEARL HIC resins increases through the ligand series: ether, PPG (polypropylene glycol), phenyl, butyl, and hexyl (Figure 2).

Table 1: Properties of TOYOPEARL HIC resins

TOYOPEARL resin	Hydrophobicity	Base bead	Pore size (nm)	Bead diameter (µm)	Ligand type	DBC (g/L)	Pressure rating
Ether-650S	+	HW-65	100	20 - 50	Ether	10-30	0.3 MPa
Ether-650M	+	HW-65	100	40 - 90	Ether	10-30	0.3 MPa
PPG-600M	++	HW-60	75	40 - 90	Polypropylene glycol	45 - 55	0.3 MPa
Phenyl-600M	+++	HW-60	75	40 - 90	Phenyl	45 - 65	0.3 MPa
Phenyl-650S	+++	HW-65	100	20 - 50	Phenyl	30 - 50	0.3 MPa
Phenyl-650M	+++	HW-65	100	40 - 90	Phenyl	30 - 50	0.3 MPa
Phenyl-650C	+++	HW-65	100	50 - 150	Phenyl	30 - 50	0.3 MPa
Butyl-650S	++++	HW-65	100	20 - 50	Butyl	30 - 50	0.3 MPa
Butyl-650M	++++	HW-65	100	40 - 90	Butyl	30 - 50	0.3 MPa
Butyl-650C	++++	HW-65	100	50 - 150	Butyl	30 - 50	0.3 MPa
Butyl-600M	++++	HW-60	75	40 - 90	Butyl	40 - 60	0.3 MPa
SuperButyl-550C	++++	HW-55	50	50 - 150	Butyl	52 - 70	0.3 MPa
Hexyl-650C	+++++	HW-65	100	50 - 150	Hexyl	30 - 50	0.3 MPa

Three HIC ligands are available in the TOYOPEARL -600 resin format: PPG, phenyl, and butyl. The selectivities of TOYOPEARL Butyl-600M, TOYOPEARL PPG-600M and the TOYOPEARL Phenyl-600M resins are shown in Figure 3. Available in the TOYOPEARL -650 series are the following four HIC ligands: hexyl, butyl, phenyl, and ether. The remaining ligand available in the TOYOPEARL HIC resin line is SuperButyl-550.

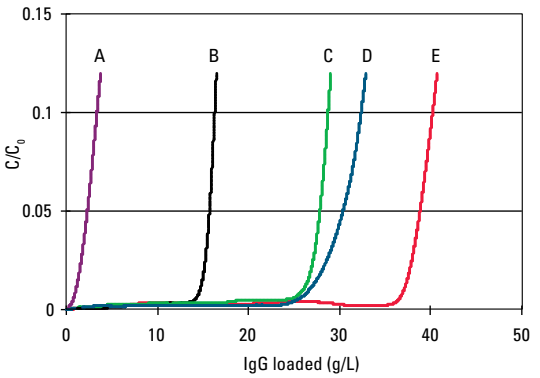
Figure 3: Comparison of TOYOPEARL -600M resins



Resins: **A. TOYOPEARL PPG-600M**
B. TOYOPEARL Phenyl-600M
C. TOYOPEARL Butyl-600M
Column size: 7.5 mm ID × 7.5 cm
Mobile phase: Buffer A: 1.8 mol/L $(NH_4)_2SO_4$ + 0.1 mol/L sodium phosphate, pH 7.0
Buffer B: 0.1 mol/L sodium phosphate, pH 7.0
Gradient: 60 min linear gradient from buffer A to B
Flow rate: 136 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Temperature: ambient
Samples: 1 g/L of: 1. ribonuclease A 2.lysozyme 3. α-chymotrypsinogen
Load volume: 100 µL

A comparison of the dynamic binding capacities (DBCs) of the TOYOPEARL -600 resins with TOYOPEARL Phenyl-650M is shown in Figure 4. Figure 5 compares the selectivities of the TOYOPEARL Phenyl-600M and TOYOPEARL Phenyl-650M resins with an agarose based phenyl resin. The narrower pore diameter of TOYOPEARL SuperButyl-550C resin (based on the 50 nm pore diameter TOYOPEARL HW-55 resin) is recommended for the analysis of smaller molecules such as lysozyme (1.2×10^4 Da). A comparison of the DBC of TOYOPEARL SuperButyl-550C resin with other TOYOPEARL HIC resins is shown in Figures 6 and 7.

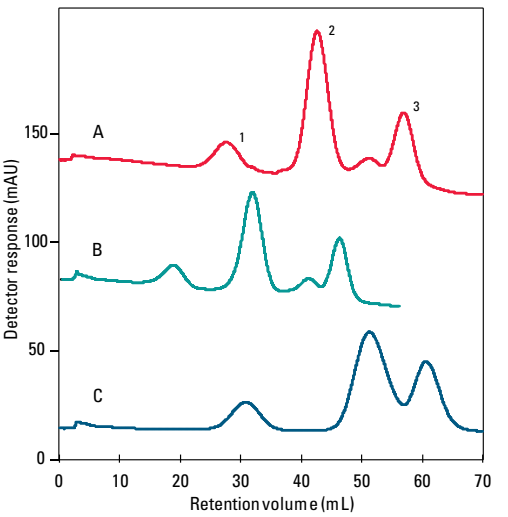
Figure 4: Breakthrough curves of polyclonal IgG on various HIC resins



Resins:	**A. TOYOPEARL PPG-600M** **B. TOYOPEARL Phenyl-650M** **C. TOYOPEARL Butyl-600M** D. Competitor Phenyl Agarose **E. TOYOPEARL Phenyl-600M**
Column size:	7.8 mm ID × 20 cm
Mobile phase:	0.1 mol/L sodium phosphate, pH 7.0 + 0.8 mol/L $(NH_4)_2SO_4$
Flow rate:	300 cm/hr (2.4 mL/min)
Detection:	UV @ 280 nm
Temperature:	25 °C
Samples:	1.0 g/L polyclonal IgG

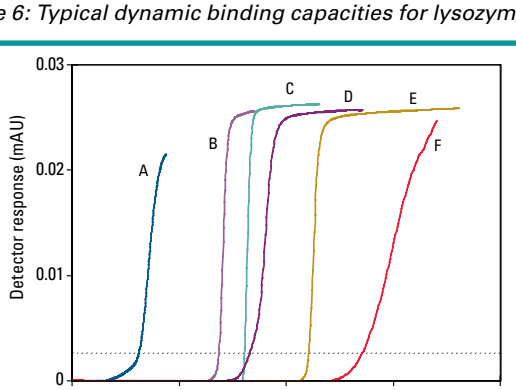
DBC was calculated at 10% breakthrough

Figure 5: Selectivity comparison of phenyl-type resins



Resins:	**A. TOYOPEARL Phenyl-600M** **B. TOYOPEARL Phenyl-650M** C. Competitor Phenyl Agarose
Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	Buffer A: 1.8 mol/L $(NH_4)_2SO_4$ + 0.1 mol/L sodium phosphate, pH 7.0 Buffer B: 0.1 mol/L sodium phosphate, pH 7.0
Gradient:	60 min linear gradient from buffer A to B
Flow rate:	136 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	1.0 g/L of: 1. ribonuclease A 2.lysozyme 3. α-chymotrypsinogen
Load volume:	100 µL

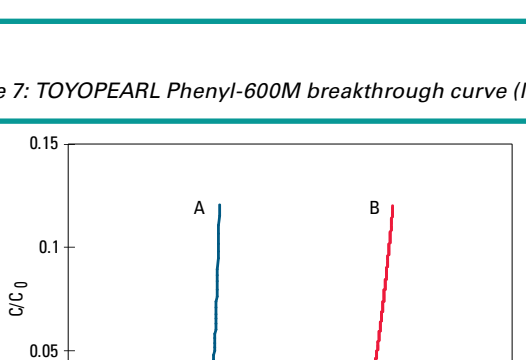
Figure 6: Typical dynamic binding capacities for lysozyme



Resins:	binding capacity (g/L) (10% breakthrough)
A. TOYOPEARL Ether-650M	12.5
B. TOYOPEARL Phenyl-650M	27.5
C. TOYOPEARL Butyl-650M	32.2
D. TOYOPEARL Hexyl-650C	33.2
E. TOYOPEARL PPG-600M	44.2
F. TOYOPEARL SuperButyl-550C	54.3

Column size: 7.8 mm ID × 20 cm
Mobile phase: 1.8 mol/L sodium sulfate + 0.1 mol/L phosphate, pH 7.0
Flow rate: 100 cm/hr (0.8 mL/min)
Detection: UV @ 280 nm
Temperature: ambient
Sample: 1 g/L lysozyme
Sample load: as indicated in figure

Figure 7: TOYOPEARL Phenyl-600M breakthrough curve (lysozyme)



Resins:	binding capacity (g/L) (10% breakthrough)
A. TOYOPEARL Phenyl-650M	27
B. TOYOPEARL Phenyl-600M	58

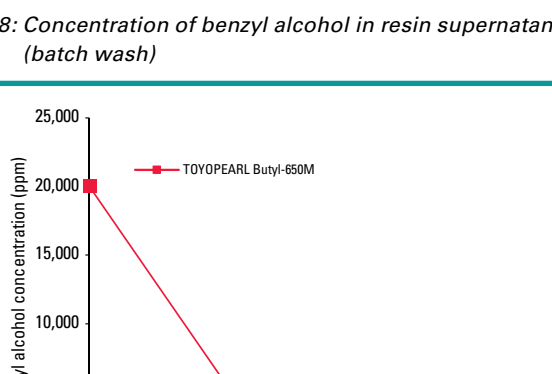
Column size: 7.8 mm ID × 20 cm
Mobile phase: 1.8 mol/L $(NH_4)_2SO_4$ + 0.1 mol/L phosphate, pH 7.0
Flow rate: 300 cm/hr (2.4 mL/min)
Detection: UV @ 280 nm
Temperature: ambient
Sample: 1 g/L lysozyme
Sample load: as indicated in figure

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

Samples of the filtered supernatant from the TOYOPEARL Butyl-650M resin was taken after the 5th, 10th, and 15th washes and analyzed for benzyl alcohol concentration (Figure 8). As demonstrated in the figure, a 2% benzyl alcohol solution can be removed from the TOYOPEARL Butyl-650M resin by thorough washing with DI water.

As benzyl alcohol is a hydrophobic molecule, it may not be possible to adequately reduce its concentration from hydrophobic interaction chromatography resins due to interactions between the preservative and the ligand. The use of benzyl alcohol (2%) with chromatography media that are un-functionalized or are functionalized with non-hydrophobic ligands is an acceptable alternative to the recommended 20% ethanol.

Figure 8: Concentration of benzyl alcohol in resin supernatant (batch wash)



The larger pore TOYOPEARL products such as TOYOPEARL Butyl-650 and TOYOPEARL Phenyl-650 resins are very useful for protein aggregate separation and removal. In addition, Tosoh Bioscience HIC resins are very effective in separating misfolded proteins from the native protein form. Because misfolded proteins will generally be more hydrophobic than the native protein, TOYOPEARL Butyl-650M resin is used frequently for the removal of misfolded proteins. In many cases, flow-through chromatography can be accomplished under eluent conditions binding the misfolded protein while allowing the native target protein to flow through the column.

Hydrophobic interaction is a very useful technique for the purification of monoclonal antibodies (mAbs), with their diverse hydrophobic nature. The range of HIC ligands of varying hydrophobicity available from Tosoh Bioscience (Figure 2) gives chromatographic developers a range of options for finding the right ligand for their target molecule.

TSKgel Hydrophobic Interaction Chromatography Resins

The same ether and phenyl ligands that are used for the TOYOPEARL resins are also available within the TSKgel HIC resin product line. Properties of TSKgel HIC resins are listed in Table 2. The TSKgel HIC resins use the same methacrylic polymer chemistry as the TOYOPEARL resins (Table 3) 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 3: Methacrylic base beads available for HIC

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

TOYOPEARL HIC resins are chemically stable from pH 1-13. 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.

Table 2: Properties of TSKgel HIC resins

TSKgel resin	Hydrophobicity	Base bead	Pore size (nm)	Bead diameter (µm)	Ligand type	DBC (g/L)	Pressure rating
Ether-5PW (20)	+	PW5000	100	15 - 25	Ether	10 - 30	2.0 MPa
Ether-5PW (30)	+	PW5000	100	20 - 40	Ether	10 - 30	2.0 MPa
Phenyl-5PW (20)	++	PW5000	100	15 - 25	Phenyl	10 - 30	2.0 MPa
Phenyl-5PW (30)	++	PW5000	100	20 - 40	Phenyl	10 - 30	2.0 MPa

Because TOYOPEARL and TSKgel HIC resins have the same backbone polymer chemistry, the selectivity for proteins and impurities will be unchanged. Table 4 shows the ligands and particle sizes available for TOYOPEARL and TSKgel HIC resins and is arranged in increasing levels of resolution by bead size (i.e. low, medium, and high resolution). The semi-rigid polymeric backbone of TOYOPEARL and TSKgel HIC resins permits high flow rates for maximum throughput and productivity. TOYOPEARL HIC resins may be operated at pressures up to 0.3 MPa and TSKgel -5PW HIC resins may be operated up to 2.0 MPa. The pressure-flow characteristics for each particle size grade of TOYOPEARL Phenyl-650 resins are shown in Figure 9.

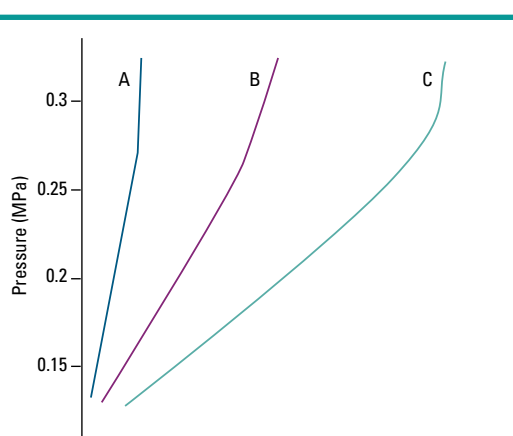
Resolution increases with decreasing particle size. Resin particle size is proportional to HETP and inversely proportional to the column efficiency and resolution of two peaks. TOYOPEARL HIC resins are available in three particle sizes, though not all ligands are available in each grade:

- S-grade = 35 µm (Superfine)
- M-grade = 65 µm (Fine)
- C-grade = 100 µm (Coarse)

Some processes, such as the purification of antibody-drug conjugates, require resins that are capable of higher resolution separations. For these separations, smaller diameter TOYOPEARL S-grade or TSKgel resins are preferred. TSKgel HIC resins are currently available in two ligands and two bead sizes:

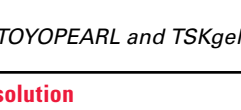
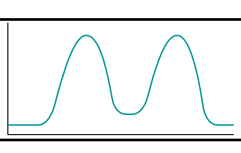
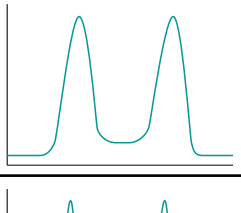
- TSKgel Ether-5PW (30) = 30 µm
- TSKgel Ether-5PW (20) = 20 µm
- TSKgel Phenyl-5PW (30) = 30 µm
- TSKgel Phenyl-5PW (20) = 20 µm

Figure 9: Pressure-flow curve for TOYOPEARL Phenyl-650 resins of various particle sizes



Resins: **A. TOYOPEARL Phenyl-650S**
B. TOYOPEARL Phenyl-650M
C. TOYOPEARL Phenyl-650C
Column size: 25 mm ID × 25 cm
Mobile phase: 2.0 mol/L $(NH_4)_2SO_4$
Flow rate: as indicated in figure

Table 4: Resolution of TOYOPEARL and TSKgel HIC resins

Resolution		Bead diameter (µm)	Pore size (nm)	HIC resin
Low		100	50 100 100 100	TOYOPEARL SuperButyl-550C TOYOPEARL Hexyl-650C TOYOPEARL Butyl-650C TOYOPEARL Phenyl-650C
Medium		65	75 75 75	TOYOPEARL Butyl-600M TOYOPEARL Phenyl-600M TOYOPEARL PPG-600M
		65	100 100 100	TOYOPEARL Butyl-650M TOYOPEARL Phenyl-650M TOYOPEARL Ether-650M
High		35	100 100 100	TOYOPEARL Butyl-650S TOYOPEARL Phenyl-650S TOYOPEARL Ether-650S
		30	100 100	TSKgel Phenyl-5PW (30) TSKgel Ether-5PW (30)
		20	100 100	TSKgel Phenyl-5PW (20) TSKgel Ether-5PW (20)

Parameters to Consider when Using Tosoh Bioscience HIC Resins

Coordinating the hydrophobicity of the therapeutic target to the resin hydrophobicity is critical for the best overall purification performance. Too hydrophobic a resin for a given protein can result in its irreversible binding to the resin or a loss of biological activity. Tables 5 and 6 show typical mass recovery and biological activity recovery data for TOYOPEARL HIC resins.

Table 5: High mass recovery (%) of proteins

Protein	TOYOPEARL resin		
	Ether-650M	Phenyl-650M	Butyl-650M
bovine serum albumin	84	62	76*
α-chymotrypsinogen	96	88*	90
cytochrome c	—	81*	87*
IgG	91	—	—
α-lactalbumin	90	—	—
lysozyme	94	92	85
ovalbumin	83	88	73
ribonuclease A	—	72*	82*

Procedure: A 200 mL sample containing 200 mg of protein was loaded onto a 7.5 mm ID × 7.5 cm column and eluted with a 60 minute gradient of 1.8 mol/L (*1.5 mol/L) to 0.0 mol/L ammonium sulfate in 0.1 mol/L sodium phosphate, pH 7.0. The mass recovery was determined spectrophotometrically at UV 280 nm and 25 °C.

Table 6: Recovery of enzymatic activity of proteins

TOYOPEARL resin	Protein	% Activity recovery
Phenyl-650	phytochrome	79
Butyl-650	halophilic protease	85
Butyl-650	poly (3-hydroxybutyrate) depolymerase	88
Butyl-650	aculeacin-A acylase	82
Butyl-650	opine dehydrogenase	81

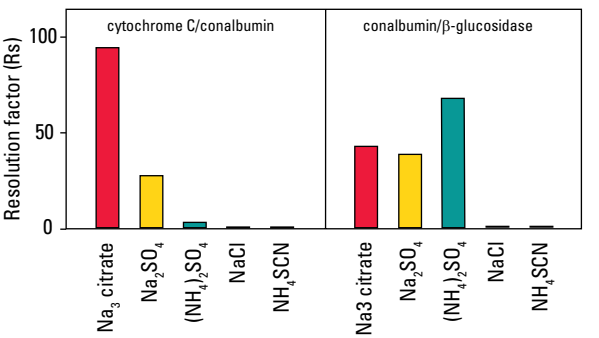
An optimum HIC process step will balance high dynamic binding capacity, adequate selectivity, good mass recovery and retention of biological activity. The wide range of selectivities for TOYOPEARL and TSKgel resins enables a developer to optimize protein separations at the extremes of the hydrophobic spectrum. The more hydrophobic ligands on TOYOPEARL Hexyl-type and TOYOPEARL Butyl-type resins are used to separate hydrophilic proteins. These two resins should also be considered for separations requiring a low salt environment.

TOYOPEARL and TSKgel Ether resins are used for the purification of very hydrophobic targets such as certain monoclonal antibodies and membrane proteins. These proteins may bind irreversibly to other more hydrophobic resins.

TOYOPEARL PPG and TOYOPEARL and TSKgel Phenyl resins complement the other HIC ligands available in the HIC series and offer alternatives for moderately hydrophobic proteins.

In addition to the hydrophobicity of the ligand, the selectivity in HIC is influenced by the eluent salt type. Figure 10 demonstrates the effect of salt type on the resolution factor of different protein pairs.[2] The Hofmeister lyotropic salt series shown in Figure 11 ranks anions and cations by their ability to promote protein precipitation. Ions on the left are referred to as "lyotropic" while the ions on the right are called "chaotropic". Lyotropic salts will precipitate or "salt out" proteins at high salt concentrations due to increased hydrophobic interaction, while chaotropic salts will promote protein denaturation at high salt concentrations. The Hofmeister lyotropic salt series indicates that the use of different salt systems may generate a variety of adsorption and desorption selectivities for each resin with a given protein. This feature of HIC provides an additional parameter for the optimization of a process step.

Figure 10: Influence of salt-type on resolution



Chromatography on a Toyopearl Butyl-substituted support

Resin: **TOYOPEARL Butyl-650M**
Column size: 4.1 mm ID × 4 cm
Mobile phase: Buffer A: 20 mmol/L phosphate buffer in 1.0 mol/L indicated salt, pH 7.0
Buffer B: buffer A with 1.0 mol/L indicated salt
Flow rate: 484 cm/hr (1 mL/min)
Detection: UV @ 280 nm

[2]Fausnaugh, J.; Kennedy, L.; Regnier, F. *J. Chromatography*, **1984**, 141, 317.

Figure 11: Hofmeiseter lyotropic salt series

for anions

$SO_4^{2-} > HPO_4^{2-} > CH_3COO^- >$ halide $> NO_3^- > ClO_4^- > SCN^-$

for cations

$(CH_3)_4N^+ > K^+ > Na^+ > Cs^+ > Li^+ > Mg^{2+} > Ca^{2+} > Ba^{2+}$

Ammonium sulfate and sodium sulfate are the most commonly used salts in HIC. NaCl is often used as well.

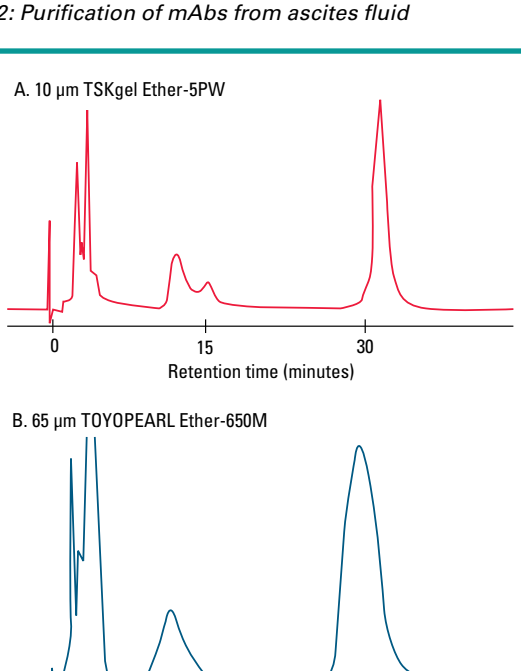
HIC is commonly used as a polishing step in monoclonal antibody purification processes. HIC offers an orthogonal selectivity to ion exchange chromatography and can be an effective step for aggregate clearance and host cell protein reduction, however, this mode of chromatography suffers from the limitation of use of high concentrations of kosmotropic salts to achieve the desired separation. Ghose et al[3] reports an unconventional way of operating HIC in the flowthrough (FT) mode with no kosmotropic salt in the mobile phase. TOYOPEARL Hexyl-650C was selected as the stationary phase and the pH of the mobile phase was modulated to achieve the required selectivity. Optimum pH conditions were chosen under which the antibody product of interest flowed through while impurities such as aggregates and host cell proteins bound to the column. The performance of the TOYOPEARL Hexyl-650C resin was comparable to that observed using conventional HIC conditions with high salt.

[3]Ghose, S.; Tao, Y.; Conley, L.; Cecchini, D. Purification of monoclonal antibodies by hydrophobic interaction chromatography under no-salt conditions. mAbs. 2013, 5, (5), 795-800.

Purification of Monoclonal Antibodies

For a very hydrophobic mAb, such as mouse anti-chicken lectin (14 kDa), the less hydrophobic TOYOPEARL Ether ligand works quite well. The purification of this mAb from ascites fluid (Figure 12) was performed with a 10 µm TSKgel Ether-5PW semi-preparative column. Identical selectivity for scale-up was found with corresponding 65 µm TOYOPEARL Ether-650M resin.

Figure 12: Purification of mAbs from ascites fluid

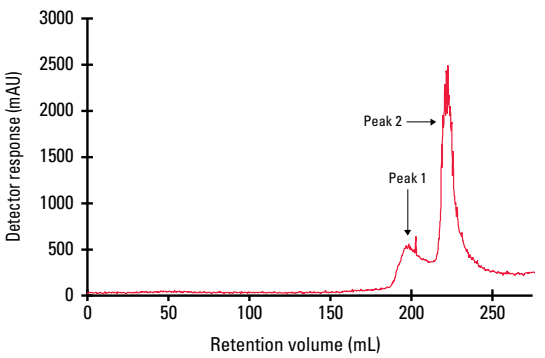


Resins:	**A: TSKgel Ether-5PW (prepacked HPLC column)**
	B: TOYOPEARL Ether-650M
Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	Buffer A: 1.5 mol/L $(NH_4)_2SO_4$ + 0.1mol/L phosphate, pH 7.0
	Buffer B: 0.1 mol/L phosphate, pH 7.0
Gradient:	60 min linear gradient from buffer A to B
Flow rate:	136 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	A: 1.5 mg/100 µL anti-chicken 14 kDa lectin
	B: 0.76 mg/50 µL diluted ascites fluid
Load volume:	150 µL

Plasmid DNA Purification

TOYOPEARL Hexyl-650C resin was used successfully for plasmid DNA purification by Cambrex, Baltimore, MD (US patent 6,953,686). The resin was shown to be the most effective among HIC resins for endotoxin removal with capacities exceeding 2 million EU/mL of resin. Additionally, RNA and protein impurities were effectively eliminated. TOYOPEARL Hexyl-650C was also effective in separating the supercoiled and open circular forms of plasmid DNA (Figure 13). Under certain binding conditions, the two forms are bound to the resin, and subsequently eluted with a simple gradient, resulting in two distinct peaks corresponding to the relaxed and supercoiled forms respectively.

Figure 13: Plasmid DNA separation

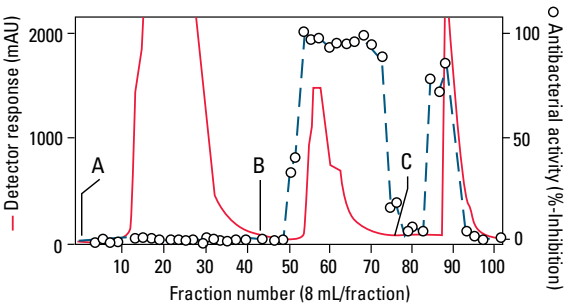


Resin:	**TOYOPEARL Hexyl-650C**
Column size:	1.0 cm ID × 30 cm (23.6 mL)
Mobile phase:	Buffer A. 3.0 mol/L ammonium sulfate, 10 mmol/L Tris, 1 mmol/L EDTA, pH 7.4
	Buffer B. 10 mmol/L Tris, 1 mmol/L EDTA, pH 7.4
Gradient:	3.0 mol/L ammonium sulfate to 1.0 mol/L ammonium sulfate (6 CV)
Flow rate:	153 cm/hr (2.0 mL/min)
Detection:	UV @ 254 nm
Sample:	Plasmid DNA in 3.0 mol/L ammonium sulfate

Purification of Glycoproteins

TOYOPEARL HIC resins can purify glycoproteins, which often bind irreversibly to saccharide-based chromatographic media. Figure 14 shows the purification of a large glycoprotein on TOYOPEARL Butyl-650S resin.

Figure 14: Large glycoprotein purified on TOYOPEARL Butyl-650S

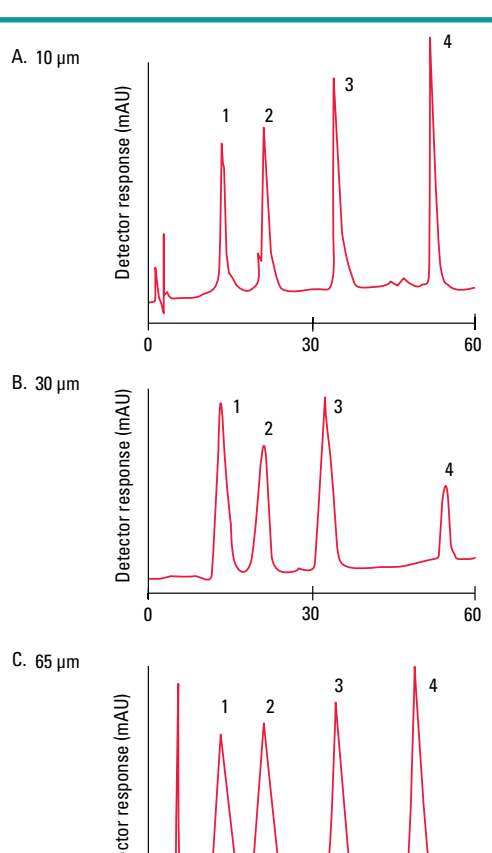


Resin:	**TOYOPEARL Butyl-650S**
Column size:	22 mm ID × 26 cm
Mobile phase:	Buffer A: 40% saturated $(NH_4)_2SO_4$ + 50 mol/L phosphate, pH 7.0
	Buffer B: 50 mol/L phosphate, pH 7.0
Gradient:	A: Load and wash in 100% buffer A
	B: 50% buffer A with 50% buffer B
	C: 100% buffer B
Detection:	UV @ 280 nm
Sample:	crude protein from sea hare *Aplysia kurodai*

Ultra Purification of Target Compound

Biopharmaceutical process development often requires a high performance step for ultra-purification of a target compound. To meet these needs, 20 and 30 µm TSKgel Phenyl-5PW and Ether-5PW are available. The selectivity of these packings is similar to the 10 µm TSKgel 5PW Phenyl-5PW and Ether-5PW analytical columns. Therefore methods can easily be transferred from analytical to preparative scale resins of the same chemistry using a seamless scale-up strategy. Figure 15 shows the similar elution pattern on 10 µm and 30 µm TSKgel packings, along with 65 µm TOYOPEARL process-scale resin.

Figure 15: Seamless scale up

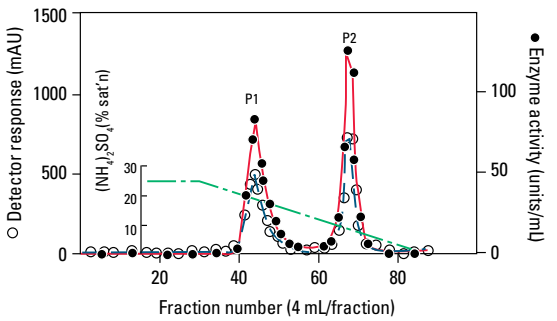


Resins:	**A and B. TSKgel Phenyl-5PW** **C. TOYOPEARL Phenyl-650M**
Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	A and B: 60 min linear gradient from 1.8 mol/L to
Gradient:	0 mol/L $(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer C: 240 min linear gradient from 1.8 mol/L to 0 mol/L$(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer
Flow rate:	A and B. 68 cm/hr (0.50 mL/min) C. 272 cm/hr (2.0 mL/min)
Detection:	UV @ 280 nm
Samples:	1. myoglobin 2. ribonuclease A 3. lysozyme 4. α-chymotrypsinogen
Load volume:	100 µL containing 1 g/L of each protein

Purification and Resolution of Pullulanase

The power of HIC is illustrated in a scheme in which pullulanase, an amylase-like enzyme responsible for hydrolysis of branched chain sugars, is purified and resolved into two closely related forms. Ion exchange and size exclusion chromatography effectively purified pullulanase. With TOYOPEARL Butyl-650S, however, two closely related proteins were resolved, based on differences in their surface hydrophobicity (Figure 16).

Figure 16: Separation of two active pullulanase forms

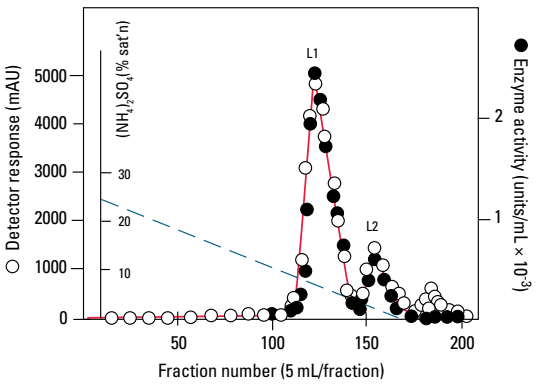


Resin:	**TOYOPEARL Butyl-650S**
Column size:	18 mm ID × 16 cm
Mobile phase:	isocratic elution, 120 mL $(NH_4)_2SO_4$, 25% saturation in 0.02 mol/L phosphate, pH 7.0, followed by a linear gradient, 224 mL $(NH_4)_2SO_4$, 25% to 0% saturation, in buffer
Flow rate:	12 cm/hr
Detection:	UV @ 280 nm
Sample:	protein from *Bacillus acidopullulyticus*
Sample load:	20 mg

Lipase Isozymes

Incorporation of HIC into a purification scheme has separated lipase isozymes that were not resolved by a previously reported method. After ion exchange and size exclusion chromatography, an additional step employing TOYOPEARL Butyl-650M, as shown in Figure 17, enabled the separation of two active lipase isozymes, L1 and L2, from an inactive impurity. Activity recovery was 93% for this step.

Figure 17: Separation of lipase isozymes from impurity



Resin:	**TOYOPEARL Butyl-650M**
Column size:	34 mm ID × 29 cm
Mobile phase:	linear gradient, 810 mL $(NH_4)_2SO_4$, 25% to 0% saturation in 0.01 mol/L acetate buffer, pH 5.6
Flow rate:	2 cm/hr
Detection:	UV @ 254nm
Sample:	lipase from *Geotrichum candidum*
Sample load:	375 mg

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

Ordering Information

TOYOPEARL HIC resins:

Part #	Product description	Container size (mL)	Bead diameter (µm)	Typical lysozyme capacity (g/L)
43151	TOYOPEARL Ether-650S	25	20 - 50	10 - 30
16172	TOYOPEARL Ether-650S	100	20 - 50	10 - 30
16174	TOYOPEARL Ether-650S	1,000	20 - 50	10 - 30
16176	TOYOPEARL Ether-650S	5,000	20 - 50	10 - 30
19805	TOYOPEARL Ether-650M	25	40 - 90	10 - 30
16173	TOYOPEARL Ether-650M	100	40 - 90	10 - 30
16175	TOYOPEARL Ether-650M	1,000	40 - 90	10 - 30
16177	TOYOPEARL Ether-650M	5,000	40 - 90	10 - 30
21301	TOYOPEARL PPG-600M	25	40 - 90	45 - 55
21302	TOYOPEARL PPG-600M	100	40 - 90	45 - 55
21303	TOYOPEARL PPG-600M	1,000	40 - 90	45 - 55
21304	TOYOPEARL PPG-600M	5,000	40 - 90	45 - 55
21305	TOYOPEARL PPG-600M	50,000	40 - 90	45 - 55
21887	TOYOPEARL Phenyl-600M	25	40 - 90	45 - 65
21888	TOYOPEARL Phenyl-600M	100	40 - 90	45 - 65
21889	TOYOPEARL Phenyl-600M	1,000	40 - 90	45 - 65
21890	TOYOPEARL Phenyl-600M	5,000	40 - 90	45 - 65
21891	TOYOPEARL Phenyl-600M	50,000	40 - 90	45 - 65
43152	TOYOPEARL Phenyl-650S	25	20 - 50	30 - 50
14477	TOYOPEARL Phenyl-650S	100	20 - 50	30 - 50
14784	TOYOPEARL Phenyl-650S	1,000	20 - 50	30 - 50
14935	TOYOPEARL Phenyl-650S	5,000	20 - 50	30 - 50
19818	TOYOPEARL Phenyl-650M	25	40 - 90	30 - 50
14478	TOYOPEARL Phenyl-650M	100	40 - 90	30 - 50
14783	TOYOPEARL Phenyl-650M	1,000	40 - 90	30 - 50
14943	TOYOPEARL Phenyl-650M	5,000	40 - 90	30 - 50
18364	TOYOPEARL Phenyl-650M	50,000	40 - 90	30 - 50
43126	TOYOPEARL Phenyl-650C	25	50 - 150	30 - 50
14479	TOYOPEARL Phenyl-650C	100	50 - 150	30 - 50
14785	TOYOPEARL Phenyl-650C	1,000	50 - 150	30 - 50
14944	TOYOPEARL Phenyl-650C	5,000	50 - 150	30 - 50

Part #	Product description	Container size (mL)	Bead diameter (µm)	Typical lysozyme capacity (g/L)
43153	TOYOPEARL Butyl-650S	25	20 - 50	30 - 50
07476	TOYOPEARL Butyl-650S	100	20 - 50	30 - 50
14701	TOYOPEARL Butyl-650S	1,000	20 - 50	30 - 50
07975	TOYOPEARL Butyl-650S	5,000	20 - 50	30 - 50
18826	TOYOPEARL Butyl-650S	50,000	20 - 50	30 - 50
19802	TOYOPEARL Butyl-650M	25	40 - 90	30 - 50
07477	TOYOPEARL Butyl-650M	100	40 - 90	30 - 50
14702	TOYOPEARL Butyl-650M	1,000	40 - 90	30 - 50
07976	TOYOPEARL Butyl-650M	5,000	40 - 90	30 - 50
18355	TOYOPEARL Butyl-650M	50,000	40 - 90	30 - 50
43127	TOYOPEARL Butyl-650C	25	50 - 150	30 - 50
07478	TOYOPEARL Butyl-650C	100	50 - 150	30 - 50
14703	TOYOPEARL Butyl-650C	1,000	50 - 150	30 - 50
07977	TOYOPEARL Butyl-650C	5,000	50 - 150	30 - 50
22826	TOYOPEARL Butyl-650C	50,000	50 - 150	30 - 50
21448	TOYOPEARL Butyl-600M	25	40 - 90	40 - 60 (γ-globulin)
21449	TOYOPEARL Butyl-600M	100	40 - 90	40 - 60 (γ-globulin)
21450	TOYOPEARL Butyl-600M	1,000	40 - 90	40 - 60 (γ-globulin)
21451	TOYOPEARL Butyl-600M	5,000	40 - 90	40 - 60 (γ-globulin)
21452	TOYOPEARL Butyl-600M	50,000	40 - 90	40 - 60 (γ-globulin)
19955	TOYOPEARL SuperButyl-550C	25	50 - 150	52 - 70
19956	TOYOPEARL SuperButyl-550C	100	50 - 150	52 - 70
19957	TOYOPEARL SuperButyl-550C	1,000	50 - 150	52 - 70
19958	TOYOPEARL SuperButyl-550C	5,000	50 - 150	52 - 70
19959	TOYOPEARL SuperButyl-550C	50,000	50 - 150	52 - 70
44465	TOYOPEARL Hexyl-650C	25	50 - 150	30 - 50
19026	TOYOPEARL Hexyl-650C	100	50 - 150	30 - 50
19027	TOYOPEARL Hexyl-650C	1,000	50 - 150	30 - 50
19028	TOYOPEARL Hexyl-650C	5,000	50 - 150	30 - 50
21973	TOYOPEARL Hexyl-650C	50,000	50 - 150	30 - 50

TSKgel HIC resins:

Part #	Product description	Container size (mL)	Bead diameter (µm)	Typical lysozyme capacity (g/L)
43276	TSKgel Ether-5PW (20)	25	15 - 25	10 - 30
16052	TSKgel Ether-5PW (20)	250	15 - 25	10 - 30
16053	TSKgel Ether-5PW (20)	1,000	15 - 25	10 - 30
18437	TSKgel Ether-5PW (20)	5,000	15 - 25	10 - 30
43176	TSKgel Ether-5PW (30)	25	20 - 40	10 - 30
16050	TSKgel Ether-5PW (30)	250	20 - 40	10 - 30
16051	TSKgel Ether-5PW (30)	1,000	20 - 40	10 - 30
18439	TSKgel Ether-5PW (30)	5,000	20 - 40	10 - 30
43277	TSKgel Phenyl-5PW (20)	25	15 - 25	15 - 35
14718	TSKgel Phenyl-5PW (20)	250	15 - 25	15 - 35
14719	TSKgel Phenyl-5PW (20)	1,000	15 - 25	15 - 35
18438	TSKgel Phenyl-5PW (20)	5,000	15 - 25	15 - 35
43177	TSKgel Phenyl-5PW (30)	25	20- 40	10 - 30
14720	TSKgel Phenyl-5PW (30)	250	20 - 40	10 - 30
14721	TSKgel Phenyl-5PW (30)	1,000	20 - 40	10 - 30
17210	TSKgel Phenyl-5PW (30)	5,000	20 - 40	10 - 30


TOYOPEARL®
Phenyl-650M
Net Vol. 100 mL
Part No. 14478
Lot No.
FOR IN VITRO USE ONLY
TOYOPEARL®
Butyl-650M
25 mL
19802
TOSOH
TSKgel®
Phenyl-5PW (20)
25 mL
43277
TOSOH

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

The Role of Affinity Chromatography in Process Purification

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

TOYOPEARL Affinity Resins

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

TOYOPEARL affinity resins are composed of hydrophilic, dimensionally stable base resins that exhibit excellent pressure-flow characteristics. These resins use the TOYOPEARL HW-65 SEC resin as a base bead. The 100 nm pore diameter of the TOYOPEARL affinity resins can accommodate large globular proteins up to 5×10^6.

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

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

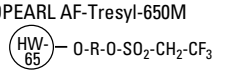
The structure of TOYOPEARL resins with group-specific ligands are shown in Figure 2. Note that due to the proprietary bonding chemistry of TOYOPEARL AF rProtein A-650F, TOYOPEARL AF-rProtein A HC-650F and TOYOPEARL AF rProtein L-650F resins, only ligand structures are shown for these resins (located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

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

Figure 1: Activated and reactive TOYOPEARL affinity resins

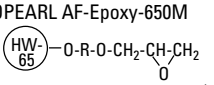
Activated TOYOPEARL affinity resins

TOYOPEARL AF-Tresyl-650M



Ligand Density: 80 µmol/g (dry)

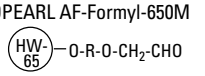
TOYOPEARL AF-Epoxy-650M



Ligand Density: 800 µmol/g (dry)

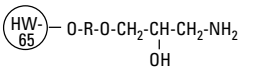
Reactive TOYOPEARL affinity resins

TOYOPEARL AF-Formyl-650M



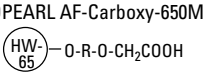
Ligand Density: 60 µeq/mL

TOYOPEARL AF-Amino-650M



Ligand Density: 100 µmol/mL

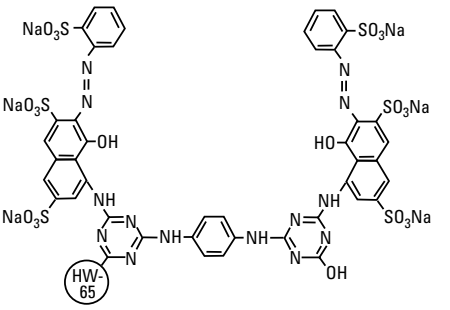
TOYOPEARL AF-Carboxy-650M



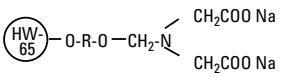
Ligand Density: 100 eq/mL

Figure 2: Group-specific TOYOPEARL affinity resins

TOYOPEARL AF-Red



TOYOPEARL AF-Chelate



Ligand Density: 20 µmol/mL

Protein A Chromatography in Process Purification

Protein A chromatography, the most widely used type of affinity chromatography, relies on the specific and reversible binding of antibodies to an immobilized ligand; in this case protein A. Protein A is a 56 kDa surface protein native to the cell wall of the bacterium *Staphylococcus aureus*. It is composed of five immunoglobulin-binding domains, each of which are able to bind proteins from many mammalian species, most notably Immunoglobulin G (IgG) through the heavy chain within the Fc region. While the native form of protein A was used as the ligand for first generation protein A resins, the recombinant form (rProtein A) produced in *E. coli* is the most prevalent today. Modifications to the protein structure of the ligand, the advent of ligands composed of single domain multimers, and multipoint attachment have given rise to the caustic stable, high capacity and extremely robust protein A resins in use today.

The protein A ligand can either bind directly to the Fc region of an antibody or to an Fc tag that has been fused to the target of interest. Protein A chromatography is a very robust purification procedure and is used as a capture step due to its specificity and, depending on the intended use for the target molecule (antibodies for diagnostic testing), might be the only chromatographic step required to achieve adequate product purity.

In protein A chromatography, crude feed stock is passed through a column under conditions that promote binding. After loading is complete, the column is washed under conditions that do not interrupt the specific interaction between the target and ligand, but that will disrupt any non-specific interactions between process impurities (host cell proteins, etc.) and the stationary phase. The bound protein is then eluted with mobile phase conditions that disrupt the target/ligand interactions. Elution of the target molecule from protein A resin is most commonly accomplished by lowering the pH of the mobile phase, creating an environment whereby the structure of the target molecule is altered in such a way as to inhibit binding. Low pH elution can have a negative effect on protein stability and it is advised that the eluted protein solution be neutralized to minimize aggregation and denaturation.

TOYOPEARL Protein A Resins

Tosoh Bioscience offers two TOYOPEARL affinity resins with a recombinant protein A ligand (Table 1). TOYOPEARL AF-rProtein A resins are composed of hydrophilic, dimensionally stable base resins that exhibit excellent pressure-flow characteristics. These resins use the TOYOPEARL HW-65 SEC resin as a base bead. The 100 nm pore diameter of the TOYOPEARL affinity resins can accommodate large globular proteins up to 5×10^6.

TOYOPEARL AF-rProtein A HC-650F is a high capacity protein A resin for monoclonal antibody purification. An enhanced rProtein A ligand (Figure 3) is bound to the TOYOPEARL HW-65F base bead via multipoint attachment resulting in excellent base (Figure 4) stability for up to 200 CIP cycle with 0.1 mol/L NaOH. TOYOPEARL AF-rProtein A HC-650F resin maintains 80% of initial dynamic binding capacity after 40 CIP cycles with 0.5 mol/L NaOH (Figure 5). TOYOPEARL AF-rProtein A HC-650F resin exhibits dynamic binding capacities of greater than 65 g/L at residence times of 5 minutes and greater than 50 g/L at 2 minutes residence time with feed stock concentrations from 1.0 g/L to 10.0 g/L (Figure 6).

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

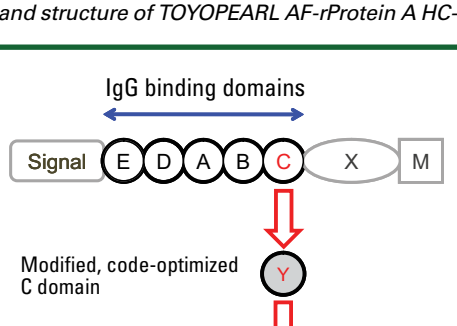
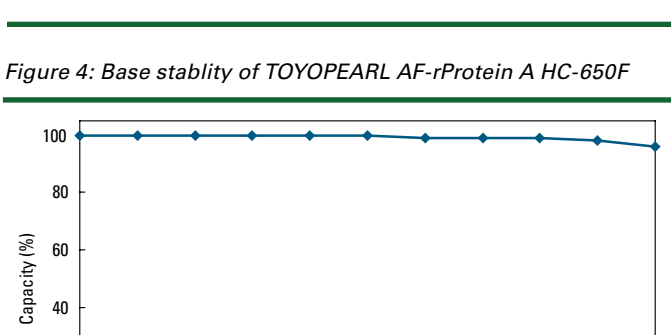


Figure 4: Base stablity of TOYOPEARL AF-rProtein A HC-650F

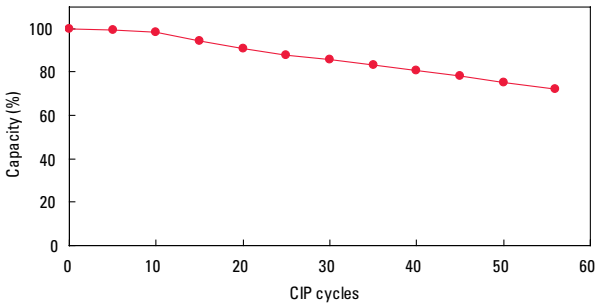


Column size: 5 mm ID × 5 cm
Wash procedure: A: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (10 CV)
B: 0.1 mol/L citrate, pH 3.0 (5CV)
C: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (7 CV)
D: 0.1 mol/L NaOH (3 CV – 15 min contact time)
E: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (5 CV)
Capacity: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

Table 1: Properties of TOYOPEARL Protein A resins

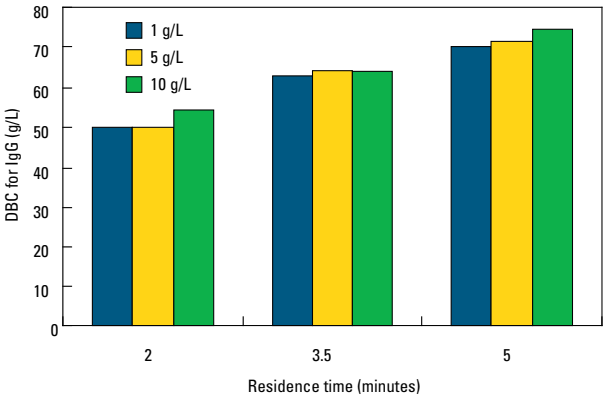
TOYOPEARL resin	Functionality	Base bead	Pore size	Bead diameter	Ligand type	Ligand leakage	DBC (g/L)	Pressure rating
AF-rProtein A-650F	Protein A	HW-65	100 nm	45 µm	rProtein A	5 - 25 ng/mg	> 30 @ 3 min	0.3 MPa
AF-rProtein A HC-650F	Protein A	HW-65	100 nm	45 µm	rProtein A	0.6 - 1.7 ng/mg	> 65 @ 5 min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

Figure 5: DBC of TOYOPEARL AF-rProtein A HC-650F resin after CIP with 0.5 mol/L NaOH



Column size:	5 mm ID × 5 cm
Wash procedure:	A: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (10 CV) B: 0.1 mol/L citrate, pH 3.0 (5 CV) C: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (7 CV) D: 0.5 mol/L NaOH (3 CV – 15 min contact time) E: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (5 CV)
Capacity:	DBC was determined at 10% breakthrough after every 5 cycles

Figure 6: DBC of of TOYOPEARL AF-rProtein A HC-650F



Resin:	**TOYOPEARL AF-rProtein A HC-650F**
Column size:	5 mm ID × 5 cm
Mobile phase:	0.02 mol/L sodium phosphate, 0.15 mol/L NaCl, pH 7.4
Residence time:	2, 3.5, 5 min
Detection:	UV @ 280 nm (10% breakthrough)
Sample:	human IgG @ 1, 5, 10 g/L in mobile phase

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

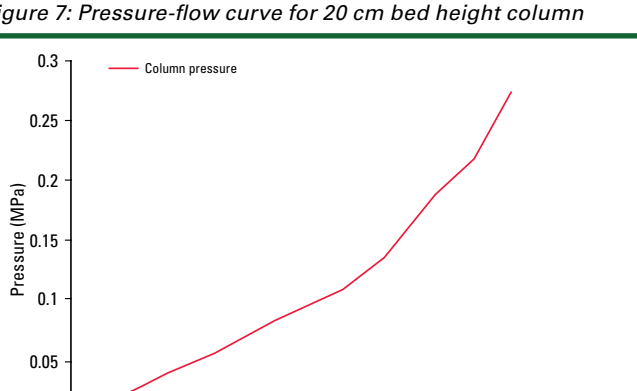
Note that this selected recombinant protein A ligand has very high affinity for mAbs from mouse, goat, rat and hybridoma cell lines.

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

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

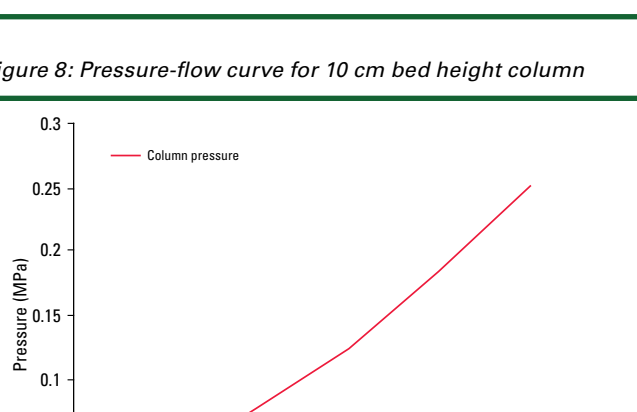
Achievement of high linear velocities at relatively low pressure enables high throughput at production scale using equipment with moderate pressure limitations (Figures 7 and 8).

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



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

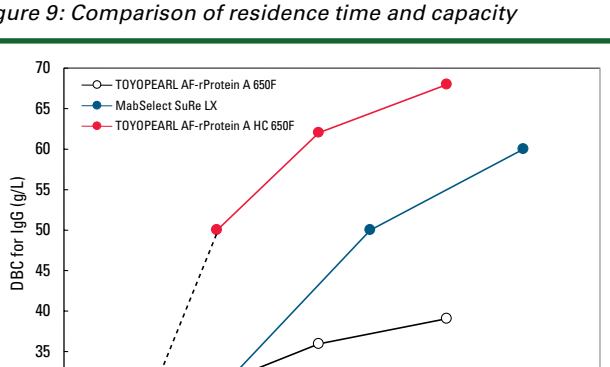
Figure 8: Pressure-flow curve for 10 cm bed height column



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

Improved mass transfer characteristics allow it to maintain a larger percent of its capacity at lower residence times (Figure 9) relative to agarose base stable resins. Typical leakage for this rProtein A ligand is 0.6 -1.7 ng rProtein A / mg eluted antibody by ELISA testing (Table 3).

Figure 9: Comparison of residence time and capacity



Resins:	**TOYOPEARL AF-rProtein A HC-650F** **TOYOPEARL AF-rProtein A-650F** MabSelect SuRe™ LX
Column size:	5 mm ID ×5 cm
Mobile phase:	0.02 mol/L sodium phosphate, 0.15 mol/L NaCl, pH 7.4
Residence time*:	2, 3.5, 5 min
Detection:	UV @ 280 nm
Sample:	human IgG @ 1 g/L in mobile phase

*MabSelect SuRe DBC data taken from product brochure(2, 4, and 6 minute residence times).

DBC was calculated at 10% breakthrough

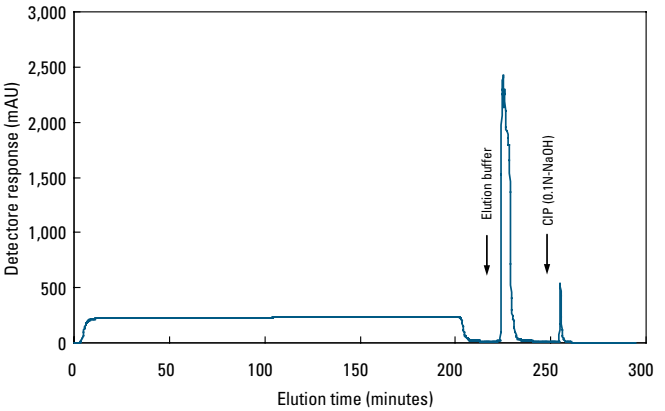
Table 3: Ligand leakage before and after CIP

Amount of ligand leakage (ppm)	Before CIP		After 200 CIP cycles	
	Elution Buffer		Elution Buffer	
	citrate (pH 3.0)	glycine-HCl (pH 3.0)	citrate (pH 3.0)	glycine-HCl (pH 3.0)
	1.7	1.6	0.6	0.5
Amount of ligand leakage was determined with TOYOPEARL AF-rProtein A HC-650F ELISA				
ppm = ng/mg IgG				

Purification of Monoclonal Antibodies

TOYOPEARL AF-rProtein A HC-650F was used for the purification of a monoclonal antibody from CHO cell culture supernatant with a concentration of 1.0 g/L (Figure 10) at 5 minutes residence time in a 5 cm bed height column. As can be seen from the chromatogram, tailing is minimal on the elution peak and the eluted mAb is > 95% pure by SEC. A second series of purification was performed to study the effects of resin loading.

Figure 10: Purification of monoclonal antibody



Resin:	**TOYOPEARL AF-rProtein A HC-650F**
Column size:	5 mm ID × 5.0 cm
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate containing 0.15 mol/L NaCl, pH 7.4
	Buffer B: 0.1 mol/L citrate, pH 3.0
Flow rate:	61 cm/hr (0.2 mL/min)
Residence time:	5 min
Sample:	40 mL of CHO cell culture, containing 1.0 gL humanized IgG_1

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

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

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

DOE Characterization of mAb Capture Step

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

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

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

Figure 11: Design space parameters

Variables: feedstock titer
resin load
HCP spiking
pH

Center point values

Four factor, central composite experimental design

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

Variable: elution buffer (citrate or acetate)

Figure 12: HCP removal for all resins evaluated

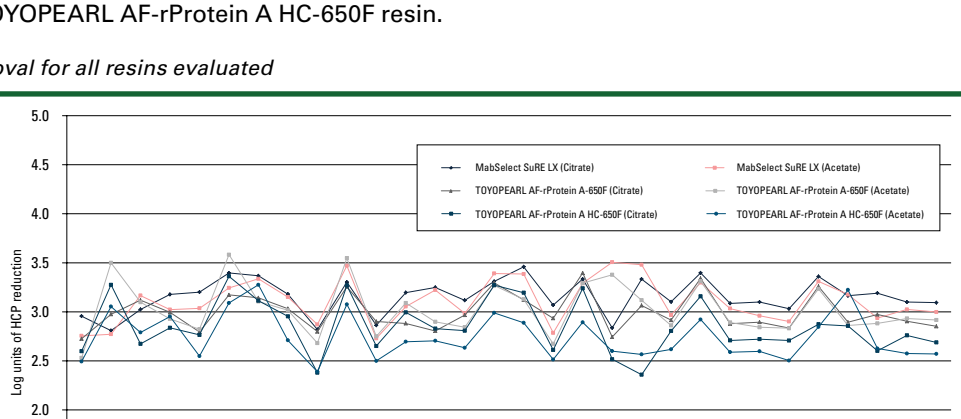


Figure 13: Effect of feedstock titer on HCP concentration in column elution

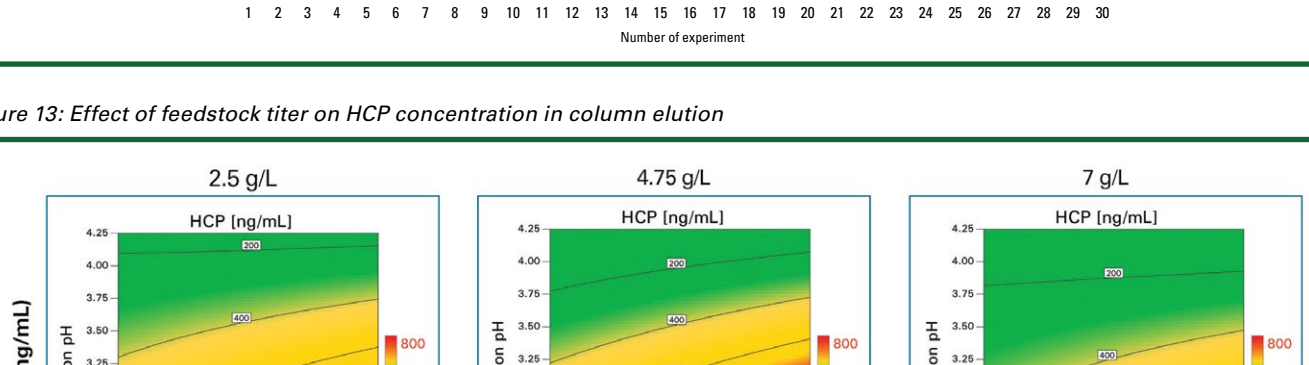


Figure 14 shows the results of the DOE experiments for ligand leakage (ng/mL) for all three resins using both citrate and acetate as an elution buffer. Acceptable levels of ligand leakage were seen for all resins tested; however, the TOYOPEARL AF-rProtein A HC-650F showed levels of leakage an order of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

Figure 14: Ligand leaching from Protein A resins tested

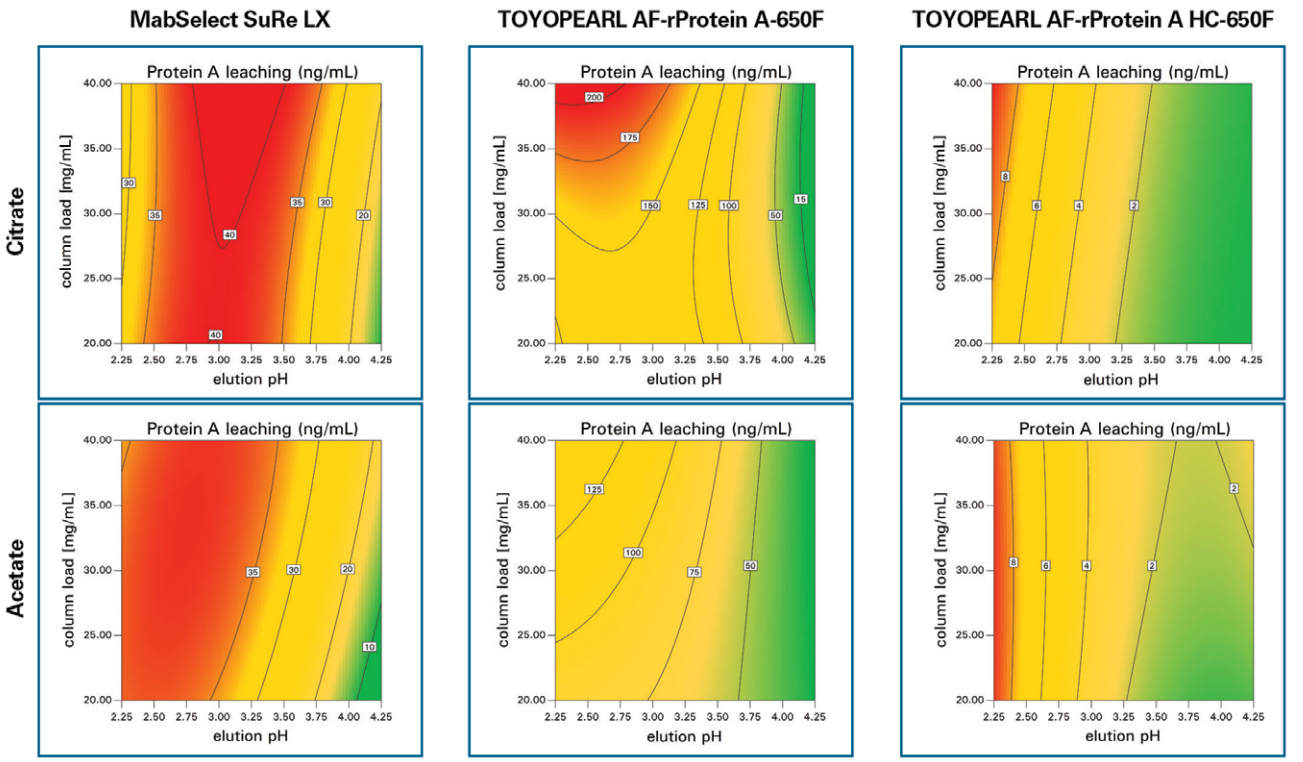
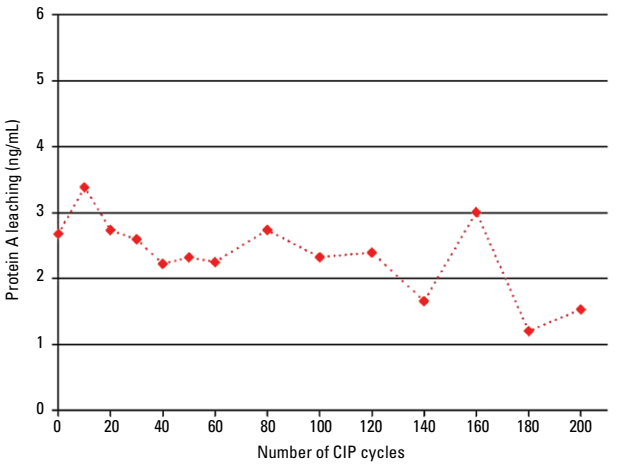


Figure 15 shows the amount of ligand eluted from the TOYOPEARL AF-rProtein A HC-650F resin over 200 cycles using 0.2 mol/L NaOH to clean in place (CIP) between each cycle. As the number of CIP cycles increased, the amount of ligand present in the eluted product decreased. This indicates that the TOYOPEARL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

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



TOYOPEARL AF-rProtein A-650F resin is an affinity resin for monoclonal antibody purification. The recombinant ligand (Figure 16) is expressed in *E. coli* and is free of animal derived products. The ligand is bound to the TOYOPEARL HW-65F base bead via multipoint attachment resulting in excellent base (Figure 17 and 18) and thermal stability (Figure 19). TOYOPEARL AF-rProtein A-650F resin exhibits dynamic binding capacities of greater than 30 g/L at residence times of 3 minutes and greater.

Figure 16: Ligand structure of TOYOPEARL AF-rProtein A-650F resin

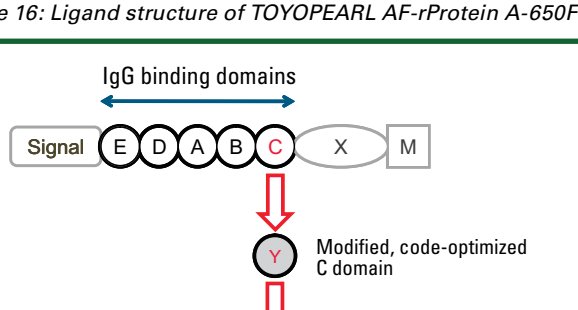
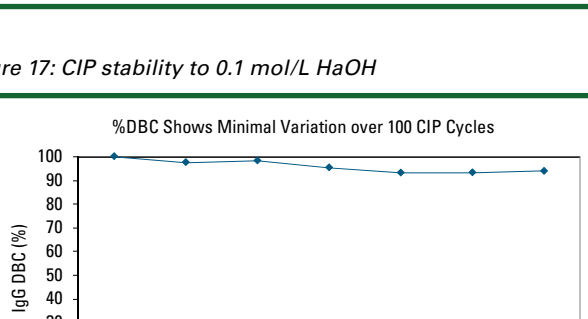


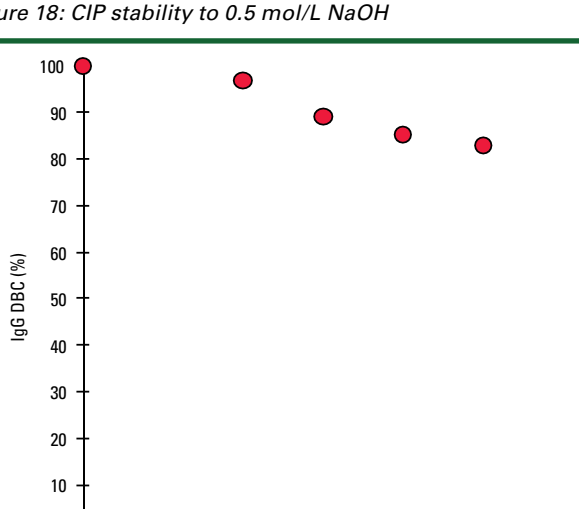
Figure 17: CIP stability to 0.1 mol/L HaOH



Resin:	**TOYOPEARL AF-rProtein A-650F**
Column size:	4.6 mm ID × 10 cm
Mobile phase:	Buffer A: 0.15 mol/L NaCl in 0.02 mol/L sodium phosphate buffer, pH 7.2 Buffer B: 0.10 mol/L citrate buffer, pH 3.0
Cycle volumes:	5 column volumes binding buffer 10 column volumes elution buffer 3 column volumes 0.1 mol/L NaOH, (16 min contact time) 5 column volumes H_2O

DBC was calculated at 10% breakthrough

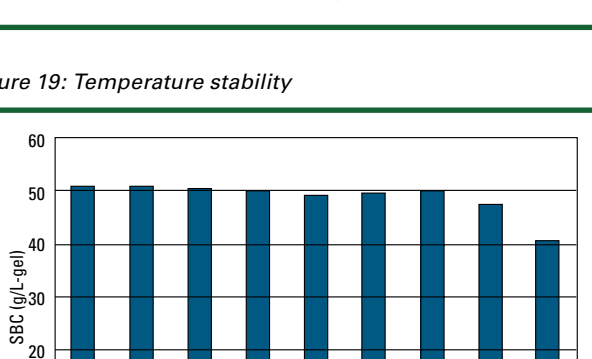
Figure 18: CIP stability to 0.5 mol/L NaOH



Resin:	**TOYOPEARL AF-rProtein A-650F**
Column size:	5.0 mm ID × 5 cm
Mobile phase:	Buffer A: 0.15 mol/L NaCl in 0.02 mol/L sodium phosphate buffer, pH 7.2 Buffer B: 0.10 mol/L citrate buffer, pH 3.0
Cycle volumes:	5 column volumes binding buffer 10 column volumes elution buffer 8 column volumes 0.5 mol/L NaOH, (16 min contact time) 5 column volumes H_2O

DBC was calculated at 10% breakthrough

Figure 19: Temperature stability

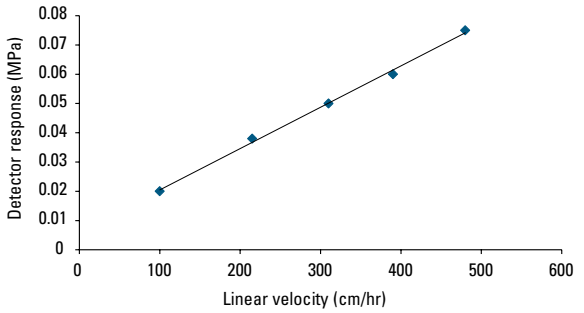


Resin:	**TOYOPEARL AF-rProtein A-650F**
Mobile phase:	deionized H_2O
Autoclave settings:	120 °C, 1.2 atm, 15 min
Heating time:	30 min

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

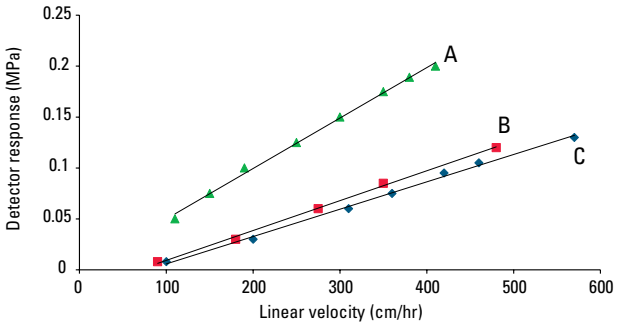
TOYOPEARL AF-rProtein A resins remain dimensionally stable within wide extremes of pH and ionic strength. Moreover, the semi-rigid TOYOPEARL AF-rProtein A particles do not distort under flow rates that generate up to 0.3 MPa pressure. These properties of the resins, combined with the narrow particle size distributions, result in superior pressure-flow characteristics for the packed TOYOPEARL bed. Linear velocities of 300 – 500 cm/hr generate a pressure of between 0.1 and 0.2 MPa in a packed bed (Figures 20 and 21).

Figure 20: Linear velocity and pressure curve



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

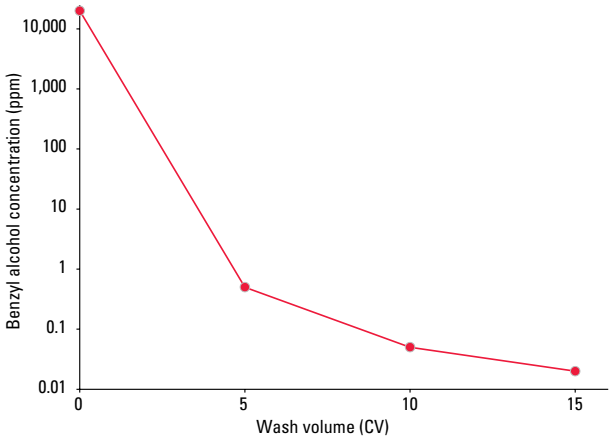
Figure 21: Comparison of linear velocity and pressure curves



Resins:	**A. TOYOPEARL AF-rProtein A -650F, 45 µm, 20 cm ID × 32 cm**
	B. TOYOPEARL AF-rProtein A -650F, 45 µm, 20 cm ID × 18 cm
	C. TOYOPEARL Butyl-650M, 65 µm, 20 cm ID × 21 cm
Column:	QuikScale®, 20 cm ID
Mobile phase:	H_2O
Linear velocity:	various
Detection:	pressure (MPa)

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

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



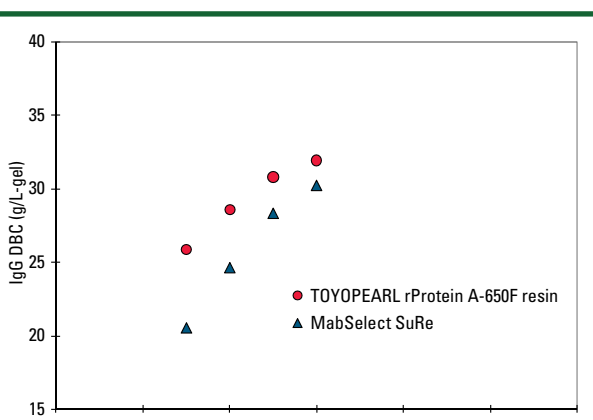
Resin:	**TOYOPEARL AF-rProtein A-650F**
Column size:	1.6 cm ID × 10 cm (20.1 mL)
Mobile phase:	DI H_2O
Flow rate:	800 cm/hr (26.8 mL/min)
Residence time:	45 sec

Improved mass transfer characteristics allow TOYOPEARL AF-rProtein A-650F to maintain a larger percent of its capacity at lower residence times (Figure 23) relative to agarose base stable resins. Typical leakage for this rProtein A ligand is 5-25 ng rProtein A /mg eluted antibody by ELISA testing.

Achievement of high linear velocities at relatively low pressure enables high throughput at production scale using equipment with moderate pressure limitations. Sanitization or cleaning may be conducted with up to 0.5 mol/L NaOH or 0.5 mol/L HCl depending upon the ligand.

An important aspect of the use of a Protein A resin in the capture step is its ability to remove host cell protein (HCP) from the feedstock. TOYOPEARL AF-rProtein A-650F addresses this key area as well (Table 5).

Figure 23: DBC at various residence times



Resins:	**TOYOPEARL AF-rProtein A-650F**
	MabSelect SuRe
Column size:	5 mm ID × 5 cm (1 mL)
Mobile phase:	0.02 mol/L sodium phosphate buffer, pH 7.2 + 0.15 mol/L NaCL
Residence time:	1.5, 2.0, 2.5, 3.0 min

Table 5: TOYOPEARL AF-rProtein A-650F resin vs. MabSelect SuRe resin

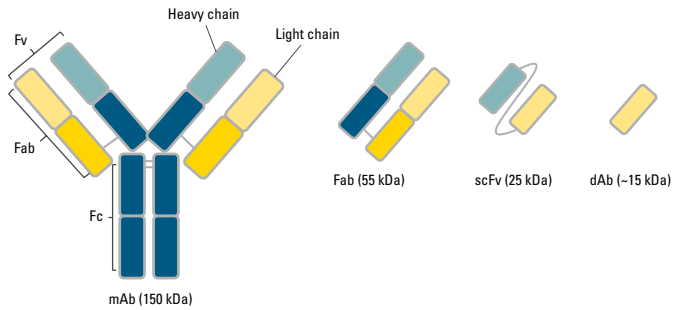
Resin	Protein load (mg/mL gel)	pH	Flow (cm/hr)	BV (µL)	Buffer	CHO (ng/mL)
Toyopearl AF-rProtein A-650F MabSelect SuRe	5 5	3,9 3,9	250 250	200 200	Tris Phosphate	9,76 30,52
Toyopearl AF-rProtein A-650F MabSelect SuRe	45 45	3,4 3,4	100 100	200 200	Tris Phosphate	0,67 36,52
Toyopearl AF-rProtein A-650F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

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

Protein L Chromatography in Process Purification

Protein L-based affinity chromatography is used for the capture of antibodies and antibody fragments that do not bind to protein A. Unlike protein A and G, which bind to the Fc region of immunoglobulins (IgGs), protein L binds through interactions with the variable region of an antibody's kappa light chain. Therefore, protein L binds a wider range of antibody classes than protein A such as IgG, IgM, IgA, IgE, and IgD. Figure 24 shows typical protein L binding regions, such as antigen binding fragments (Fabs), single-chain variable fragments (scFvs) and domain antibodies (dAbs).

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



TOYOPEARL AF-rProtein L-650F Resin

TOYOPEARL AF-rProtein L-650F is an affinity chromatography resin that combines a rigid polymer matrix with a recombinant ligand, which is derived from the B4 domain of native protein L from *Peptostreptococcus magnus* and is expressed in *E.coli* (Figure 25). Code optimization of the domain results in higher binding capacity and an improved stability of the ligand compared to the native molecule. The key characteristics of TOYOPEARL AF-rProtein L-650F resin are listed in Table 6.

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

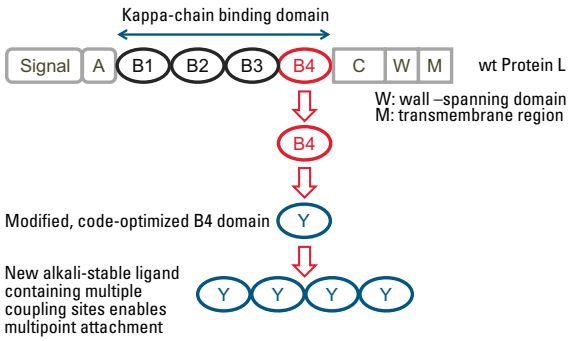


Table 6: Properties of TOYOPEARL AF-rProtein L-650F resin

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

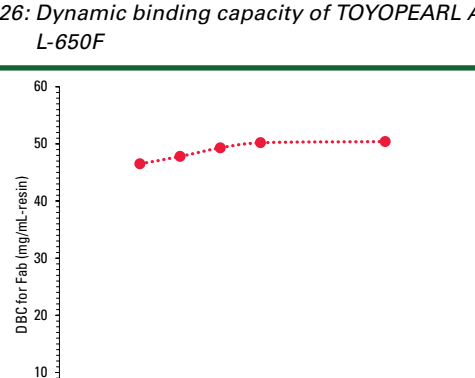
The selected recombinant Protein L ligand used in the TOYOPEARL AF-rProtein L-650F resin has an affinity for a broad range of antibody subclasses, as demonstrated in Table 7.

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

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

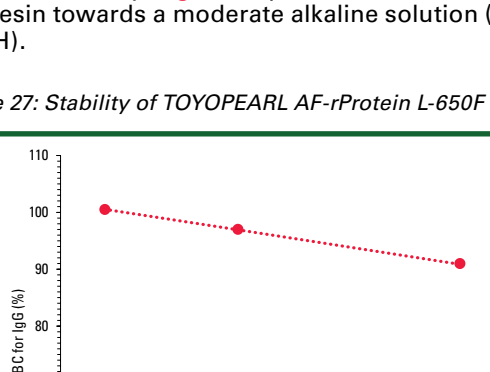
The combination of an optimized recombinant ligand and the proven TOYOPEARL base matrix results in a resin that provides the highest binding capacity available on the market for Fab molecules. Figure 26 shows the excellent binding capacity of TOYOPEARL AF-rProtein L-650F for a Fab fragment at various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

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



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

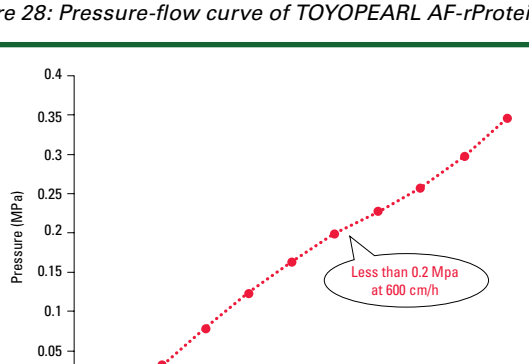
Figure 27: Stability of TOYOPEARL AF-rProtein L-650F



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

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

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

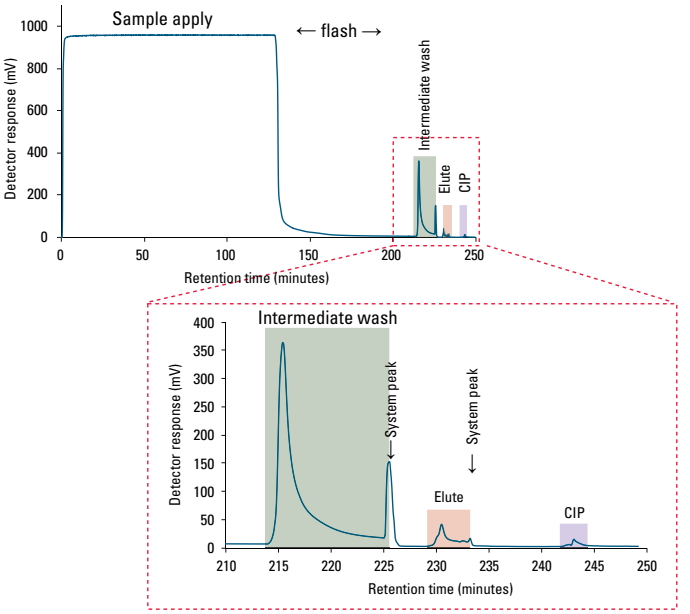


The protein L ligand is immobilized to the highly cross bead matrix via a multi-point coupling that also gives the TOYOPEARL AF rProtein L-650F resin a low ligand leakage. The analysis of the protein L ligand leakage is determined by using a commercially available ELISA-protein L ligand leakage kit in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

Purification and Analysis of scFv Fragment of hIgG$_1$

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

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



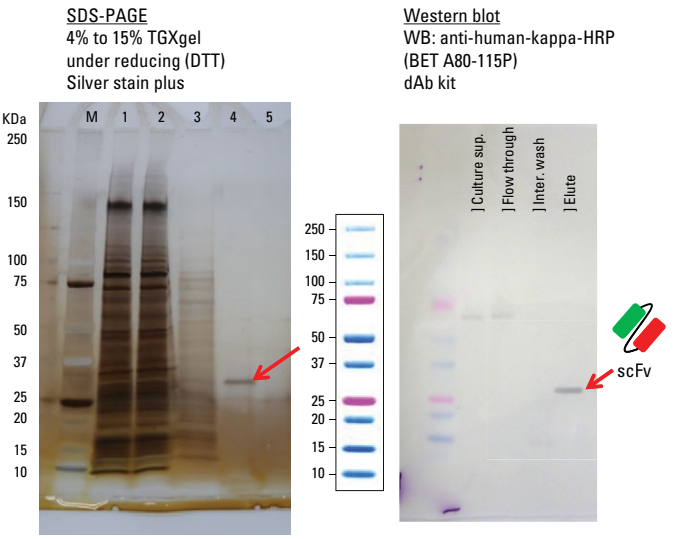
Resin:	**TOYOPEARL AF-rProtein L-650F**
Column size:	5 mm ID × 2.5 cm (0.5 mL)
Equib & flash wash:	0.1 mol/L Na-citrate, pH 6.5
Intermediate wash:	0.1 mol/L Na-citrate, pH 6.5 + 1 mol/L Arg-HCl, 10% iPrOH
Elute:	0.1 mol/L Citrate, pH 2.3
CIP:	0.05 mol/L NaOH
Flow rate:	0.35 mL/min (residence time = 1.4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

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

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

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

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

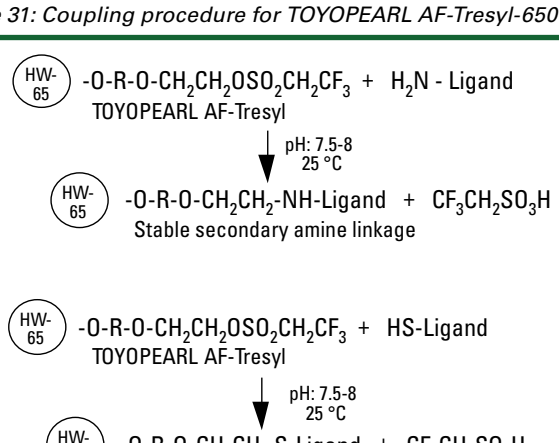


M prestained size marker (Bio-Rad 1610374)
1 Applied sample (hu_scFv_1)
2 Flow through
3 Intermediate wash (Arg+iPrOH)
4 Elute (pH 2.3) 1114series
5 CIP (50 mmol/L NaOH)

Activated resins – ready for direct ligand attachment

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

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



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

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

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

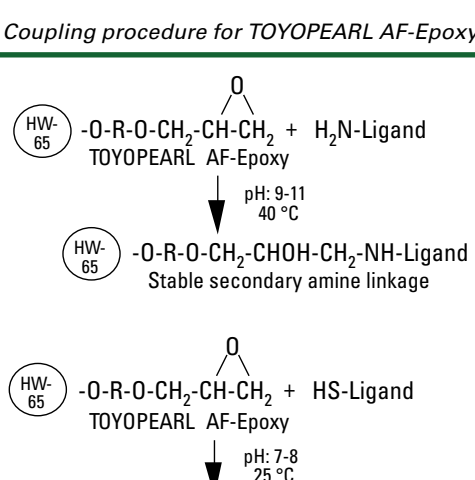


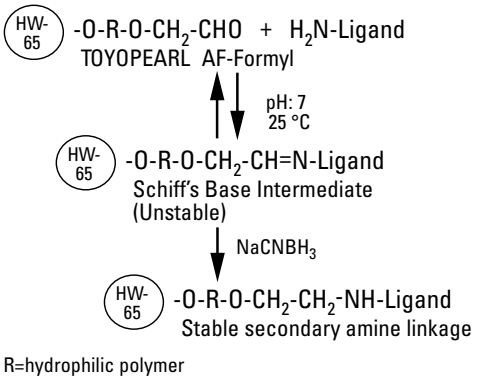
Table 8: Representative coupling densities for activated and reactive TOYOPEARL media

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

Reactive resins - require activation for ligand attachment

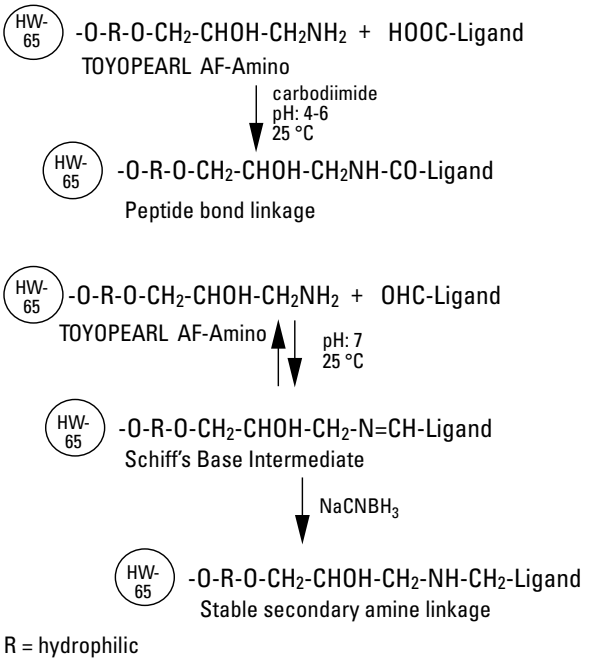
Ligands may be coupled to TOYOPEARL AF-Formyl-650M (aldehyde-bearing) resin under mild conditions exclusively using primary amines. The ligand is bound to the resin by a stable secondary amine linkage (Figure 33). A wide variety of industrial enzymes have been immobilized on aldehyde-bearing supports.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

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



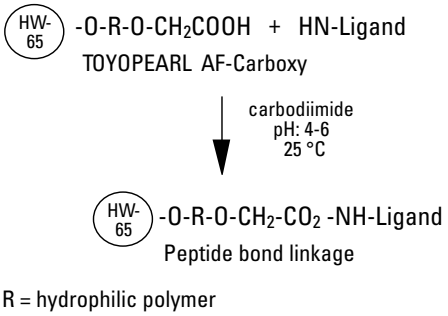
TOYOPEARL AF-Amino-650M resin may be used to couple ligands using their carboxyl groups through peptide bond formation or aldehyde groups by reductive amination as shown in Figure 34. Aldehyde groups may be present in a carbohydrate or glycoprotein ligand or may be introduced into the ligand by mild,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

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



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

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



Resins with group specific ligands

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

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

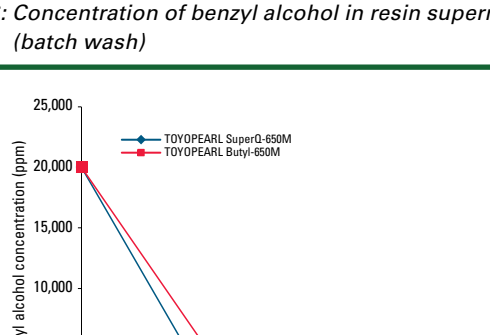
Table 9: Representative binding capacities for TOYOPEARL AF-Red-650ML

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

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

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

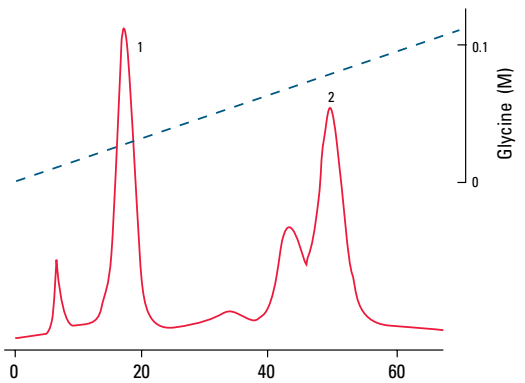
Figure 36: Concentration of benzyl alcohol in resin supernatant (batch wash)



Separation of Two Proteins

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

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

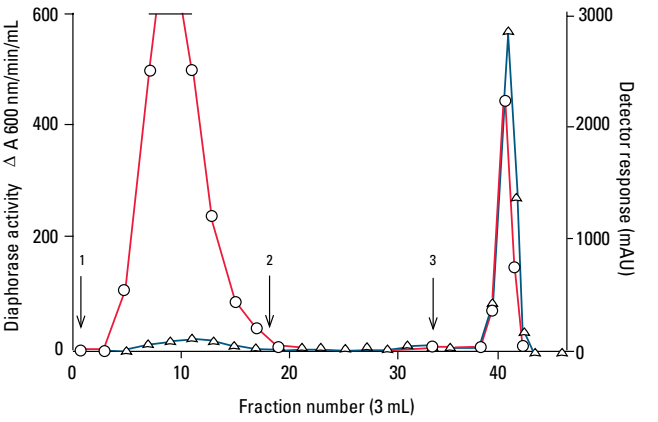


Resin:	**TOYOPEARL AF-Chelate-650M**
Column size:	8 mm ID × 7.5 cm
Metal ion:	Zn^{+2}
Mobile phase:	Buffer A: 20 mmol/L Tris-HCl, 0.5 mol/L NaCl, pH 8.0 Buffer B: buffer A + 0.2 mol/L glycine
Gradient:	0 - 100% B (120 min)
Flow rate:	60 cm/hr (0.5 mL/min)
Detection:	UV @ 280 nm
Samples:	1. ribonuclease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

Purification of Ferredoxin-NADP Reductase

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

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



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

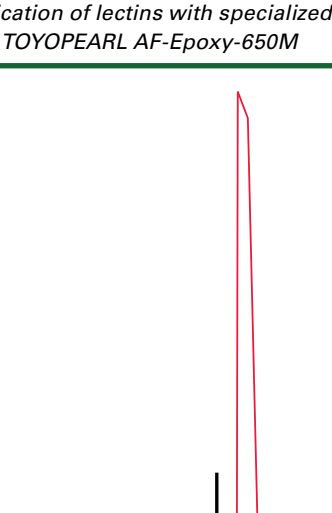
[1]Sakihama, N.; Nagai, K.; Ohmori, H.; Tomizawa, H.; Tsujita, M.; Shin, M. Immobilized ferredoxins for affinity chromatography of ferredoxin-dependent enzymes. *J. Chroma. A.* **1992**, *597*, 147-153.

Purification of Lectins

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

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

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



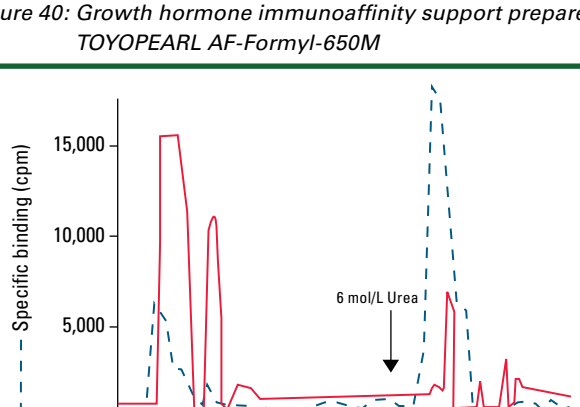
Resin:	**GalNAc-Epoxy TOYOPEARL**
Column size:	10 mm ID × 5 cm
Mobile phase:	Buffer A: 10 mmol/L phosphate-buffered saline, pH 7.4
	Buffer B: buffer A + 0.1mol/L lactose
Gradient:	equilibrate/load/wash 100% buffer A isocratic elution 100% buffer B
Detection:	UV @ 275 nm
Sample:	4.0 mg impure *Grifola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

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

Purification of GH Receptor Protein

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

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



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

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

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

Ordering Information

TOYOPEARL Affinity resins:

Part #	Product description	Container size (mL)
	TOYOPEARL Protein A Resins	
22803	TOYOPEARL AF-rProtein A-650F	10
22804	TOYOPEARL AF-rProtein A-650F	25
22805	TOYOPEARL AF-rProtein A-650F	100
22806	TOYOPEARL AF-rProtein A-650F	1,000
22807	TOYOPEARL AF-rProtein A-650F	5,000
23425	TOYOPEARL AF-rProtein A HC-650F	10
23426	TOYOPEARL AF-rProtein A HC-650F	25
23427	TOYOPEARL AF-rProtein A HC-650F	100
23428	TOYOPEARL AF-rProtein A HC-650F	1,000
23429	TOYOPEARL AF-rProtein A HC-650F	5,000

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

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

HSA = Human Serum Albumin

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

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

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


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

TOYOPEARL MX-Trp-650M

The Role of Mixed-Mode Chromatography in Process Purification

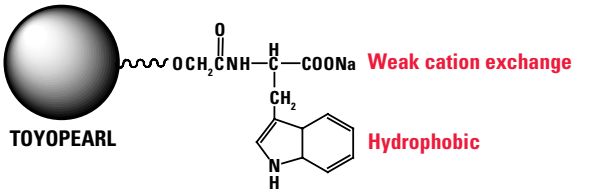
Multimodal or mixed-mode chromatography resins are based on media that have been functionalized with ligands inherently capable of several different types of interaction: ion exchange, affinity, size exclusion, and hydrophobic. The ability to merge and take advantage of these modes of protein separations can enhance overall selectivity in a purification process. This enhanced selectivity can be used to remove process impurities in a single column step that would otherwise require multiple processing steps to remove. Mixed-mode resins are in effect an amalgamation of complementary approaches to chromatographic separation on a single platform.

Unlike monomodal chromatographic methods where molecules are separated based on a single characteristic (activity, charge, hydrophobicity), with mixed-mode chromatography and mixed-mode ligands there is no known single specific interaction between the ligand and the molecule of interest. As such, screening mixed-mode resins becomes an exploration for sites on the target molecule that will deliver suitable selectivity and capacity. It is recommended that chromatographers screen for pH and conductivity as well as loading conditions when optimizing a purification process that incorporates mixed-mode resins. Protein-ligand interactions are not independent of one another on mixed-mode resins. For example, when using a mixed-mode resin having both hydrophobic interaction and ion exchange components, increasing conductivity will interrupt ionic bonds while at the same time enhancing any hydrophobic interactions. Because multiple dependent and independent variables are involved in using mixed-mode chromatography, the use of Design-of-Experiments (DoE) is recommended to characterize and optimize chromatographic conditions.

TOYOPEARL Mixed-Mode Chromatography Resin

TOYOPEARL MX-Trp-650M resin is a functionalized version of the TOYOPEARL HW size exclusion resin and is therefore based on a hydroxylated polymethacrylic polymer bead. Tosoh Bioscience offers one mixed-mode ligand, the amino acid tryptophan, which has both indole hydrophobic and weak carboxyl cationic functional groups (Figure 1).

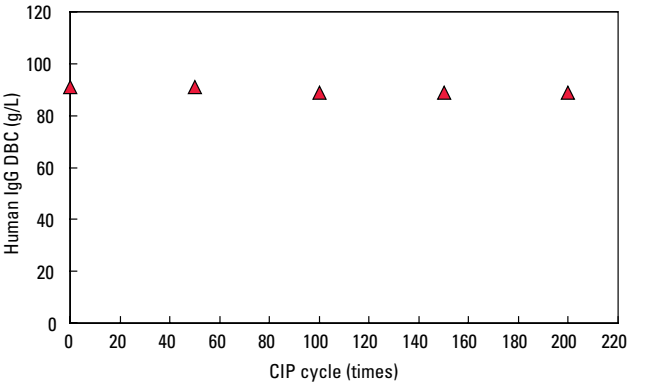
Figure 1: TOYOPEARL MX-Trp-650M structure



Product name: TOYOPEARL MX-Trp-650M
Particle size: 50-100 µm

The semi-rigid polymeric backbone of TOYOPEARL MX-Trp-650M permits high flow rates for maximum throughput and productivity. This mixed-mode resin may be operated at pressures up to 0.3 MPa and is 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. As shown in Figure 2, TOYOPEARL MX-Trp-650M has excellent stability to 0.5 mol/L NaOH and can be run for many CIP cycles without decreasing dynamic binding capacity (DBC).

Figure 2: Stability in 0.5 mol/L NaOH



Resin: **TOYOPEARL MX-Trp-650M**

Alkaline cleaning (CIP) conditions
- 3CV: 0.5 mol/L NaOH,
- 5CV: 0.1 mol/L Tris-HCl, pH 8.5 + 0.3 mol/L NaCl
- Flow rate: 212 cm/hr (1.0 mL/min)

DBC Measurement
- Column size: 6 mm ID × 4 cm
- Binding buffer: 0.05 mol/L acetate buffer, pH 4.7 + 0.1 mol/L NaCl
- Flow rate: 212 cm/hr (1 mL/min)
- Detection: UV @ 280 nm
- Sample: polyclonal human IgG
- Sample Load: 1 mg/mL

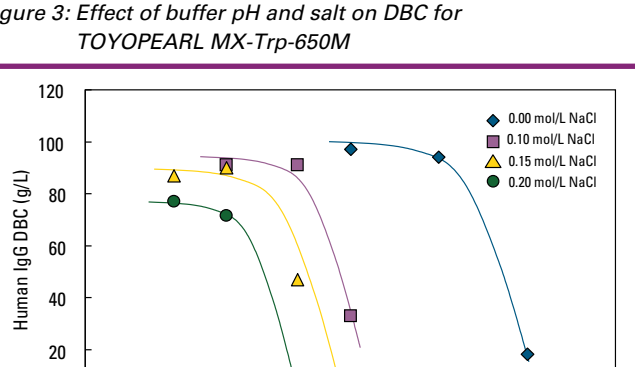
Table 1: Properties of TOYOPEARL MX-Trp-650M resin

TOYOPEARL resin	Functionality	Base bead	Pore size	Bead diameter	Ligand type	Ligand pKa ($-CO_2H$)*	DBC (g/L)	Pressure rating
MX-Trp-650M	cationic/HIC	HW-65	100 nm	75 µm	HIC/ weak cation	2.38	90 - 100	0.3 MPa

**Ligand pKa value is the pKa of the α-carboxyl group on the amino acid itself.*

TOYOPEARL MX-Trp-650M is a high capacity mixed-mode resin used for the purification of monoclonal antibodies and other proteins. The multimodal resin maintains DBC at elevated feedstock or buffer conductivities (Figure 3). Table 2 shows the DBC of TOYOPEARL MX-Trp-650M at two feedstock conductivities: 12 mS/cm and 17 mS/cm. For comparison purposes, data for an agarose based resin is also shown. For the 12 mS/cm and 17 mS/cm measurements, the TOYOPEARL MX-Trp-650M resin shows almost 7× higher and 4× higher DBC, respectively, than the agarose based resin. Superior product recovery over the agarose based resin is also demonstrated in Table 3.

Figure 3: Effect of buffer pH and salt on DBC for TOYOPEARL MX-Trp-650M



Resin: **TOYOPEARL MX-Trp-650M**
Column: 6 mm ID × 4 cm
Mobile phase: Buffer A: 0.05 mol/L acetate buffer, pH 4.0 - 6.0 + 0 - 0.2 mol/L NaCl
Buffer B: 0.1 mol/L Tris-HCl buffer, pH 8.5 + 0.3 mol/L NaCl
Flow rate: 212 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Sample: human polyclonal IgG (1 mg/mL)

Dynamic binding capacity (DBC) calculated from 10% height of breakthrough curve

Table 2: Dynamic binding capacities at high conductivities

Resin	Particle size(µm)	Ion exchange capacity (meq)	DBC (g/L)	Recovery %
TOYOPEARL MX-Trp-650M (12 mS/cm)	50 - 100	0.12	95	97
TOYOPEARL MX-Trp-650M (17 mS/cm)	50 - 100	0.12	48	96
Brand M (Agarose 12 mS/cm)	75 (median)	0.24	14	86
Brand M (Agarose 17 mS/cm)	75 (median)	0.24	11	85

Resins: **TOYOPEARL MX-Trp-650M**
Brand M
Column size: 6 mm ID × 4 cm
Mobile phase: Buffer (12 mS/cm): 0.05 mol/L acetate buffer, pH 4.3, 4.7, 5.0 + 0.10 mol/L NaCl
Buffer (17 mS/cm): 0.05 mol/L acetate buffer, pH 4.3, 4.7, 5.0 + 0.15 mol/L NaCl
Flow rate: 212 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Sample: human polyclonal IgG (1 mg/mL)

Dynamic binding capacity (DBC) calculated from 10% height of breakthrough curve.

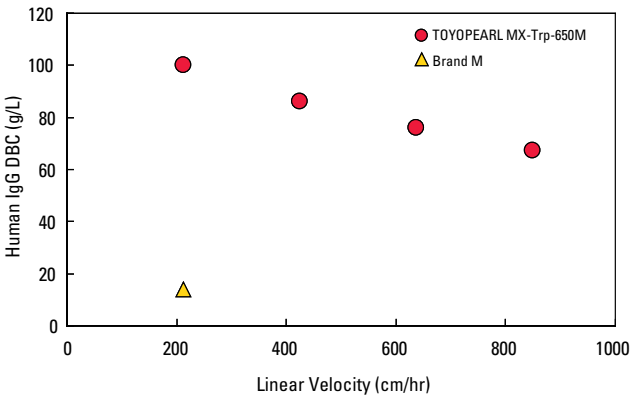
Table 3: Recovery comparison at conductivities of 12 and 17 mS/cm

Resin	IgG DBC 12 mS/cm	Recovery 12 mS/cm	IgG DBC 17 mS/cm	Recovery 17 mS/cm
TOYOPEARL MX-Trp-650M	95	97%	48	96%
Capto MMC	14	86%	11	85%

Resins: **TOYOPEARL MX-Trp-650M**
Capto MMC
Column size: 6 mm ID × 4 cm
Mobile phase: Buffer (12 mS/cm): 0.05 mol/L acetate buffer, pH 4.7 + 0.1 mol/L NaCl
Buffer (17 mS/cm): 0.05 mol/L acetate buffer, pH 4.7 + 0.15 mol/L NaCl
Flow rate: 212 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Sample: polyclonal IgG

The mass transfer properties of a resin influence the economics of the loading and elution stages of a capture step and the degree of resolution for intermediate purification. Good mass transfer kinetics enables the resin to maintain its DBC at increased linear velocities (Figure 4). In keeping with the exceptional target binding and eluting properties of TOYOPEARL GigaCap ion exchange resins, TOYOPEARL MX-Trp-650M also shows a narrow elution peak width to complement its higher capacity (Figure 5). The mass transfer properties also contribute to minimal peak broadening. Figure 6 shows the excellent peak shape for TOYOPEARL MX-Trp-650M and the much broader tailing associated with the Brand M agarose material.

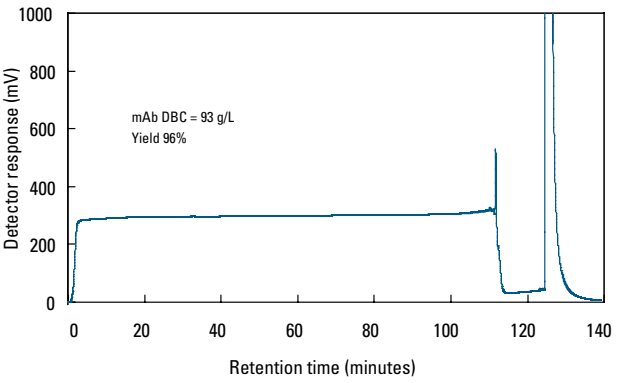
Figure 4: DBC at higher linear velocities



Resins:	**TOYOPEARL MX-Trp-650M**
	Brand M
Column size:	0.6 mm ID × 4.0 cm
Mobile phase:	Buffer A: 0.05 mol/L sodium acetate buffer, pH 4.7 + 0.1 mol/L sodium chloride
	Buffer B: 0.1 mol/L Tris-HCl buffer, pH 8.5 + 0.3 mol/L sodium chloride
Flow rates:	212, 425, 637, 849 cm/hr (1, 2, 3, 4 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	polyclonal human IgG (1 mg/mL)
Sample load:	1 mg/mL

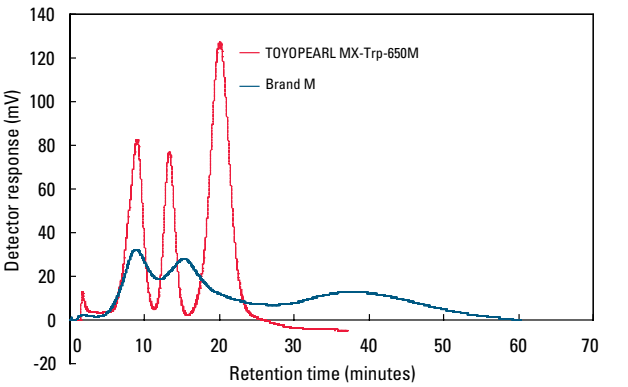
Dynamic binding capacities (DBC) were determined at 10% breakthrough

Figure 5: Narrow elution peak widths



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 0.05 mol/L acetate buffer, pH 4.7 + 0.1 mol/L NaCl (12 mS/cm)
	Buffer B: 0.1 mol/L Tris-HCl buffer, pH 8.5 + 0.3 mol/L NaCl
Flow rate:	A: 212 cm/hr (1.0 mL/min)
	B: 424 cm/hr (2.0 mL/min) started at 124 min
Detection:	UV @ 280 nm
Sample:	CHO cell culture media, monoclonal antibody (1 mg/mL) diluted with buffer A

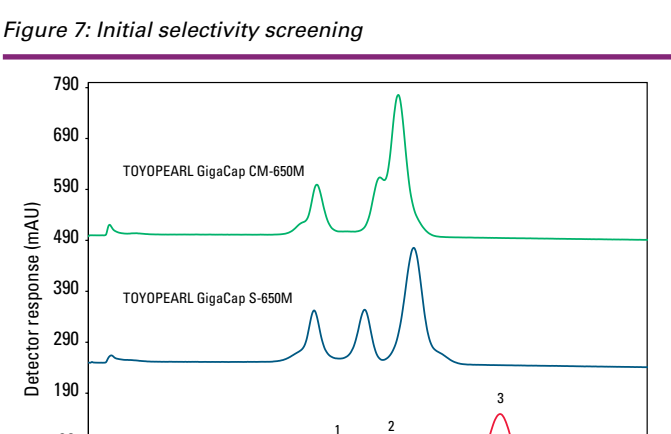
Figure 6: Good resolution for intermediate purification



Resins:	**TOYOPEARL MX-Trp-650M, Brand M**
Column size:	7.5 mm ID × 7.5 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:	30 min. linear gradient from buffer A to buffer B
Flow rate:	136 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Sample:	trypsinogen (6.6 mg/mL)
	cytochrome C (3.6 mg/mL)
	lysozyme (6.6 mg/mL)
Load volume:	25 µL

Selectivity of TOYOPEARL MX-Trp-650M, when compared to a traditional weak cation exchange (TOYOPEARL GigaCap CM-650M) and a traditional strong cation exchange (TOYOPEARL GigaCap S-650M) resin, is noticeably different. A three protein mixture (trypsinogen, cytochrome C, and lysozyme) was loaded onto each resin in 20 mmol/L sodium phosphate buffer (pH 7.0) and eluted with a linear salt gradient (Figure 7). Resolution between the peaks was measured and recorded for comparison (Table 4). Further selectivity comparisons were done at decreasing pH levels for all three resins with the same protein mixture at pH 6.0 (20 mmol/L sodium acetate) and pH 5.0 (20 mmol/L sodium citrate) and were compared to the initial screening at pH 7.0 (Figures 8-10). Resolution between the peaks was likewise measured and recorded for comparison (Tables 5-7).

Figure 7: Initial selectivity screening

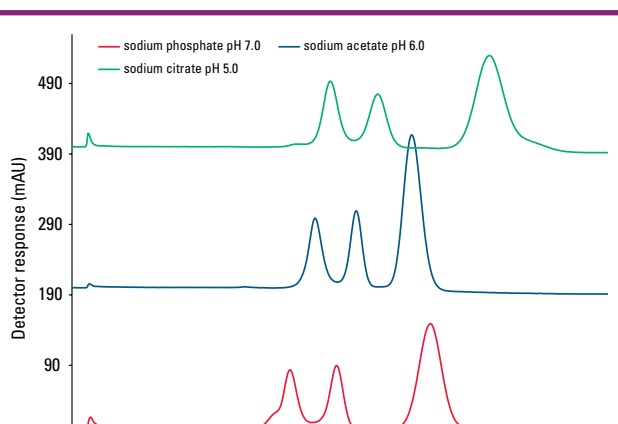


Resin:	**As Indicated**
Column size:	6.6 mm ID × 15.5 ± 1.0 cm
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate, pH 7.0 Buffer B: buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Samples:	1. trypsinogen (6.6 mg/mL) 2. cytochrome C (3.6 mg/mL) 3. lysozyme (6.6 mg/mL)
Sample load:	5% CV (4.02 - 4.60 mg total protein)

Table 4: Initial selectivity screening peak resolutions

	Peak resolution	
Resin	**trypsinogen/ cytochrome C**	**cytochrome C/ lysozyme**
TOYOPEARL MX-Trp-650M	0.81	1.50
TOYOPEARL GigaCap S-650M	0.94	0.82
TOYOPEARL GigaCap CM-650M	1.40	0.43

Figure 8: TOYOPEARL MX-Trp-650M pH scouting

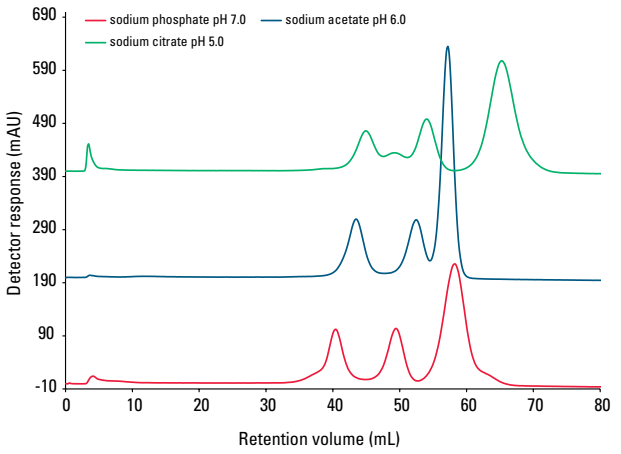


Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate, pH 7.0 Buffer A: 20 mmol/L sodium acetate, pH 6.0 Buffer A: 20 mmol/L citrate, pH 5.0 Buffer B: buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Samples:	1. trypsinogen (6.6 mg/mL), 2. cytochrome C (3.6 mg/mL) 3. lysozyme (6.6 mg/mL)
Sample load:	5% CV (4.45 mg total protein)

Table 5: TOYOPEARL MX-Trp-650M pH scouting peak resolutions

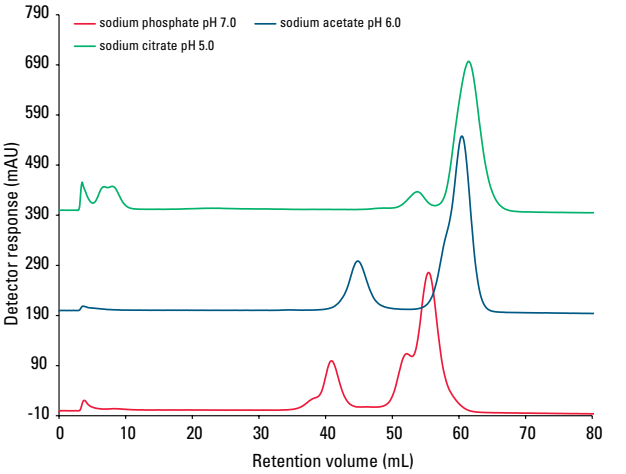
	Trypsinogen retention (mL)	Cytochrome C retention (mL)	Trypsinogen/ cytochrome C resolution (Rs)	Lysozyme retention (mL)	Cytochrome C/ lysozyme resolution (Rs)
Phosphate pH 7.0	44.88	54.36	0.81	73.63	1.50
Acetate pH 6.0	50.01	58.45	0.89	69.87	1.04
Citrate pH 5.0	53.08	62.94	1.07	85.97	1.57

Figure 9: TOYOPEARL GigaCap S-650M pH scouting



Resin:	**TOYOPEARL GigaCap S-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate, pH 7.0 Buffer A: 20 mmol/L sodium acetate, pH 6.0 Buffer A: 20 mmol/L citrate, pH 5.0 Buffer B: buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	1. trypsinogen (6.6 mg/mL), 2. cytochrome C (3.6 mg/mL) 3. lysozyme (6.6 mg/mL)
Sample load:	5% CV (4.31 mg total protein)

Figure 10: TOYOPEARL GigaCap CM-650M pH scouting



Resin:	**TOYOPEARL GigaCap CM-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate, pH 7.0 Buffer A: 20 mmol/L sodium acetate, pH 6.0 Buffer A: 20 mmol/L citrate, pH 5.0 Buffer B: buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	1. trypsinogen (6.6 mg/mL), 2. cytochrome C (3.6 mg/mL) 3. lysozyme (6.6 mg/mL)
Sample load:	5% CV (4.31 mg total protein)

Table 6: TOYOPEARL GigaCap S-650M pH scouting peak resolutions

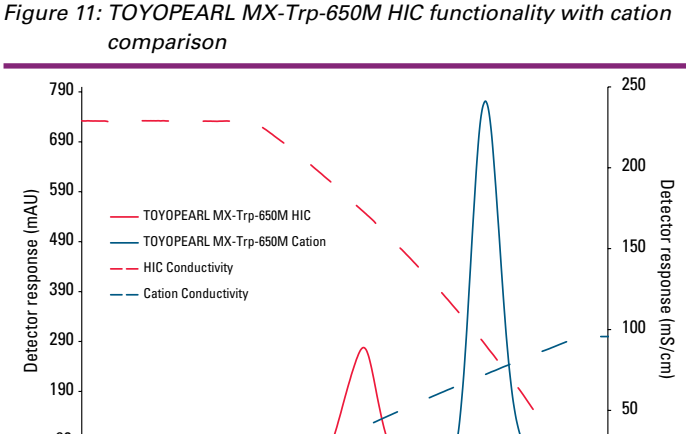
	Trypsinogen retention (mL)	Cytochrome C retention (mL)	Trypsinogen/ cytochrome C resolution (Rs)	Lysozyme retention (mL)	Cytochrome C/ lysozyme resolution (Rs)
Phosphate pH 7.0	40.38	49.46	0.94	58.27	0.82
Acetate pH 6.0	43.44	52.46	1.16	57.20	0.75
Citrate pH 5.0	44.96	54.05	1.23	65.29	1.00

Table 7: TOYOPEARL GigaCap CM-650M pH scouting peak resolutions

	Trypsinogen retention (mL)	Cytochrome C retention (mL)	Trypsinogen/ cytochrome C resolution (Rs)	Lysozyme retention (mL)	Cytochrome C/ lysozyme resolution (Rs)
Phosphate pH 7.0	40.89	52.20	1.40	55.45	0.43
Acetate pH 6.0	44.81	60.46	1.18	60.46	0
Citrate pH 5.0	53.71	61.46	0.84	61.46	0

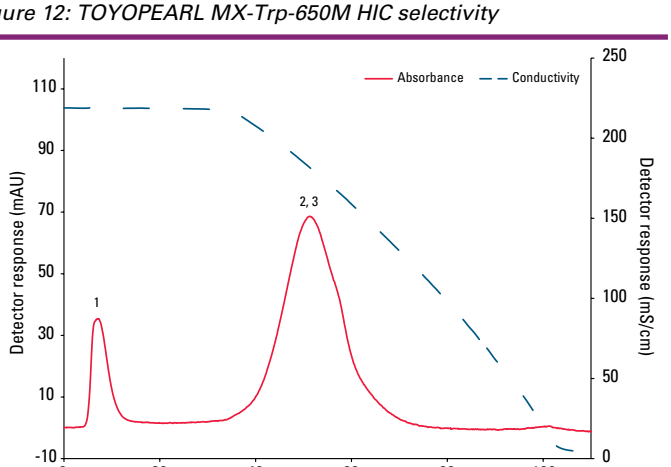
To examine the role the hydrophobic region of the tryptophan ligand can play in protein separations on TOYOPEARL MX-Trp-650M, the resin was tested to determine if it was possible to be used solely in HIC mode by loading lysozyme onto the column in 10 mmol/L sodium citrate, 1.8 mol/L ammonium sulfate, pH 5.0. The bound lysozyme was eluted with a decreasing linear gradient of 10 mmol/L sodium citrate, pH 5.0 (Figure 11). Comparison of resin selectivity in HIC mode and weak cation mode was done using a three protein mix (ribonuclease A, α α-chymotrypsinogen, and lysozyme) at pH 5.0 with sodium citrate as the mobile phase buffering salt (Figure 12 and 13). Further selectivity experiments with TOYOPEARL MX-Trp-650M can be found in *AN44: TOYOPEARL MX-Trp-650M Salt Selectivity and Tolerance.*

Figure 11: TOYOPEARL MX-Trp-650M HIC functionality with cation comparison



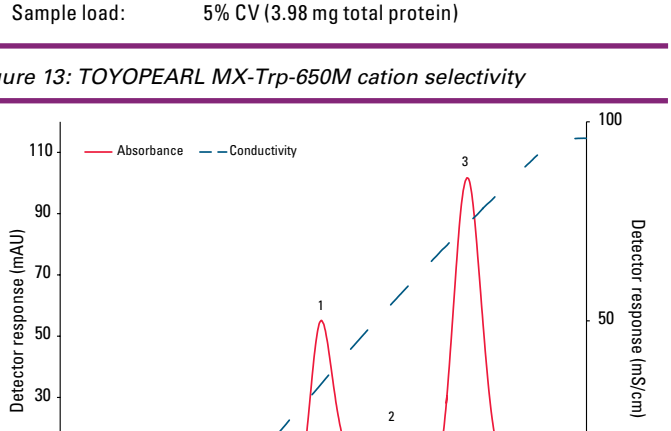
Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A (HIC): 10 mmol/L sodium citrate, 1.8 mol/L ammonium sulfate, pH 5.0 Buffer B (HIC): 10 mmol/L sodium citrate, pH 5.0 Buffer A (cation): 20 mmol/L sodium citrate, pH 5.0 Buffer B (cation): buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Temperature:	ambient
Sample:	lysozyme (cation – 10 mg/mL; HIC – 4 mg/mL)
Sample load:	5% CV (1.06 and 2.65 mg total protein)

Figure 12: TOYOPEARL MX-Trp-650M HIC selectivity



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A (HIC): 10 mmol/L sodium citrate, 1.8 mol/L ammonium sulfate, pH 5.0 Buffer B (HIC): 10 mmol/L sodium citrate, pH 5.0
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Temperature:	ambient
Sample:	1. ribonuclease A (4.0 mg/mL), 2. α-chymotrypsinogen (5.0 mg/mL) 3. lysozyme (6.0 mg/mL)
Sample load:	5% CV (3.98 mg total protein)

Figure 13: TOYOPEARL MX-Trp-650M cation selectivity



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A (cation): 20 mmol/L sodium citrate, pH 5.0 Buffer B (cation): buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Temperature:	ambient
Sample:	1. ribonuclease A (4.0 mg/mL), 2. α-chymotrypsinogen (5.0 mg/mL) 3. lysozyme (6.0 mg/mL)
Sample load:	5% CV (3.98 mg total protein)

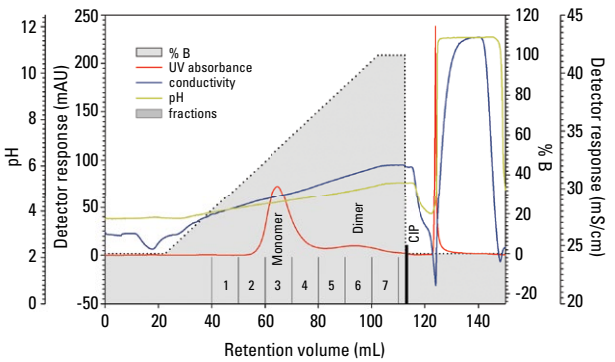
Parameters to Consider when Using TOYOPEARL MX-Trp-650M

Coordinating the hydrophobicity and charge of the therapeutic target to TOYOPEARL MX-Trp-650M is critical for the best overall purification performance. Operating at the extremes of hydrophobicity or charge for a given protein can result in drastically reduced performance of the resin or in some cases, a loss of biological activity. An optimum mixed-mode process step will balance high dynamic binding capacity, adequate selectivity, good mass recovery, and retention of biological activity. Execution of a DoE protocol during the screening process will enable developers to optimize protein separations by fine tuning mobile phase pH, conductivity and product load parameters.

Separation of Aggregates from mAbs

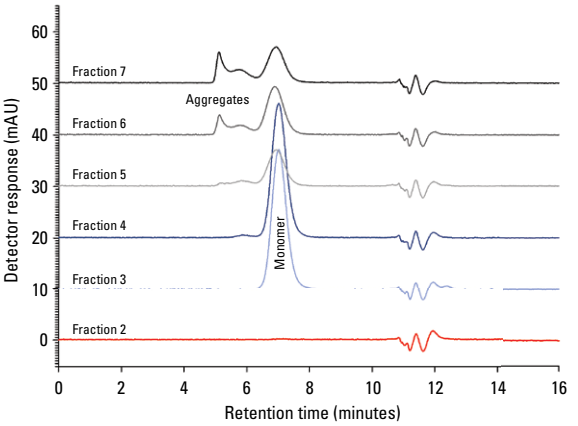
TOYOPEARL MX-Trp-650M successfully removes mAb aggregate from monomer using a narrow combination gradient of pH and conductivity (the pH and salt concentration range from pH 4.0 to 6.0 and 0.2 mol/L NaCl to 0.4 mol/L NaCl) respectively (Figure 14). The aggregate content in the monomer pool is below 1%, as shown in SEC chromatograms of the collected fractions analyzed in Figure 15. From these results it can be seen that TOYOPEARL MX-Trp-650M can be utilized as a highly efficient tool for aggregate removal of mAbs, as it offers capacities comparable to IEX, high recovery, and excellent selectivity.

Figure 14: Separation of mAb monomers and aggregates



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 2.0 cm
Mobile phase:	Buffer A: 0.1 mol/L acetate + 0.2 mol/L NaCl, pH 4.3 Buffer B: 0.1 mol/L acetate + 0.4 mol/L NaCl, pH 5.6
Flow rate:	150 cm/hr (0.86 mL/min)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Sample:	10 mg mAb + mAb aggregates
Sample load:	1 g/L

Figure 15: SEC chromatograms of the collected fractions



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 2.0 cm
Mobile phase:	Buffer A: 0.1 mol/L acetate + 0.2 mol/L NaCl, pH 4.3 Buffer B: 0.1 mol/L acetate + 0.4 mol/L NaCl, pH 5.6
Flow rate:	150 cm/hr (0.86 mL/min)
Detection:	UV @ 280 nm
Sample:	10 mg mAb + mAb aggregates
Sample load:	1 g/L

A selection of screening tools are available for TOYOPEARL Mixed-Mode resin. See the Process Development Products section of this Product Guide for details.

Ordering Information

TOYOPEARL Mixed-mode resin:

Part #	Product description	Container size (mL)	Bead diameter (µm)	IgG capacity (g/L)
22817	TOYOPEARL MX-Trp-650M	25	50 - 100	90 - 100
22818	TOYOPEARL MX-Trp-650M	100	50 - 100	90 - 100
22819	TOYOPEARL MX-Trp-650M	1,000	50 - 100	90 - 100
22820	TOYOPEARL MX-Trp-650M	5,000	50 - 100	90 - 100


TOYOPEARL®
MX-Trp-650M
FOR IN VITRO USE ONLY / FOR PACKED-BED USE ONLY
Net Vol. 100 mL
Part No. 22818
Lot No.
TOSOH
Made in Japan
S1048, Rev. 0.0

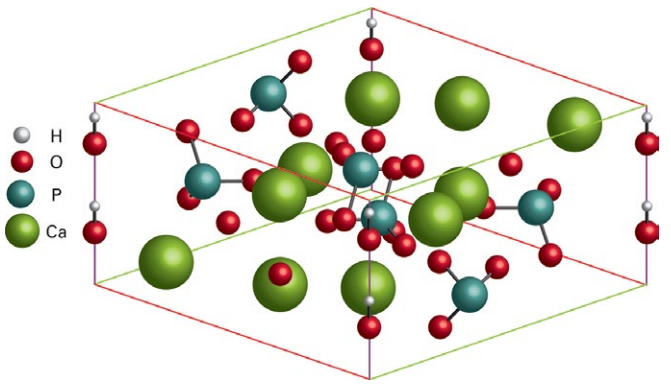
Ca^{++}Pure-HA
(Hydroxyapatite)

Ca++Pure-HA Resin

Ca++Pure-HA resin is a hydroxyapatite resin which has unique separation properties for biomolecules. This resin is specifically developed for the purification of antibodies and DNA; the separation of impurity antibody and its fragments from the native antibody (intact/monomer), and the isolation of single-stranded from double-stranded DNA.

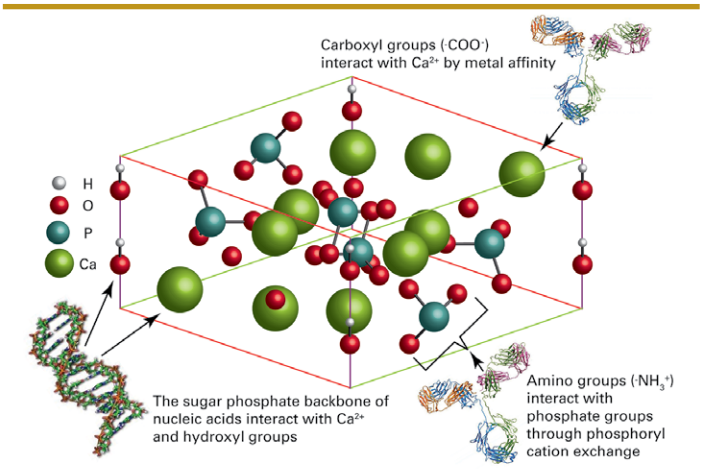
Ca++Pure-HA (hydroxyapatite: $Ca_{10}(PO_4)_6(OH)_2$) resin is a spherical, macroporous form of the hexagonal crystalline structure of hydroxyapatite (Figure 1). This resin has been sintered at high temperatures for increased mechanical and chemical stability, allowing it to withstand the rigors of industrial-scale applications. The robust nature of Ca++Pure-HA allows for it to be used reproducibly for many cycles at high flow rates and in large columns. Unlike other resins available from Tosoh Bioscience, the formation of the Ca++Pure-HA particle, both the ligand and the base bead, is created simultaneously from the same source of materials.

Figure 1: Ca++Pure-HA crystalline structure



Ca++Pure-HA resin has multiple mechanisms of interaction (Figure 2): calcium metal affinity interaction and phosphate group interaction.

Figure 2: Types of interactions with Ca++Pure-HA resin



The calcium metal affinity interactions occur between carboxyl (-OOC) groups on proteins and/or phosphate groups (e.g., sugar phosphate backbone of nucleic acids). These carboxyl and phosphate groups are repulsed by the negatively charged groups in the Hydroxyapatite structure. Elution of proteins that are bound by Ca^{2+} affinity (molecules that are composed mostly of acidic residues) will require increasing levels of phosphate in the mobile phase. Phosphate will out-compete proteins for the Ca^{2+} functional sites due to its strong affinity for calcium. Increasing concentrations of neutral salts, such as NaCl, will not typically have much effect on elution from the Hydroxyapatite resin when calcium metal affinity is the driving mechanism.

Cation exchange on Hydroxyapatite resin occurs when positively charged amino groups (^+H_3N) are ionically attracted to the negatively charged ***phosphate groups*** and are repulsed by the (Ca^{2+}) calcium groups. Elution of proteins bound by Hydroxyapatite resin through phosphoryl cation exchange (molecules that are composed mostly of basic residues), requires the addition of neutral salts, such as sodium chloride, to the mobile phase. Basic proteins may be eluted with either phosphates or neutral salts, depending on the selectivity of the resin for your target molecule and its impurities. Cationic interactions can also be disrupted by increasing mobile phase pH. Hence, the addition of salt or phosphate, or an increase in pH, can be used to weaken the interaction. In this way, Hydroxyapatite resin will behave in a similar fashion to a traditional cation exchange resin.

With its multiple mechanism of interaction and its unique particle formation, Ca++Pure-HA resin is used in the chromatographic separation of biomolecules and offers unparalleled selectivity and resolution for process scale operations. Its highly selective nature often separates proteins otherwise shown to be homogeneous by electrophoresis and other chromatographic techniques.

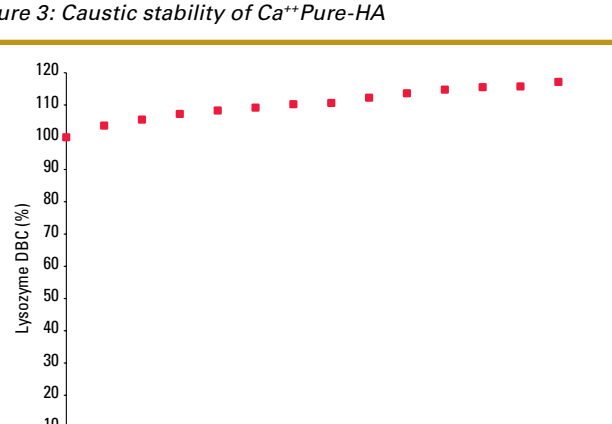
The key characteristics of Ca++Pure-HA resin are listed in Table 1.

Table 1: Properties of Ca++Pure-HA resin

Bead matrix	Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$
Particle size (mean)	39 µm
Dynamic binding capacity	> 30 g/L human IgG (2 min residence time) > 25 g/L lysozyme (2 min residence time)
Pressure rating	10 MPa (max)
Bead density	≥ 0.5 g/mL
Caustic stability	> 65 CIP cycles in 1.0 mol/L NaOH
Storage solution	Dry powder or 0.1 to 0.5 mol/L NaOH

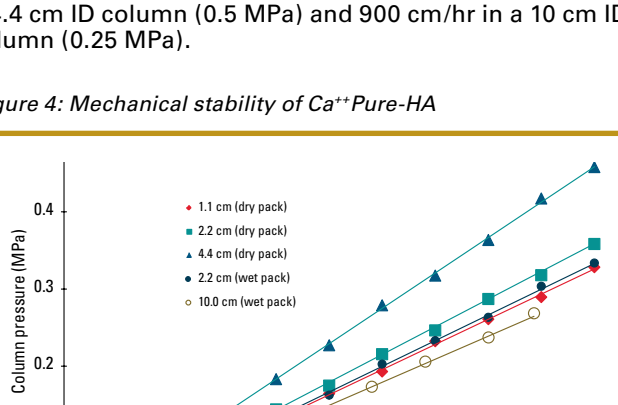
Ca⁺⁺Pure-HA is alkaline stable in 1.0 mol/L NaOH for greater than 65 CIP cycles with no appreciable loss of dynamic binding capacity (DBC). Figure 3 illustrates the caustic stability of Ca⁺⁺Pure-HA, with the DBC of lysozyme measured after every 5th CIP cycle with 1.0 mol/L NaOH.

Figure 3: Caustic stability of Ca⁺⁺Pure-HA



Ca⁺⁺Pure-HA is a rigid, crystalline support and can operate under very high flow rates and pressures when packed in a column. Ca⁺⁺Pure-HA was packed in columns of various ID to a height of 20 cm in a neutral pH mobile phase. Figure 4 demonstrates that Ca⁺⁺Pure-HA resin has excellent mechanical stability at linear velocities up to 1,000 cm/hr in a 4.4 cm ID column (0.5 MPa) and 900 cm/hr in a 10 cm ID column (0.25 MPa).

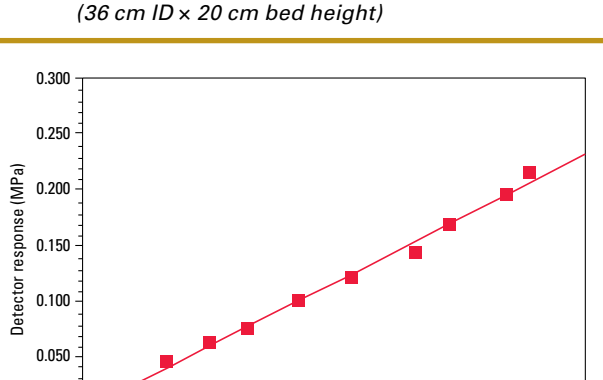
Figure 4: Mechanical stability of Ca⁺⁺Pure-HA



Resin:	**Ca⁺⁺Pure-HA**
Column size:	1.1 cm ID × 19.3 cm (dry pack) 2.2 cm ID × 20.3 cm (dry pack) 4.4 cm ID × 19.3 cm (dry pack) 2.2 cm ID × 20.6 cm (wet pack) 10.0 cm ID × 21.3 cm (wet pack)
Mobile phase:	5 mmol/L phosphate buffer, pH 7.2
Linear velocity:	as noted
Detection:	pressure (MPa)

To demonstrate the excellent pressure-flow in a process-scale column, Ca⁺⁺Pure-HA resin was packed in a 36 mm ID × 20 cm column. Data in Figure 5 shows that a flow rate of <0.3 MPa is achieved at a pressure drop at 600 cm/hr.

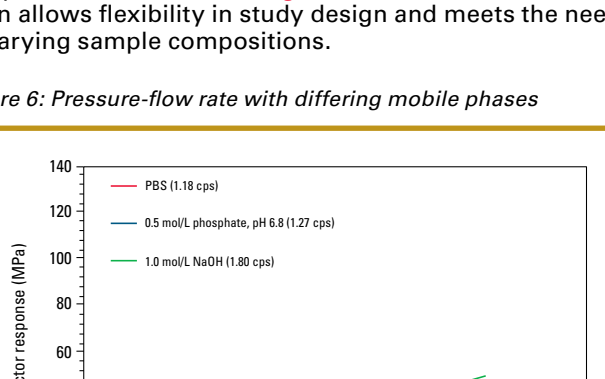
Figure 5: Pressure-flow rate curve on large process column (36 cm ID × 20 cm bed height)



Resin:	**Ca⁺⁺Pure-HA**
Column size:	36 mm ID x 20 cm
Mobile phase:	20 mmol/L phosphate buffer, 150 mmol/L chloride, pH 6.8
Linear velocity:	various
Detection:	pressure (MPa)

With solutions of differing viscosities, Ca⁺⁺Pure-HA exhibits low pressure, as shown in Figure 6. This characteristic of the resin allows flexibility in study design and meets the needs of varying sample compositions.

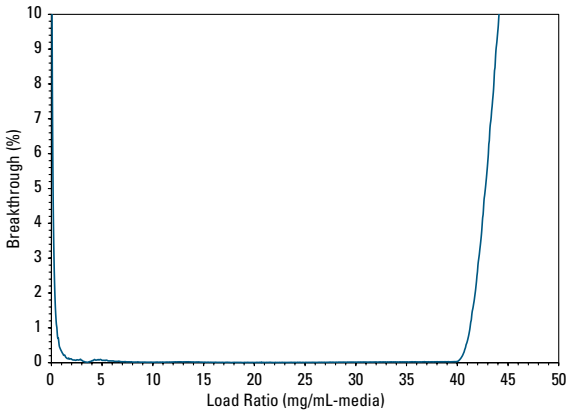
Figure 6: Pressure-flow rate with differing mobile phases



Resin:	**Ca⁺⁺Pure-HA**
Column size:	36 mm ID x 20 cm
Mobile phase:	as indicated in figure
Linear velocity:	various
Detection:	pressure (MPa)
Temperature:	23 °C

Ca++Pure-HA has a demonstrated a dynamic binding capacity (DBC), at 5% breakthrough, of greater than 40 g/L human IgG_1 at residence times as low as 4 minutes as shown in Figure 7.

Figure 7: Ca++Pure-HA dynamic binding capacity



Resin:	**Ca++Pure-HA , lot CPBL122716A**
Column size:	5 mm × 5 cm (1.0 mL)
Equilibration:	20 mmol/L MES, 5 mmol/L KPO_4, pH 6.5
Elution/Strip:	500 mmol/L KPO_4, pH 6.5
Sanitization:	1.0 mol/L KOH
Flow rate:	75 cm/hr (4 min residence time)
Detection:	UV @ 280 nm, Conductivity (mS/cm)
Temperature:	ambient
Sample:	IgG_1 @ 2.00 g/L
Instrument:	ÄKTA avant 25

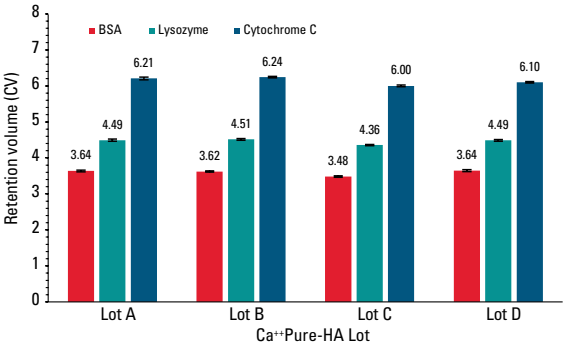
Table 2 shows the dynamic binding capacity (DBC) of IgG_1 at various residence times. Data shows that Ca++Pure-HA resin can achieve a DBC of greater than 30 g/L for human IgG_1 at 2 minutes residence time at 10% breakthrough.

Table 2: Dynamic binding capacity of Ca++Pure-HA for human IgG

	2 min	5 min
Human IgG	32.4 g/L	51.6 g/L

Ca++Pure-HA resin performs consistently from lot-to-lot, as shown in Figure 8. Separation of a protein mixture containing 1 g/L BSA, 0.2 g/L lysozyme, and 0.5 g/L cytochrome C was carried out in triplicate for four (4) individual lots of Ca++Pure-HA. Reproducible separation of the 3 standard proteins could be obtained under phosphate conditions.

Figure 8: Ca++Pure-HA lot-to-lot variability



Resin:	**Ca++Pure-HA**
Column size:	1.0 cm ID ×10 cm (7.9 mL)
Mobile phase	A: 1 mmol/L phosphate buffer, pH 6.8 B: 500 mmol/L phosphate buffer, pH 6.8
Gradient:	0–100% B (6 CV)
Linear velocity:	300 cm/hr (3.93 mL/min)
Residence time:	2.0 min
Detection:	UV @ 280 nm, conductivity
Temperature:	ambient
Injection vol.:	0.1 CV (0.8–0.9 mL)
Samples:	1 g/L BSA, 0.2 g/L lysozyme, 0.5 g/L cytochrome C in mobile phase A

Removal of mAb Aggregates

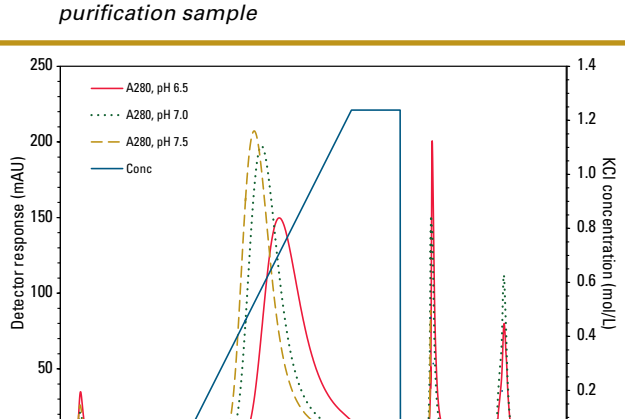
Downstream process chromatography scientists are constantly searching for better and more selective ways to remove aggregates and other process related impurities from a monoclonal antibody (mAb) monomer. Making use of chromatography resins with better selectivity, resolution and capacity is one approach to solving the problem of aggregate removal in monoclonal antibody production.

The following data demonstrates the capabilities of Ca^{++}Pure-HA media operated with potassium salts such as potassium phosphate and potassium chloride, to remove dimer and higher order aggregates from the monomer of a protein A purified IgG_1 monoclonal antibody.

To remove mAb aggregates from a post-protein A purified sample, Ca^{++}Pure-HA media was used in a polishing chromatography step. The below protocol was used.

Figure 9 shows a high resolution separation between the monomer peak and the aggregate peak across three different pH conditions using Ca^{++}Pure-HA media. The elution of the monomer peak at pH 6.5 was delayed and broader.

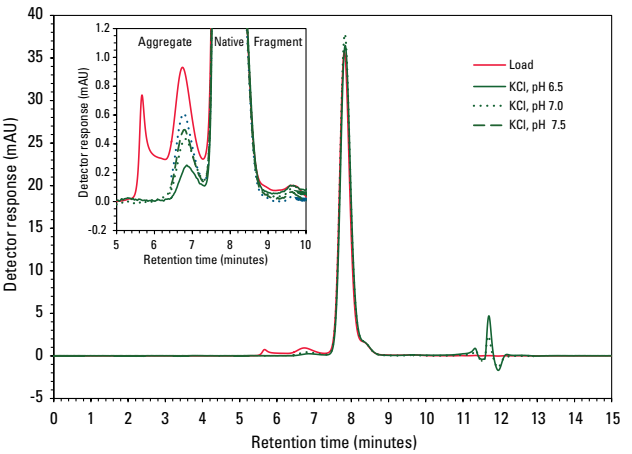
Figure 9: Removal of mAb aggregates from a post-protein A purification sample



Resin:	**Ca^{++}Pure-HA**
Column size:	5 mm × 5 cm (1.0 mL)
Mobile phase:	A: 50 mmol/L HEPES, 5 mmol/L KPO_4, pH as indicated B: mobile phase A + 2.0 mol/L potassium chloride, pH as indicated C: 500 mmol/L KPO_4, pH as indicated D: 1.0 mol/L KOH
Gradient:	69.4% B (chloride), 10 CV
Gradient:	delay, 5 CV
Flow rate:	300 cm/hr (1 min residence time)
Detection:	UV @ 280 nm, Conductivity (mS/cm), pH
Temperature:	ambient
Injection vol.:	5 µL
Sample:	2.0 mg/mL-media partially-purified mAb-01 (0.2 mL injection)
Instrument:	ÄKTA avant 25
Method:	Pre-equilibrate, mobile phase C, 3 CV
Equilibrate:	mobile phase A, 10 CV
	Load
	Wash, mobile phase A, 5 CV
	Elution, gradient as indicated, 25 CV
	Strip, mobile phase C, 5 CV
Sanitize:	mobile phase D, 5 CV

Size exclusion chromatography data analysis in Figure 10 show that after the sample passed through Ca^{++}Pure HA media under potassium phosphate buffer and potassium chloride operating conditions, mAb aggregates were reduced significantly. In fact, at pH 6.5 operating conditions, the aggregate amount was reduced from 6.6 to as low as 1.3%. Analytical HPLC peak integration data is shown in Table 3.

Figure 10: Aggregate analysis of pooled mAb monomer peaks eluted from different pH buffers using size exclusion chromatography



Column:	**TSKgel G3000SW$_{XL}$, 7.8 mm ID × 30 cm**
Mobile phase:	0.1 mol/L phosphate, 0.1 mol/L Na_2SO_4, 0.3% sodium azide, pH 6.7
Flow rate:	1.0 mL/min
Gradient:	isocratic
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection:	10 µg native
Instrument:	HPLC (400 bar pressure)

Table 3: Aggregate analysis data of pooled mAb monomer peak eluted from different pH conditions

Salt	pH	Peak molarity (mmol/L)	Recovery (% native)	Aggregate (%)	Fragment (%)
Load				**6.6**	**0.6**
KCl	6.5	814	72.9	1.3	0.5
	7.0	615	80.0	1.8	0.3
	7.5	509	81.0	2.2	0.3

Ordering Information

Ca^{++}Pure-HA resin:

Part #	Product description	Container size (g)
45045	Ca^{++}Pure-HA	50
45039	Ca^{++}Pure-HA	100
45040	Ca^{++}Pure-HA	250
45041	Ca^{++}Pure-HA	500
45042	Ca^{++}Pure-HA	1,000
45043	Ca^{++}Pure-HA	5,000

ToyoScreen process development columns

ToyoScreen RoboColumns®

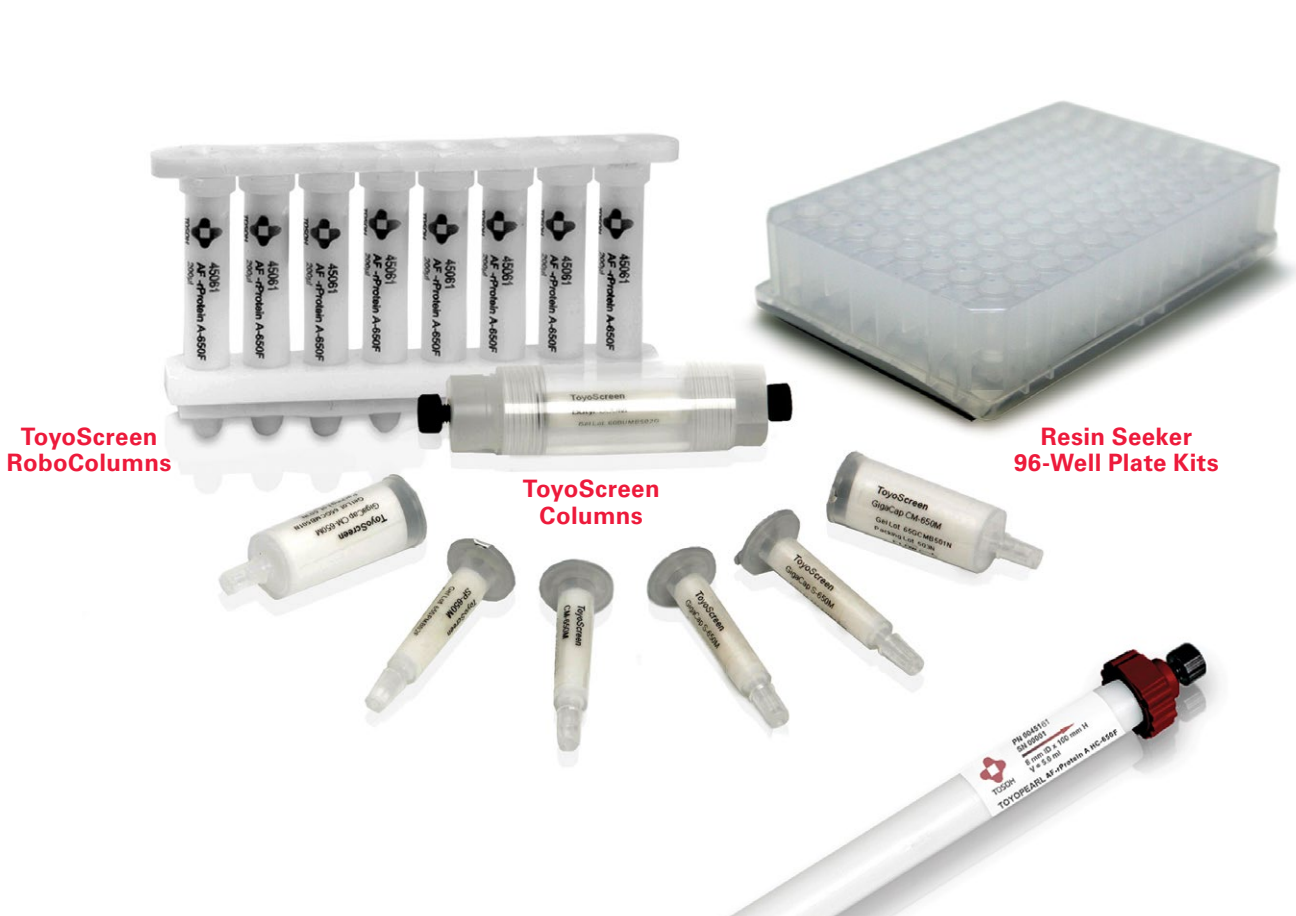
TOYOPEARL and TSKgel LabPak media

Resin Seeker 96-well plate kits

MiniChrom columns

TOYOPEARL protein A ELISA kit

TOYOPEARL protein L ELISA kit



The Role of Resin Screening in Process Chromatography

Resin screening and selection is an integral part of chromatographic optimization in process manufacturing. Due to the diversity in available ligand chemistries and base matrices offered by different vendors (e.g., agarose, methacrylate, styrene/divinylbenzene, etc.), it is prudent at the first part of the development process to screen as many resins as possible. A thorough evaluation is a necessity as each target molecule has very different physical and chromatographic properties. Very often a resin that worked in the past for a similar molecule will not work as effectively for the new target molecule. In addition, performance parameters such as selectivity, binding capacity, recovery, etc. are mainly influenced by the properties of the chromatographic resin. Therefore, selection of the most suitable resin is the significant key point to succeed in purification.

Tosoh Bioscience offers a wide variety of screening tools composed of TOYOPEARL and TSKgel process media. In addition, bulk media volumes of <1 L are available for process development.

TOYOPEARL and TSKgel Process Media

TOYOPEARL resins are hydrophilic macroporous methacrylic resins for large-scale chromatographic applications. Their rigid polymeric backbone has better pressure-flow properties than most other commercially made materials. Therefore, higher linear operating velocities can be used for faster process throughput and decreased recycling times.

TOYOPEARL resins are stable over the pH 2-12 range for normal operating conditions and pH 1-13 for cleaning conditions. The resins are available in average particle sizes of 35 µm, 65 µm, 75 µm, and 100 µm for high resolution, intermediate purification, or capture chromatography. In most modes, TOYOPEARL is available in three grades: S (superfine) for highest performance, F (fine), and M (medium) for economical purification. Two additional grades, C (coarse) and EC (extra coarse), are available for capture.

TOYOPEARL resins are also offered in many different pore diameters for size exclusion, ion exchange, hydrophobic interaction, mixed-mode, and affinity chromatography. Pore diameter and surface area can be optimized to ensure excellent kinetic access and binding capacity of a target regardless of molecular size.

For predictable results in scale-up, TOYOPEARL resins are based on the same chemistries as the prepacked TSKgel columns. This allows the seamless direct scale-up of methods developed on TSKgel columns to TOYOPEARL resins.

TSKgel resins are larger particle size versions of the chemically equivalent methacrylic packing of analytical scale TSKgel columns. The polymeric resins with particle sizes of 20 µm and 30 µm used in TSKgel columns are also available in bulk quantities for large scale ion exchange and hydrophobic interaction chromatography. Their mechanical stability and permeability make them excellent for use when increased separation performance and plate count are needed for optimum preparative or process chromatography.

ToyoScreen Process Development Columns

In order to improve the efficiency of resin screening experiments, pre-packed process development columns are available from Tosoh Bioscience. The 1 mL and 5 mL ToyoScreen columns are packed with various TOYOPEARL process resins and are a convenient and affordable alternative to self-packing. Advantages of ToyoScreen columns are summarized in Table 1.

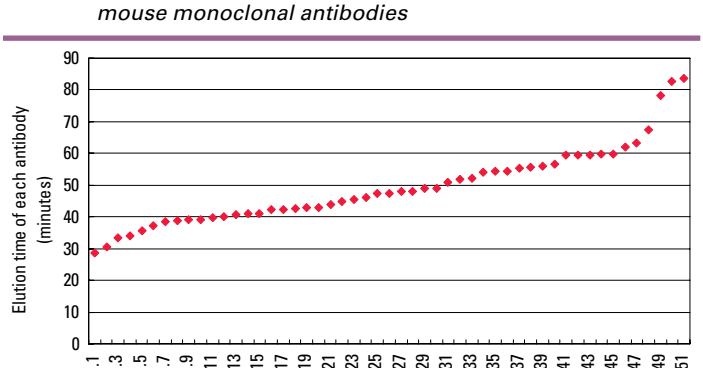
Table 1: Features and benefits of ToyoScreen columns

Features	Benefits
Packed with TOYOPEARL hydrophobic interaction, ion exchange or affinity chemistries	Added flexibility in determining the optimum purification protocol
1 mL and 5 mL formats available	For sample limited applications up to milligram purifications
Cartridge and holder design	Provides a low cost, efficient alternative to self packing with bulk resin
Easy connections with ÄKTA®, FPLC, and even HPLC systems	Seamless integration with any platform
Offered in mixed or single chemistry packages	For cost savings in screening or process optimization experiments

Resin Screening with ToyoScreen Columns

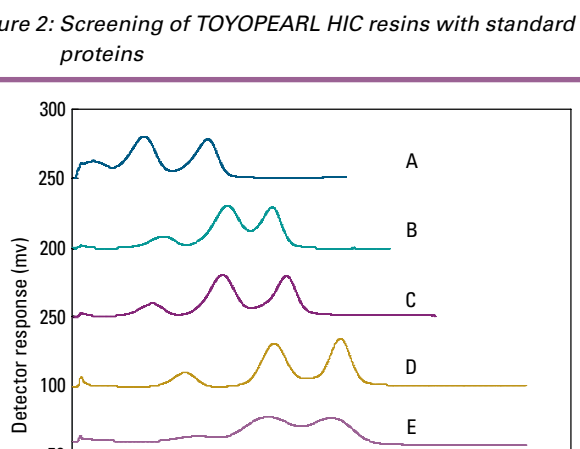
TOYOPEARL hydrophobic interaction media **(HIC)** is available in six different chemistries ranging in hydrophobicity from Ether-650 (low) to Hexyl-650 (high). Depending on a target's feedstock and impurity profile, the determination of the best selectivity is an empirical process. As shown in Figure 1, hydrophobicities can vary widely within a class of similar biologics like mAbs. Figure 2 shows the selectivity differences of the ToyoScreen HIC chemistries on the separation of protein standards. Figure 3 demonstrates the selectivity differences on the separation of anti-IgG from albumin in mouse ascites fluid.

Figure 1: Hydrophobic diversity of mouse monoclonal antibodies - plot of chromatographic elution times for 51 different mouse monoclonal antibodies



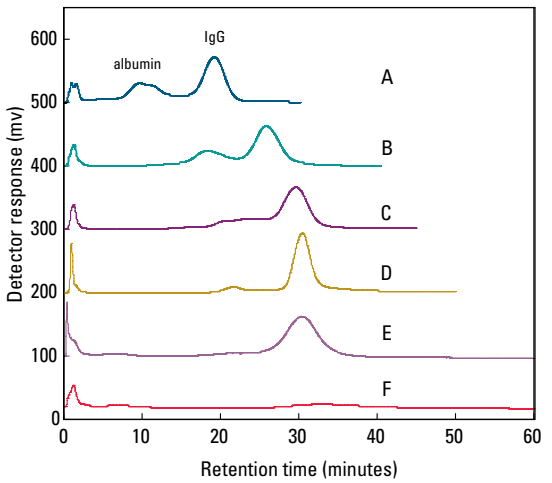
Column: **TSKgel Phenyl-5PW**
Mobile phase: Buffer A: 0.1 mol/L phosphate buffer + 1.8 mol/L ammonium sulfate, pH 7.0
Buffer B: 0.1 mol/L phosphate buffer, pH 7.0
Gradient: 65 minutes, 0 - 100% B
Flow rate: 1 mL/min
Samples: 51 unique mouse monoclonal antibodies

Figure 2: Screening of TOYOPEARL HIC resins with standard proteins



Resins:
A. TOYOPEARL Ether-650M
B. TOYOPEARL PPG-600M
C. TOYOPEARL Phenyl-650M
D. TOYOPEARL Butyl-650M
E. TOYOPEARL SuperButyl-550C
F. TOYOPEARL Hexyl-650C
Column size: ToyoScreen, 1 mL
Mobile phase: Buffer A: 0.1 mol/L phosphate buffer + 1.8 mol/L sodium sulfate, pH 7.0
Buffer B: 0.1 mol/L phosphate buffer, pH 7.0
Gradient: 30 min linear gradient buffer A to buffer B
Flow rate: 1 mL/min
Samples: ribonuclease A, lysozyme, α-chymotrypsinogen, 1 mg/mL each

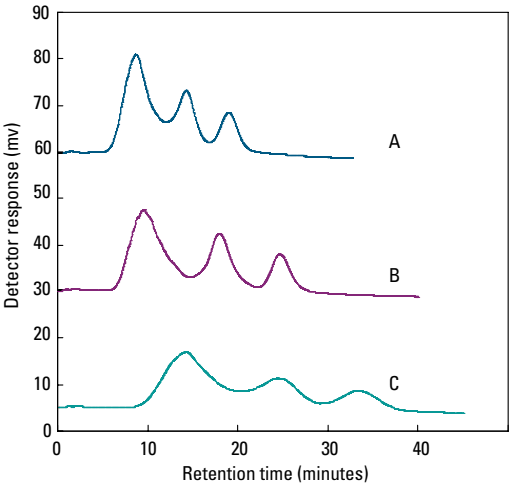
Figure 3: Screening of TOYOPEARL HIC resins with mouse ascites fluid (Anti-IgE)



Resins:
A. TOYOPEARL Ether-650M
B. TOYOPEARL PPG-600M
C. TOYOPEARL Phenyl-650M
D. TOYOPEARL Butyl-650M
E. TOYOPEARL SuperButyl-550C
F. TOYOPEARL Hexyl-650C
Column size: ToyoScreen, 1 mL
Mobile phase: Buffer A: 0.1 mol/L phosphate buffer + 1.8 mol/L sodium sulfate, pH 7.0
Buffer B: 0.1 mol/L phosphate buffer, pH 7.0
Gradient: 30 min linear gradient buffer A to buffer B
Flow rate: 1 mL/min
Samples: mouse ascites fluid: A:B = 1:1:2

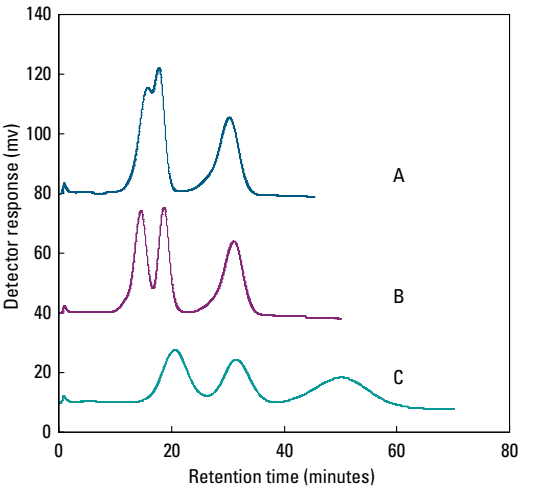
Ion exchange chromatography **(IEX)** separates molecules based on the ionic interaction of the molecule with the charged support. The individual functional group and its pKa can be used to evaluate different selectivities in chromatographic separations. ToyoScreen columns are offered in both strong and weak functionalities for both cation and anion ligand types. Figures 4 and 5 detail the effect on the separation for the available TOYOPEARL anion and cation exchange chemistries when screening protein standards.

Figure 4: Screening of TOYOPEARL anion exchange resins with standard proteins



Resins:
A. TOYOPEARL DEAE-650M
B. TOYOPEARL SuperQ-650M
C. TOYOPEARL QAE-550C
Column size: ToyoScreen, 1 mL
Mobile phase: Buffer A: 20 mmol/L Tris-HCl, pH 8.0
Buffer B: 20 mmol/L Tris-HCl + 0.5 mol/L NaCl, pH 8.0
Gradient: 60 minutes, 0 - 100% B
Flow rate: 1 mL/min
Samples: transferrin, ovalbumin, trypsin inhibitor, 1 mg/mL each

Figure 5: Screening of TOYOPEARL cation exchange resins with standard proteins



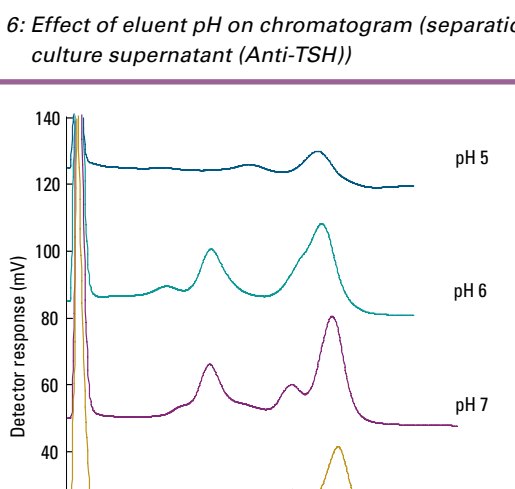
Resins:
A. TOYOPEARL CM-650M
B. TOYOPEARL SP-650M
C. TOYOPEARL SP-550C
Column size: ToyoScreen, 1 mL
Mobile phase: Buffer A: 20 mmol/L phosphate buffer, pH 6.0
Buffer B: 20 mmol/L phosphate buffer + 0.5 mol/L NaCl, pH 6.0
Gradient: 60 minutes, 0 - 100% B
Flow rate: 1 mL/min
Samples: α-chymotrypsinogen A, cytochrome C, lysozyme, 1 mg/mL each

In affinity chromatography **(AFC)**, the ligands employed are specific to a particular protein class or functional group on the accessible surface of the target molecule. ToyoScreen affinity columns are offered in three group specific ligand chemistries: AF-rProtein A-650F, AF-Chelate-650M, and AF-Red 650ML. AF-rProtein A-650F is used for the purification of monoclonal antibodies. AF-Red-650ML is specific for dehydrogenases and other proteins such as plasminogen. AF-Chelate-650M can be converted to either the Ni^{++} or Ca^{++} form. When converted to the Ni^{++} form it is an excellent resin for metal ligand affinity for molecules containing His-tags. ToyoScreen affinity columns allow for the quick assessment of optimum binding conditions for any of these columns.

Method Optimization

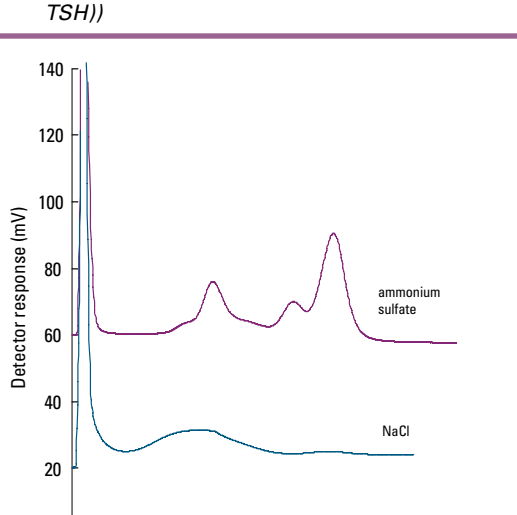
Beyond the determination of "what sticks" during resin screening experiments, ToyoScreen columns can be used to quickly establish optimum elution conditions. Varying pH, salt type, salt gradients, and flow rate are common experimental parameters explored. The effect of varying pH is shown in Figure 6 and the effect of varying salt type is shown in Figure 7 for Anti-TSH in cell culture supernatant on ToyoScreen Phenyl-650M.

Figure 6: Effect of eluent pH on chromatogram (separation of cell culture supernatant (Anti-TSH))



Resin:	**TOYOPEARL Phenyl-650M**
Column size:	ToyoScreen, 1 mL
Mobile phase:	Buffer A: 0.1 mol/L phosphate buffer + 1.8 mol/L ammonium sulfate, pH 7.0 Buffer B: 0.1 mol/L phosphate buffer, pH 7.0
Gradient:	30 min linear gradient, 30 CV
Flow rate:	1 mL/min
Injection vol.:	200 µL
Samples:	cell culture supernatant (× 4 diluted) (antibody: Anti-TSH)

Figure 7: Comparison of chromatogram under differing salt conditions (separation of cell culture supernatant (Anti-TSH))

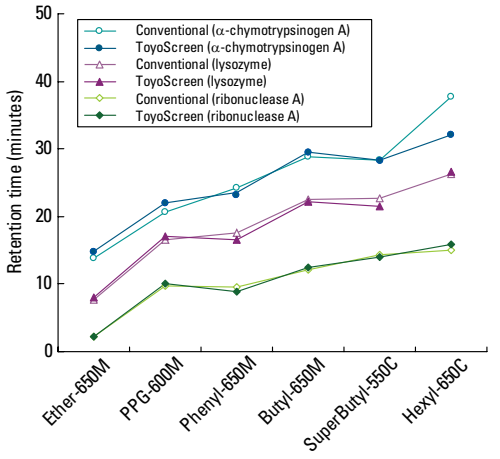


Resin:	**TOYOPEARL Phenyl-650M**
Column size:	ToyoScreen, 1 mL
Mobile phase:	Buffer A: 0.1 mol/L phosphate buffer containing 1.8 mol/L each salt, pH 7.0 Buffer B: 0.1 mol/L phosphate buffer, pH 7.0
Gradient:	30 min linear gradient, 30 CV
Flow rate:	1 mL/min
Injection vol.:	200 µL
Samples:	cell culture supernatant (× 4 diluted) (antibody: Anti-TSH)

Scalability

Initial results from resin screening and optimization with ToyoScreen columns accurately predict the separation behavior at larger scales. Figure 8 illustrates the similar retention time behavior between 1 mL ToyoScreen columns and conventional 7.5 mm ID × 7.5 cm analytical columns. Additionally, Figure 9 depicts a practical antibody scale-up in which conditions were set using a 1 mL ToyoScreen column and applied to a 10 mL semi-preparative column with a different inner diameter and length.

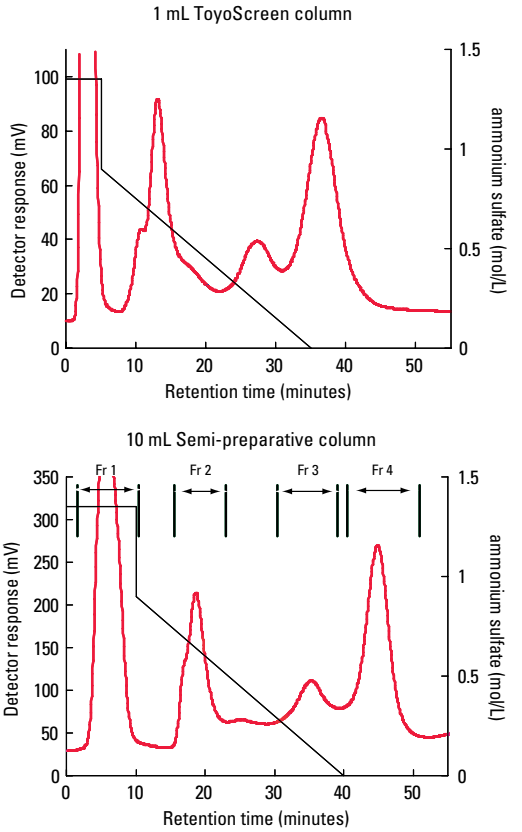
Figure 8: Comparison of selectivity between ToyoScreen and conventional column



Resins: **TOYOPEARL Ether-650M**
TOYOPEARL PPG-600M
TOYOPEARL Phenyl-650M
TOYOPEARL Butyl-650M
TOYOPEARL SuperButyl-550C
TOYOPEARL Hexyl-650C
Column size: ToyoScreen, 1 mL
Conventional column, 7.5 mm ID × 7.5 cm
Mobile phase: Buffer A: 0.1 mol/L phosphate buffer + 1.8 mol/L sodium sulfate, pH 7.0
Buffer B: 0.1 mol/L phosphate buffer, pH 7.0
Gradient: 30 min linear gradient, 30 CV
Flow rate: 1 mL/min
Injection vol.: 50 µL
Samples: ribonuclease A, lysozyme, α-chymotrypsinogen A 1 mg/mL

Retention time of conventional column was plotted after converting following equation: plotted value = actual measurement value - 4.82

Figure 9: Comparison chromatograms between ToyoScreen and semi-preparative columns



Resin: **TOYOPEARL Phenyl-650M**
Mobile phase: Buffer A: 0.1 mol/L phosphate buffer containing 1.8 mol/L $(NH_4)_2SO_4$, pH 7.0
Buffer B: 0.1 mol/L phosphate buffer, pH 7.0
Sample: anti-TSH from cell culture supernatant (× 4 diluted)

	1 mL ToyoScreen	**10 mL semi-preparative**
Column dimensions	6.4 mm ID × 3 cm	14.6 mm ID × 6 cm
Injection vol.	500 µL	5000 µL
Flow rate	0.5 mL/min; 0.5 CV/min; 93 cm/hr	2.5 mL/min; 0.25 CV/ min; 90 cm/hr
Gradient profile	25% B; 0-5 min (isocratic) 50% B: 5 min (step) 50% to 100% B; 5-35 min (linear)	25% B; 0-10 min (isocratic) 50% B: 10 min (step) 50% to 100% B; 10-40 min (linear)
Gradient slope*	0.06 mol/L/mL	0.012 mol/L/mL

* The gradient slope is the change in ionic strength per unit volume. Gradient volume is the product of flow rate and gradient time.

Ordering Information - ToyoScreen process development columns

Please note that a ToyoScreen column holder is needed with each ToyoScreen column.

Part #	Description	Matrix	Container size
Ion Exchange			
23472	ToyoScreen Sulfate-650F, 1 mL	polymer	1 mL × 6 ea
23473	ToyoScreen Sulfate-650F, 5 mL	polymer	5 mL × 6 ea
23443	ToyoScreen NH_2-750F, 1 mL	polymer	1 mL × 6 ea
23444	ToyoScreen NH_2-750F, 5 mL	polymer	5 mL × 6 ea
21366	ToyoScreen CM-650M, 1 mL	polymer	1 mL × 6 ea
21367	ToyoScreen CM-650M, 5 mL	polymer	5 mL × 6 ea
21360	ToyoScreen DEAE-650M, 1 mL	polymer	1 mL × 6 ea
21361	ToyoScreen DEAE-650M, 5 mL	polymer	5 mL × 6 ea
22872	ToyoScreen GigaCap DEAE-650M, 1 mL	polymer	1 mL × 6 ea
22873	ToyoScreen GigaCap DEAE-650M, 5 mL	polymer	5 mL × 6 ea
21859	ToyoScreen GigaCap Q-650M, 1 mL	polymer	1 mL × 6 ea
21860	ToyoScreen GigaCap Q-650M, 5 mL	polymer	5 mL × 6 ea
21868	ToyoScreen GigaCap S-650M, 1 mL	polymer	1 mL × 6 ea
21869	ToyoScreen GigaCap S-650M, 5 mL	polymer	5 mL × 6 ea
21951	ToyoScreen GigaCap CM-650M, 1 mL	polymer	1 mL × 6 ea
21952	ToyoScreen GigaCap CM-650M, 5 mL	polymer	5 mL × 6 ea
21870	ToyoScreen MegaCap II SP-550EC, 1 mL	polymer	1 mL × 6 ea
21871	ToyoScreen MegaCap II SP-550EC, 5 mL	polymer	5 mL × 6 ea
21362	ToyoScreen SuperQ-650M, 1 mL	polymer	1 mL × 6 ea
21363	ToyoScreen SuperQ-650M, 5 mL	polymer	5 mL × 6 ea
21992	ToyoScreen Q-600C AR, 1 mL	polymer	1 mL × 6 ea
21993	ToyoScreen Q-600C AR, 5 mL	polymer	5 mL × 6 ea
21364	ToyoScreen QAE-550C, 1 mL	polymer	1 mL × 6 ea
21365	ToyoScreen QAE-550C, 5 mL	polymer	5 mL × 6 ea
21370	ToyoScreen SP-550C, 1 mL	polymer	1 mL × 6 ea
21371	ToyoScreen SP-550C, 5 mL	polymer	5 mL × 6 ea
21368	ToyoScreen SP-650M, 1 mL	polymer	1 mL × 6 ea
21369	ToyoScreen SP-650M, 5 mL	polymer	5 mL × 6 ea
Hydrophobic Interaction			
21494	ToyoScreen Butyl-600M, 1 mL	polymer	1 mL × 6 ea
21495	ToyoScreen Butyl-600M, 5 mL	polymer	5 mL × 6 ea
21376	ToyoScreen Butyl-650M, 1 mL	polymer	1 mL × 6 ea
21377	ToyoScreen Butyl-650M, 5 mL	polymer	5 mL × 6 ea
21372	ToyoScreen Ether-650M, 1 mL	polymer	1 mL × 6 ea
21373	ToyoScreen Ether-650M, 5 mL	polymer	5 mL × 6 ea
21378	ToyoScreen Hexyl-650C, 1 mL	polymer	1 mL × 6 ea
21379	ToyoScreen Hexyl-650C, 5 mL	polymer	5 mL × 6 ea
21892	ToyoScreen Phenyl-600M, 1 mL	polymer	1 mL × 6 ea
21893	ToyoScreen Phenyl-600M, 5 mL	polymer	5 mL × 6 ea
21374	ToyoScreen Phenyl-650M, 1 mL	polymer	1 mL × 6 ea
21375	ToyoScreen Phenyl-650M, 5 mL	polymer	5 mL × 6 ea
21380	ToyoScreen PPG-600M, 1 mL	polymer	1 mL × 6 ea
21381	ToyoScreen PPG-600M, 5 mL	polymer	5 mL × 6 ea

Part #	Description	Matrix	Container size
21382	ToyoScreen SuperButyl-550C, 1 mL	polymer	1 mL × 6 ea
21383	ToyoScreen SuperButyl-550C, 5 mL	polymer	5 mL × 6 ea
Mixed-Mode			
22824	ToyoScreen MX-Trp-650M, 1 mL	polymer	1 mL × 6 ea
22825	ToyoScreen MX-Trp-650M, 5 mL	polymer	5 mL × 6 ea
Affinity			
21384	ToyoScreen AF-Chelate-650M, 1 mL	polymer	1 mL × 6 ea
21385	ToyoScreen AF-Chelate-650M, 5 mL	polymer	5 mL × 6 ea
21390	ToyoScreen AF-Heparin HC-650M, 1 mL	polymer	1 mL × 6 ea
21391	ToyoScreen AF-Heparin HC-650M, 5 mL	polymer	5 mL × 6 ea
21388	ToyoScreen AF-Red-650M, 1 mL	polymer	1 mL × 6 ea
21389	ToyoScreen AF-Red-650M, 5 mL	polymer	5 mL × 6 ea
Protein A			
22809	ToyoScreen AF-rProtein A-650F, 1 mL	polymer	1 mL × 5 ea
22810	ToyoScreen AF-rProtein A-650F, 5 mL	polymer	5 mL × 1 ea
22811	ToyoScreen AF-rProtein A-650F, 5 mL	polymer	5 mL × 5 ea
23430	ToyoScreen AF-rProtein A HC-650F, 1 mL	polymer	1 mL × 5 ea
23431	ToyoScreen AF-rProtein A HC-650F, 5 mL	polymer	5 mL × 1 ea
23432	ToyoScreen AF-rProtein A HC-650F, 5 mL	polymer	5 mL × 5 ea
Protein L			
23494	ToyoScreen AF-rProtein L-650F, 1 mL	polymer	1 mL × 5 ea
23495	ToyoScreen AF-rProtein L-650F, 5 mL	polymer	5 mL × 1 ea
23496	ToyoScreen AF-rProtein L-650F, 5 mL	polymer	5 mL × 5 ea
Anion Mix Pack (DEAE-650M, SuperQ-650M, QAE-550C, GigaCap Q-650M, Q-600C AR)			
21392	ToyoScreen IEC Anion Mix Pack, 1 mL	polymer	1 mL × 5 grades × 1 each
21393	ToyoScreen IEC Anion Mix Pack, 1 mL	polymer	5 mL × 5 grades × 1 each
Cation Mix Pack (CM-650M, SP-650M, SP-550C, GigaCap CM-650M, GigaCap S-650M)			
21394	ToyoScreen IEC Cation Mix Pack, 1 mL	polymer	1 mL × 5 grades × 1 each
21395	ToyoScreen IEC Cation Mix Pack, 5 mL	polymer	5 mL × 5 grades × 1 each
IEX Mix Pack (GigaCap Q-650M, SuperQ-650M, Q-600C AR, GigaCap CM-650M, GigaCap S650M, SP-550C)			
21396	ToyoScreen IEC Mix Pack, 1 mL	polymer	1 mL × 5 grades × 1 each
21397	ToyoScreen IEC Mix Pack, 5 mL	polymer	5 mL × 5 grades × 1 each
HIC Mix Pack (PPG-600M, Phenyl-600M, Phenyl-650M, Butyl-600M, Butyl-650M, Hexyl-650C)			
21398	ToyoScreen HIC Mix Pack, 1 mL	polymer	1 mL × 5 grades × 1 each
21399	ToyoScreen HIC Mix Pack, 5 mL	polymer	5 mL × 5 grades × 1 each
ToyoScreen Accessories			
21400	ToyoScreen Column Holder		
42194	ToyoScreen Holder with fittings		Incl. 1 × 21400, 2 × 42196, 1 × 42195
42195	Column Coupler, 10-32, 0.03”ID SS Tubing		
42196	Adapter, M6 interior to 10-32 exterior, PEEK		
42197	Adapter, 1/4-28 interior to 10-32 exterior, PEEK		

ToyoScreen RoboColumns

ToyoScreen RoboColumns are miniaturized chromatographic columns pre-packed with TOYOPEARL ion exchange, mixed-mode, hydrophobic interaction or affinity media. They are packed with TOYOPEARL to our specifications by Atoll GmbH and are supplied in strips of 8 columns. Available in different volumes, ToyoScreen RoboColumns are designed to operate with a robotic liquid handling system, such as the Freedom EVO® from TECAN.

This approach allows automated high throughput, small-scale biochromatographic separations of protein samples by running up to eight individual columns simultaneously. Liquid flow in the columns is driven by positive pressure liquid displacement, rather than by air pressure, thus mimicking the situation in columns individually connected to a conventional standalone chromatography system.

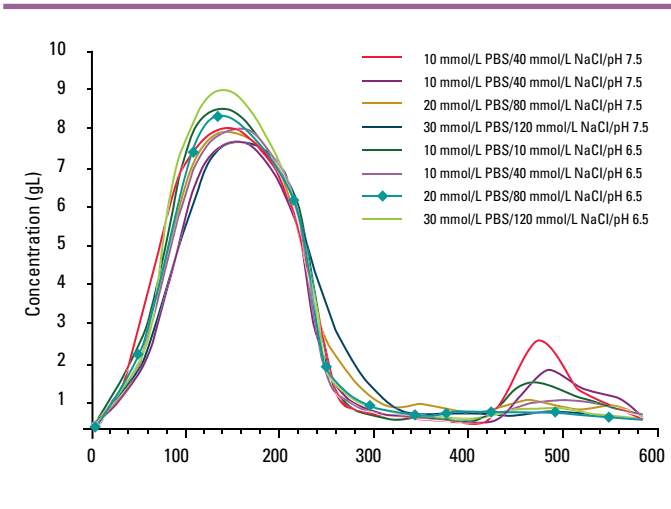
ToyoScreen RoboColumns can be used in a wide range of applications, including individual and parallel resin screening, optimization of separation conditions, scale-down experiments, as well as high throughput sample preparation.

Resin Screening with ToyoScreen RoboColumns

Binding and elution conditions, washing parameters, etc. can be investigated to explore the design space of the particular molecule's purification process. Design of Experiments (DoE), a statistical approach used to define those factors having the greatest impact on the process, is a suitable tool to minimize the number of experiments needed.

Figure 10 shows a screening experiment to optimize the chromatographic parameters for the intermediate flow through anion exchange step in a purification platform for monoclonal antibodies (mAbs). Protein binding of a protein A capture eluate on ToyoScreen RoboColumns packed with TOYOPEARL SuperQ-650M resin was analyzed by varying salt concentration and pH of loading and washing buffer. Best results were achieved using 20 mmol/L sodium phosphate, 80 mmol/L sodium chloride, pH 6.5.

Figure 10: Optimization of anion exchange conditions



Elution profile of a protein A capture eluate on ToyoScreen RoboColumns packed with TOYOPEARL SuperQ-650M at various conditions. Data kindly provided by T. Schröder, Atoll GmbH.

Separation with ToyoScreen RoboColumns

ToyoScreen RoboColumns can be used to perform small scale purifications/separations by applying either an isocratic or step gradient. Examples are small scale mAb purification using protein A affinity for in-process monitoring of fermentation or sample preparation prior to subsequent analysis by MS, ELISA or CGE/SDS-Page.

Formats of ToyoScreen RoboColumns

ToyoScreen RoboColumns are available in two formats with 200 µL (bed height of 10 mm) and 600 µL (bed height of 30 mm) resin volume, respectively. They are supplied in a row of eight units pre-packed with the same TOYOPEARL resin and sealed with two removable silicon cover seals for proper storage.

They can be individually arranged on a 96 position array plate. All chromatographic media used in the ToyoScreen RoboColumns are also available in larger pre-packed ToyoScreen columns of 1 mL or 5 mL volume and as bulk resins for use at all scales.

Ordering Information - ToyoScreen RoboColumns

Part #	Packed with:	Package Description
Ion Exchange		
45027	TOYOPEARL Sulfate-650F	8 × 200 µL
45028	TOYOPEARL Sulfate-650F	8 × 600 µL
45021	TOYOPEARL NH_2-750F	8 × 200 µL
45022	TOYOPEARL NH_2-750F	8 × 600 µL
45023	TOYOPEARL GigaCap S-650S	8 × 200 µL
45024	TOYOPEARL GigaCap S-650S	8 × 600 µL
45001	TOYOPEARL GigaCap S-650M	8 × 200 µL
45002	TOYOPEARL GigaCap S-650M	8 × 600 µL
45025	TOYOPEARL GigaCap Q-650S	8 × 200 µL
45026	TOYOPEARL GigaCap Q-650S	8 × 600 µL
45003	TOYOPEARL GigaCap Q-650M	8 × 200 µL
45004	TOYOPEARL GigaCap Q-650M	8 × 600 µL
45005	TOYOPEARL GigaCap CM-650M	8 × 200 µL
45006	TOYOPEARL GigaCap CM-650M	8 × 600 µL
45007	TOYOPEARL GigaCap DEAE-650M	8 × 200 µL
45008	TOYOPEARL GigaCap DEAE-650M	8 × 600 µL
45011	TOYOPEARL Q-600C AR	8 × 200 µL
45012	TOYOPEARL Q-600C AR	8 × 600 µL
45013	TOYOPEARL SuperQ-650M	8 × 200 µL
45014	TOYOPEARL SuperQ-650M	8 × 600 µL
Hydrophobic Interaction		
45031	TOYOPEARL Phenyl-600M	8 × 200 µL
45032	TOYOPEARL Phenyl-600M	8 × 600 µL
45033	TOYOPEARL Butyl-600M	8 × 200 µL
45034	TOYOPEARL Butyl-600M	8 × 600 µL
45089	TOYOPEARL Butyl-650M	8 × 200 µL
45090	TOYOPEARL Butyl-650M	8 × 600 µL
45035	TOYOPEARL PPG-600M	8 × 200 µL
45036	TOYOPEARL PPG-600M	8 × 600 µL
45037	TOYOPEARL Phenyl-650M	8 × 200 µL
45038	TOYOPEARL Phenyl-650M	8 × 600 µL
45091	TOYOPEARL Hexyl-650C	8 × 200 µL
45092	TOYOPEARL Hexyl-650C	8 × 600 µL
Mixed-Mode		
45051	TOYOPEARL MX-Trp-650M	8 × 200 µL
45052	TOYOPEARL MX-Trp-650M	8 × 600 µL
Protein A		
45061	TOYOPEARL AF-rProtein A-650F	8 × 200 µL
45062	TOYOPEARL AF-rProtein A-650F	8 × 600 µL
45063	TOYOPEARL AF-rProtein A HC-650F	8 × 200 µL
45064	TOYOPEARL AF-rProtein A HC-650F	8 × 600 µL
Protein L		
45065	TOYOPEARL AF-rProtein L-650F	8 × 200 µL
45066	TOYOPEARL AF-rProtein L-650F	8 × 600 µL

Size Exclusion		
45071	TOYOPEARL HW-40F	8 × 200 µL
45072	TOYOPEARL HW-40F	8 × 600 µL
Accessories		
45099	Array plate	

TOYOPEARL and TSKgel LabPak Media

LabPak products are multi-milliliter containers of TOYOPEARL and TSKgel bulk media products. Typically they contain 3 or 4 different ligand types offered for a particular chromatography mode.

They are useful for developmental engineers who wish to familiarize themselves with resin physical properties in different buffer systems:

- slurry and reslurry mechanics
- resin handling during column packing
- mechanical strength relative to agarose
- degree of compressibility
- flow adaptor regimen

The larger resin amounts in LabPak products allow the packing of wider ID and longer columns than available in the ToyoScreen products. This helps the developmental chemist or engineer to better measure under actual packing conditions the following properties:

- dynamic binding capacity
- selectivity
- column efficiency

Ordering Information - TOYOPEARL LabPak media

Part #	Description	Package Description
Size Exclusion		
19820	SECPAK HP (HW-40, 50, 55, 65S), 30 µm	4 × 150 mL
19821	SECPAK LMW (HW-40, 50, 55F), 45 µm	3 × 150 mL
19819	SECPAK HMW (HW-55, 65, 75F), 45 µm	3 × 150 mL
Ion Exchange		
19817	IEXPAK HP (DEAE-650S, SP-650S, CM-650S, SuperQ-650S), 35 µm	4 × 25 mL
43210	AIEXPAK (GigaCap Q-650M, SuperQ-650M, Q-600C AR) , 65/75/100 µm	3 × 25 mL
43220	CIEXPAK (GigaCap CM-650M, GigaCap S-650M, SP-550C), 75/100 µm	3 × 25 mL
Hydrophobic Interaction		
43150	HICPAK HP (Ether, Phenyl, Butyl-650S), 35 µm	3 × 25 mL
19806	HICPAK (Ether, Phenyl, Butyl-650M), 65 µm	3 × 25 mL
43125	HICPAK-C (Phenyl, Butyl, Hexyl-650C), 100 µm	3 × 25 mL
Affinity		
43400	AFFIPAK ACT (AF-Epoxy, Tresyl-650M), 65 µm	2 × 5 g*
43410	AFFIPAK (AF-Amino, Carboxyl, Formyl-650 M), 65 µm	3 × 10 mL

*1 g is approximately 3.5 mL

Ordering Information - TSKgel LabPak media

Part #	Description	Package Description
Ion Exchange		
43380	IEXPAK PW (DEAE-5PW, SP-5PW, SuperQ-5PW), 20 µm	3 × 25 mL
43280	IEXPAK PW (DEAE-5PW, SP-5PW, SuperQ-5PW), 30 µm	3 × 25 mL
Hydrophobic Interaction		
43278	HICPAK PW (Ether-5PW, Phenyl-5PW) , 20 µm	2 × 25 mL
43175	HICPAK PW (Ether-5PW, Phenyl-5PW) , 30 µm	2 × 25 mL

Resin Seeker 96-Well Plate Kits

Resin Seeker 96-well plates are disposable filter plates packed with TOYOPEARL and Ca^{++}Pure-HA resins and are available in several configurations for ion exchange, HIC, mixed-mode, hydroxyapatite, and protein A chromatography. Resin Seeker 96-well plates can be used to screen multiple steps of the purification process including binding, wash, and elution conditions in addition to resin selectivity, binding kinetics, purity, and recovery of your target molecule.

Resin Seeker 96-well plate kits are manufactured by Orochem and sold by Tosoh Bioscience. All components necessary to run an experiment are included in each kit: a wash plate and collection plate. Resin Seeker plates can be operated manually using a multi-channel pipette or in an automated system designed for high throughput screening in a 96-well plate format.

TOYOPEARL resins used in the Resin Seeker 96-well plates are also available in ToyoScreen pre-packed columns and as bulk media. This allows seamless scale-up and process optimization once resin screening is complete.

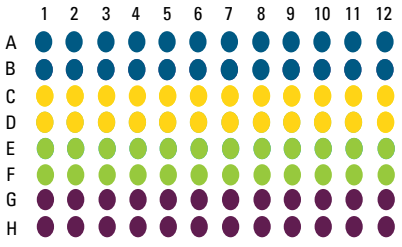
Ordering Information - Resin Seeker 96-well plate kits

Part #	Description	Package Description
OC41MDAEX-96	AIEX kit	Mixed anion exchange plate (20 µL resin beds)
OC41MDGCDE-650M	GigaCap DEAE-650M kit	TOYOPEARL GigaCap DEAE-650M plate(20 µL resin beds)
OC41MDGCQ-650M	GigaCap Q-650M kit	TOYOPEARL GigaCap Q-650M plate (20 µL resin beds)
OC41MDCEX-96	CIEX kit	Mixed cation exchange plate (20 µL resin beds)
OC41MDGCCM-650M	GigaCap CM-650M kit	TOYOPEARL GigaCap CM-650M plate (20 µL resin beds)
OC41MDGCS-650M	GigaCap S-650M kit	TOYOPEARL GigaCap S-650M plate (20 µL resin beds)
OC41MDHIC-96	HIC kit	Mixed hydrophobic interaction plate (20 µL resin beds)
OC41MDTRP-96	MMC kit	TOYOPEARL MX-Trp-650M plate (20 µL resin beds)
OC41MDAFPA-650F	Protein A HC kit	TOYOPEARL AF-rProtein A HC-650F plate (20 µL resin beds)
OC41MDAFPL-650F	Protein L kit	TOYOPEARL AF-rProtein L-650F plate (20 µL resin beds)
OC41MDNH2-750F	NH_2-750F kit	TOYOPEARL NH_2-750F plate (20 µL resin beds)
OC41MDCPHA	Ca^{++}Pure-HA kit	Ca^{++}Pure-HA plate (20 µL resin beds)
OC41MDCPHA-500	Ca^{++}Pure-HA kit	Ca^{++}Pure-HA plate (500 µL resin beds)
OC41MDLSFT-650F	Sulfate-650F kit	TOYOPEARL Sulfate-650F plate (20 µL resin beds)

Plate configurations available for Resin Seeker mixed plate offerings:

Mixed Anion Exchange Plate:

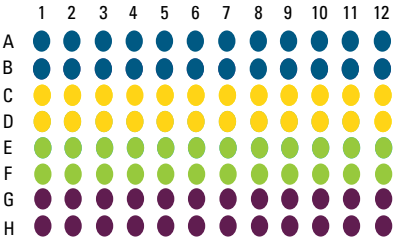
- TOYOPEARL GigaCap Q-650M
- TOYOPEARL SuperQ-650C
- TOYOPEARL Q-600C AR
- TOYOPEARL DEAE-650C



20 µL resin volumes

Mixed Cation Exchange Plate:

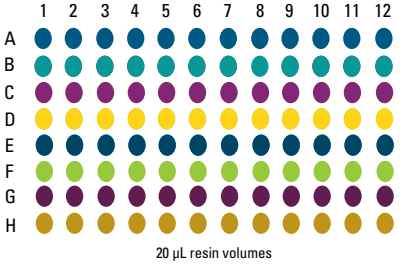
- TOYOPEARL GigaCap S-650M
- TOYOPEARL GigaCap CM-650M
- TOYOPEARL SP-550C
- TOYOPEARL CM-650C



20 µL resin volumes

Mixed Hydrophobic Interaction Plate:

- TOYOPEARL Hexyl-650C
- TOYOPEARL Butyl-650M
- TOYOPEARL Butyl-600M
- TOYOPEARL SuperButyl-550C
- TOYOPEARL Phenyl-650M
- TOYOPEARL Phenyl-600M
- TOYOPEARL PPG-600M
- TOYOPEARL Ether-650M



20 µL resin volumes

MiniChrom Columns

MiniChrom columns are small, pre-packed columns (8 mm ID x 10 cm) with 5 mL resin volume designed for fast method development or resin screening with TOYOPEARL, TSKgel and Ca^{++}Pure-HA resins. They guarantee optimal performance and can be connected to common high or medium pressure liquid chromatography systems.

The 5 mL MiniChrom columns are the ideal tool to further optimize a purification method and/or to confirm operational parameters after having selected a resin for a certain purification task by resin screening, e.g. with ToyoScreen cartridges on conventional LC systems or by high throughput screening using RoboColumns on robotic workstations. Two columns can be connected in series to increase the column height in order to model real conditions in pilot scale or for scale down experiments.

MiniChrom columns are packed by Atoll GmbH. They are reproducibly and individually flow-packed to account for the varying compressibility of each resin. Therefore, each column provides an accurate representation of resin performance that translates to full scale production columns.

Ordering Information - MiniChrom columns

Part #	Description	Column Dimensions
45101	TOYOPEARL GigaCap S-650M, 75 µm	8 mm ID × 10 cm
45102	TOYOPEARL GigaCap S-650S, 35 µm	8 mm ID × 10 cm
45103	TOYOPEARL GigaCap CM-650M, 75 µm	8 mm ID × 10 cm
45104	TOYOPEARL GigaCap Q-650M, 75 µm	8 mm ID × 10 cm
45105	TOYOPEARL GigaCap Q-650S, 35 µm	8 mm ID × 10 cm
45106	TOYOPEARL GigaCap DEAE-650M, 75 µm	8 mm ID × 10 cm
45107	TSKgel SuperQ-5PW (20), 20 µm	8 mm ID × 10 cm
45108	TOYOPEARL NH_2-750F, 45 µm	8 mm ID × 10 cm
45109	TOYOPEARL SuperQ-650M, 65 µm	8 mm ID × 10 cm
45110	TOYOPEARL SP-650M, 65 µm	8 mm ID × 10 cm
45111	TOYOPEARL SP-650S, 35 µm	8 mm ID × 10 cm
45112	TOYOPEARL DEAE-650M, 65 µm	8 mm ID × 10 cm
45113	TOYOPEARL DEAE-650S, 35 µm	8 mm ID × 10 cm
45114	TOYOPEARL SuperQ-650S, 35 µm	8 mm ID × 10 cm
45115	TOYOPEARL Q-600C AR, 100 µm	8 mm ID × 10 cm
45116	TSKgel SP-5PW, 20 µm	8 mm ID × 10 cm
45117	TOYOPEARL Sulfate-650F, 45 µm	8 mm ID × 10 cm
45119	TOYOPEARL QAE-550C, 100 µm	8 mm ID × 10 cm
45121	TOYOPEARL Phenyl-650M, 65 µm	8 mm ID × 10 cm
45122	TOYOPEARL Phenyl-650S, 35 µm	8 mm ID × 10 cm
45123	TOYOPEARL Phenyl-600M, 65 µm	8 mm ID × 10 cm
45124	TOYOPEARL PPG-600M, 65 µm	8 mm ID × 10 cm
45125	TOYOPEARL Butyl-650M, 65 µm	8 mm ID × 10 cm
45126	TOYOPEARL Butyl-650S, 35 µm	8 mm ID × 10 cm
45127	TOYOPEARL Butyl-600M, 65 µm	8 mm ID × 10 cm
45128	TOYOPEARL SuperButyl-550C, 100 µm	8 mm ID × 10 cm
45129	TOYOPEARL Hexyl-650C, 100 µm	8 mm ID × 10 cm
45130	TSKgel Phenyl-5PW (20), 20 µm	8 mm ID × 10 cm
45151	TOYOPEARL MX-Trp-650M, 75 µm	8 mm ID × 10 cm
45152	Ca^{++}Pure-HA, 39 µm	8 mm ID × 10 cm
45161	TOYOPEARL AF-rProtein A HC-650F, 45 µm	8 mm ID × 10 cm
45162	TOYOPEARL AF-rProtein L-650F, 45 µm	8 mm ID × 10 cm
45171	TOYOPEARL HW-40F, 45 µm	8 mm ID × 10 cm

Part #	Description	Column Dimensions
45181	TOYOPEARL CM-650M, 65 µm	8 mm ID × 10 cm
45182	TOYOPEARL CM-650S, 35 µm	8 mm ID × 10 cm
45183	TSKgel SP-3PW, 30 µm	8 mm ID × 10 cm
45184	TSKgel DEAE-5PW, 20 µm	8 mm ID × 10 cm
45185	TOYOPEARL SP-550C, 100 µm	8 mm ID × 10 cm
45186	TOYOPEARL MegaCap II SP-550EC, >100 µm	8 mm ID × 10 cm

About: TOYOPEARL Protein A ELISA Kit

An ELISA (enzyme-linked immunosorbent assay) kit is available for TOYOPEARL AF-rProtein A-650F and TOYOPEARL AF-rProtein A HC-650F resins from Cygnus Technologies. The TOYOPEARL ELISA kit is used for the quantitation of leached protein A ligand present in eluted product.

Please note that this kit is specifically prepared for TOYOPEARL AF-rProtein A-650F and TOYOPEARL AF-rProtein A HC-650F resins respectively. Test kits for other commercially available protein A products may not work properly for these TOYOPEARL protein A resins.

Ordering Information - TOYOPEARL protein A ELISA kit

Part #	Description
F910	Tosoh R40 and R28 Protein A, Mix-N-Go ELISA Kit

Please contact Cygnus Technologies directly for pricing and to order:

Phone: 910-454-9442
Email: orders@cygnustechnologies.com

About: TOYOPEARL Protein L ELISA Kit

The following ELISA (enzyme-linked immunosorbent assay) kit is available for TOYOPEARL AF-rProtein L-650F resin.

The TOYOPEARL ELISA kit is used for the quantitation of leached protein L ligand present in eluted product.

Ordering Information - TOYOPEARL protein L ELISA kit

Part #	Description
23497	ELISA Kit for Protein L-T36

Where to Order

Direct from Tosoh Bioscience:

Website: www.tosohbioscience.com
E-mail: info.tbl@tosoh.com
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