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ToyoScreen®

#438

INTRODUCTION

Large scale commercial purifications begin at a small scale in methods development. In some cases the developers wish to use bulk resin to pack their initial small columns. By doing so, they experience how the resin handles physically and they can use this knowledge later during scale up as the column volumes become larger. In other cases the speed and convenience of having a pre-packed column for resin evaluation is needed. Tosoh Bioscience is pleased to offer pre-packed ToyoScreen Process Development Columns containing our popular TOYOPEARL® resins for evaluation. The ToyoScreen Series consist of small screening columns packed with TOYOPEARL, a packing material for semi-preparative and preparative liquid chromatography. These columns are suitable for evaluating different TOYOPEARL resins or for developing the purification conditions of biological target molecules such as proteins or nucleic acids. The ToyoScreen Series is available in two column volumes (1 mL and 5 mL formats).

HIGHLIGHTS

- Packed with TOYOPEARL hydrophobic interaction, ion exchange, mixed mode or affinity chemistries.
- Low cost, efficient alternative to self packing.
- Easy connections with ÄKTA®, FPLC and HPLC.
- Offered in mixed or single chemistry packages of 5 or 6

SCREENING

TOYOPEARL, for example, is available in four different particle sizes and three different pore sizes. So optimal selection of a particular resin could involve screening of several resins.

HIC - HYDROPHOBIC INTERACTION

Hydrophobic Interaction Chromatography (HIC) sorts biomolecules by degree of their surface hydrophobicity. Samples are adsorbed to the resin at relatively high salt concentrations and eluted with a decreasing salt gradient. The mild conditions used in HIC separation typically maintain protein structure and biologic activity. Separation can either be optimized by varying the mobile phase or by using different HIC packings. TOYOPEARL HIC media are available in six different chemistries ranging in hydrophobicity from Ether-650 (low) to Hexyl-650 (high), see Figure 1. Depending on the target feedstock and impurity profile, the determination of the best selectivity is an empirical process. Figure 2 shows the selectivity differences of the ToyoScreen HIC chemistries on the separation of protein standards and antibodies from albumin in mouse ascites fluid.

TOYOSCREEN SERIES PACKED WITH TOYOPEARL

TOYOPEARL	Particle size (µm)	IEX (eq/L-gel)	SBC (g/L-gel)
IEC type			
DEAE-650M	40 - 90	0.08 - 0.12	25 - 35[1)]
GigaCap Q-650M	50 - 100	0.10 - 0.20	≥ 162[1)]
SuperQ-650M	40 - 90	0.20 - 0.30	105 - 155[1)]
Q-600C AR	50 - 150	0.15 - 0.20	> 120[1)]
QAE-550C	50 - 150	0.28 - 0.38	60 - 80[1)]
GigaCap CM-650M	50 - 150	0.17 - 0.28	≥ 110[6)]
CM-650M	40 - 90	0.08 - 0.12	30 - 50[2)]
GigaCap S-650M	50 - 100	0.10 - 0.20	136 - 176[6)]
SP-650M	40 - 90	0.13 - 0.17	40 - 60[2)]
SP-550C	50 - 150	0.14 - 0.18	80 - 120[2)]
HIC type			
Ether-650M	40 - 90	-	10 - 30[2)]
Phenyl-650M	40 - 90	-	30 - 50[2)]
Phenyl-600M	40 - 90	-	45 - 65[2)]
Butyl-650M	40 - 90	-	30 - 50[2)]
Butyl-600M		-	40 - 60[2)]
Hexyl-650C	50 - 150	-	30 - 50[2)]
PPG-600M	40 - 90	-	20 - 35[3)]
SuperButyl-550C	50 - 150	-	52 - 70[2)]
MX-type			
MX-Trp-650M	50 - 100	-	> 75[6)]
AFC type			
AF-rProtein A-650F	30 - 60	-	> 45[6)]
AF-Chelate-650M	40 - 90	0.025 - 0.045	-
AF-Blue HC-650M	40 - 90	-	≥ 18[4)]
AF-RED-650M	40 - 90	-	2.5 - 4.5[4)]

Measured with [1)] Bovine serum albumin, [2)] Lysozyme, [3)] γ-Globulin, [4)] Human serum albumin, [5)] Antithrombin-III (Tosoh original method.), [6)] IgG

Table 1

HIC LIGAND CANDIDATES

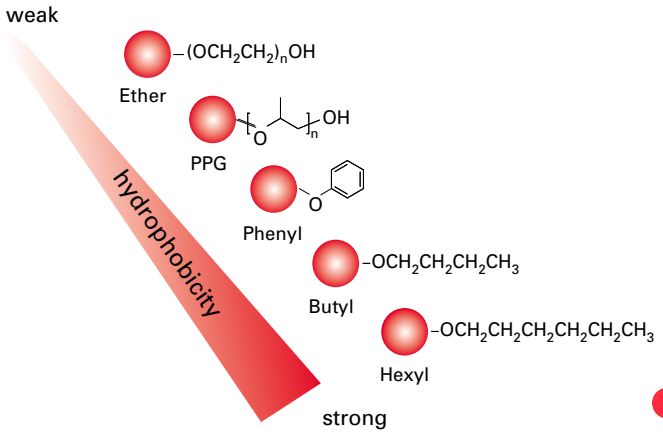
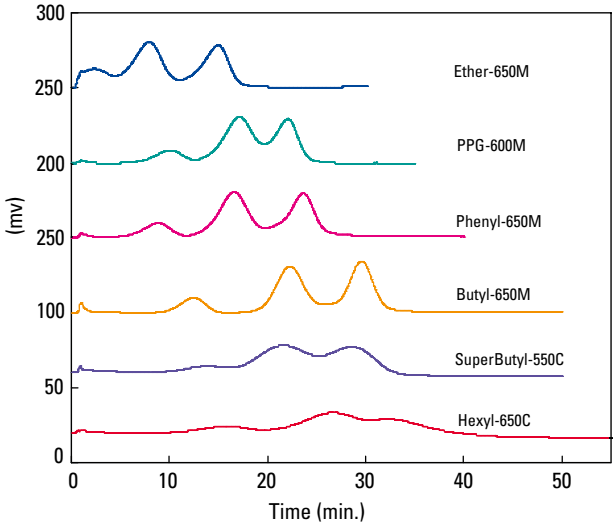


Figure 1

TOSOH

SCREENING OF TOYOPEARL HIC RESINS - STANDARD PROTEINS



SCREENING OF TOYOPEARL HIC RESINS - MOUSE ASCITES FLUID

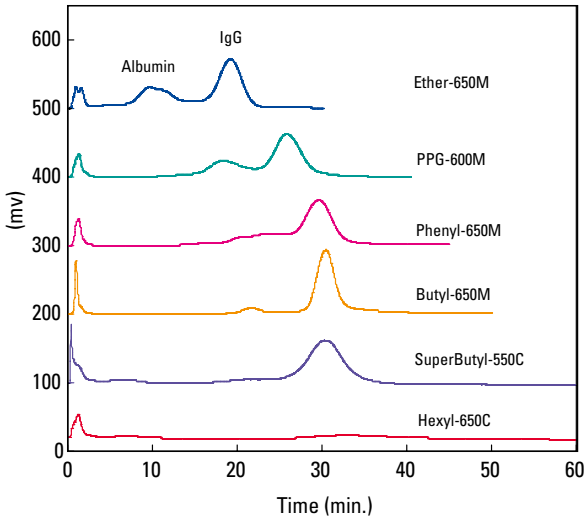


Figure 2 Column: ToyoScreen, 1 mL

Column: ToyoScreen, 1 mL; Mobile phase A: 0.1 mol/L phosphate buffer +1.8 mol/L sodium sulfate, pH 7.0 ; B: 0.1 mol/L phosphate buffer, pH 7.0; Gradient: 30 min linear; Flow Rate: 1 mL/min; Injection Vol.: 50 µL; Samples: Ribonuclease A, Lysozyme, γ-Chymotrypsinogen 1 mg/mL

Column: ToyoScreen, 1 mL; Mobile phase A: 0.1 mol/L phosphate buffer +1.8 mol/L sodium sulfate, pH 7.0; B: 0.1 mol/L phosphate buffer, pH 7.0; Gradient: 30 min linear; Flow Rate: 1 mL/min; Injection Vol.: 50 µL; Samples: Mouse Ascites Fluid:A:B=1:1:2

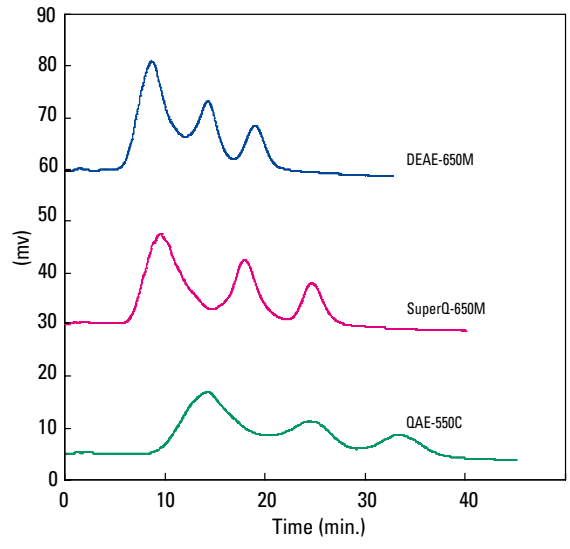
IEX - ION EXCHANGE

Ion exchange chromatography (IEX) separates molecules based on the ionic interaction of the molecule with the charged support. The net surface charge of proteins is dependent on the pH and ionic strength of the mobile ph
The development of optimum chromatographic conditions requires knowledge of both the protein's pI and the pKa of the ion exchange media. In biopurification IEC is used either in 'bind/elute mode' or in 'flow-through mode'. Ion exchange media should be selected according to the properties of the feedstock and the objective of the process step. Factors influencing the final choice are binding capaci
the resin, target scale and speed of the purification step.

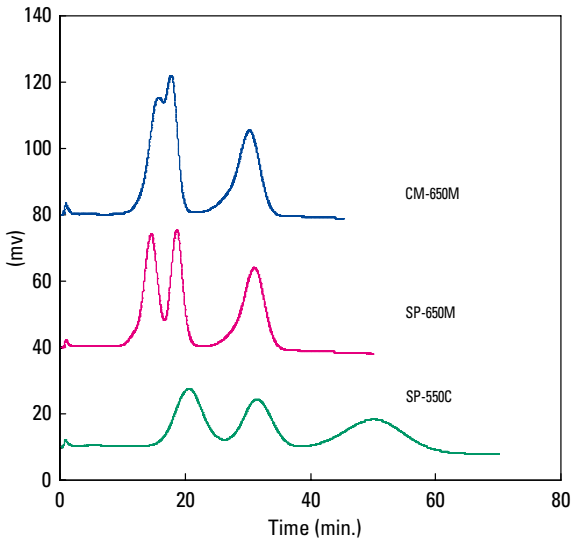
TOYOPEARL IEC resins are available in pore sizes of 1000 Å, 750 Å and 500 Å. TOYOPEARL-550 and -600 resins are designed for the purification of small to medium size proteins. The smaller pores result in increased surface area, thus offering more binding sites and high binding capac

ToyoScreen columns are offered in strong and weak functionalities for both c
tional groups comprise of sulfopropyl and carboxymethyl groups for cation exchange or quaternary ammonium or diethylaminoethyl groups for anion exchange resins, respectively.

SREENING OF TOYOPEARL ANION IEC RESINS - STANDARD PROTEIN



SCREENING OF TOYOPEARL CATION IEC RESINS - STANDARD PROTEINS



of **Screening of TOYOPEARL Cation IEC Resins Standard Proteins**

Figure 3

Column: ToyoScreen, 1 mL; Mobile phase A: 20 mmol/ ris-HCl, pH 8.0 Mobile phase B: 20 mmol/L Tris-HCl + 0.5 mol/L NaCl, pH 8.0; Gradient: B 0-->100% 60min linear; Flow Rate: 1 mL/min; Samples: Transferrin, Ovalbumin, Trypsin Inhibitor 1 mg/mL ea

Column: ToyoScreen, 1 mL; Mobile phase A: 20 mmol/L phosphate bu , pH 6.0; B: 20 mmol/L phosphate buffer + 0.5 mol/L NaCl, pH 6. 0-->100% 60min linear; Flow Rate: 1 mL/min; Samples: α-Chymotrypsinogen A, CytochromeC, Lysozyme 1 mg/mL each

The particle surfaces are modified either by traditional or network bonding chemistries. Network attachment chemistry improves the accessibility of the ligand groups. This significantly improves binding capacity and mass transfer. This technology is applied in TOYOPEARL GigaCap and Super Q resins. Figure 3 shows the separation of protein standards for some TOYOPEARL ion exchange chemistries.

MX – MIXED MODE

Mixed mode media combine ion exchange with hydrophobic interaction functionalities. They bind the target based on the hydrophobic interaction and elute the target when ionic interactions more precise electrostatic repulsion takes the lead. The multimodal cation exchanger TOYOPEARL MX-Trp-650M is salt tolerant and shows unique selectivity towards specific targets.

Since the ionic and hydrophobic properties of the ligand vary with salt concentration and pH, optimization of eluents for adsorption, washing and elution is crucial. ToyoScreen MX-Trp cartridges are ideally suited to determine proper conditions for this multimodal cation exchanger.

AFC - AFFINITY

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 four group specific ligand chemistries: AF-rProtein A-650F, AF-Blue HC-650M, AF-Chelate-650M, and AF-Red 650M.

AF-rProtein A-650F is used for the purification of monoclonal antibodies.

AF-Blue HC-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.

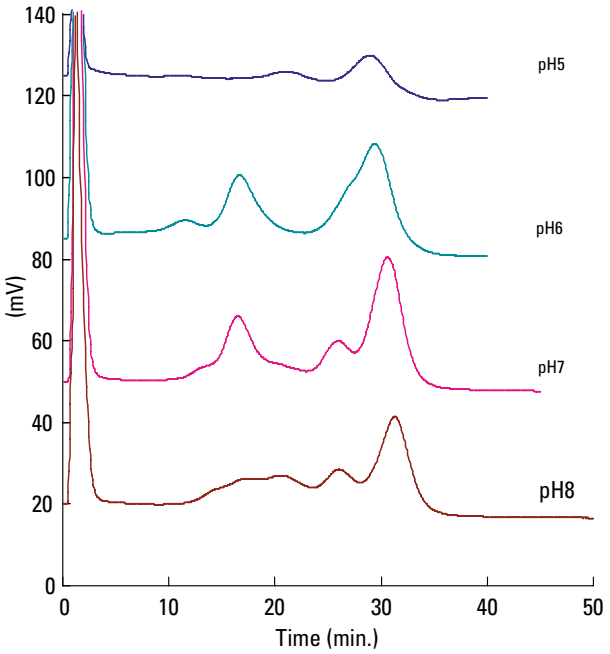
AF-Chelate-650M can be converted to either the $Ni^{++,}$ Ca^{++} or Zn^{++} form. When converted to the Ni^{++} form it is an excellent resin for metal ligand affinity for molecules containing His-tags.

ToyoScreen affinity columns allow for the quick assessment of optimum binding conditions for any of these columns.

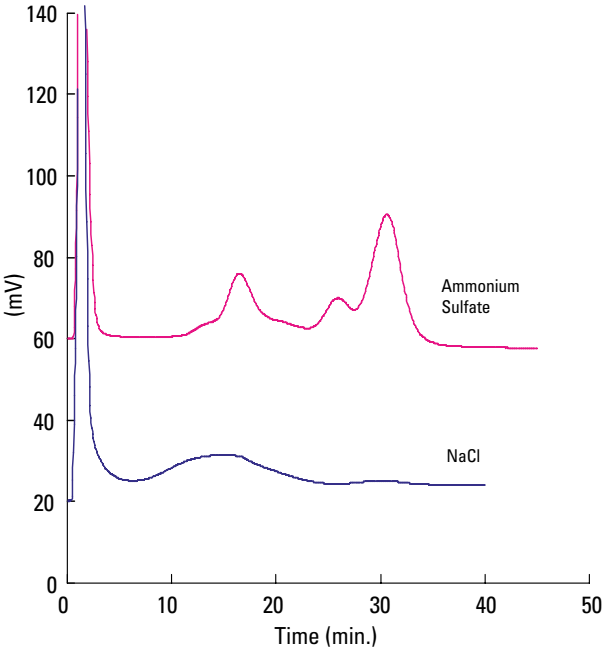
METHOD OPTIMIZATION

Beyond the determination of selectivity and capacity for the target molecules 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 Figure 4 for Anti-TSH in cell culture supernatant on ToyoScreen Phenyl-650M.

EFFECT OF ELUENT PH AND SALT TYPE ON SEPARATION OF CELL CULTURE SUPERNATANT



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



Figur

Column: ToyoScreen Phenyl-650M, 1 mL , Mobile phase A: 0.1 mol/L phosphate buffer containing 1.8 mol/L each salt, pH 7.0; B: 0.1 mol/L phosphate buffer, pH 7.0; Flow Rate: 1 mL/min Gradient: , 30 CV; Injection Vol.: 200 L; Samples: Cell culture supernatant (x 4 diluted) (antibody: Anti-TSH)

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SCALABILITY

Initial results from resin screening and optimization with ToyoScreen columns accurately predict the separation behavior at larger scales. Figure 5, illustrates the similar retention time behavior between 1 mL ToyoScreen columns and conventional 7.5 mm ID x 7.5 cm analytical columns. Additionally, Figure 6 depicts a practical antibody scale up in which conditions were set using a 1 mL ToyoScreen and applied to a 10 mL conventional column with a different inner diameter and length.

COMPARISON OF SELECTIVITY BETWEEN TOYOSCREEN AND CONVENTIONAL COLUMN

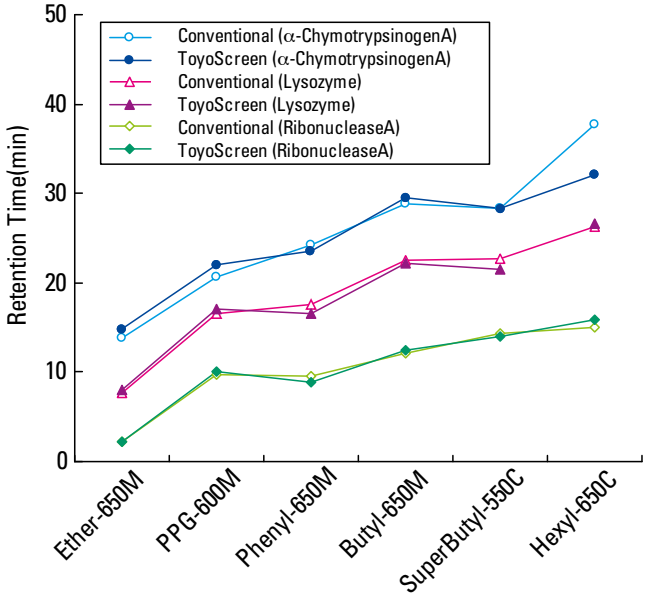

Figure 5

Columns: ToyoScreen (1 mL), Conventional column (7.5 mm ID x 7.5 cm)
Mobile phase A: 0.1 mol/L phosphate buffer + 1.8 mol/L sodium sulfate, pH 7.0; B: 0.1 mol/L phosphate buffer, pH 7.0; Gradient: 30 min linear, 30 CV
Flow Rate: 1 mL/min; Injection 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

COMPARISON OF CHROMATOGRAMS BETWEEN TOYOSCREEN AND CONVENTIONAL COLUMNS

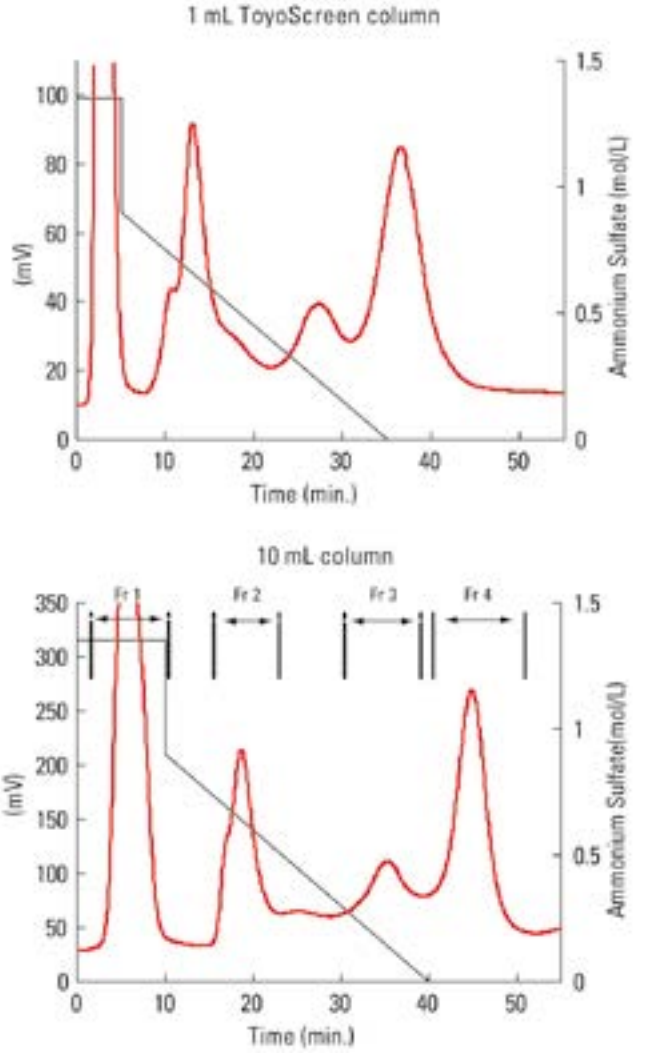

Figure 6

Packing: TOYOPEARL Phenyl-650M; Mobile phase: (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, pH 7.0; Sample: Anti-TSH from cell culture supernatant (x 4 diluted)

	1 mL ToyoScreen	10 mL column
Column:	6.4 mm ID x 3 cm L	14.6 mm ID x 6 cm L
Injection Volume:	500 µL	5,000 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)	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 mol/L/min	0.012 mol/L/min

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

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LABORATORY BENCHTOP PURIFICATIONS

Some ToyoScreen affinity columns can be used in simple one step laboratory purifications. This can result in either the isolation of a target molecule or the removal of an overly abundant impurity such as human serum albumin in blood.

REMOVAL OF HUMAN SERUM ALBUMIN

ToyoScreen AF-Blue HC-650M has a very high capacity for HSA as shown in Figure 7. It can be used to remove HSA or to purify albumin conjugated molecules.

COMPARISON OF HUMAN SERUM ALBUMIN BINDING CAPACITIES AT VARIOUS pHs OF AF-BLUE HC-650M AND AGAROSE (BLUE FUNCTIONALIZED AGAROSE) RESINS

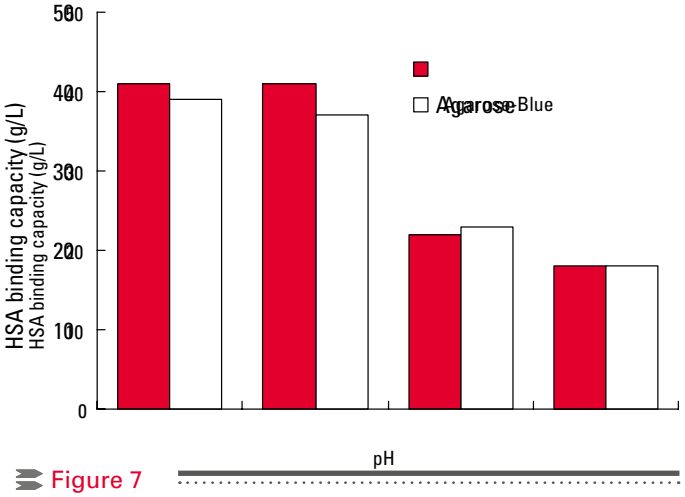

Figure 7

PURIFICATION OF HIS-TAG NusG FUSION PROTEIN

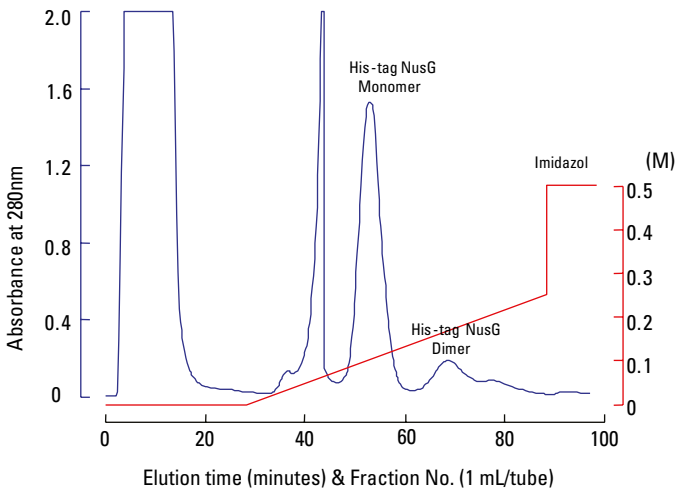

Figure 8

Column: ToyoScreen Chelate-650M (Ni-chelate), 5 mL

Column: ToyoScreen Chelate-650M (Ni-chelate), 5 mL; Starting Buff 20 mmol/L NaPi, pH 8.2, 0.01%NaN_3; Buffer B: 0.5 mol/L Imidazol, 20 mmol/L NaPi, pH 7.4, 0.01% Na_3; Flow rate: 1 mL/min, at RT, Gradient: 0-25 min=0% B, 25-85 min=0-50% B, 85-90 min=100%, 90
Sample: crude cell extract (5 mL, 10 mg, 20 mmol/L NaPi, pH 8.2, 0.01%NaN_3)

PURIFICATION OF HISTIDINE TAGGED (HIS-TAGGED) PROTEINS

A popular technique for bench scale purification of recombinant proteins is the expression of the protein with a poly-histidine tag and using a chelate column in the Ni^{++} form to selectively bind and elute the fusion protein. The histidine tag is subsequently cleaved from the protein for further work. As shown in Figure 8, ToyoScreen AF-Chelate-650M can be placed into the Ni^{++} form and used to purify his-tagged proteins.

MONOCLONAL ANTIBODY PURIFICATION

ToyoScreen AF-rProtein A-650F has a new recombinant protein A ligand attached to it as shown in Figure 9. It has a high binding capacity. It is very base stable and can be cleaned with 0.1 - 0.5 mol/L NaOH. Figure 10 shows the purification of a humanized IgG1 from a Chinese Hamster Ovary (CHO) cell lysate. ELISA tests prove that remaining host cell proteins and leached protein A ligand amounts were very low, resulting in a very high product purity.

TOYOPEARL AF-rPROTEIN A-650F LIGAND STRUCTURE

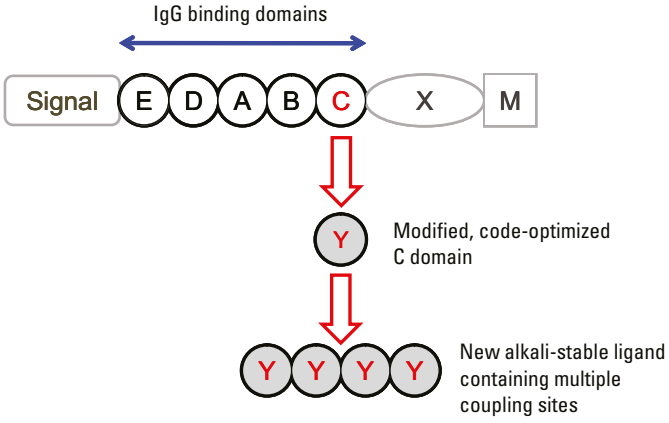

Figure 9

PURIFICATION OF HUMANIZED IgG1

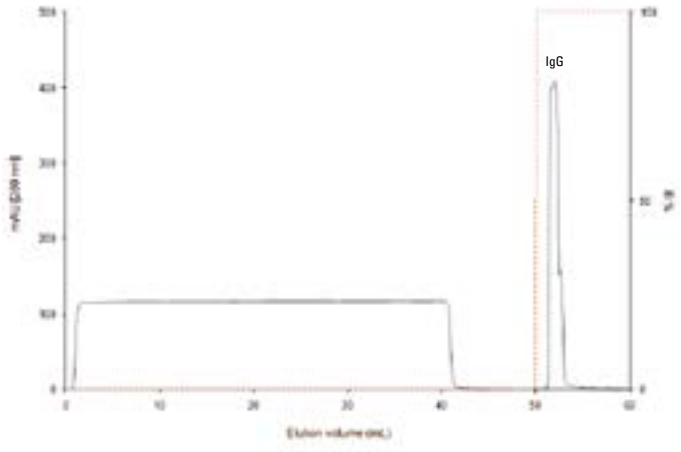

Figure 10

Column: 5 mm ID x 4.9 cm L; Flow rate: 0.5 mL/min (150 cm/h, 2 min residence time); Sample: 40 mL CHO cell culture lysate containing 0.5 mg / mL humanized IgG1; Binding buffer: 20 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.4; Elution buffer: 100 mmol/L glycine-HCl, pH 3.0

TOSOH

Ordering information

ToyoScreen®

Part-No	Description	Comment
ION EXCHANGE		
21360	ToyoScreen DEAE-650M, 1 mL	1 mL x 6 each
21361	ToyoScreen DEAE-650M, 5 mL	5 mL x 6 each
21362	ToyoScreen SuperQ-650M, 1 mL	1 mL x 6 each
21363	ToyoScreen SuperQ-650M, 5 mL	5 mL x 6 each
21364	ToyoScreen QAE-550C, 1 mL	1 mL x 6 each
21365	ToyoScreen QAE-550C, 5 mL	5 mL x 6 each
21859	ToyoScreen GigaCap Q-650M, 1 mL	1 mL x 6 each
21860	ToyoScreen GigaCap Q-650M, 5 mL	5 mL x 6 each
21366	ToyoScreen CM-650M, 1 mL	1 mL x 6 each
21367	ToyoScreen CM-650M, 5 mL	5 mL x 6 each
21368	ToyoScreen SP-650M, 1 mL	1 mL x 6 each
21369	ToyoScreen SP-650M, 5 mL	5 mL x 6 each
21370	ToyoScreen SP-550C, 1 mL	1 mL x 6 each
21371	ToyoScreen SP-550C, 5 mL	5 mL x 6 each
21868	ToyoScreen GigaCap S-650M, 1 mL	1 mL x 6 each
21869	ToyoScreen GigaCap S-650M, 5 mL	5 mL x 6 each
21870	ToyoScreen MegaCap II SP-550EC, 1 mL	1 mL x 6 each
21871	ToyoScreen MegaCap II SP-550EC, 5 mL	5 mL x 6 each
21992	ToyoScreen Q-600C AR	1 mL x 6 each
21923	ToyoScreen Q-600C AR	5 mL x 6 each
HYDROPHOBIC INTERACTION		
21372	ToyoScreen Ether-650M, 1 mL	1 mL x 6 each
21373	ToyoScreen Ether-650M, 5 mL	5 mL x 6 each
21374	ToyoScreen Phenyl-650M, 1 mL	1 mL x 6 each
21375	ToyoScreen Phenyl-650M, 5 mL	5 mL x 6 each
21376	ToyoScreen Butyl-650M, 1 mL	1 mL x 6 each
21377	ToyoScreen Butyl-650M, 5 mL	5 mL x 6 each
21378	ToyoScreen Hexyl-650C, 1 mL	1 mL x 6 each
21379	ToyoScreen Hexyl-650C, 5 mL	5 mL x 6 each
21380	ToyoScreen PPG-600M, 1 mL	1 mL x 6 each
21381	ToyoScreen PPG-600M, 5 mL	5 mL x 6 each
21892	ToyoScreen Phenyl-600M, 1 mL	1 mL x 6 each
21893	ToyoScreen Phenyl-600M, 5 mL	5 mL x 6 each
21494	ToyoScreen Butyl-600M, 1 mL	1 mL x 6 each
21495	ToyoScreen Butyl-600M, 5 mL	5 mL x 6 each
21382	ToyoScreen SuperButyl-550C, 1 mL	1 mL x 6 each
21383	ToyoScreen SuperButyl-550C, 5 mL	5 mL x 6 each
MIXED MODE		
22824	ToyoScreen MX-Trp-650M	1 mL x 6 each
22825	ToyoScreen MX-Trp-650M	5 mL x 6 each

Part-No	Description	Comment
AFFINITY		
21384	ToyoScreen AF-Chelate-650M, 1 mL	1 mL x 6 each
21385	ToyoScreen AF-Chelate-650M, 5 mL	5 mL x 6 each
21386	ToyoScreen AF-Blue HC-650M, 1 mL	1 mL x 6 each
21387	ToyoScreen AF-Blue HC-650M, 5 mL	5 mL x 6 each
21388	ToyoScreen AF-Red-650M, 1 mL	1 mL x 6 each
21389	ToyoScreen AF-Red-650M, 5 mL	5 mL x 6 each
22809	ToyoScreen AF-rProtein A-650F, 5 mL	1 mL x 5 each
22810	ToyoScreen AF-rProtein A-650F, 1 mL	5 mL x 1 each
22811	ToyoScreen AF-rProtein A-650F, 5 mL	5 mL x 5 each
21390	ToyoScreen AF-HeparinHC-650M	1 mL x 6 each
21391	ToyoScreen AF-HeparinHC-650M	5 mL x 6 each
MIX PACKS		
21392	ToyoScreen IEC Anion Mix Pack, 1 mL (DEAE-650M, SuperQ-650M, QAE-550C, GigaCap Q-650M, Q-600C AR)	1 mL x 5 Grades x 1 each
21393	ToyoScreen IEC Anion Mix Pack, 5 mL (DEAE-650M, SuperQ-650M, QAE-550C, GigaCap Q-650M, Q-600C AR)	5 mL x 5 Grades x 1 each
21394	ToyoScreen IEC Cation Mix Pack, 1 mL (CM-650M, SP-650M, SP-550C, , GigaCap CM-650M, GigaCap S-650M)	1 mL x 5 Grades x 1 each
21395	ToyoScreen IEC Cation Mix Pack, 5 mL (CM-650M, SP-650M, SP-550C, GigaCap CM-650M, GigaCap S-650M)	5 mL x 5 Grades x 1 each
21396	ToyoScreen IEC Mix Pack, 1 mL (GigaCap Q-650M, SuperQ-650M, Q-600 AR, GigaCap CM-650M, GigaCap S-650M, SP-550C)	1 mL x 6 Grades x 1 each
21397	ToyoScreen IEC Mix Pack, 5 mL (GigaCap Q-650M, SuperQ-650M, Q-600 AR, GigaCap CM-650M, GigaCap S-650M, SP-550C)	5 mL x 6 Grades x 1 each
21398	ToyoScreen HIC Mix Pack, 1 mL (PPG-600M, Phenyl-600M, Phenyl-650M Butyl-600M, Butyl-650M, Hexyl-650C)	1 mL x 6 Grades x 1 each
21399	ToyoScreen HIC Mix Pack, 5 mL (PPG-600M, Phenyl-600M, Phenyl-650M, Butyl-600M, Butyl-650M, Hexyl-650C)	5 mL x 6 Grades x 1 each
TOYOSCREEN COLUMN ACCESSORIES		
21400	ToyoScreen Column Holder	

Please note that all of the ToyoScreen packages shown above require a ToyoScreen column holder either PN 21400 for operation.

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