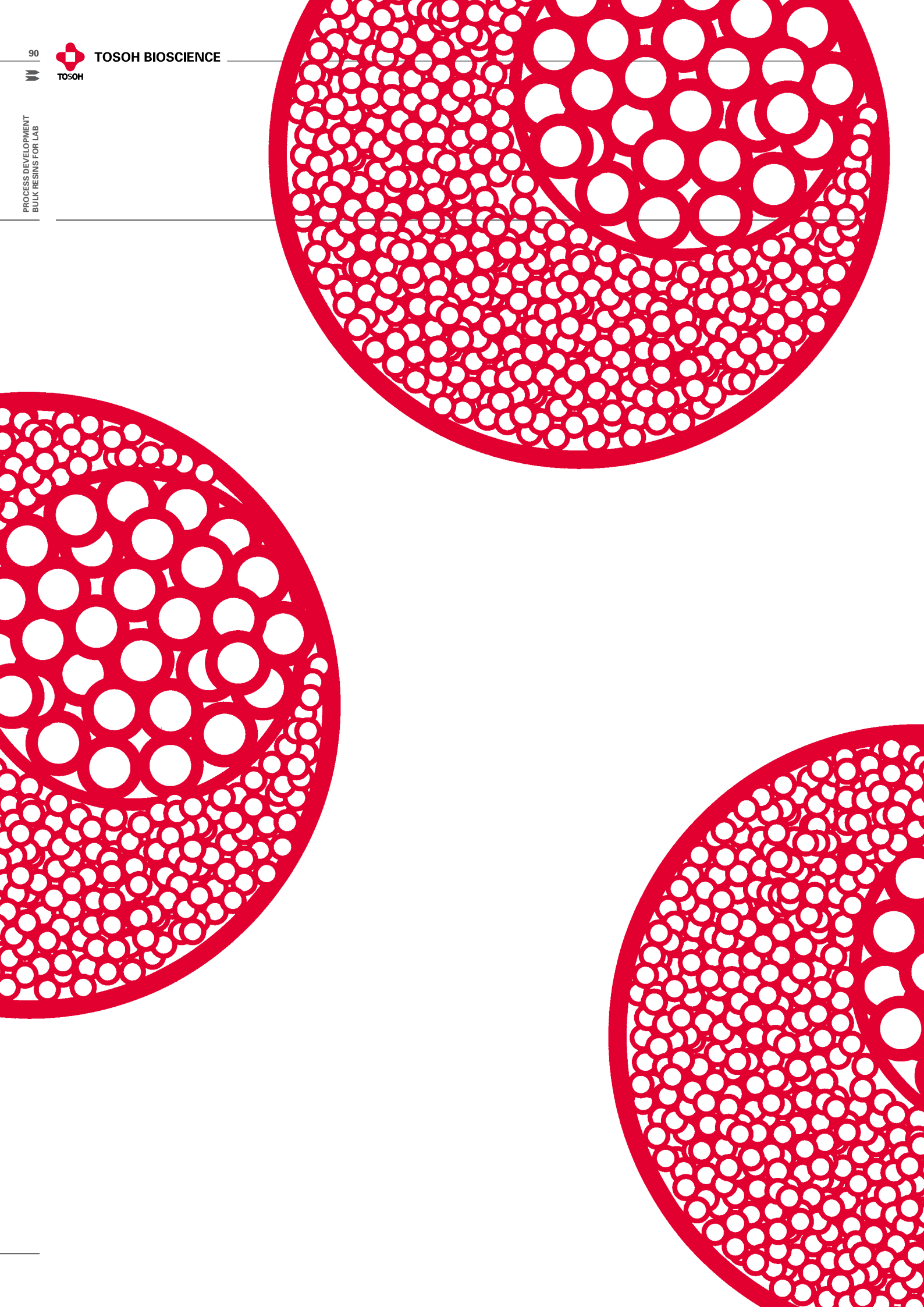
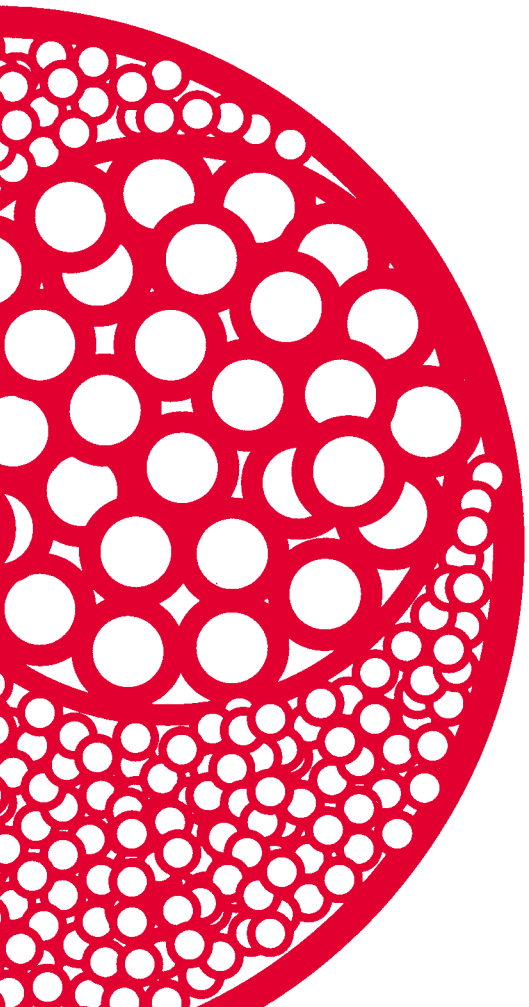


PROCESS DEVELOPMENT PRODUCTS AND BULK RESINS FOR LABORATORY SCALE PURIFICATION

PROCESS DEVELOPMENT & RESINS

- TOYOSCREEN PROCESS DEVELOPMENT COLUMNS
  TOYOPEARL AND TSKgel LABPAK
  TOYOPEARL AND TSKgel BULK RESINS



TOSOH FACT

Tosoh Bioscience offers a range of technical support services to our TSKgel, ToyoScreen, and TOYOPEARL chromatography products.

Whether you need help developing an HPLC assay for the analysis of a new therapeutic target, want to know how to monitor drug metabolites in the human body or need regulatory files to support a submission to the FDA, our technical support specialists will provide assistance in all of these areas and more.

We offer on-site training and application-specific seminars and are committed to providing prompt and courteous service for these and other requests.

TOYOSCREEN PROCESS DEVELOPMENT COLUMNS

ToyoScreen Process Development columns are easy-to-use, pre-packed columns containing Tosoh Bioscience's most popular TOYOPEARL resins. These columns provide a convenient, low-cost method for the evaluation of TOYOPEARL ligand chemistries. ToyoScreen Process Development columns are available in packages of 6 x 1 mL and 6 x 5 mL volumes for affinity, ion exchange and hydrophobic interaction chromatography. For the new TOYOSCREEN AF-rProtein A-650F package sizes are 5 x 1 mL, 1 x 5 mL and 5 x 5 mL. See the chapter on bulk resins for detailed information on TOYOPEARL resins.

SCREENING

Historically, resin screening was accomplished by manually packing various bulk resins into small columns requiring a significant investment in time and cost. In order to improve the efficiency of resin screening experiments, pre-packed ToyoScreen Process Development columns were developed for the evaluation of different TOYOPEARL resins.

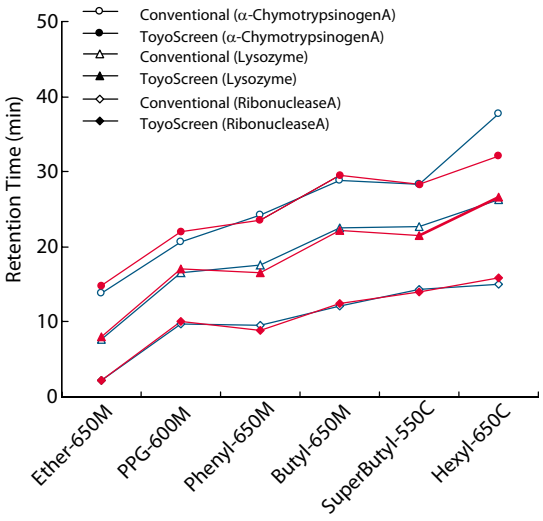
SCALABILITY

Initial results from resin screening and optimization with ToyoScreen columns can accurately predict the separation behavior at larger scales. FIGURE 1 illustrates the similar retention time behavior between 1 mL ToyoScreen columns and con-ventional 7.5 mm ID x 7.5 cm L analytical columns. Additionally, FIGURE 2 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. Similar resolution results are predicted by the following equation:

$$Rs \propto \frac{1}{dp} \frac{z^{1/2}}{u^{1/2}(g(V_t\text{-}V_o))^{1/2}}$$

FIGURE 1

Comparison of selectivity between Toyoscreen and Conventional Column



Columns: ToyoScreen (6.4 mm ID x 3 cm L), Conventional Column (7.5 mm ID x 7.5 cm L);
Eluent A: 0.1 mol/L phosphate buffer + 1.8 mol/L sodium sulfate (pH 7.0),
Eluent B: 0.1 mol/L phosphate buffer (pH 7.0); Flow Rate: 1 mL/min
Gradient: 30 min linear; Inj. Vol.: 50 µL; Samples: Ribonuclease A, Lysozyme, α-Chymotrypsinogen, 1 mg/mL

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

METHOD OPTIMIZATION

Besides the determination of what sticks during resin screening experiments, ToyoScreen Process Development 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 salt type and pH are shown in FIGURES 3 & 4 for anti-TSH in cell culture supernatant on ToyoScreen Phenyl-650M.

FEATURES

- Pre-packed columns
- 1 mL and 5 mL bed volume
- Cartridge design
- Ready to connect to ÄKTA, FPLC and HPLC systems
- Six pieces offered in mixed or single chemistry

BENEFITS

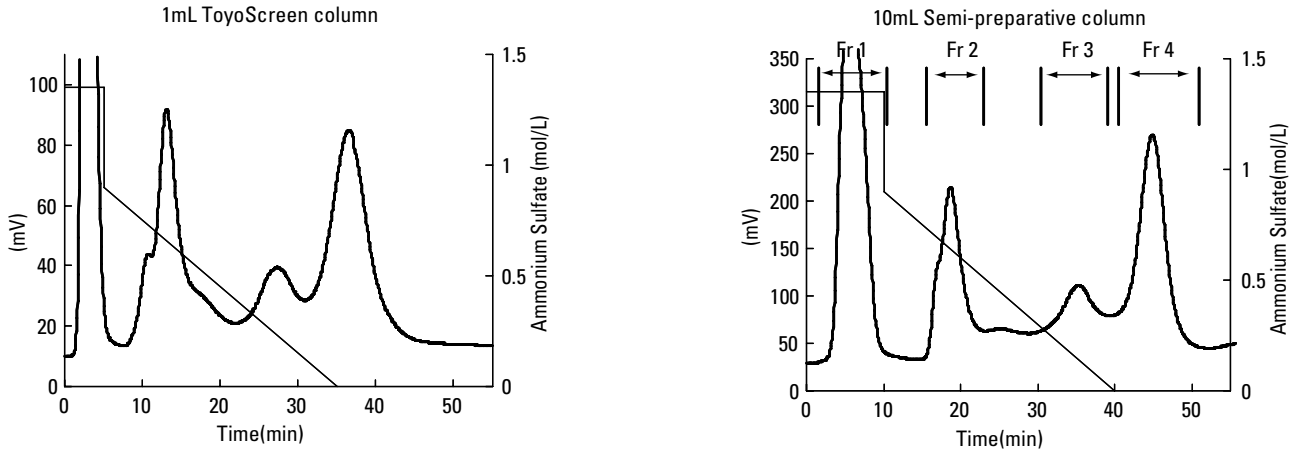
- Easy to set up and screen an entire resin series for a specific chromatographic mode
- For sample limited applications with up to milligram purifications
- Provides low cost, efficient alternative to hand packing with bulk resin
- Seamless integration into any platform
- For cost savings in screening or process experiments

PROCESS DEVELOPMENT

APPLICATIONS - TOYOSCREEN PROCESS DEVELOPMENT COLUMNS

FIGURE 2

Comparison chromatograms between ToyoScreen and Semi-preparative columns



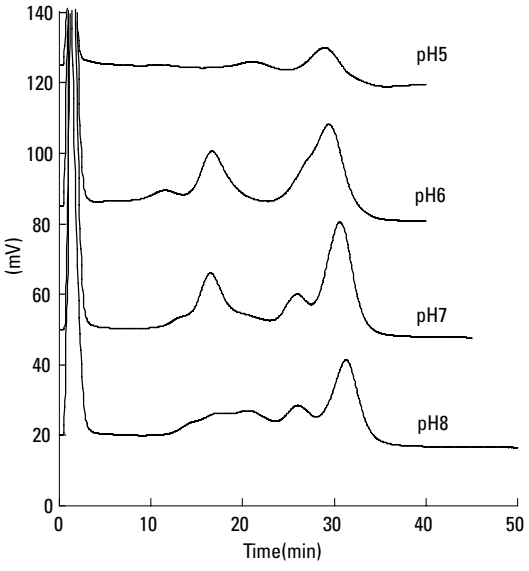
Packing: TOYOPEARL Phenyl-650M; Eluent: (A) 0.1 mol/L phosphate buffer containing 1.8 mol/L $(NH_4)_2SO_4$, pH 7.0 (B) 0.1 mol/L phosphate buffer, pH7.0; Sample: Anti-TSH from cell culture supernatant (x4 diluted)

	1 mL ToyoScreen	10 mL Semi-preparative
Column Dimensions:	6.4 mm ID x 3 cm L	14.6 mm ID x 6 cm L
Injection Volume:	500 µL	5000 µL
Flow Rate:	0.5 mL/min; 0.5 CV/min; 93 cm/h	2.5 mL/min; 0.25 CV/min; 90 cm/h
Gradient Profile:	25% B; 0-5 min (isocratic)	25% B; 0-10 min (isocratic)
	50% B: 5 min (step)	50% B: 10 min (step)
	50% to 100% B; 5-35 min (linear)	50% to 100% B; 10-40 min (linear)
Gradient Slope*:	0.06 M/mL	0.012 M/mL

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

FIGURE 3

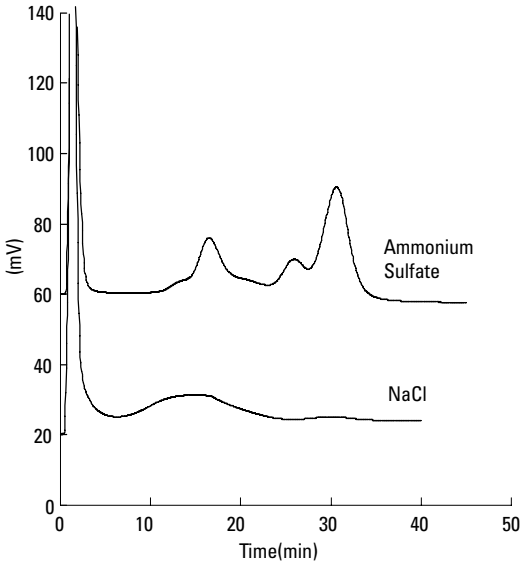
Optimizing eluent pH in HIC



Column: ToyoScreen Phenyl-650M (1 mL); Eluent A: 0.1 mol/L phosphate buffer + 1.8 mol/L ammonium sulfate (pH7.0); Eluent B: 0.1 mol/L phosphate buffer (pH 7.0); Flow Rate: 1 mL/min; Gradient: 30 min linear (30 CV); Inj. Vol.: 200 µL; Sample: Cell culture supernatant (x4 diluted) (antibody: Anti-TSH)

FIGURE 4

Optimizing salt conditions in HIC

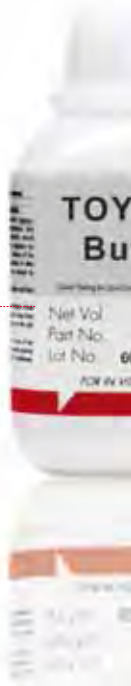


Column: ToyoScreen Phenyl-650M (1 mL); Eluent A: 0.1 mol/L phosphate buffer containing 1.8 mol/L each salt (pH7.0); Eluent B: 0.1 mol/L phosphate buffer (pH 7.0); Flow Rate: 1 mL/min; Gradient: 30 min linear (30 CV); Inj. Vol.: 200 µL; Sample: Cell culture supernatant (x 4 diluted) (antibody: Anti-TSH)

ORDERING INFORMATION

Part #	Description	Package description
Ion Exchange		
21360	ToyoScreen DEAE-650M, 1 mL	1 mL x 6 ea
21361	ToyoScreen DEAE-650M, 5 mL	5 mL x 6 ea
21362	ToyoScreen SuperQ-650M, 1 mL	1 mL x 6 ea
21363	ToyoScreen SuperQ-650M, 5 mL	5 mL x 6 ea
21364	ToyoScreen QAE-550C, 1 mL	1 mL x 6 ea
21365	ToyoScreen QAE-550C, 5 mL	5 mL x 6 ea
21993	ToyoScreen Q-600C AR, 5 mL	5 mL x 6 ea
21992	ToyoScreen Q-600C AR, 1 mL	1 mL x 6 ea
21859	ToyoScreen GigaCap Q-650M, 1 mL	1 mL x 6 ea
21860	ToyoScreen GigaCap Q-650M, 5 mL	5 mL x 6 ea
21871	ToyoScreen MegaCapII SP-550EC, 5 mL	5 mL x 6 ea
21870	ToyoScreen MegaCapII SP-550EC, 1 mL	1 mL x 6 ea
21392	ToyoScreen IEC Anion Mix Pack, 1 mL (DEAE-650M, SuperQ-650M, QAE-550C, GigaCap Q-650M, Q-600C AR)	1mL x 5 Grades
21393	ToyoScreen IEC Anion Mix Pack, 5 mL (DEAE-650M, SuperQ-650M, QAE-550C, GigaCap Q-650M, Q-600C AR)	5mL x 5 Grades
21366	ToyoScreen CM-650M, 1mL	1 mL x 6 ea
21367	ToyoScreen CM-650M, 5mL	5 mL x 6 ea
21951	ToyoScreen GigaCap CM 650M, 1 mL	1 mL x 6 ea
21952	ToyoScreen GigaCap CM 650M, 5 mL	5 mL x 6 ea
21368	ToyoScreen SP-650M, 1mL	1 mL x 6 ea
21369	ToyoScreen SP-650M, 5mL	5 mL x 6 ea
21370	ToyoScreen SP-550C, 1mL	1 mL x 6 ea
21371	ToyoScreen SP-550C, 5mL	5 mL x 6 ea
21868	ToyoScreen GigaCap S-650M, 1 mL	1 mL x 6 ea
21869	ToyoScreen GigaCap S 650M, 5 mL	5 mL x 6 ea
21394	ToyoScreen IEC Cation Mix Pack, 1 mL (CM-650M, SP-650M, SP-550C, GigaCap CM-650M /S-650M)	1 mL x 5 Grades
21395	ToyoScreen IEC Cation Mix Pack, 5 mL (CM-650M, SP-650M, SP-550C, GigaCap CM-650M /S-650M)	5 mL x 5 Grades
21396	ToyoScreen IEC Mix Pack, 1 mL (GigaCap Q-650M/ CM-650M/S-650M, SuperQ-650M, Q-600C AR)	1mL x 6 Grades x 1 ea
21397	ToyoScreen IEC Mix Pack, 5 mL (GigaCap Q-650M/ CM-650M/S-650M, SuperQ-650M, Q-600C AR)	5mL x 6 Grades x 1 ea
Hydrophobic Interaction		
21372	ToyoScreen Ether-650M, 1 mL	1 mL x 6 ea
21373	ToyoScreen Ether-650M, 5 mL	5 mL x 6 ea
21892	ToyoScreen Phenyl-600M, 1 mL	1 mL x 6 ea
21893	ToyoScreen Phenyl-600M, 5 mL	5 mL x 6 ea
21374	ToyoScreen Phenyl-650M, 1 mL	1 mL x 6 ea
21375	ToyoScreen Phenyl-650M, 5 mL	5 mL x 6 ea
21494	ToyoScreen Butyl-600M, 1 mL	1 mL x 6 ea
21495	ToyoScreen Butyl-600M, 5 mL	5 mL x 6 ea
21376	ToyoScreen Butyl-650M, 1 mL	1 mL x 6 ea
21377	ToyoScreen Butyl-650M, 5 mL	5 mL x 6 ea
21378	ToyoScreen Hexyl-650C, 1 mL	1 mL x 6 ea
21379	ToyoScreen Hexyl-650C, 5 mL	5 mL x 6 ea
21380	ToyoScreen PPG-600M, 1 mL	1 mL x 6 ea
21381	ToyoScreen PPG-600M, 5 mL	5 mL x 6 ea
21382	ToyoScreen SuperButyl-550C, 1 mL	1 mL x 6 ea
21383	ToyoScreen SuperButyl-550C, 5 mL	5 mL x 6 ea
21398	ToyoScreen HIC Mix Pack, 1 mL (PPG-600M, Butyl-600M/-650M, Phenyl-600M/-650M, Hexyl-650C)	1 mL x 6 Grades x 1 ea
21399	ToyoScreen HIC Mix Pack, 5 mL (PPG-600M, Butyl-600M/-650M, Phenyl-600M/-650M, Hexyl-650C)	5 mL x 6 Grades x 1 ea
Affinity		
22809	ToyoScreen AF-rProtein A-650F, 1 mL	1 mL x 5 ea
22810	ToyoScreen AF-rProtein A-650F, 5 mL	5 mL x 1 ea
22811	ToyoScreen AF-rProtein A-650F, 5 mL	5 mL x 5 ea
21386	ToyoScreen AF-Blue HC-650M, 1 mL	1 mL x 6 ea
21387	ToyoScreen AF-Blue HC-650M, 5 mL	5 mL x 6 ea
21384	ToyoScreen AF-Chelate-650M, 1 mL	1 mL x 6 ea
21385	ToyoScreen AF-Chelate-650M, 5 mL	5 mL x 6 ea
21390	ToyoScreen AF-Heparin HC-650M, 1 mL	1 mL x 6 ea
21391	ToyoScreen AF-Heparin HC-650M, 5 mL	5 mL x 6 ea
21388	ToyoScreen AF-Red-650M, 1 mL	1 mL x 6 ea
21389	ToyoScreen AF-Red-650M, 5 mL	5 mL x 6 ea
ToyoScreen Accessories		
21400	ToyoScreen column holder	

ToyoScreen columns are cartridge columns. They require a column holder (P/N 21400) to run the column in a LC system.



PROCESS DEVELOPMENT

TOYOPEARL AND TSKgel LABPAK MEDIA

TOYOPEARL and TSKgel LabPak media products are small package sizes of TOYOPEARL and TSKgel bulk media products. Typically they contain three or four different ligand types offered for a particular chromatography mode.

They are useful for developmental scientists and engineers who wish to familiarize themselves with the physical properties of resins in different buffer systems:

- slurry and reslurry mechanics
- resin handling during column packing
- mechanical strength relative to other resin backbones
- degree of compressibility

The larger resin amounts in LabPak products allow the packing of wider bore and longer columns than available in the ToyoScreen products. This helps the developmental scientist or engineer to more accurately determine the resin's:

- dynamic binding capacity
- selectivity
- column efficiency
- operating conditions

ORDERING INFORMATION

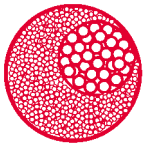
TSKgel LABPAKS

Part #	Description	Container size
IEC		
43380	IEXPAK PW, 20 µm (DEAE-5PW, SP-5PW, SuperQ-5PW)	3 x 25 mL
43280	IEXPAK PW, 30 µm (DEAE-5PW, SP-5PW, SuperQ-5PW)	3 x 25 mL
HIC		
43278	HICPAK PW, 20 µm (Ether-5PW, Phenyl-5PW)	2 x 25 mL
43175	HICPAK PW, 30 µm (Ether-5PW, Phenyl-5PW)	2 x 25 mL

TOYOPEARL LABPAKS

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

***1 g is approximately 3.5 mL**



INTRODUCTION TO BULK RESINS FOR LABORATORY PURIFICATION

Tosoh Bioscience offers TOYOPEARL and TSKgel resins (media) in bulk quantities for laboratory-scale applications.

Although the resins can be applied to the purification of small as well as large MW compounds, TOYOPEARL and TSKgel resins are most useful for the separation of peptides, proteins, and oligonucleotides.

The focus of this section is on the use of bulk resins in laboratory applications. Please request the Process Chromatography catalog for information about the use of TOYOPEARL and TSKgel for larger scale separations or visit our website at: www.tosohbioscience.com.

TOYOPEARL BULK RESIN

TOYOPEARL resins are hydrophilic, macroporous media for medium pressure liquid chromatographic applications.

The polymethacrylate backbone structure of TOYOPEARL packings assure excellent pressure/flow characteristics. TOYOPEARL is mechanically stable up to 0,3 MPa, which simplifies column packing by reducing the setup time and improving reproducibility from column to column.

The media is stable over the range of pH 2-12 for normal operating conditions and pH 1-13 for cleaning conditions. In most modes, TOYOPEARL is available in three grades, S (superfine) for highest performance, F (fine) and M (medium) for economical purification, and C (coarse) and EC (extra coarse) for capture. Consult TABLE I for particle sizes associated with the various chemistries and pore sizes.

FEATURES	BENEFITS
chemistries available in Size Exclusion, Ion Exchange, Hydrophobic Interaction and Affinity chromatography	added flexibility during method development
methacrylate backbone has hydrophilic surface properties	less non-specific adsorption
	high recovery of proteins, enzymes, glycoproteins
TSKgel and TOYOPEARL bulk resin product lines feature the same ligand and backbone chemistries from 20 µm to 150 µm particle sizes	simplified scale up from laboratory separation to process
SEC product line available in 5 pore sizes	suitable for fractionation of large and small biopolymers
IEC, HIC and AFC products are based on 1000 Å, 750 Å and 500 Å pore size particles.	high capacity and efficient chromatography of small protein and large biopolymers due to unrestricted access of available surface area
chemical stability	cleanable resins in strong base or acid (pH 1-13)
	compatible with all water soluble organic solvents
	stable in chaotropic agents such as: guanidine hydrochloride, sodium dodecyl sulfate and urea
thermal stability	autoclavable at 120°C
	wide range of operating temperature (4-60°C)
mechanical stability	linear relationship between flow rate and pressure drop
column bed stability	constant bed volume over a wide range of salt concentrations

PROCESS DEVELOPMENT

PROCESS DEVELOPMENT BULK MEDIA

TOYOPEARL HW-type resins, available in pore sizes ranging from 50 Å to >1000 Å, are employed in size exclusion chromatography (SEC). TOYOPEARL HW-65 and HW-55 resins are used as starting materials for the production of all other functionalized TOYOPEARL resins. The large pore size of HW-65 (1000 Å) allows unhindered access of large proteins to the stationary phase, resulting in faster separation and shorter recycling times.

For predictable results during scale up, TOYOPEARL resins are based on the same chemistry as the prepacked TSKgel columns. This allows for seamless scale up from the laboratory to manufacturing.

TSKgel BULK RESINS

TSKgel resins are larger particle size versions of the chemically equivalent methacrylic packing of analytical-scale TSKgel columns used for protein analysis and purification. The TSKgel resin product line consists of DEAE-5PW, SuperQ-5PW, SP-5PW and SP-3PW resins for ion exchange, Tresyl-5PW resins for afffinity chromatography and Ether-5PW and Phenyl-5PW resins for HIC. TSKgel resins are often employed to simplify scale-up from analytical columns, as only the particle size is different. Their small particle sizes, high degree of cross-linking and high mechanical stability make TSKgel resins the preferred choice for high efficiency purifications.

TABLE I

Characteristics of TOYOPEARL and TSKgel media

Mode	Resin	Grade/particle size (µm)	Pore size (Å)**	MW range Proteins (Da)	Operating pH range	Max. pressure (MPa)
SEC	TOYOPEARL HW-40	S (20-40), F (30-60), C (50-100)	50	1×10^2 - 1×10^4	2–12	0.3
	TOYOPEARL HW-50	S (20-40), F (30-60)	125	5×10^2 - 8×10^4	2–12	0.3
	TOYOPEARL HW-55	S (20-40), F (30-60)	500	1×10^3 - 7×10^5	2–12	0.3
	TOYOPEARL HW-65	S (20-40), F (30-60)	1000	4×10^4 - 5×10^6	2–12	0.3
	TOYOPEARL HW-75	S (20-40), F (30-60)	> 1000	5×10^5 - 5×10^7	2–12	0.3
IEC	TSKgel SuperQ-5PW	20 and 30	1000	$< 5 \times 10^6$	2–12	2.0
	TSKgel DEAE-5PW	20 and 30	1000	$< 5 \times 10^6$	2–12	2.0
	TSKgel SP-5PW	20 and 30	1000	$< 5 \times 10^6$	2–12	2.0
	TSKgel SP-3PW	30	250	$< 1 \times 10^4$	2–12	2.0
	TOYOPEARL SuperQ-650	S (20-50), M (40-90), C (50-150)	1000	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL DEAE-650	S (20-50), M (40-90), C (50-150)	1000	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL GigaCap M-650	M (50-100)	1000	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL SP-650	S (20-50), M (40-90), C (50-150)	1000	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL CM-650	S (20-50), M (40-90), C (50-150)	1000	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL GigaCap S-650	M (50-100)	1000	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL GigaCap CM-650	M (50-100)	1000	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL QAE-550	C (50-150)	500	$< 5 \times 10^5$	2–12	0.3
	TOYOPEARL Q-600C AR	C (50-150)	750	$< 2.5 \times 10^6$	2-12	0.3
	TOYOPEARL SP-550	C (50-150)	500	$< 5 \times 10^5$	2–12	0.3
	TOYOPEARL MegaCap II SP-550	EC (100-300)	500	$<5 \times 10^5$	2–12	0.3
HIC	TSKgel Ether-5PW	20 and 30	1000	$< 5 \times 10^6$	2–12	2.0
	TSKgel Phenyl-5PW	20 and 30	1000	$< 5 \times 10^6$	2–12	2.0
	TOYOPEARL Ether-650	S (20-50), M (40-90)	1000	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL PPG-600	M (40-90)	750	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL Phenyl-600	M (40-90)	750	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL Butyl-600	M (40-90)	750	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL Phenyl-650	S (20-50), M (40-90), C (50-150)	1000	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL Butyl-650	S (20-50), M (40-90), C (50-150)	1000	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL Super Butyl-550	C (50-150)	500	$< 5 \times 10^5$	2–12	0.3
	TOYOPEARL Hexyl-650	C (50-150)	1000	$< 5 \times 10^6$	2–12	0.3
AFC	TSKgel Tresyl-5PW	10	1000	$< 5 \times 10^6$	2–12	1.0
	TOYOPEARL AF-Chelate-650	M (40-90)	1000	$< 5 \times 10^6$	2–12	0.3
	TOYOPEARL Protein A	F (30-60)	1000	$< 5 \times 10^6$	N/A	0.3
	TOYOPEARL AF-Tresyl-650	M (40-90)	1000	$< 5 \times 10^6$	N/A	0.3
	TOYOPEARL AF-Epoxy-650	M (40-90)	1000	$< 5 \times 10^6$	N/A	0.3
	TOYOPEARL AF-Formyl-650	M (40-90)	1000	$< 5 \times 10^6$	6-9	0.3
	TOYOPEARL AF-Amino-650	M (40-90)	1000	$< 5 \times 10^6$	2-12	0.3
	TOYOPEARL AF-Carboxy-650	M (40-90)	1000	$< 5 \times 10^6$	2-12	0.3
	TOYOPEARL AF-Red-650	M (40-90)	1000	$< 5 \times 10^6$	4-9	0.3
	TOYOPEARL AF-Blue HC-650	M (40-90)	1000	$< 5 \times 10^6$	4-9	0.3
	TOYOPEARL AF-Heparin HC-650	M (40-90)	1000	$< 5 \times 10^6$	5-10	0.3

** nominal values; Pore size of base matrix

TOYOPEARL BULK RESINS FOR SEC

HIGHLIGHTS

- Pore sizes ranging from 50 Å to >1000 Å
- Three particle sizes (S, F, C)
- HW-40 is ideal for desalting applications
- Easy to pack in semi-preparative and process scale columns

Size exclusion chromatography (SEC) is a common technique for separating molecules based on their apparent molecular size. For nearly twenty-five years, TOYOPEARL SEC bulk resins, with their macroporous packings, have been used for laboratory and production-scale biochromatography.

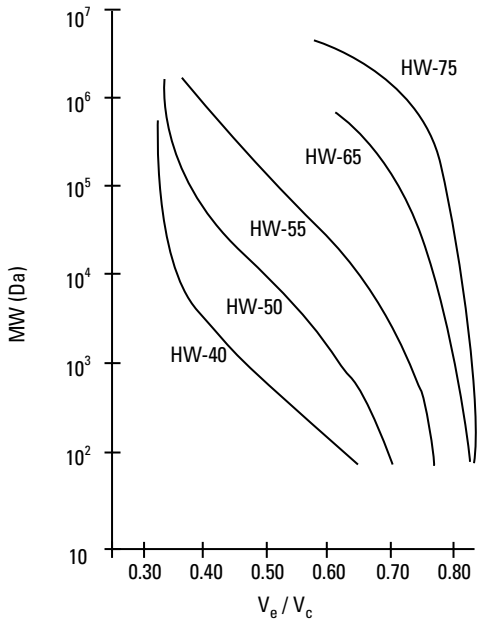
TOYOPEARL SEC resins are semi-rigid, spherical polymethacrylate beads. The resins have hydrophilic surfaces due to the presence of ether and hydroxyl groups. The numerous surface hydroxyl groups provide attachment points for other functional groups and ligands. TABLE II provides an overview of the TOYOPEARL SEC resin product line including corresponding molecular weight ranges of common target samples. Calibration curves of the TOYOPEARL HW-type resins determined with globular proteins are presented in FIGURE 5.

Ordering information for quantities <1 L is provided at the end of this section. For larger quantities, please contact customer service at +49 (0)711 13257 0. LABPAK kits are also available in popular combinations of TOYOPEARL media. See the page 99 for additional information.

Applications: proteins, peptides, amino acids, nucleic acids, and small molecular weight molecules. Please visit our website: www.tosohbioscience.com for extensive data on applications.

FIGURE 5

Calibration curves for globular proteins on TOYOPEARL HW-type resins



Column: 22 mm ID x 30 cm L; Sample: protein standards; Elution: 0.06 mol/L phosphate buffer, pH 7, in 0.06 mol/L KCl; Legend: Ve=elution volume, Vc=column volume

TABLE II

Properties and molecular weight separation ranges for TOYOPEARL HW-type resins (HW = Hydrophilic, Water-compatible polymeric base resins)

			Molecular weight of sample (Da)		
TOYOPEARL resin	**Particle size (µm)**	**Pore size (Å)**	**PEG and PEO**	**Dextrans**	**Globular proteins**
HW-40S HW-40F HW-40C	20 - 40 30 - 60 50 - 100	50 50 50	1×10^2 - 3×10^3	1×10^2 - 7×10^3	1×10^2 - 1×10^4
HW-50S HW-50F	20 - 40 30 - 60	125 125	1×10^2 - 1.8×10^4	5×10^2 - 2×10^4	5×10^2 - 8×10^4
HW-55S HW-55F	20 - 40 30 - 60	500 500	1×10^2 - 1.5×10^5	1×10^3 - 2×10^5	1×10^3 - 7×10^5
HW-65S HW-65F	20 - 40 30 - 60	1000 1000	5×10^2 - 1×10^6	1×10^4 - 1×10^6	4×10^4 - 5×10^6
HW-75F	30 - 60	>1000	4×10^3 - 5×10^6	1×10^5 - 1×10^7	5×10^5 - 5×10^7

PROCESS DEVELOPMENT

TOYOPEARL AND TSKgel BULK RESINS FOR IEC

HIGHLIGHTS

- TOYOPEARL GigaCap®S-650M, CM-650M and Q-650M resins are high capacity ion exchange resins featuring high dynamic binding capacities for both small molecules like insulin and larger proteins like monoclonal antibodies.
- Weak and strong anion and cation exchangers are offered in both product lines.
- Standard 1,000 Å pore size for large biopolymers and 500 Å pore size packing for optimal binding capacity are available.
- High efficiency TSKgel resins scale up directly from TSKgel analytical columns.

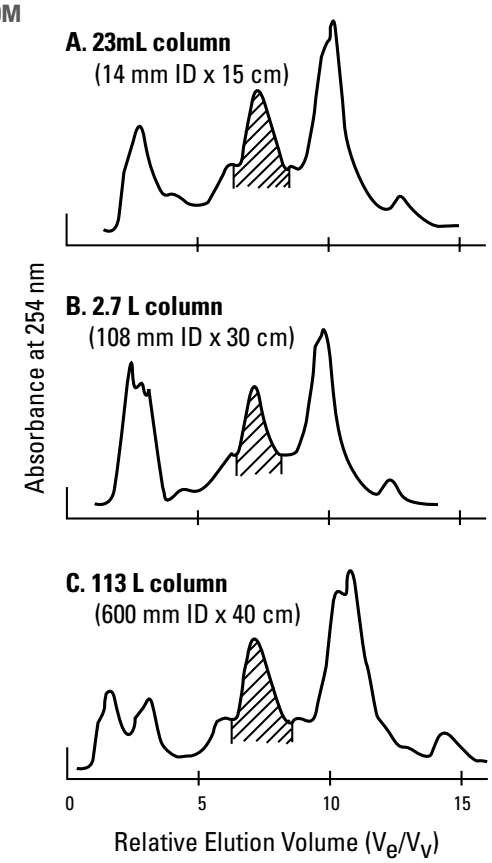
For separating mixtures of biomolecules, Ion Exchange Chromatography (IEC) is known for its high resolution and high capacity. It is very effective in the initial capture step of a chromatography process. IEC is also useful for further purification and/or polishing. It can complement other chromatographic techniques in the design of an economical downstream purification process. IEC is often used as a purification step before HIC, SEC, and RPC. IEC will also purify and concentrate the target molecule in one step when the sample is diluted. This also allows it to be used as a concentration step after SEC.

A 5000-fold scale-up of a α-galactosidase enzyme purification was accomplished using TOYOPEARL DEAE-650M. The chromatograms in FIGURE 6 demonstrate the excellent scale up characteristics of TOYOPEARL ion exchange media. Gradient slope and particle diameter remained unchanged. Linear velocity was reduced by 15% in the largest scale separation, and resolution actually improved relative to the smallest scale separation. This may be partly attributed to increased bed height and the slower linear velocity. Although the column volume was increased in part by increasing the bed height, the principal change in column volume was a result of the greater column diameter (1.4 cm to 60 cm L). This example illustrates how TOYOPEARL media can be conveniently scaled up from laboratory to production scale applications using the same particle size if desired.

Because the correct choice of an ion exchange resin can have a considerable impact on the economy of a process, Tosoh Bioscience provides many product options in both TOYOPEARL and TSKgel IEC bulk polymeric media. See TABLE III for a complete listing of available particle sizes. Ordering information for quantities < 1 L is provided at the end of this section.

FIGURE 6

Process scale-up purification of β-galactosidase with TOYOPEARL DEAE-650M



Column: TOYOPEARL DEAE-650 M; Sample: 1% β-galactosidase: A. 8 mL; B. 1L; C. 40L Elution: linear gradient from 0.03 to 0.10 mol/L NaCl in 0.014 mol/L Tris-HCl (pH7.7); Flow rate: A. 1.0 mL/min; B. 60 mL/min; C. 1.6 L/min; Linear velocity: A. 39 cm/h; B. 40 cm/h; C. 34 cm/h; Detection: UV@254nm

TABLE III

TOYOPEARL and TSKgel Ion Exchange Resins

Description	Type*	Part. size (µm)
Anion Exchange		
TSKgel DEAE-5PW	W	20, 30
TSKgel SuperQ-5PW	S	20, 30
TOYOPEARL DEAE-650	W	35, 65, 100
TOYOPEARL SuperQ-650	S	35, 65, 100
TOYOPEARL QAE-550	S	100
TOYOPEARL Q-600 AR	S	100
TOYOPEARL GigaCap Q-650M	S	75
Cation Exchange		
TSKgel SP-5PW	S	20, 30
TSKgel SP-3PW	S	30
TOYOPEARL CM-650	W	35, 65, 100
TOYOPEARL GigaCap CM-650M	W	75
TOYOPEARL SP-550	S	100
TOYOPEARL SP-650	S	35, 65, 100
TOYOPEARL MegaCap II SP-550EC	S	100-300
TOYOPEARL GigaCap S-650M	S	75

**W = Weak; S = Strong*

TOYOPEARL AND TSKgel BULK RESINS FOR HIC

HIGHLIGHTS

- A wide range of hydrophobicities is suitable for most proteins.
- Standard 1,000 Å pore size is available for large biopolymers, and three Butyl pore sizes (500 Å, 750 Å and 1,000 Å) are available.
- TOYOPEARL "600M" series of HIC resins with optimized pore size of 750 Å for antibody separation. Phenyl-600M and Butyl-600M with highest DBCs for IgG.
- Seamless scale up from high efficiency TSKgel 5PW-type analytical columns is possible.

Hydrophobic Interaction Chromatography (HIC) has become a popular mode of chromatography for the purification of biopolymers at analytical as well as preparative scale. This is accomplished by the interaction of hydrophobic ligands on the base matrix with the hydrophobic areas located on the surface of proteins. HIC is an excellent complement to size exclusion and ion exchange chromatography in difficult separations, particularly those where the contaminants are of similar pI or molecular weight. It is often preferred over reversed phase chromatography when preservation of biological activity of the protein is of utmost importance.

Tosoh Bioscience offers both the TSKgel and TOYOPEARL resin product lines for HIC. See TABLE IV for a complete listing of functionalities. Each product line has similar backbone chemistry. TSKgel 5PW-type resins possess a higher degree of cross-linking than the corresponding TOYOPEARL resins. Additionally, choices in particle size are offered to match the desired resolution and throughput. A variety of HIC bulk media are offered as LABPAK kits in quantities < 1 L and in a combination of resins with varying functionalities. Additionally, HIC media are available in ToyoScreen process development columns for convenient scouting and methods development.

Ordering information for quantities < 1 L is provided at the end of this section.

APPLICATIONS: proteins with similar chemical or structural properties, plasmids and monoclonal antibodies. See FIGURE 7 for separation of large glycoprotein from crude extract on TOYOPEARL Butyl-650S. Please visit our website: www.tosohbioscience.com for extensive application data.

TABLE IV

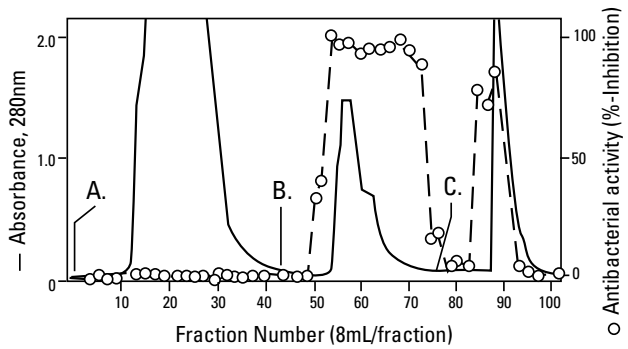
TOYOPEARL and TSKgel HIC Resins

Description	Strength*	Part. size grades (µm)
TSKgel Ether-5PW	1	20, 30
TOYOPEARL Ether-650	1	35, 65
TOYOPEARL PPG-600	2	65, 100
TSKgel Phenyl-5PW	3	20, 30
TOYOPEARL Phenyl-650	3	35, 65, 100
TOYOPEARL Phenyl-600	4	65
TOYOPEARL Butyl-600	4	65
TOYOPEARL Butyl-650	4	35, 65, 100
TOYOPEARL SuperButyl-550	4	100
TOYOPEARL Hexyl-650	5	100

* Relative scale: 1 = least hydrophobic, 5 = most hydrophobic.

FIGURE 7

Large glycoprotein purified on TOYOPEARL Butyl-650S



Column: TOYOPEARL Butyl-650S, 22 mm ID x 26 cm L;
Sample: crude protein from sea hare *Aplysia kurodai*;
Elution: multi-step $(NH_4)_2SO_4$ in 50 mmol/L phosphate buffer, pH 7.0 A. load & wash: 40 % saturated $(NH_4)_2SO_4$
B. 20% saturated $(NH_4)_2SO_4$
C. 0% saturated $(NH_4)_2SO_4$

PROCESS DEVELOPMENT

TOYOPEARL RESINS FOR AFC

HIGHLIGHTS

- New AF-rProtein A-650F resin for antibody purification.
- Active, reactive and group specific resins
- Provided in standard 1000 Å pore size for high capacity of large biopolymers.
- TOYOPEARL AF-Blue HC-650M is available for albumin and interferon applications with the lowest leaching blue.
- TOYOPEARL AF-Heparin HC-650M high capacity resin exhibits an Antithrombin III dynamic capacity of 4 mg/mL.

TOYOPEARL media for Affinity Chromatography (AFC) are based on TOYOPEARL HW-65 resin and functionalized with either chemically active groups or group-specific ligands. Resins with activated functional groups are ready for direct coupling of a protein or other ligand, while resins with reactive groups employ coupling or reductive amination to achieve covalent bonding. The 1000 Å pore size common to all TOYOPEARL affinity resins accommodates proteins up to 5,000,000 Da.

In general, TOYOPEARL AF-Tresyl-650M and AF-Formyl-650M are recommended for coupling proteins, while AF-Epoxy-650M is suited for coupling low molecular weight ligands. TOYOPEARL AF-Amino-650M and TOYOPEARL AF-Carboxy-650M may be used in either application. TOYOPEARL AF-Heparin HC-650M interacts with a wide range of biomolecules including plasma components, lipoprotein lipase, collagenase, and DNA polymerase. The structures of TOYOPEARL activated and reactive ligands are given in FIGURE 8, while the structures of TOYOPEARL group-specific ligands are listed in FIGURE 9.

FIGURE 8

Activated and reactive TOYOPEARL affinity resins

Toyopearl AF-Tresyl-650M (1) HW-65—O-R-O-SO_2-CH_2-CF_3
Ligand Density: 80 mol/g (dry)

Toyopearl AF-Epoxy-650M (1) HW-65—O-R-O-CH_2-CH-CH_2 (O)
Ligand Density: 800 mol/g (dry)

Toyopearl AF-Formyl-650M (2) HW-65—O-R-O-CH_2-CHO
Ligand Density: 60 eq/mL

Toyopearl AF-Amino-650M (3) HW-65—O-R-O-CH_2-CHOH-CH_2NH_2
Ligand Density: 100 eq/mL

Toyopearl AF-Carboxy-650M (3) HW-65—O-R-O-CH_2-COOH
Ligand Density: 100 eq/mL

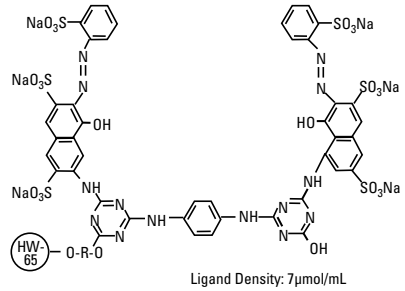
(1) Provided as dry, free-flowing powder.
One gram of dry powder produces about 3.5 mL of hydrated resin.
(2) Provided as aqueous slurry, containing 1% gluteraldehyde.
(3) Provided as aqueous slurry, containing 20% ethanol.

TOYOPEARL AF-rProtein A-650F is designed for efficient and robust purification of antibodies. The newly developed recombinant protein A ligand is derived from one of the IgG-binding domains of the staphylococcus aureus protein A (FIGURE 10). TOYOPEARL AF-rProtein-650F binds human and mouse immunoglobulin G with high binding capacity and at high flow rates. This reduces column and buffer volumes and allows fast loading procedures.

FIGURE 9

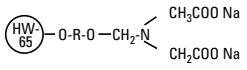
Group-specific TOYOPEARL affinity resins

Toyopearl AF-Red-650M (1)



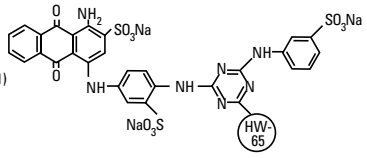
Ligand Density: 7µmol/mL

Toyopearl AF-Chelate-650M (2)

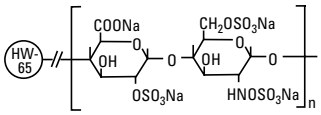


Ligand Density: 20µmol/mL

Toyopearl AF-Blue HC-650M (1)



Toyopearl AF-Heparin HC-650

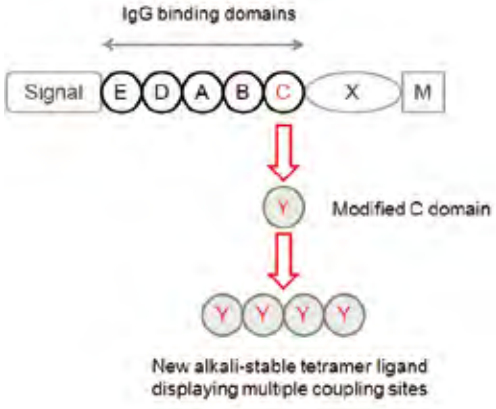


Approximate Ligand Density: 5mg/mL

(1) Provided as an aqueous slurry containing 20% ethanol, v/v in 1mol/L NaCl.
(2) Provided as an aqueous slurry containing 20% ethanol.

FIGURE 10

Recombinant Protein A derived ligand



ORDERING INFORMATION

A. Size Exclusion Chromatography

TOYOPEARL Bulk Resins

Part #	Description	Container size
19809	HW-40S, 30 µm	150 mL
07451	HW-40S, 30 µm	250 mL
19808	HW-40F, 45 µm	150 mL
07448	HW-40F, 45 µm	500 mL
19807	HW-40C, 75 µm	150 mL
07449	HW-40C, 75 µm	500 mL
19811	HW-50S, 30µm	150 mL
07455	HW-50S, 30µm	250 mL
19810	HW-50F, 45 µm	150 mL
07453	HW-50F, 45 µm	500 mL
19813	HW-55S, 30 µm	150 mL
07459	HW-55S, 30 µm	250 mL
19812	HW-55F, 45 µm	150 mL
07457	HW-55F, 45 µm	500 mL
19815	HW-65S, 30 µm	150 mL
07467	HW-65S, 30 µm	250 mL
19814	HW-65F, 45 µm	150 mL
07465	HW-65F, 45 µm	500 mL
21481	HW-65C, 75 µm	150mL
07466	HW-65C, 75 µm	500mL
19816	HW-75F, 45 µm	150 mL
07469	HW-75F, 45 µm	500 mL

B. Anion Exchange Chromatography

TSKgel Bulk Resins

43381	DEAE-5PW (20)	25 mL
14710	DEAE-5PW (20)	250 mL
43281	DEAE-5PW (30)	25 mL
14712	DEAE-5PW (30)	250 mL
43383	SuperQ-5PW (20)	25 mL
18535	SuperQ-5PW (20)	250 mL
43283	SuperQ-5PW (30)	25 mL
18536	SuperQ-5PW (30)	250 mL

TOYOPEARL Bulk Resins

19823	SuperQ-650S, 35 µm	25 mL
17223	SuperQ-650S, 35 µm	250 mL
43205	SuperQ-650M, 65 µm	100 mL
17227	SuperQ-650M, 65 µm	250 mL
43275	SuperQ-650C, 100 µm	100 mL
17231	SuperQ-650C, 100 µm	250 mL
43271	QAE-550C, 100 µm	100 mL
14026	QAE-550C, 100 µm	250 mL
21985	Q-600C AR, 100 µm - NEW -	100 mL
21986	Q-600C AR, 100 µm - NEW -	250 mL
19804	DEAE-650S, 35 µm	25 mL
07472	DEAE-650S, 35 µm	250 mL
43201	DEAE-650M, 65 µm	100 mL
07473	DEAE-650M, 65 µm	250 mL
07988	DEAE-650C, 100 µm	250 mL
21854	GigaCap Q-650M, 75 µm	100 mL
21855	GigaCap Q-650M, 75 µm	250 mL

C. Cation Exchange Chromatography

TSKgel Bulk Resins

43382	SP-5PW (20)	25 mL
14714	SP-5PW (20)	250 mL
43282	SP-5PW (30)	25 mL
14716	SP-5PW (30)	250 mL
21976	SP-3PW (30)	25 mL
21977	SP-3PW (30)	250 mL

TOYOPEARL Bulk Resins

19803	CM-650S, 35 µm	25 mL
07474	CM-650S, 35 µm	250 mL
43203	CM-650M, 65 µm	100 mL
07475	CM-650M, 65 µm	250 mL
07991	CM-650C, 100 µm	250 mL
19822	SP-650S, 35 µm	25 mL
08437	SP-650S, 35 µm	250 mL
43202	SP-650M, 65 µm	100 mL
07997	SP-650M, 65 µm	250 mL
07994	SP-650C, 100 µm	250 mL
43272	SP-550C, 100 µm	100 mL
14028	SP-550C, 100 µm	250 mL
21804	MegaCap II SP-550EC, 100-300 µm	100 mL
21805	MegaCap II SP-550EC, 100-300 µm	250 mL
21833	GigaCap S-650M, 75 µm	100 mL
21834	GigaCap S-650M, 75 µm	250 mL
21946	GigaCap CM-650M, 75 µm	100 mL
21947	GigaCap CM-650M, 75 µm	250 mL

PROCESS DEVELOPMENT BULK RESINS

ORDERING INFORMATION

D. Hydrophobic Interaction Chromatography

Part #	Description	Container size
TSKgel Bulk Resins		
43276	Ether-5PW (20)	25 mL
16052	Ether-5PW (20)	250 mL
43176	Ether-5PW (30)	25 mL
16050	Ether-5PW (30)	250 mL
43277	Phenyl-5PW (20)	25 mL
14718	Phenyl-5PW (20)	250 mL
43177	Phenyl-5PW (30)	25 mL
14720	Phenyl-5PW (30)	250 mL
TOYOPEARL Bulk Resins		
19955	SuperButyl-550C, 100 µm	25 mL
19956	SuperButyl-550C, 100 µm	100 mL
21448	Butyl-600M, 65 µm	25 mL
21449	Butyl-600M, 65 µm	100 mL
43153	Butyl-650S, 35 µm	25 mL
07476	Butyl-650S, 35 µm	100 mL
19802	Butyl-650M, 65 µm	25 mL
07477	Butyl-650M, 65 µm	100 mL
43127	Butyl-650C, 100 µm	25 mL
07478	Butyl-650C, 100 µm	100 mL
43151	Ether-650S, 35 µm	25 mL
16172	Ether-650S, 35 µm	100 mL
19805	Ether-650M , 65 µm	25 mL
16173	Ether-650M , 65 µm	100 mL
44465	Hexyl-650C, 100 µm	25 mL
19026	Hexyl-650C, 100 µm	100 mL
21887	Phenyl-600M, 65 µm	25 mL
21888	Phenyl-600M, 65 µm	100 mL
43152	Phenyl-650S, 35 µm	25 mL
14477	Phenyl-650S, 35 µm	100 mL
19818	Phenyl-650M, 65 µm	25 mL
14478	Phenyl-650M, 65 µm	100 mL
43126	Phenyl-650C, 100 µm	25 mL
14479	Phenyl-650C, 100 µm	100 mL
21301	PPG-600M, 65 µm	25 mL
21302	PPG-600M, 65 µm	100 mL

E. Affinity Chromatography

Part #	Description	Container size
TSKgel Bulk Resins		
16208	Tresyl-5PW (10)	2 g*
TOYOPEARL Bulk Resins		
22803	AF-rProtein A-650F, 45 µm - NEW -	10 mL
22804	AF-rProtein A-650F, 45 µm - NEW -	25 mL
22805	AF-rProtein A-650F, 45 µm - NEW -	100 mL
43411	AF-Amino-650M, 65 µm	10 mL
08002	AF-Amino-650M, 65 µm	25 mL
08039	AF-Amino-650M, 65 µm	100 mL
19688	AF-Blue HC-650M,65 µm	25 mL
19689	AF-Blue HC-650M, 65 µm	100 mL
43412	AF-Carboxy-650M, 65 µm	10 mL
08006	AF-Carboxy-650M, 65 µm	25 mL
08041	AF-Carboxy-650M, 65 µm	100 mL
14475	AF-Chelate-650M, 65 µm	25 mL
19800	AF-Chelate-650M, 65 µm	100 mL
43402	AF-Epoxy-650M, 65 µm	5 g*
08000	AF-Epoxy-650M, 65 µm	10 g*
08038	AF-Epoxy-650M, 65 µm	100 g*
43413	AF-Formyl-650M, 65 µm	10 mL
08004	AF-Formyl-650M, 65 µm	25 mL
08040	AF-Formyl-650M, 65 µm	100 mL
20030	AF-Heparin-HC-650M, 65 µm	10 mL
20031	AF-Heparin-HC-650M, 65 µm	100 mL
08651	AF-Red-650M, 65 µm	25 mL
19801	AF-Red-650M, 65 µm	100 mL
14471	AF-Tresyl-650M, 65 µm	5 g*
14472	AF-Tresyl-650M, 65 µm	100 g*

*1 g is approximately 3.5 mL