

Process Development Products

ToyoScreen Columns

Resin Screening

Method Optimization

Scalability

Ordering Information

LabPak

Ordering Information

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TOSOH BIOSCIENCE

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Fax: (215) 283-5035

www.tosohbioscience.com

email: info.tbl@tosoh.com

Process Development Products

Tosoh Bioscience offers 2 product lines to support your process development needs:



ToyoScreen Process Development Columns

ToyoScreen Process Development Columns are easy-to-use, pre-packed columns containing our most popular Toyopearl resins. They provide a convenient, low-cost alternative for the evaluation of Toyopearl ligand chemistries to our traditional LabPaks.



Toyopearl and TSK-GEL LabPak Media

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

Process Development Products

ToyoScreen Process Development Columns

In manufacturing, chromatography unit operations are some of the most critical in the purification of a new recombinant therapeutic protein. In many cases two to four chromatographic steps are needed to achieve the desired purity level for the target molecule. Each step can require up to multi-hundred and perhaps even thousands of liters of a specific resin for success.

The first part of the development process is the laboratory scale screening and selection of a resin. 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 this stage 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.

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 columns containing the most popular Toyopearl resins, now provide a low cost and convenient alternative for the evaluation of Toyopearl ligand chemistries.

<i>Features</i>	<i>Benefits</i>
Pre-packed columns	Easy to set up and screen an entire resin series for a specific chromatographic mode
1mL and 5mL columns	For sample limited applications with up to milligram purifications
Cartridge design	Provides low cost, efficient alternative to self packing with bulk resin
Easy connections with AKTA [®] , FPLC [®] and HPLC systems	Seamless integration with any platform
Offered in mixed or single chemistry in 6-packs	For cost savings in screening or process optimization experiments

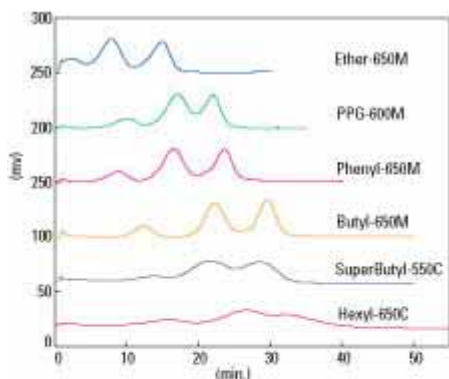
Process Development Products

Resin Screening with ToyoScreen Process Development Columns

HIC

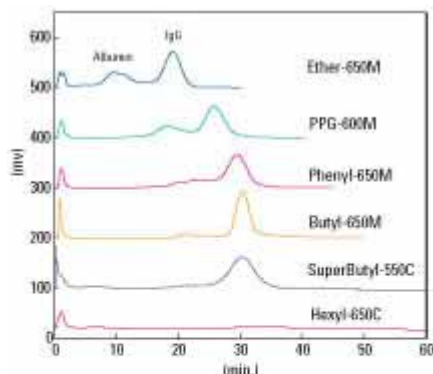
Toyopearl hydrophobic interaction Chromatography media (HIC) are available in six different chemistries ranging in hydrophobicity from Ether-650 (low) to Hexyl-650 (high). Depending on the feedstock and the impurity profile, the determination of the best selectivity is largely an empirical process. The figures below show the selectivity differences of the ToyoScreen HIC Mix Pack chemistries on the separation of protein standards and Anti-IgG from albumin in mouse ascites fluid.

HIC resins - Protein Standards



Column: ToyoScreen (1mL)
 Eluent A: 0.1M Phosphate Buffer +
 1.8M Sodium Sulfate (pH7.0)
 Eluent B: 0.1M Phosphate Buffer (pH7.0)
 Flow Rate: 1mL/min Gradient: 30min linear
 Inj. Vol.: 50L
 Samples: Ribonuclease A, Lysozyme,
 α-Chymotrypsinogen, 1mg/mL

HIC resins - Mouse Ascites Fluid (Anti-IgE)



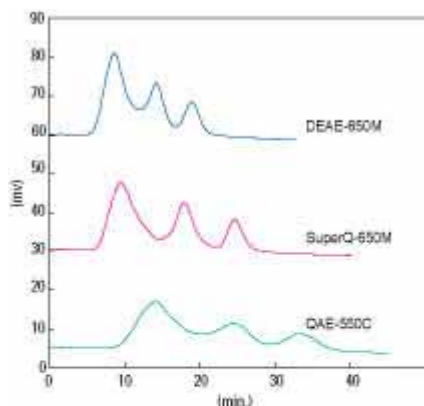
Column: ToyoScreen (1mL)
 Eluent A: 0.1M Phosphate Buffer +
 1.8M Sodium Sulfate (pH7.0)
 Eluent B: 0.1M Phosphate Buffer (pH7.0)
 Flow Rate: 1mL/min Gradient: 30min linear
 Inj. Vol.: 50L
 Samples: Mouse Ascites Fluid:A :B = 1:1:2

Process Development Products

IEC

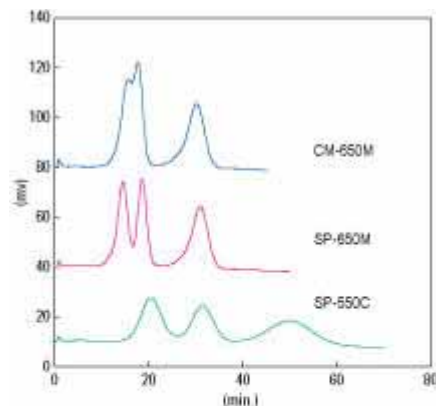
Ion exchange chromatography (IEC) separates molecules based on the ionic interaction of the molecule with the charged support. The individual functional group and its pKa can be used to evaluate different selectivities in chromatographic separations. ToyoScreen columns are offered in both strong and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

Anion Exchange Resins - Protein Standards



Column: ToyoScreen (1mL)
Eluent A: 20mM Tris-HCl (pH 8.0)
Eluent B: 20mM Tris-HCl + 0.5M NaCl (pH 8.0)
Flow Rate: 1mL/min
Gradient: B 0-->100% 60min Linear
Samples: Transferrin, Ovalbumin, Trypsin Inhibitor
1mg/mL each

Cation Exchange Resins - Protein Standards



Column: ToyoScreen (1mL)
Eluent A: 20mM Phosphate Buffer (pH 6.0)
Eluent B: 20mM Phosphate Buffer +
0.5M NaCl (pH 6.0)
Flow Rate: 1mL/min
Gradient: B 0-->100% 60min Linear
Samples: α -ChymotrypsinogenA, CytochromeC,
Lysozyme, 1mg/mL each

AFC

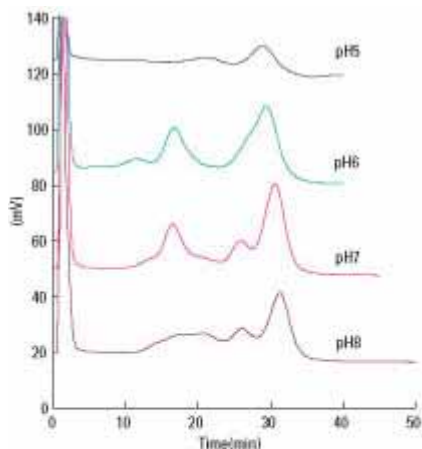
In affinity chromatography (AFC), the ligands employed are specific to a particular protein class or functional group on the accessible surface of the target molecule. ToyoScreen affinity columns are offered in three group specific ligand chemistries: AF-Blue HC-650M, AF-Chelate 650M, and AF-Red 650M. AF-Blue 650M is specific for kinases, phosphatases, dehydrogenases and other molecules such as albumin and blood coagulation factors. AF-Red-650M is specific for dehydrogenases and other proteins such as plasminogen. AF-Chelate 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.

Process Development Products

Method Optimization with ToyoScreen Process Development Columns

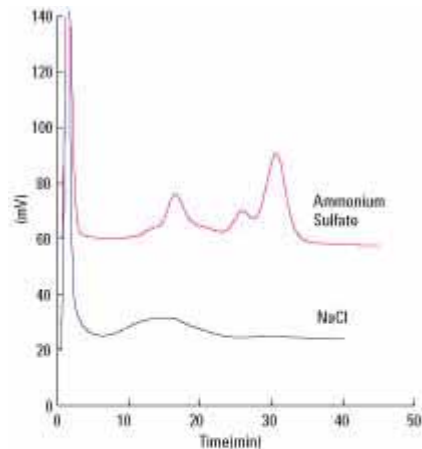
Besides determining of “what sticks” during resin screening experiments, ToyoScreen columns can be used to quickly establish optimum elution conditions. Varying pH, salt type, salt gradients and flow rate are common experimental parameters explored. The effect of varying salt type and pH are shown in the figures below for Anti-TSH in cell culture supernatant on ToyoScreen Phenyl-650M.

Optimizing eluent pH in HIC



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

Optimizing salt conditions in HIC



Column: ToyoScreen Phenyl-650M (1mL)
Eluent A: 0.1M phosphate buffer containing
1.8M each salt (pH7.0)
Eluent B: 0.1M phosphate buffer (pH7.0)
Flow Rate: 1mL/min Gradient: 30min linear (30CV)
Inj. Vol.: 200L
Samples: Cell culture supernatant (x4 diluted)
(antibody: Anti-TSH)

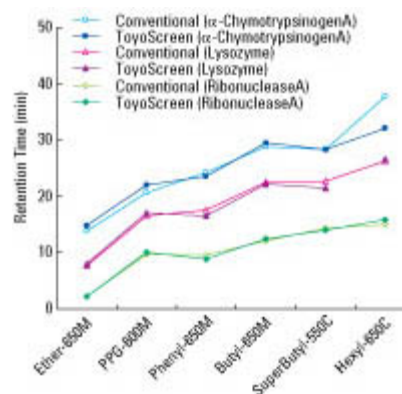
Process Development Products

Scalability

Initial results from resin screening and optimization with ToyoScreen columns can accurately predict the separation behavior at larger scales. The figure to the right illustrates the similar retention time behavior between 1mL ToyoScreen columns and conventional 7.5mmID x 7.5cm analytical columns. Additionally, the figure below depicts a practical antibody scale up in which conditions were set using a 1mL ToyoScreen and applied to a 10mL semi-preparative column with a different inner diameter and length. Similar resolution results were predicted by the following equation:

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

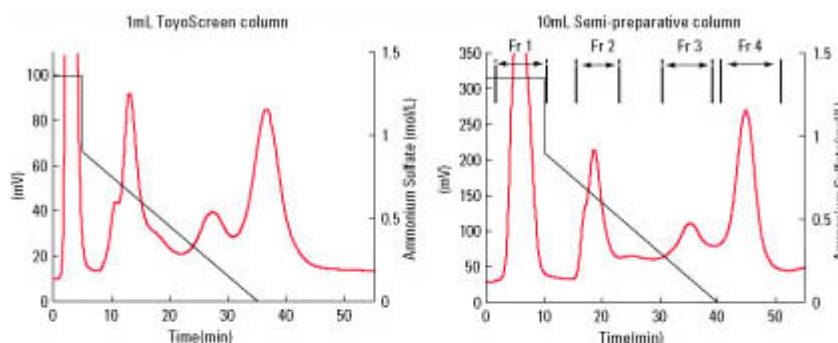
Selectivity comparison between ToyoScreen and Conventional column



Columns: ToyoScreen (1mL),
Conventional column (7.5mmID x 7.5cm)
Eluent A: 0.1M phosphate buffer +
1.8M sodium sulfate (pH7.0)
Eluent B: 0.1M phosphate buffer (pH7.0)
Flow Rate: 1mL/min
Gradient: 30min linear (30CV)
Inj. Vol.: 50L
Samples: Ribonuclease A, Lysozyme,
α-Chymotrypsinogen, 1mg/mL

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

Comparison chromatograms between ToyoScreen and Semi-preparative columns



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

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

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

Process Development Products

Ordering information:

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Ion Exchange

P/N	Description	Package Description	Price (\$)
21360	ToyoScreen DEAE-650M, 1mL	1mL x 6 ea.	113
21361	ToyoScreen DEAE-650M, 5mL	5mL x 6 ea.	240
21362	ToyoScreen SuperQ-650M, 1mL	1mL x 6 ea.	113
21363	ToyoScreen SuperQ-650M, 5mL	5mL x 6 ea.	240
21364	ToyoScreen QAE-550C, 1mL	1mL x 6 ea.	113
21365	ToyoScreen QAE-550C, 5mL	5mL x 6 ea.	240
21859	ToyoScreen GigaCap Q-650M	1mL x 6 ea.	113
21860	ToyoScreen GigaCap Q-650M	5mL x 6 ea.	240
21366	ToyoScreen CM-650M, 1mL	1mL x 6 ea.	113
21367	ToyoScreen CM-650M, 5mL	5mL x 6 ea.	240
21368	ToyoScreen SP-650M, 1mL	1mL x 6 ea.	113
21369	ToyoScreen SP-650M, 5mL	5mL x 6 ea.	240
21370	ToyoScreen SP-550C, 1mL	1mL x 6 ea.	113
21371	ToyoScreen SP-550C, 5mL	5mL x 6 ea.	240
21870	ToyoScreen MegaCap II SP-550EC	1mL x 6 ea.	113
21871	ToyoScreen MegaCap II SP-550EC	5mL x 6 ea.	240
21868	ToyoScreen GigaCap S-650M	1mL x 6 ea.	113
21869	ToyoScreen GigaCap S-650M	5mL x 6 ea.	240
21392	ToyoScreen IEC Anion Mix Pack, 1mL (DEAE, SuperQ, QAE)	1mL x 3 grades x 2 ea.	113
21393	ToyoScreen IEC Anion Mix Pack, 5mL (DEAE, SuperQ, QAE)	5mL x 3 grades x 2 ea.	240
21394	ToyoScreen IEC Cation Mix Pack, 1mL (CM, SP-650, SP-550)	1mL x 3 grades x 2 ea.	113
21395	ToyoScreen IEC Cation Mix Pack, 5mL (CM, SP-650, SP-550)	5mL x 3 grades x 2 ea.	240
21396	ToyoScreen IEC Mix Pack, 1mL (DEAE, SuperQ, QAE, CM, SP650, SP-550)	1mL x 6 grades x 1 ea.	113
21397	ToyoScreen IEC Mix Pack, 5mL (DEAE, SuperQ, QAE, CM, SP650, SP-550)	5mL x 6 grades x 1 ea	240

Process Development Products

Hydrophobic Interaction

P/N	Description	Package Description	Price (\$)
21372	ToyoScreen Ether-650M, 1mL	1mL x 6 ea.	147
21373	ToyoScreen Ether-650M, 5mL	5mL x 6 ea.	315
21374	ToyoScreen Phenyl-650M, 1mL	1mL x 6 ea.	147
21375	ToyoScreen Phenyl-650M, 5mL	5mL x 6 ea.	315
21494	ToyoScreen Butyl-600M, 1mL	1mL x 6 ea.	147
21495	ToyoScreen Butyl-600M, 5mL	5mL x 6 ea.	315
21376	ToyoScreen Butyl-650M, 1mL	1mL x 6 ea.	147
21377	ToyoScreen Butyl-650M, 5mL	5mL x 6 ea.	315
21378	ToyoScreen Hexyl-650C, 1mL	1mL x 6 ea.	147
21379	ToyoScreen Hexyl-650C, 5mL	5mL x 6 ea.	315
21380	ToyoScreen PPG-600M, 1mL	1mL x 6 ea.	147
21381	ToyoScreen PPG-600M, 5mL	5mL x 6 ea.	315
21382	ToyoScreen SuperButyl-550C, 1mL	1mL x 6 ea.	147
21383	ToyoScreen SuperButyl-550C, 5mL	5mL x 6 ea.	315
21398	ToyoScreen HIC Mix Pack, 1mL (Ether, Butyl-650, Hexyl, Phenyl, PPG, SuperButyl)	1mL x 6 grades x 1 ea.	147
21399	ToyoScreen HIC Mix Pack, 5mL (Ether, Butyl-650, Hexyl, Phenyl, PPG, SuperButyl)	5mL x 6 grades x 1 ea.	315

Affinity

P/N	Description	Package Description	Price (\$)
21384	ToyoScreen AF-Chelate-650M, 1mL	1mL x 6 ea.	115
21385	ToyoScreen AF-Chelate-650M, 5mL	5mL x 6 ea.	617
21386	ToyoScreen AF-Blue HC-650M, 1mL	1mL x 6 ea.	115
21387	ToyoScreen AF-Blue HC-650M, 5mL	5mL x 6 ea.	617
21388	ToyoScreen AF-Red-650M, 1mL	1mL x 6 ea.	115
21389	ToyoScreen AF-Red-650M, 5mL	5mL x 6 ea.	617
21390	ToyoScreen AF-Heparin HC-650M, 1mL	1mL x 6 ea.	166
21391	ToyoScreen AF-Heparin HC-650M, 5mL	5mL x 6 ea.	485

Process Development Products

Accessories

P/N	Description	Package Description	Price (\$)
21400	Toyoscreen Column Holder		34
42194	Toyoscreen Column Holder with Fittings	includes 1 x 21400, 2 x 42196, 1 x 42195	61
42195	Column Coupler, 10-32, 0.03"ID SS Tubing		15
42196	Adapter, M6 interior to 10-32 exterior, PEEK		14
42197	Adapter, 1/4-28 interior to 10-32 exterior, PEEK		15

Process Development Products

Toyopearl and TSK-GEL LabPak Products for Resin Physical Property Evaluation

LabPak products are multi-milliliter containers of Toyopearl and TSK-GEL 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 I.D. and longer columns than available in the ToyoScreen products. This helps the developmental chemist or engineer to better measure under actual packing conditions the resin's:

- dynamic binding capacity
- selectivity
- column efficiency

Process Development Products

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Toyopearl LabPak Kits

Size Exclusion Chromatography

P/N	Description	Package Description	Price (\$)
19820	SECPAK HP (HW-40, 50, 55, 65S), 30 μ m	4 x 150mL	917
19821	SECPAK LMW (HW-40, 50, 55F), 45 μ m	3 x 150mL	324
19819	SECPAK HMW (HW-55, 65, 75F), 45 μ m	3 x 150mL	324

Ion Exchange Chromatography

P/N	Description	Package Description	Price (\$)
19817	IEXPAK HP (DEAE-650S, SP-650S, CM-650S, SuperQ-650S), 35 μ m	4 x 25mL	310
43210	AIEXPAK (DEAE-650M, SuperQ-650M, QAE-550C), 65/100 μ m	3 x 150mL	292
43220	CIEXPAK (SP-650M, CM-650M, SP-550C), 65/100 μ m	3 x 150mL	284

Hydrophobic Interaction Chromatography

P/N	Description	Package Description	Price (\$)
43150	HICPAK HP (Ether, Phenyl, Butyl-650S), 35 μ m	3 x 25mL	435
19806	HICPAK (Ether, Phenyl, Butyl-650M), 65 μ m	3 x 25mL	239
43125	HICPAK-C (Phenyl, Butyl, Hexyl-650C), 100 μ m	3 x 25mL	368

Affinity Chromatography

P/N	Description	Package Description	Price (\$)
43400	AFFIPAK ACT (AF-Epoxy, Tresyl-650M), 65 μ m	2 x 5g*	242
43410	AFFIPAK (AF-Amino, Carboxyl, Formyl-650 M), 65 μ m	3 x 10mL	242

*1g is approximately 3.5mL

Process Development Products

TSK-GEL LabPak Kits

Ion Exchange Chromatography

P/N	Description	Package Description	Price (\$)
43380	IEXPAK PW (20) (DEAE-5PW (20), SP-5PW (20), SuperQ-5PW (20))	3 x 25mL	693
43280	IEXPAK PW (DEAE-5PW, SP-5PW, SuperQ-5PW), 30µm	3 x 25mL	467

Hydrophobic Interaction Chromatography

P/N	Description	Package Description	Price (\$)
43278	HICPAK PW (20) (Ether-5PW (20), Phenyl-5PW (20))	2 x 25mL	552
43175	HICPAK PW (Ether-5PW, Phenyl-5PW), 30µm	2 x 25mL	340