



TOYOPEARL® PPG-600M Toyopearl Polypropylenglycol-600

The newly developed Toyopearl PPG 600 is a resin for Hydrophobic Interaction Chromatography especially of

- monoclonal antibodies (Mab's)
- hydrophobic proteins
- pegylated (polyethyleneglycol modified) targets

Toyopearl PPG-600M is functionalized with a novel polyether phase.

Toyopearl is a methacrylic polymer incorporating high mechanical stability. Resins are available as non-functionalized resins for SEC or derivatised with surface chemistries for alternative modes of chromatography such as IEC, HIC and AFC.

Product Highlights

- Pore size optimized for monoclonal antibody separations
- High dynamic binding capacity with a typically greater mass recovery than 95%
- Retention of high biological activity
- Excellent selectivity between albumin and monoclonals

Applications for Toyopearl PPG-600:

Toyopearl PPG-600 with a pore size of 750 Å is optimized for monoclonal antibody separations and is ideal to be used as intermediate stage of antibody manufacturing.

For monoclonal antibody purification, the main prerequisite is a high dynamic binding capacity. The new Toyopearl PPG-600 shows a higher dynamic binding capacity compared to Toyopearl Phenyl-650M for IgG (see Figure 1).

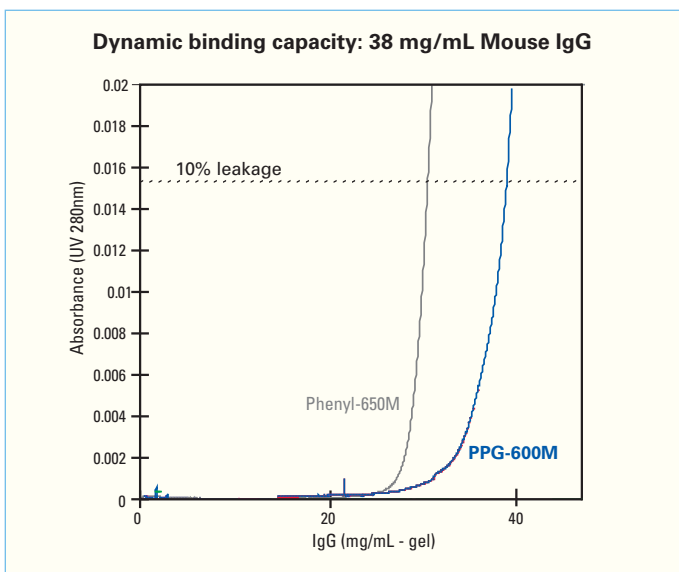


Figure 1: Dynamic Binding Capacity of IgG

Column Size : 7.8 mm ID X 20 cm L
 Linear Velocity : 300 cm/h
 Feed : MAb IgG (Anti-LH) 1 mg/mL
 in 0.1 M Phosphate Buffer + 0.8 M Ammonium Sulfate (pH 7.0)

However, not only the high binding capacity is significant also the efficient recovery for IgG and the excellent selectivity in removing impurities from antibodies of ascites samples. Figure 2 shows the complete separation of albumin and IgG from mouse ascites.

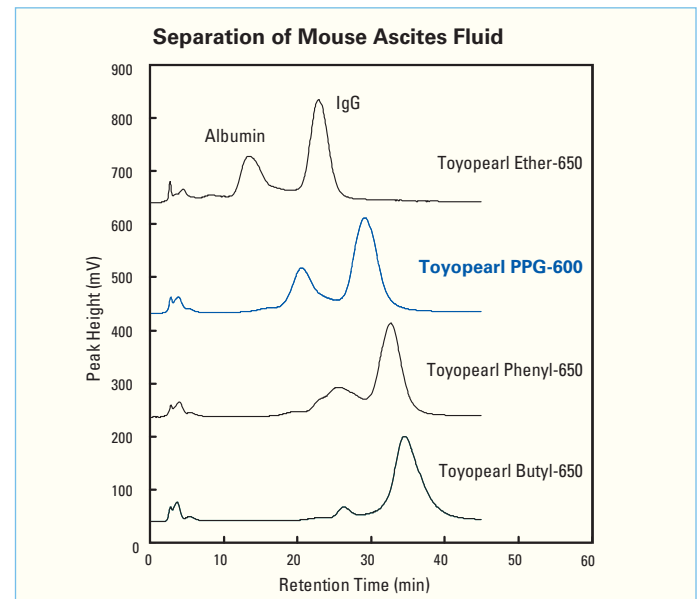


Figure 2: Separation Conditions of Toyopearl HIC Media

Column Size: 7.5 mm ID x 7.5 cm L
 Eluent: (A) 0.1 M phosphate buffer containing 1.8 M ammonium sulfate (pH 7.0)
 (B) 0.1 M phosphate buffer (pH 7.0)
 Gradient: 0% B - 100% B in 30 min.
 Flow Rate: 1 mL/min
 Injection Vol.: 100 µL

Product	Dynamic Binding Capacity (mg/ml-Gel, 10% Leakage)	Eluted amount (mg/ml-Gel)	Recovery (%)
PPG-600M	38	39	100
Phenyl-650M	30	29	95

The polyether ligand results in higher hydrophobicity of the resin than Toyopearl Ether-650 and less hydrophobicity than Toyopearl Phenyl-650 (see Figure 3).

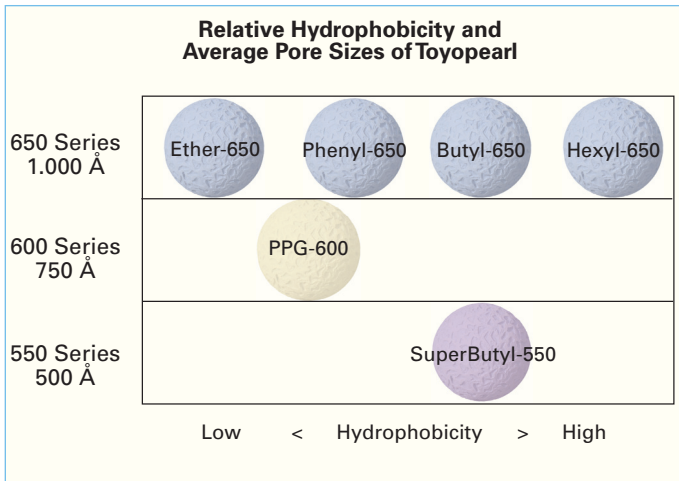


Figure 3: Product Portfolio of Toyopearl HIC Resins

With the new Toyopearl PPG-600 resin Tosoh Bioscience now offers a comprehensive product portfolio regarding different grades of hydrophobicity.

As demonstrated in figure 4, Toyopearl PPG-600 shows a narrow elution peak of IgG, so the sample is more pure and concentrated.

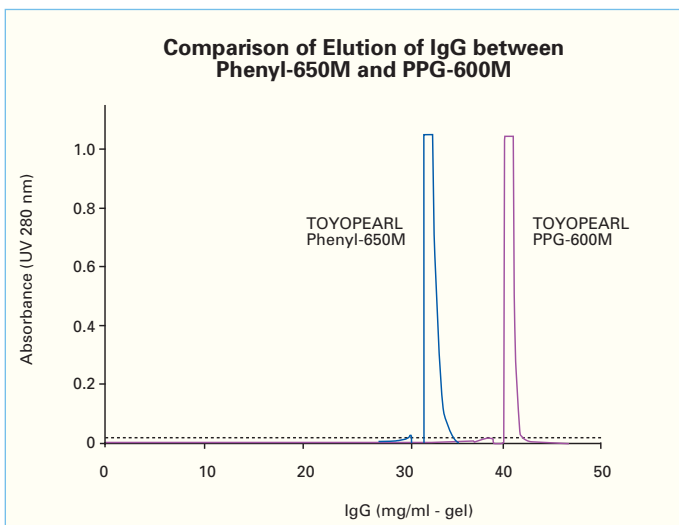


Figure 4: Elution of IgG on Toyopearl Phenyl-650M vs. Toyopearl PPG-600M

Column Size: 7.8 mm ID x 20 cm L
 Flow Rate: 2.39 ml/min (linear velocity: 300 cm/h)
 Feed: MAb IgG (Anti-LH) 1 mg/ml in 0.1 M phosphate buffer containing 0.8 M ammonium sulfate (pH 7.0)
 Wash: 0.1 M phosphate buffer containing 1.1 M ammonium sulfate (pH 7.0)
 Elute: 0.1 M phosphate buffer (pH 7.0)
 Feed until 10% Leak => Wash 6 min => Elute

In addition to the monoclonal applications, the Toyopearl PPG-600 polyether ligand also excels with hydrophobic proteins. Certain pegylated molecules also may take advantage of its unique selectivity.

Characteristics:

Excl. limit MW:	approx. 8×10^5 Dalton
Ligand:	Polypropylenglycol PPG
Particle size:	M grade (45-90 μ m) for intermediate process
Hydrophobicity:	Ether > PPG > Phenyl
Dynamic Binding capacity (MAb: Anti LH):	38mg/mL-gel

Summary:

Now PPG-600 enhances the range of the HIC-products from Tosoh Bioscience. By offering six different ligands and pore size combinations a large range of selectivities is given for customer evaluation.

- High binding capacity for Mab's
- Efficient recovery
- Removing impurities

For immediate start or screening, please request our new ToyoScreen kit for PPG-600 (6 x 1ml or 6 x 5ml) or the complete ToyoScreen HIC kit (materials included as in Figure 3, available in 6 x 1ml and 6 x 5ml).

For further details of choice and selection of the Toyopearl® resin that best suits your particular process purification needs, please contact us:

Tel. + 49 (0) 711 13257 0

or

info.sep.eu@tosoh.com

or

www.toyopearl.com

TOYOPEARL® SuperButyl-550C

Toyopearl is a methacrylic polymer incorporating high mechanical stability. Resins are available as non-functionalized "HW-" series resins for SEC or derivatised with surface chemistries for alternative modes of chromatography such as IEC, HIC and AFC.

The newly developed Toyopearl SuperButyl-550C HIC resin incorporates higher binding capacity for low molecular weight proteins (MW < 30 KDa), high recovery and low pressure drop.

Usage of Toyopearl SuperButyl-550C

The molecular weight of a wide variety of proteins is in the range of 12-36 KDa. Very small molecules like insulin and larger proteins such as antibodies are less common.

Insulin can be purified by RP chromatography. Nevertheless, this mode is less appropriate for proteins, due to the risk of denaturation. In such cases milder hydrophobic interaction chromatography (HIC) is preferable, with which insulin can also be successfully purified.

Dynamic Binding Capacity as a Function of Pore Size:

Resin	Mean Pore Size [nm]	Mean Dynamic Binding Capacity for Lysozyme [mg/ml]
Toyopearl Butyl-650C	100	40
Toyopearl SuperButyl-550C	50	61

Table 1

Mass Recovery (% of proteins)

Protein	Toyopearl SuperButyl 550C	Agarose Based Butyl	Agarose Based Phenyl (highly subst.)
Ribonuclease A	98	98	106
Lysozyme	93	94	90
Chymotrypsinogen	92	86	96
Bovine Serum Albumin	86	82	51

Table 2

Loading: 0.1 M phosphate buffer / 1.8 M ammonium sulfate (pH 7)

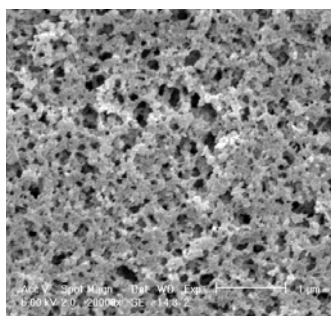
Sample: 1 mg/ml protein in loading buffer

After column saturation the unbound protein was removed with the loading buffer without salt.

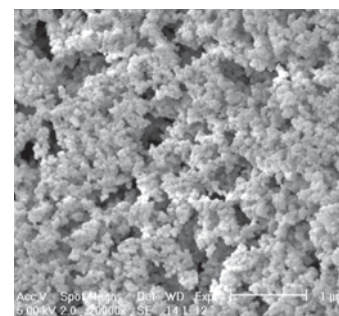
The protein elutes in 0.1 M phosphate (pH 7)

See below 50 and 100 nm pore sizes under the TEM with 20.000 fold magnification

Toyopearl with 50 nm pore size



Toyopearl with 100 nm pore size



For smaller proteins the mass transfer is fast, and resins with smaller pores (e.g. 50 nm) can be used. Such resins provide a large internal surface area and this results in a high protein binding capacity.

The recovery for different proteins is comparable to competitive resins. Examination of BSA recovery shows that Toyopearl SuperButyl-550C performs better than all other resins (Table 2).

Toyopearl SuperButyl-550C is a coarse grade resin with a particle size range between 50-150 µm, and is well suited to capture steps. The pressure drop of a packed bed remains low over a wide flow-rate range. Pressure is lower than competitive HIC resins in aqueous mobile phase (Figure 1).

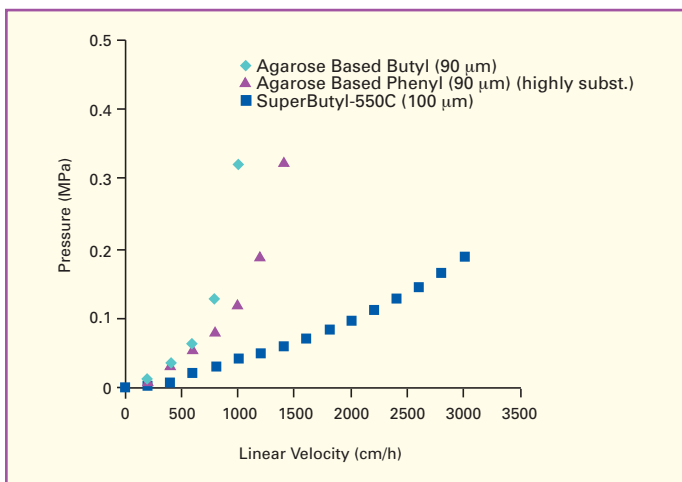


Figure 1

Fast mass transfer action is desired during the capture step. Dynamic binding capacity of four different resins is plotted in Figure 2. Toyopearl Butyl-650C and the agarose based Butyl behave very similarly, whereas Toyopearl SuperButyl-550C compares with the agarose based Phenyl offering high dynamic capacity. Toyopearl SuperButyl-550C however shows higher overall capacity. The resolving power of the new Toyopearl SuperButyl-550C resin was compared to other Toyopearl HIC resins with 100 µm particle size (Figure 3).

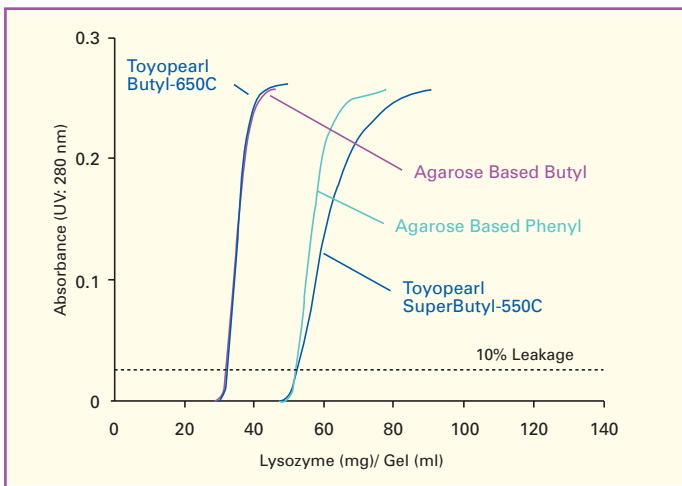


Figure 2

Conditions for binding capacity tests:

Loading: 1 mg/ml lysozyme in 0.1 M phosphate buffer/1.8 M ammonium sulfate (pH 7)
 Flow rate: 300 cm/hour

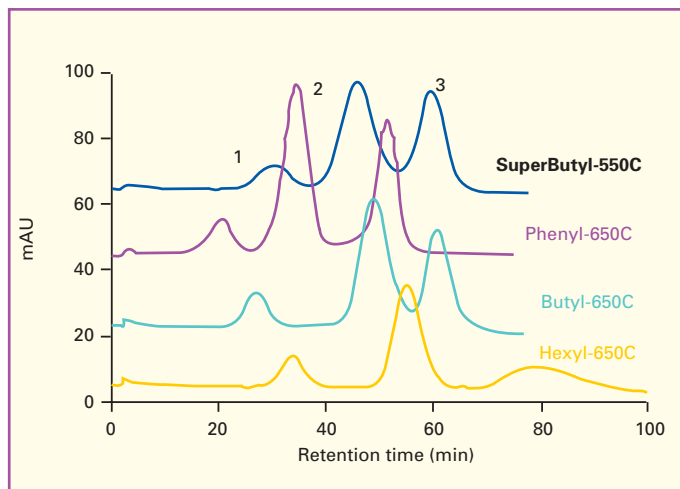


Figure 3

Conditions:

Column: 7.5 mm ID X 7.5 cm length
 Eluent A: 0.1 M phosphate buffer / 1.8 M ammonium sulfate, pH 7
 Eluent B: 0.1 M phosphate buffer, pH 7
 Linear gradient from A to B in 60 min
 Flow rate: 1 ml/min (136 cm/hour)
 UV-Monitor: 280 nm
 Sample: 1. Ribonuclease A
 2. Lysozyme
 3. a-Chymotrypsinogen A (1mg/ml each)
 Injection vol.: 100 µl

Summary:

- Toyopearl SuperButyl-550C offers a high dynamic binding capacity for smaller proteins.
- The coarse grade resin generates low pressures even at high linear flow rates.

**For further details
of choice and selection of
the Toyopearl® resin
that best
suits your particular
process purification needs,
please contact us:**

**Tel. + 49 (0) 711 13257 0
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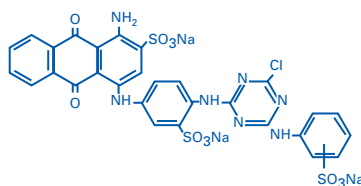
TOYOPEARL® AF-BlueHC-650M

Toyopearl is a methacrylic polymer incorporating high mechanical stability. Resins are available as non-functionalized "HW" series resins for SEC or derivatised with surface chemistries for alternative modes of chromatography such as IEC, HIC and AFC.

Toyopearl AF-Blue is functionalized with Cibacron Blue F3GA, useful in the purification of NAD/NADP dependant enzymes and blood components, like Interferon, Coagulation factors and Albumin (Table 1).

Advantages: Toyopearl AF-BlueHC provides enhanced protein binding capacity and reduced dye leakage for preparative applications. The original Toyopearl AF-Blue product remains available in support of existing processes.

Formula of Cibacron Blue F3GA:



Applications for Affinity Blue Resin:

The ligand attached is Cibacron Blue F3GA. This is a monochlorinated triazine dye, which is immobilised by an elimination reaction involving the chlorine atom attached to the triazine ring and hydrogen atoms at the resin surface.

Some proteins bind biospecifically with this dye ligand due to its structural similarity to cofactors such as NAD/NADP, while others, such as albumin and interferon bind in a less specific manner through ionic and/or hydrophobic interactions.

Biospecifically adsorbed proteins may be eluted by low concentrations of free cofactor (1-20mM). Less specifically adsorbed molecules require the use of higher concentrations of cofactor (> 50 mM), salt (Table 2) or a pH-step.

Regeneration of the gel is achieved using alternate low and high pH (e.g. pH 5 followed by pH 11) or by using high salt (2M KCl or 3M NaCl). Tightly bound lipids can be removed with 4M Urea.

Table 2

Solvents recommended for elution and regeneration of Toyopearl® AF-Blue HC-650M

Solvent	Concentration
Potassium chloride	2 M
Sodium chloride	3 M
Urea	4 M
Ammonium sulfate (saturated)	4,2 M
Sodium thiocyanate	1 M
Sodium hydroxide	0,1 N
Triton X-100	1 %
Ethylene Glycol	75 %
Chloroform / Methanol	50/50%

Table 1

Commercial Processes using the Blue ligand

<i>Protein Source</i>	<i>Lactoferrin</i> Human Whey
<i>Binding buffer</i>	5 mM Na-barbital/HCl, pH 7.4; 50 mM NaCl
<i>Eluent</i>	0.05 - 1M NaCl /linear gradient)
<i>Protein Source</i>	<i>Albumin</i> Human Serum
<i>Binding buffer</i>	50 mM Tris-HCl, pH 8.0, 50 mM NaCl
<i>Eluent</i>	0.2M NaSCN
<i>Protein Source</i>	<i>Interferon</i> Human Fibroblasts or Leucocytes
<i>Binding buffer</i>	20mM Sodium phosphate, pH 7.4
<i>Eluent</i>	0-50 % (v) ethylene glycol or 0,15-1,0 m NaCl
<i>Protein Source</i>	<i>Lactate dehydrogenase</i> Rabbit muscle
<i>Binding buffer</i>	50mM Phosphate, pH 7.0
<i>Eluent</i>	1mM NAD ⁺ and 2mM Na ₂ SO ₃

(Protein Liquid Chromatography. Kastner, 1999 Elsevier)

Caustic Stability:

The caustic stability of AF-Blue HC-650M was tested in 0.1 N and 0.5 N NaOH solutions at 25°C.

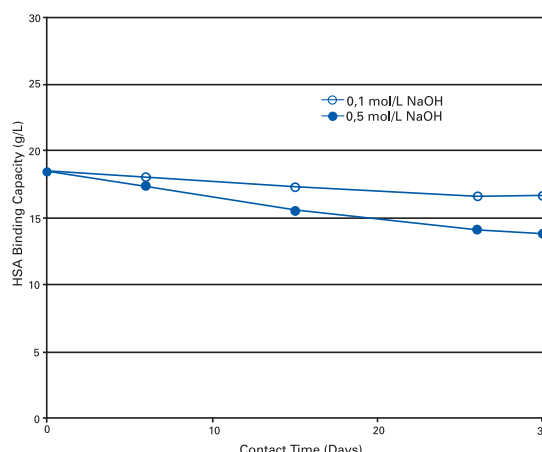


Figure 1

1 ml of adsorbent was suspended with 5 ml of each solution. Stability was determined by measuring HSA equilibrium binding capacity.

Toyopearl AF-BlueHC-650M withstands exposure to 0.1N NaOH for 30 days. When contamination is severe, 0.5 N NaOH may be used for short exposure times. Longer contact times decrease binding capacity for HSA.

Ligand Leakage:

The dye can be measured spectrophotometrically at 620 nm. It also strongly adsorbs at UV280. Toxicity investigations for the dye in vitro with eukaryotic and prokaryotic cells demonstrated zero or slight toxicity only [Mohammad, J. (1995), J. Chrom. 510, 155-164]

Competitive leakage study

Conditions:

200 mg of each resin was suspended in 4 ml of solvent (Table 3) and incubated at RT on a shaker for 24h. Absorption of the supernatant at 620 nm was measured after adjustment to neutrality. Dye concentration was estimated assuming a molar extinction coefficient of 12.750 (L/M cm⁻¹).

Thermal Stability:

The resin can be autoclaved at 121°C for 20 min.

Binding Capacity:

The binding capacity for HSA is > 18 mg/ml swollen gel and is dependant from pH (Figure 2) and from linear flowrate (Figure 3).

Packing

Table 3

Solvent	Concentration of Cibacron Blue F3GA (µmol/l)	
	Toyopearl AF-Blue HC-650M (TOSOH)	Affinity Blue Resin Agarose based resin
0,1 N NaOH	1,1	1,9
0,5 N NaOH	3,1	11,7
0,1 M HCl	4,8	6,7
0,5 M HCl	4,1	11,5

Please refer to the brochure "Toyopearl Packing Instructions and Use".

HSA Capacity vs. pH

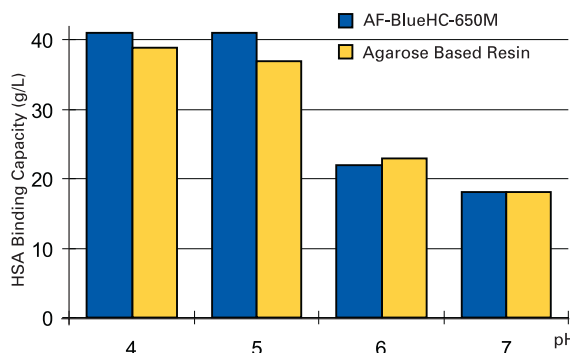


Figure 2

Ligand Leakage

HSA Capacity vs Flow

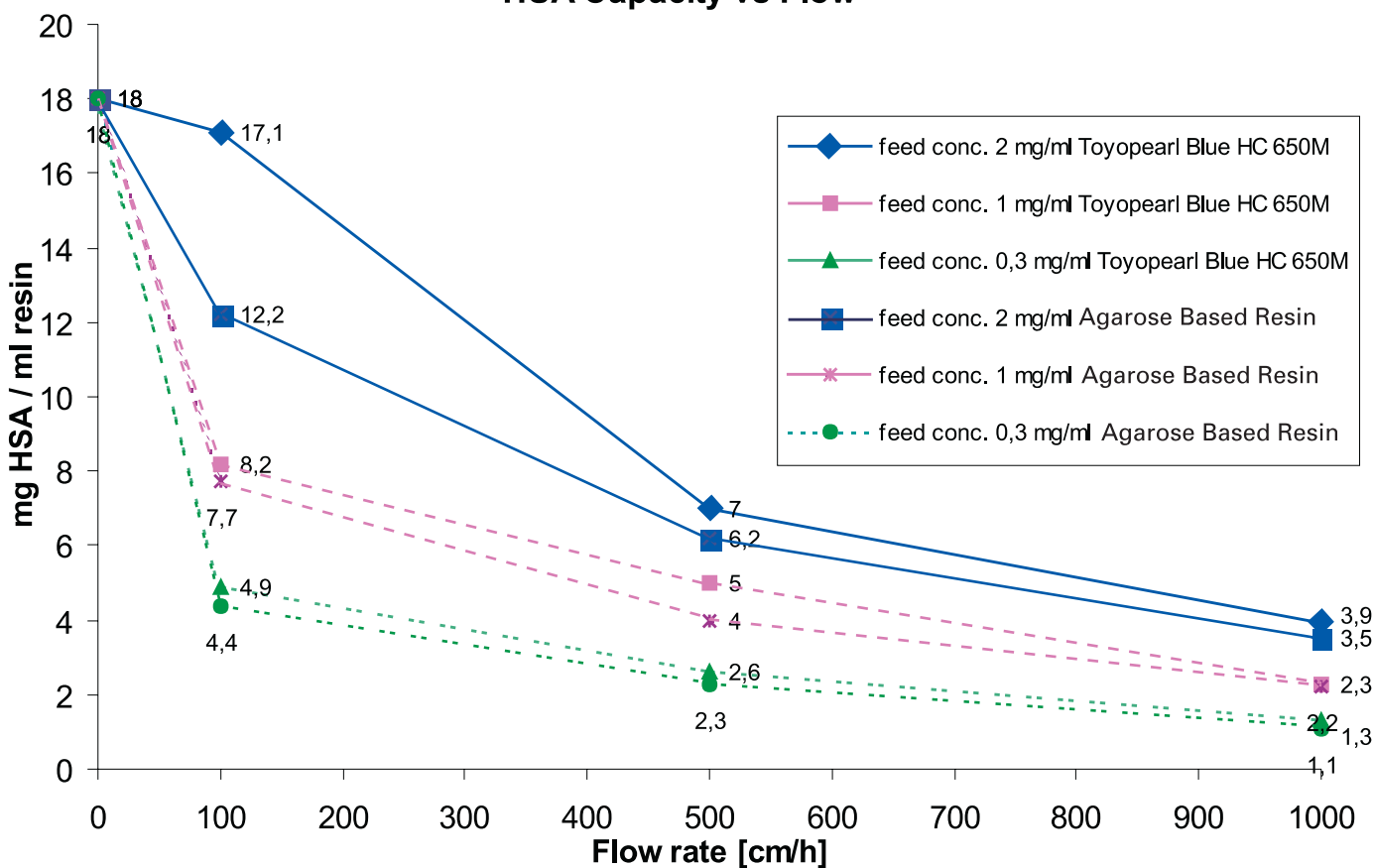


Figure 3



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TOYOPEARL® Butyl-600M

The newly developed Toyopearl Butyl-600M is a resin for Hydrophobic Interaction Chromatography

especially designed for:

- monoclonal antibodies (mAb's)
- hydrophobic proteins
- high recovery

TOYOPEARL is a methacrylic polymer incorporating high mechanical stability. Resins are available as non-functionalized resins for SEC or derivatised with surface chemistries for alternative modes of chromatography such as IEC, HIC and AFC.

Product highlights

- Optimized pore size for monoclonal antibody separations
- High dynamic binding capacity with a typically greater mass recovery of more than 90% for mAb's
- Retention of high biological activity
- Excellent selectivity between monoclonals and proteins

The HIC portfolio

The Tosoh Bioscience HIC resin portfolio has been enhanced by the Toyopearl PPG-600M and Butyl-600M intermediate hydrophobic resins, which complement the whole HIC resin range of products (see Figure 1). Both resins incorporate a pore size which is especially designed for the mAb purification process.

Butyl-600M exhibits extended capacity and better hydrophobic selectivity compared to the already well-known HIC resins from Tosoh Bioscience.

The new Butyl-600M and the PPG-600M resins both provide prominent alternatives to Affinity Chromatography for antibody purification.

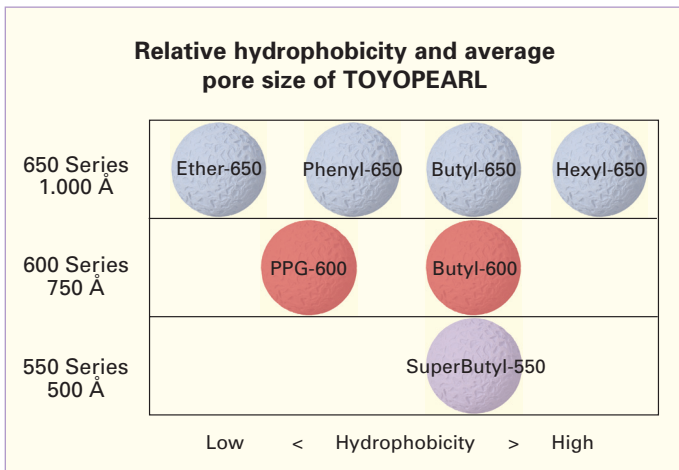


Figure 1 Product portfolio of TOYOPEARL HIC resins

Applications for Toyopearl Butyl-600M:

Butyl-600M exhibits excellent separation of IgG and alpha-fetoprotein (AFP), contained in fetal calf serum culture medium. As can be seen in Figure 2, the separation shows one peak for Phenyl-650M, using Butyl-600M selectivity is enhanced and the two substances elute separately.

The high dynamic binding capacity of Butyl-600M is shown in Figure 3. In comparison to Phenyl-650M and PPG-600M, Butyl-600M has the highest dynamic binding capacity of Anti Lutening Hormone (Mouse LH).

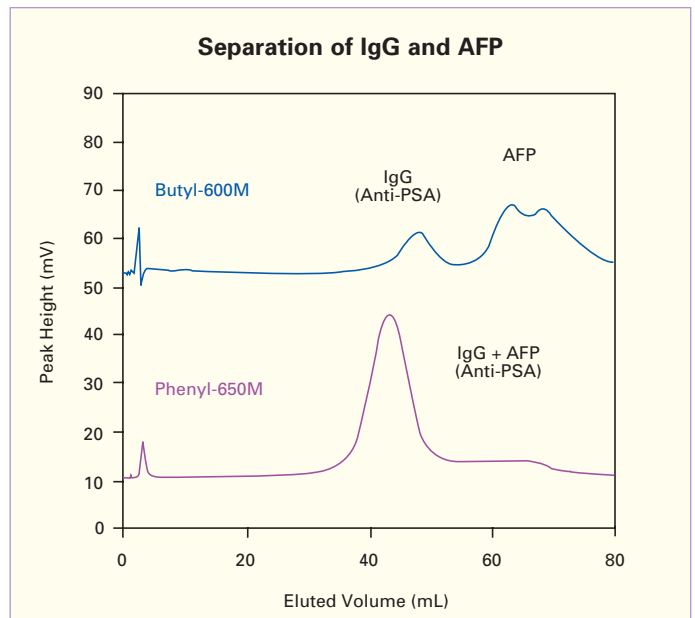


Figure 2

Column size : 7.5 mm ID x 7.5 cm L
 Eluent : (A) phosphate buffer + 1.0 mol/L ammonium sulfate (pH 7.4)
 (B) phosphate buffer (pH 7.4)
 Flow Rate : 1 mL/min
 Gradient : (B) 0% (5 min) - 100% (65 min) linear
 Sample : IgG Anti-PSA (prostate specific antigene) + AFP (alpha-fetoprotein) in 1.0 mol/L ammonium sulfate
 Injection Vol. : 200 µL
 Detection: UV @ 280 nm

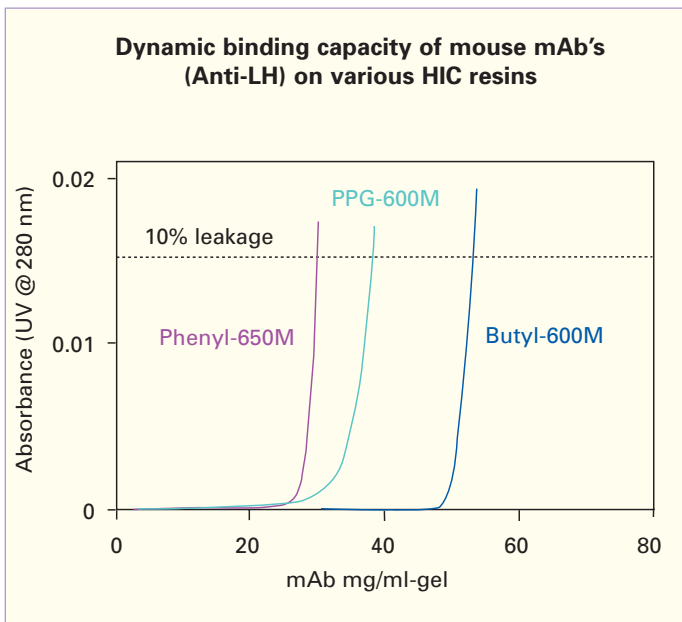


Figure 3

Column size : 7.8 mm ID x 20 cm L
 Feed : Mouse monoclonal antibody (Anti-LH)
 1 mg/mL in 0.1 mol/L phosphate buffer
 + 0.8 mol/L ammonium sulfate (pH 7.0)
 Linear velocity : 300 cm/h
 Temperature : 25 °C
 Detection: UV @ 280 nm

Table 1: Dynamic binding capacity

Product	Binding capacity, 10% leakage (mg/mL-gel)	Recovered capacity (mg/mL-gel)	Recovery (%)
Butyl-600M	54	49	91
PPG-600M	38	39	100
Phenyl-650M	30	29	95

The different hydrophobicities in comparison to Butyl-600M and PPG-600M are compared in Figure 4. The peaks of the separation of IgG and Albumin in cell culture supernatant show an inverse retention.

This inverse retention offers the choice to optimize your purification strategy.

Summary

The new Butyl-600M provides you with:

- High binding capacity for mAb's
- Efficient recovery
- Removing impurities

For further details
 of choice and selection of
 the TOYOPEARL[®] resin
 that best
 suits your particular
 process purification needs,
 please contact us:

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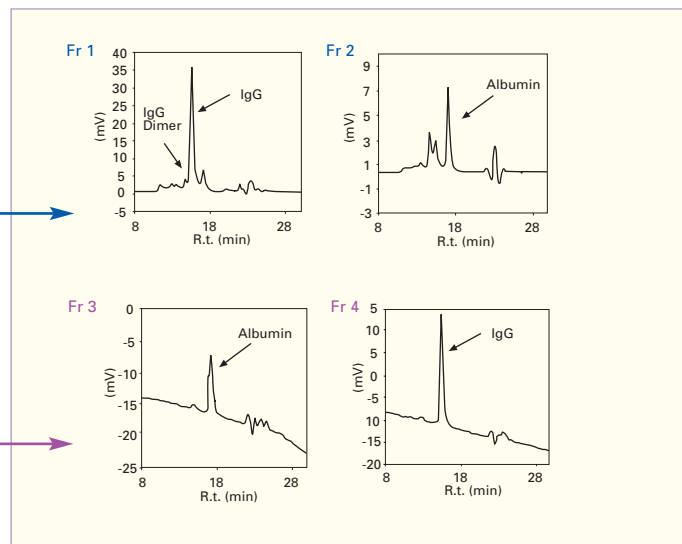
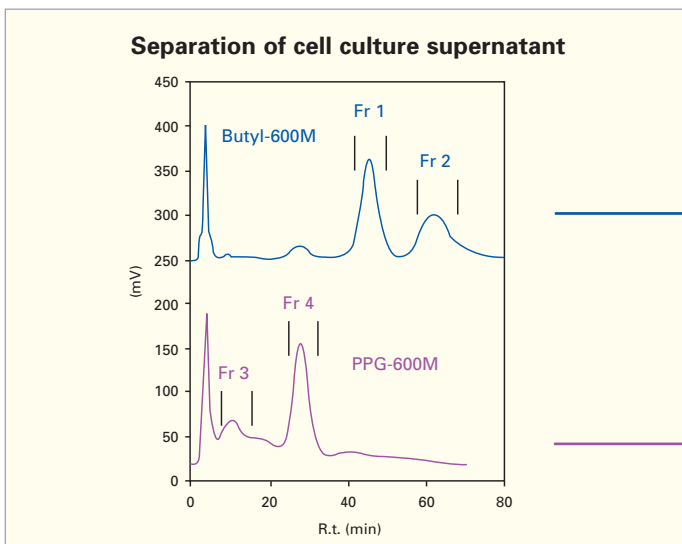


Figure 4

Separation on HIC resins

Column size: 7.5 mm ID x 7.5 cm L
 Eluent : (A) 0.1 mol/L phosphate buffer + 1.0 mol/L ammonium sulfate (pH 7.0)
 (B) 0.1 mol/L phosphate buffer (pH 7.0)
 Flow rate : 1 mL/min
 Gradient : 0% B - 100% B in 60 min linear
 Sample : Cell culture supernatant
 (x 2 diluted with 1.8 mol/L ammonium sulfate)
 Injection Vol. : 100 µL
 Detection: UV @ 280 nm

SEC assay of collected fractions

Column : TSKgel BioAssist G3SWXL, 7.8 mm ID x 30 cm L
 Eluent : 0.3 mol/L NaCl in 50 mmol/L phosphate buffer (pH 7.0)
 Flow rate : 1.0 mL/min

TOYOPEARL® Phenyl-600M

The newly developed Toyopearl Phenyl-600M is a resin for Hydrophobic Interaction Chromatography especially designed for:

- ❖ high dynamic binding capacity
- ❖ high protein load
- ❖ high antibody recovery

TOYOPEARL is a methacrylic polymer incorporating high mechanical stability. Resins are available as non-functionalized resins for SEC or derivatised with surface chemistries for alternative modes of chromatography such as IEC, HIC and AFC.

Toyopearl Phenyl-600M Highlights:

- ❖ HIC resin with high density of phenyl ligands
- ❖ High dynamic binding capacities (DBC) for both large and small proteins
- ❖ Optimized pore size of 750 Å for antibody separation
- ❖ Antibody recoveries greater than 90%
- ❖ Polymethacrylate based resin with excellent flow characteristics

Hydrophobic Interaction Chromatography (HIC) is a widely-used technique for the separation and purification of proteins and peptides. The mild conditions used in HIC typically maintain protein structure and biologic activity.

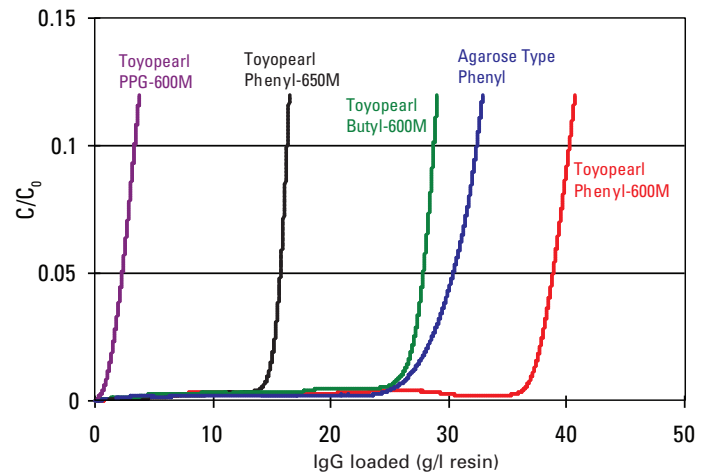
Tosoh Bioscience offers a broad range of HIC resins with different ligands and pore sizes to increase the probability of matching a resin best to the given target.

Dynamic binding capacities (DBC) at 300 cm/h

	Polyclonal IgG		Lysozyme
	DBC (g/L)	Recovery %	DBC (g/L)
Toyopearl Phenyl-600M	40	91	58
Agarose Type Phenyl	32	87	52
Toyopearl Butyl-600M	29	87	ND
Toyopearl Phenyl-650M	16	95	27
Toyopearl PPG-600M	3	ND	43

Table 1

Breakthrough curves of a polyclonal IgG on various HIC resins



Column: 7.8 mm ID x 20 cm L
 Sample: polyclonal human IgG
 Binding buffer: 1 g/l IgG in 0.8 mol/l (NH₄)₂SO₄ + 0,1 mol/l sodium phosphate (pH 7.0)
 Linear velocity: 300 cm/h
 Temperature: 25 °C

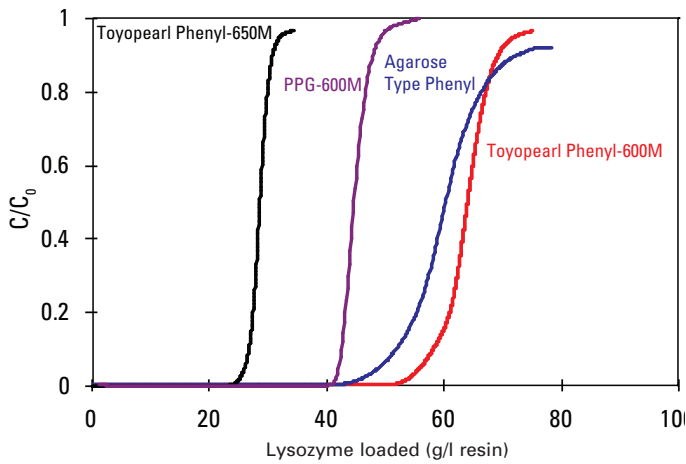
Figure 1

Toyopearl Phenyl-600M is the third product in our Toyopearl "600M" series of resins optimized for mAbs.

It complements Toyopearl PPG-600M and Butyl-600M. The order of hydrophobicities for the three ligands from lowest to highest is:

PPG < Phenyl < Butyl

Breakthrough curves of lysozyme on various resins



Column: 7.8 mm ID x 20 cm L
 Sample: lysozyme (from Sigma)
 Binding buffer: 1 g/l lysozyme in 1.8 mol/l (NH₄)₂SO₄ + 0,1 mol/l sodium phosphate (pH 7.0)
 Linear velocity: 300 cm/h
 Temperature: 25 °C

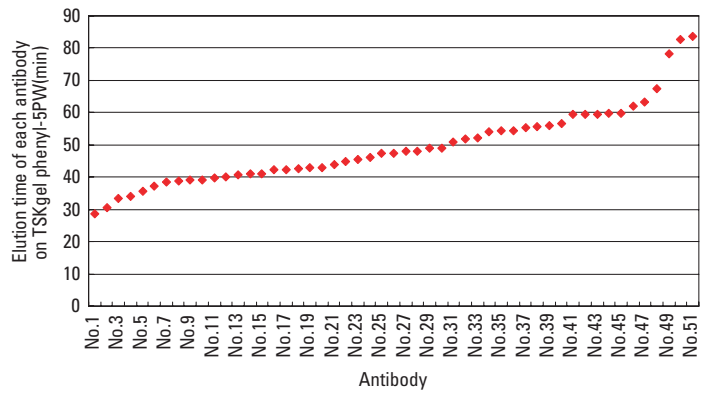
Figure 2

Toyopearl Phenyl-600M offers high dynamic binding capacities (DBC) for both small and large proteins (Table 1, Figure 2). In addition to the DBC of typically 40 g/liter resin (Figure 1), recoveries for IgG are also high, mostly greater than 90%.

Antibodies can vary greatly in their hydrophobicities (Figure 3). Having a diverse selection of ligands available can be very beneficial for the development process. For very hydrophobic antibodies less hydrophobic HIC resins like PPG-600M might be favourable. Therefore it is recommended that all three resins be screened when developing a mAb purification.

In order to simplify the resin screening, Tosoh Bioscience offers sets of prepacked ToyoScreen® columns.

Hydrophobic diversity of mouse monoclonals



Column: TSKgel Phenyl-5PW
 Eluent: (A) 0.1 mol/l phosphate buffer containing 1.8 mol/l ammonium sulfate (pH 7.0)
 (B) 0.1 mol/l phosphate buffer (pH 7.0)
 Flow rate: 1 mol/min
 Gradient: 0 min --> 0% (B)
 5 min --> 0% (B)
 65 min --> 100% (B)
 Samples: 51 kinds of mouse monoclonal antibodies

Figure 3

For further details of choice and selection of the TOYOPEARL® resin that best suits your particular process purification needs, please contact us:

Tel. + 49 (0) 711 132570

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info.tb@tosoh.com

or

www.toyopearl.com

Ordering information

Toyopearl Phenyl-600M

Particle size: 40 - 90 µm
 Pore size: 750 Å

Part-No	Description	Resin Volume
21887	Toyopearl Phenyl-600M	25 ml
21888	Toyopearl Phenyl-600M	100 ml
21889	Toyopearl Phenyl-600M	1 l
21890	Toyopearl Phenyl-600M	5 l
21891	Toyopearl Phenyl-600M	50 l
21892	ToyoScreen Phenyl-600M	1 ml x 6 ea.
21893	ToyoScreen Phenyl-600M	5 ml x 6 ea.



TOYOPEARL® Q-600C AR

INTRODUCTION

Ion exchange Chromatography (IEC) is one of the most frequently used chromatographic modes for the separation and purification of biomolecules. It is used at all stages and scales of purification of therapeutic proteins: from laboratory scale purification to industrial scale downstream processing. Toyopearl IEC resins are hydrophilic, macroporous media available with various ligands and in different particle and pore sizes.

Due to the high dynamic binding capacities of ion exchange resins relative to those of the other chromatographic modes, IEC is frequently used for the capture chromatographic step. Toyopearl Q-600C AR is a strong anion exchange resin designed for efficient and robust capturing in large scale biopurification. It is more alkaline resistant (AR) than other quaternary amino type anion exchange resins and offers high dynamic binding capacities.

HIGHLIGHTS

- Excellent tolerance to caustic exposure up to 1 N NaOH for improved CIP robustness
- High dynamic binding capacities for efficient capturing out of high-titer feedstocks
- Fast elution kinetics generating low elution pool volumes for improved process efficiency

FEATURES

Toyopearl Q-600C AR is a highly alkaline stable capture resin. It was designed to be more alkaline resistant than the well established Toyopearl QAE-550C resin. Toyopearl Q-600C AR tolerates caustic cleaning solutions up to 1N NaOH with essentially no change in dynamic binding capacity (DBC) for BSA (Figure1). Both, retention (Figure 2) and ion exchange capacity (Figure 3) are not significantly altered after 100 days storage in 1N NaOH as well.

Toyopearl Q-600C AR has a slightly larger pore diameter (750 Å) than Toyopearl QAE-550C, but nevertheless a higher dynamic binding capacity for proteins. It has the third highest DBC for bovine serum albumin among all Toyopearl anion exchange resins (Table 1).

FEATURES OF ANION EXCHANGE RESINS

Resin	Particle size (µm)	IEC (meq/mL)	DBC BSA 66 kDa (g/L)	DBC h IgG 160 kDa (g/L)	DBC Thyroglobulin 660 kDa (g/L)
TOYOPEARL Q-600C AR	50-150	0.18	108	90	26
TOYOPEARL QAE-550C	50-150	0.36	29	32	6
TOYOPEARL GigaCap Q-650M	50-100	0.17	173	108	71
TOYOPEARL SuperQ-650M	40-90	0.24	145	13	3
TOYOPEARL DEAE-650M	40-90	0.11	25	31	3

➤ Table 1

Dynamic binding capacities were determined at 10% breakthrough
 Column size: 6 mm ID x 40 mm L
 Sample: bovine serum albumin BSA, human IgG, thyroglobulin (1 mg/mL)
 Loading buffer (BSA): 0.05 M Tris-HCl buffer (pH 8.5)
 Loading buffer (h IgG): 0.015 M Tris-HCl buffer (pH 8.7)
 Loading buffer (thyroglobulin): 0.015 M Tris-HCl buffer (pH 8.7) + 0.15 M NaCl
 Elution buffer: Loading buffer + 1.0 M NaCl
 Linear velocity: 212 cm/hr
 Detection: UV @ 280 nm

DYNAMIC BINDING CAPACITY AFTER EXPOSURE TO 1 N NaOH

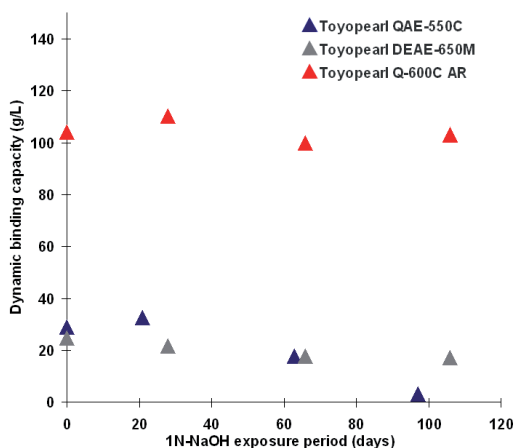


Figure 1

RETENTION TIME STABILITY AFTER EXPOSURE TO 1 N NaOH

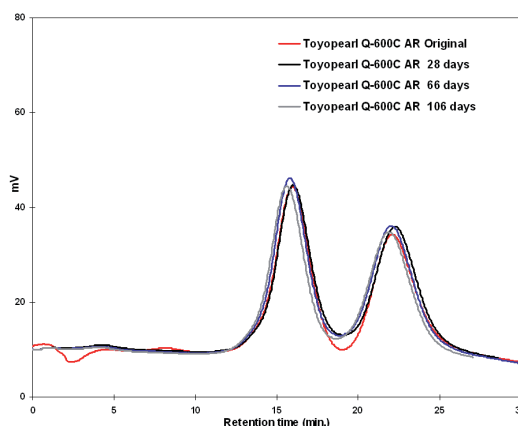


Figure 2

ION EXCHANGE CAPACITY AFTER EXPOSURE TO 1 N NaOH

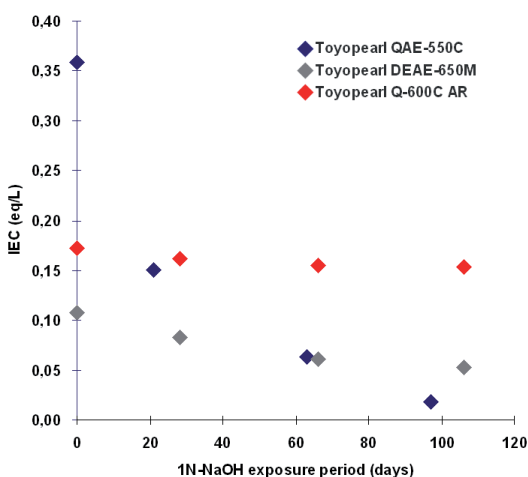


Figure 3

Column: 6.0 mm ID x 4 cm L
 Flow rate: 1.0 mL/min
 Elution: Buffer A: 0.05 M Tris-HCl buffer (pH 8.5)
 Buffer B: 0.05 M Tris-HCl buffer + 1.0 M NaCl (pH 8.5)
 Gradient: 60-min linear gradient from buffer A to buffer B
 Detection: UV @ 280 nm

Similar to the recently introduced Toyopearl GigaCap IEC resins, Toyopearl Q-600C AR shows fast elution kinetics resulting in low pool volumes for the target molecule. The combination of high binding capacity, low elution pool volume and high caustic stability supports both, high process efficiency and improved robustness. This makes Toyopearl Q-600C AR the ideal choice for anion exchange capture steps.

For further details of choice and selection of the TOYOPEARL® resin that best suits your particular separation needs, please contact us:

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sales&marketing.sep@tosoh.com

www.toyopearl.com

Ordering information

TOYOPEARL Q-600C AR

Part-No	Description	Resin volume	Pore size	Particle size
21985	Toyopearl Q-600C AR	100 mL	750 Å	100 µm
21986	Toyopearl Q-600C AR	250 mL	750 Å	100 µm
21987	Toyopearl Q-600C AR	1 L	750 Å	100 µm
21988	Toyopearl Q-600C AR	5 L	750 Å	100 µm
21989	Toyopearl Q-600C AR	50 L	750 Å	100 µm
21992	ToyoScreen Q-600C AR	1 mL x 6 (each)	750 Å	100 µm
21993	ToyoScreen Q-600C AR	5 mL x 6 (each)	750 Å	100 µm



TSK-GEL® SP-3PW

INTRODUCTION

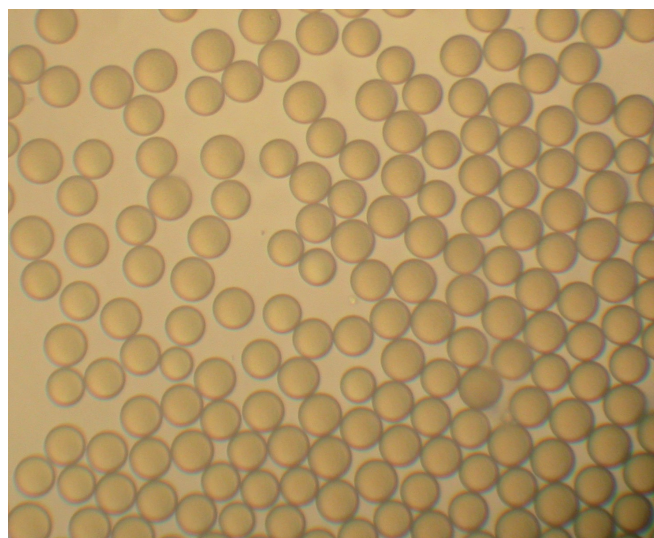
Ion exchange chromatography (IEC) is one of the most frequently used chromatographic modes for the separation and purification of biomolecules. It is used at all stages and scales of purification of therapeutic proteins: from laboratory scale purification to industrial scale downstream processing. TSK-GEL IEC resins are hydrophilic, macroporous media available with various ligands and in different particle and pore sizes.

TSK-GEL resins are based on a highly cross-linked polymethacrylate particle showing even higher pressure tolerance than the well known Toyopearl bioprocess resins. Their smaller particle sizes of 20 or 30 µm make TSK-GEL resins an excellent choice for polishing and high performance resolution steps. TSKgel SP-3PW (30) is a strong cation exchange resin designed for efficient purification of small proteins or peptides.

HIGHLIGHTS

- Strong cation exchange resin
- Typical insulin dynamic binding capacity of 49 g/L
- Small, rigid polymethacrylate beads
- Narrow particle size distribution

MICROSCOPIC IMAGE TSK-GEL SP-3PW



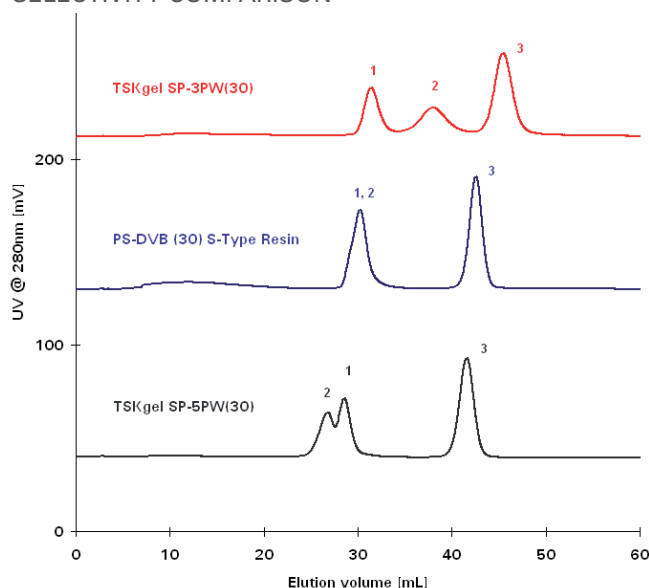
➤ Figure 1

FEATURES

TSKgel SP-3PW (30) is a strong cation exchange resin having a smaller pore size than the corresponding TSKgel SP-5PW (30) material. The technologies used in the manufacturing processes of the two resins are different. Therefore the particle size distribution of the new TSKgel SP-3PW (30) resin is more narrow than the particle size distribution of TSKgel SP-5PW (30). Due to the small - 30 µm - mean diameter and the narrow particle size distribution, TSKgel SP-3PW (30) is ideally suited when resolution is an issue. Figure 1 shows a microscopic image of TSKgel SP-3PW (30) particles.

TSKgel SP-3PW (30) shows a unique selectivity. Elution order is different when compared to TSKgel SP-5PW (30). It also may offer more resolution than other commercial small particle resins (Figure 2). TSKgel SP-3PW (30) was developed to provide high

SELECTIVITY COMPARISON



➤ Figure 2

Column: 7.5 mm ID x 7.5 cm L
 Mobile Phase: A: 20 mM sodium citrate buffer (pH 3.2)/ethanol = 8/2 (v/v)
 B: 1.0 M NaCl in 20 mM sodium citrate buffer (pH 3.2)/ethanol = 8/2 (v/v)
 Gradient: 60 min linear gradient from Buffer A to Buffer B
 Flow rate: 1.0 ml/min
 Detection: UV @ 280 nm
 Temperature: RT
 Sample: 1. trypsinogen, 2. insulin, 3. lysozyme; 100 µL (0.5 mg/ml each)

dynamic binding capacities (DBC) for peptides and small proteins. Table 1 shows a comparison of insulin dynamic binding capacities of various resins.

A typical DBC of 49 g/L for insulin makes TSKgel SP-3PW (30) attractive for all peptide and small protein purification tasks that involve a cation exchange step.

INSULIN DYNAMIC BINDING CAPACITIES

	TSKgel SP-3PW (30)	TSKgel SP-5PW (30)	PS-DVB (30) S Type Resin
Matrix	polymethacrylate	polymethacrylate	polystyrene divinylbenzene
Particle size	30 µm	30 µm	30 µm
Insulin DBC	49 g/L	24 g/L	45 g/L
Ion exchange capacity	0.12 eq/L	0.09 eq/L	0.08 eq/L
Pore size	250 Å	1000 Å	NR

Table 1

Column: 4.6 mm ID x 7.5 cm L
 Eluent: Acidic buffer (pH 3.0) containing neutral salt and 1-propanol
 Flow rate: 0.75 ml/min (270 cm/h)
 Sample: Recombinant insulin (7.2 g/L)

DBC calculated at 10% breakthrough

For further details of choice and selection of the TOYOPEARL® or TSK-GEL® resin that best suits your particular separation needs, please contact us:

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www.tskgel.com

Ordering information

TSK-GEL SP-3PW (30)

Part-No	Description	Resin volume	Pore size	Particle size
21976	TSKgel SP-3PW (30)	25 mL	250 Å	30 µm
21977	TSKgel SP-3PW (30)	250 mL	250 Å	30 µm
21978	TSKgel SP-3PW (30)	1 L	250 Å	30 µm
21979	TSKgel SP-3PW (30)	5 L	250 Å	30 µm



TOYOPEARL® AF-rProtein A-650F

INTRODUCTION

Protein A based affinity chromatography is the industry standard for the capture of monoclonal antibodies (MAbs) in downstream processing. Affinity media offer high selectivity thereby yielding a high purity of the target molecule in one step. The antibodies are captured at near neutral pH and eluted using acidic pH. The rising fermentation titres in the production of monoclonal antibodies drive the focus on developing resins that increase downstream capacity and efficiency for these processes.

Toyopearl AF-rProtein A-650F is an affinity resin designed for efficient and robust large-scale purification of monoclonal antibodies. A newly developed recombinant protein A ligand (*E. coli*) was linked to the well proven methacrylic polymer backbone of Toyopearl media. The ligand is derived from one of the IgG-binding domains of the *staphylococcus aureus* protein A (Figure 1). Its amino acid sequence was optimized in order to increase its stability towards alkaline solutions. Multipoint attachment of the ligand to the Toyopearl matrix further enhances chemical and thermal stability of the resin. In practice this pays off for a low level of protein A leaching and also for a high resistance to alkaline solutions employed in cleaning-in-place (CIP) procedures.

Toyopearl AF-rProtein-650F binds human and mouse immunoglobulin G, as well as immunoglobulin M and Fab fragments.

RECOMBINANT PROTEIN A DERIVED LIGAND

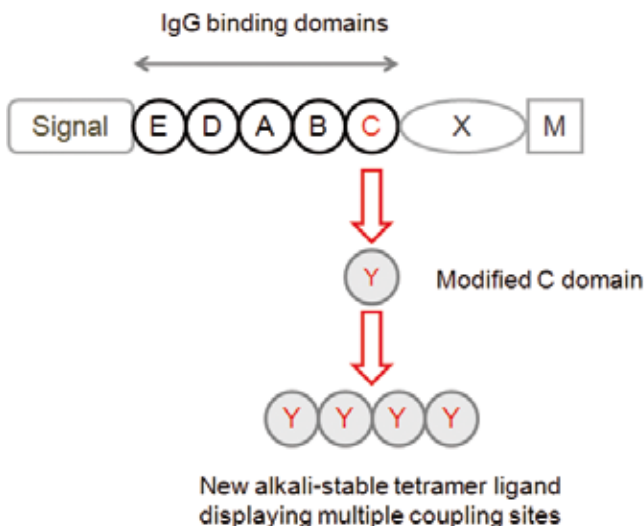


Figure 1

HIGHLIGHTS

- Efficient MAb capturing from high titer feedstocks
- High IgG binding capacity over a wide range of velocities
- High alkaline stability for effective cleaning and sanitization
- Rigid polymethacrylate base matrix with ideal pressure/flow characteristics

FEATURES

HIGH BINDING CAPACITY AT SHORT RESIDENCE TIME

The particle size of 45 µm and the ligand density of Toyopearl AF-rProtein A-650F were optimized in order to reach high dynamic binding capacities (DBC) for immunoglobulins. Typical static IgG binding capacity is > 45 mg/mL resin and typical dynamic IgG binding capacity at 10 % breakthrough is > 30 mg/mL resin at 2 minutes residence time (1 mg/mL protein load).

DYNAMIC BINDING CAPACITY

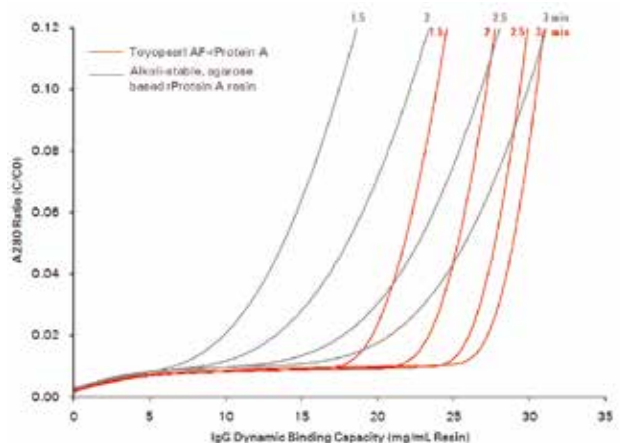


Figure 2

Breakthrough curves for hIgG loading (polyclonal, 10 mg/ml)
 Typical DBC at 10% breakthrough:
 30,5 mg/mL @ 100 cm/hr (3 min residence time) - 24 mg/mL @ 200 cm/hr (1.5 min residence time)
 Column: 5 mm ID x 5 cm L
 Mobile phase: 20 mM sodium phosphate buffer pH 7.2 containing 150 mM NaCl
 Sample conc.: 10 mg/mL
 Residence time: 1.5, 2.0, 2.5, 3.0 min

Fast mass transfer kinetics support high binding capacities even when applying high titer feedstocks at high flow rates. IgG breakthrough curves (Figure 2) at various linear velocities demonstrate the high IgG DBC at high velocities and the superior kinetic performance of Toyopearl AF-rProtein A-650F.

CIP STUDY WITH 0.1 AND 0.5 M NaOH

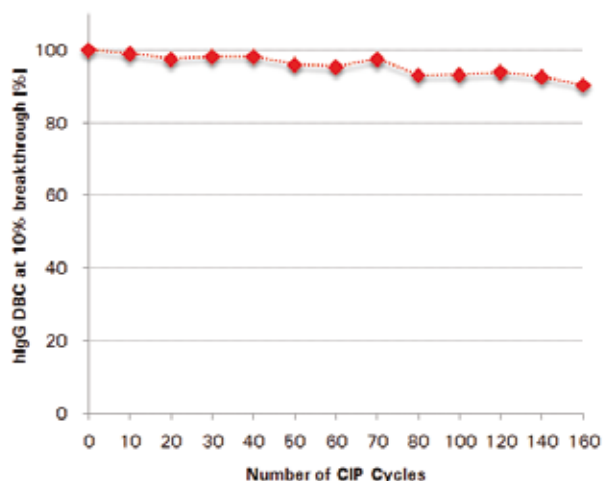


Figure 3A

Cleaning-in-place with 0.1 M NaOH:
 Column: 5 mm ID x 5 cm L
 10 column volumes binding buffer pH 7.4
 5 column volumes elution buffer pH 3.0
 3 column volumes binding buffer containing 0.1 M NaOH,
 16 min contact time
 3 column volumes binding buffer pH 7.4

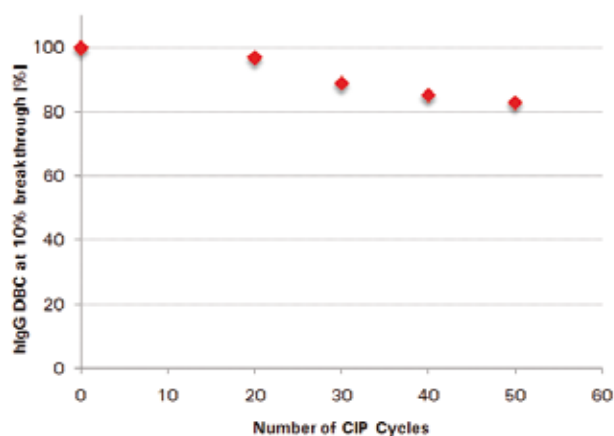


Figure 3B

Cleaning-in-place with 0.5 M NaOH:
 Column 4.6 mm ID x 10 cm L
 10 column volumes binding buffer pH 7.2
 5 column volumes elution buffer pH 3.0
 3 column volumes binding buffer containing 0.5 M NaOH,
 17 min contact time
 3 column volumes binding buffer pH 7.2

DBC of hlgG determined at 10% breakthrough

HIGH CIP AND SANITIZATION STABILITY

The structure of the recombinant ligand and its multipoint attachment to the base matrix enhances the stability of Toyopearl AF-rProtein A-650F in 0.1 - 0.5 M NaOH. The dynamic binding capacity remains high after repeated CIP cycles. After more than 150 CIP cycles with 0.1 M NaOH at 16 min contact time per cycle more than 90 % of initial dynamic binding capacity was retained (Figure 3 a). When performing cleaning-in-place with 0.5 M NaOH about 80 percent of IgG binding capacity remain after 50 cycles with 17 min contact time (Figure 3b).

Toyopearl AF-rProtein A-650F is also stable in ethanol, 6 M urea, 6 M guanidinium chloride, and 1% phosphoric acid, respectively. Static binding capacity of the resin is not impaired when heated for 30 minutes to temperatures of up to 90°C. It can be stored at room temperature at production site. Recommended conditions for long term storage are a storage solution of 20 % ethanol and temperature of 4 - 8 °C.

PURIFICATION OF HUMANIZED IgG1

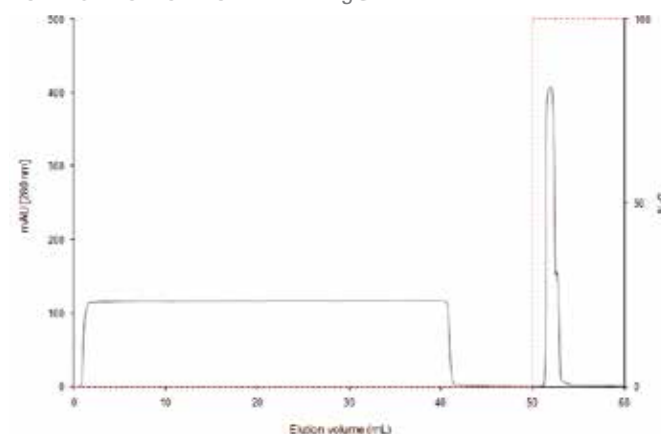


Figure 4

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

HIGH PURITY OF CAPTURED ANTIBODY

Figure 4 shows the purification of a humanized IgG1 from a Chinese Hamster Ovary (CHO) cell lysate. Elisa tests prove that both, remaining host cell proteins and leached protein A ligand amounts, were very low resulting in a very high product purity.

Table 1 shows the removal of host cell protein obtained in a high throughput screening approach to evaluate protein A affinity resins for MAb capture steps. Protein A affinity resins packed in MediaScout® MiniColumns (Atoll GmbH, Weingarten, Germany) were tested by varying the binding buffer pH, the sample load and the residence time. In order to simulate a real feedstock sample a pure monoclonal antibody (IgG1) was spiked into a CHO cell lysate. The purity of the antibody fraction was tested by measuring the amount of CHO host cell proteins

(HCP) and the amount of leached protein A by immunoassays. Table 1 exemplarily shows the HCP removal when varying the flow rate (residence time) at fixed bed volume and pH of the elution buffer. Fractions purified by Toyopearl AF-rProteinA showed a lower amount of remaining host cell proteins under all conditions tested when compared to those purified by a widely used competitive rProtein A resin providing a similar IgG binding capacity. The ligand leaching from Toyopearl AF-rProtein A-650F is generally low with typical values of leached protein A ligand between 5 - 25 ppm (ng ligand/mg antibody; determined with Protein A-R28 ELISA kit). Leached recombinant protein A can be removed from the final product through subsequent ion exchange chromatography steps.

Table 2 shows the results of lot-to-lot variation of Toyopearl AF-rProtein A-650F with regard to static and dynamic binding capacity and purity.

RIGID POLYMER MATRIX ALLOWS HIGH VELOCITIES

Toyopearl AF-rProtein A-650F is based on the well proven polymethacrylate matrix used for all Toyopearl resins. The unique pressure flow characteristics allow the use of large production scale columns. Figure 5 shows the pressure/flow curve for Toyopearl AF-rProtein A-650F up to linear velocities of more than 1000 cm/hr.

OPERATION

Typically the clarified feedstock is loaded onto the column at a neutral pH. After sufficient washing with the loading buffer, the antibody is eluted at pH 3-4. However, the physicochemical properties of different MAbs are varying, depending on the expression system and antibody subclass. Therefore a generic method needs to be optimized for each individual target in order to establish conditions that will bind the highest amount

HCP REMOVAL

	Bed volume (µl)	Protein load (mg/ml gel)	pH	Flow rate (cm/h)	HCP (ppm)
Toyopearl AF-rProtein A	200	5	3.9	100	19.2
Competitive rProtein A resin	200	5	3.9	100	59.7
Toyopearl AF-rProtein A	200	5	3.9	250	9.8
Competitive rProtein A resin	200	5	3.9	250	30.5
Toyopearl AF-rProtein A	200	25	3.9	250	47.3
Competitive rProtein A resin	200	25	3.9	250	629.6

Table 1

Effect of residence time on the CHO host cell protein (HCP) removal for two alkali-stable rProtein A resins: Toyopearl AF-rProtein A-650F and a competitive agarose-based resin. HCP content detected by ELISA (Cygnus Technologies). Data kindly provided by U. Breuninger, University of Applied Science Esslingen.

PRESSURE/FLOW CURVE

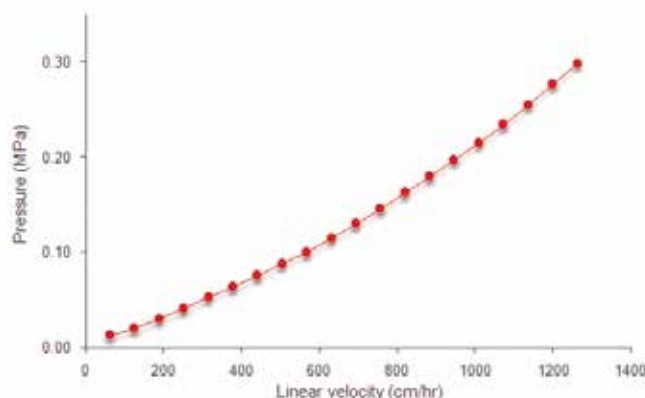


Figure 5

Column: 2.2 mm ID x 20 cm L

of the target molecule in the shortest time and elute it with the highest purity. For initial scouting of method parameters we recommend using pre-packed ToyoScreen columns or high throughput screening devices.

Suitable load/wash buffers are 20-100 mM sodium phosphate, 150 mM NaCl, pH 7.2 - 7.5 or 100 mM Tris-HCl, 150 mM NaCl, pH 7.2 - 7.5. Washing at reduced pH (e.g. pH 6) might further improve host cell protein reduction. Suitable elution buffers are 100 mM citrate, pH 3 - 4, 100 mM acetate, pH 3 - 4, or 100 mM glycine-HCl, pH 3 - 4. Due to the strong binding of IgGs to the recombinant protein A ligand it might be necessary to modify the elution conditions (lowering pH by 0.1 - 0.3 units) when transferring methods from protein A affinity methods developed on other protein A resins.

For cleaning and sanitization the use of 0.1 to 0.5 molar NaOH is recommended. Depending on the origin and subclass of the antibody, contact time, concentration, and frequency of CIP cycles should be optimized.

LOT-TO-LOT VARIATION

	Lot A	Lot B	Lot C
IgG capacity (SBC)	51.0 g/L	50.1 g/L	53.1 g/L
IgG capacity (DBC)	35.7 g/L	33.6 g/L	35.6 g/L
IgG purity (by SEC)	98.8 %	98.5 %	98.5 %

➤ **Table 2**

DBC measured at 10 % breakthrough (polyclonal hIgG, 1mg/ml, 2 min residence time)

For further details of choice and selection of the TOYOPEARL® or TSK-GEL® resin that best suits your particular separation needs, please contact us:

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www.tskgel.com

Ordering information

TOYOPEARL® AF-rProtein A-650F

Part-No	Description	Resin volume	Pore size	Particle size
22803	TOYOPEARL AF-rProtein A-650F	10 mL	1000 Å	45 µm
22804	TOYOPEARL AF-rProtein A-650F	25 mL	1000 Å	45 µm
22805	TOYOPEARL AF-rProtein A-650F	100 mL	1000 Å	45 µm
22806	TOYOPEARL AF-rProtein A-650F	1 L	1000 Å	45 µm
22807	TOYOPEARL AF-rProtein A-650F	5 L	1000 Å	45 µm
22808	TOYOPEARL AF-rProtein A-650F	50 L	1000 Å	45 µm
22809	ToyoScreen AF-rProtein A-650F	1 mL x 5	1000 Å	45 µm
22810	ToyoScreen AF-rProtein A-650F	5 mL x 1	1000 Å	45 µm
22811	ToyoScreen AF-rProtein A-650F	5 mL x 5	1000 Å	45 µm
45061	ToyoScreen RoboColumn AF-rProtein A-650F	0.2 mL x 8 col	1000 Å	45 µm
45062	ToyoScreen RoboColumn AF-rProtein A-650F	0.2 mL x 8 col	1000 Å	45 µm
22815	Protein A-R28 ELISA Kit	Kit		



TOSOH



ToyoScreen®

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.

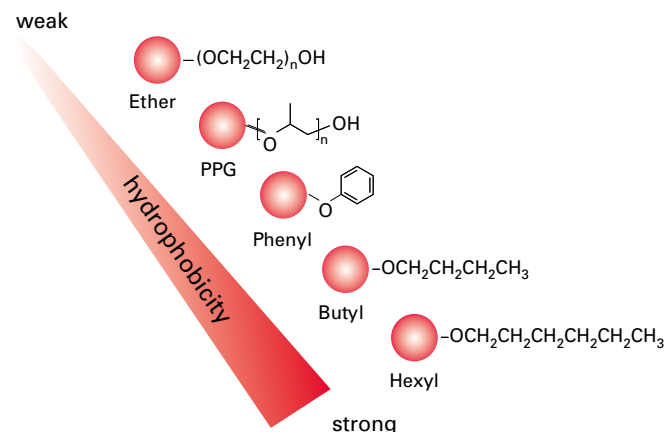
TOYOSCREEN SERIES PACKED WITH TOYOPEARL

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

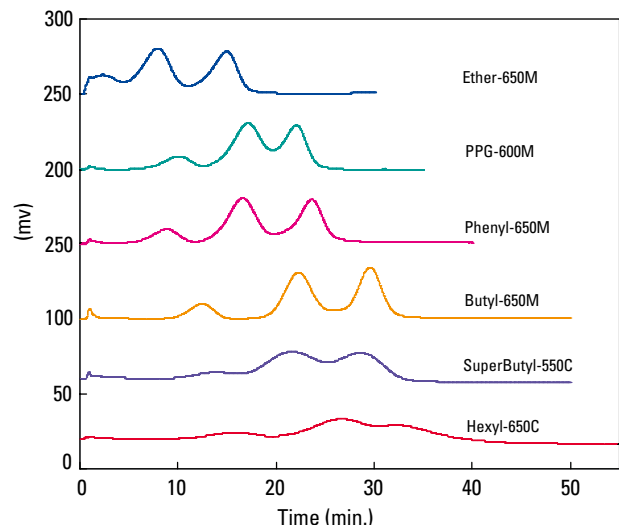
➤ Table 1

HIC LIGAND CANDIDATES



➤ Figure 1

SCREENING OF TOYOPEARL HIC RESINS - STANDARD PROTEINS



SCREENING OF TOYOPEARL HIC RESINS - MOUSE ASCITES FLUID

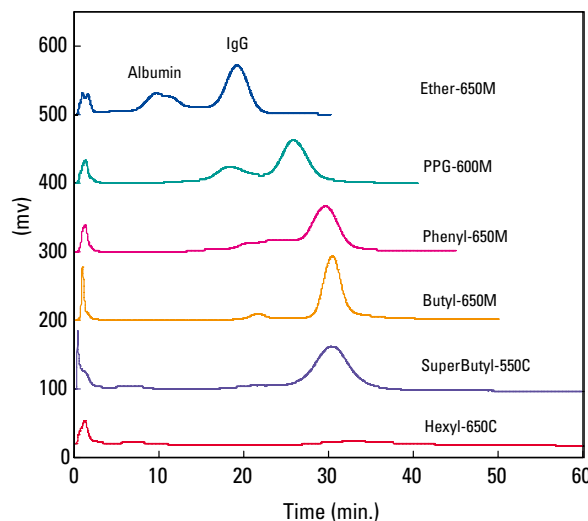


Figure 2

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

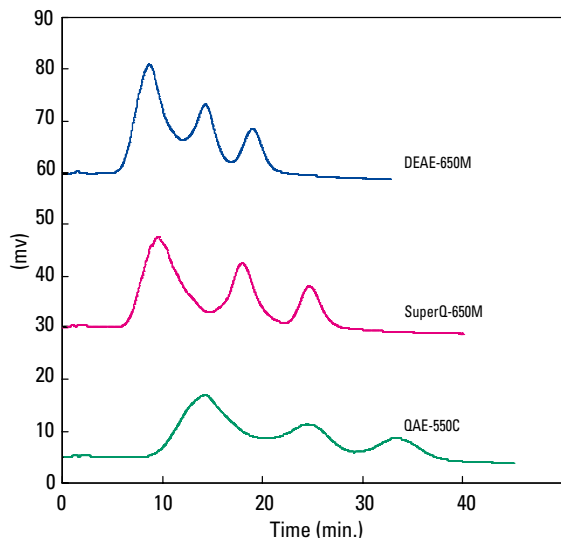
IEX - ION EXCHANGE

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 phase. 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 capacity of 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 capacities.

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

SCREENING OF TOYOPEARL ANION IEC RESINS - STANDARD PROTEIN



SCREENING OF TOYOPEARL CATION IEC RESINS - STANDARD PROTEINS

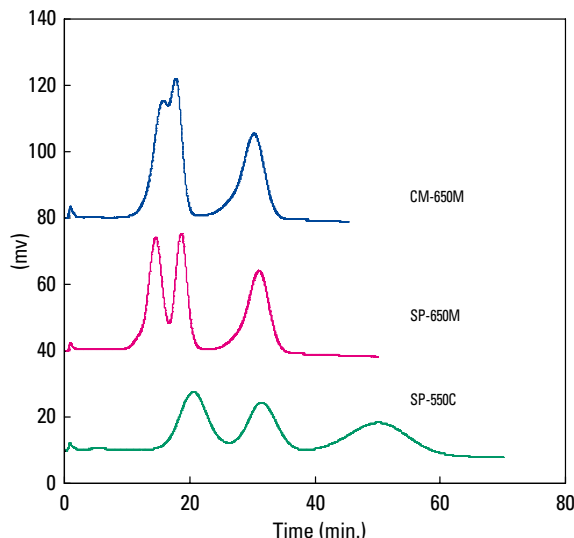


Figure 3

Column: ToyoScreen, 1 mL; Mobile phase A: 20 mmol/L Tris-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 each

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

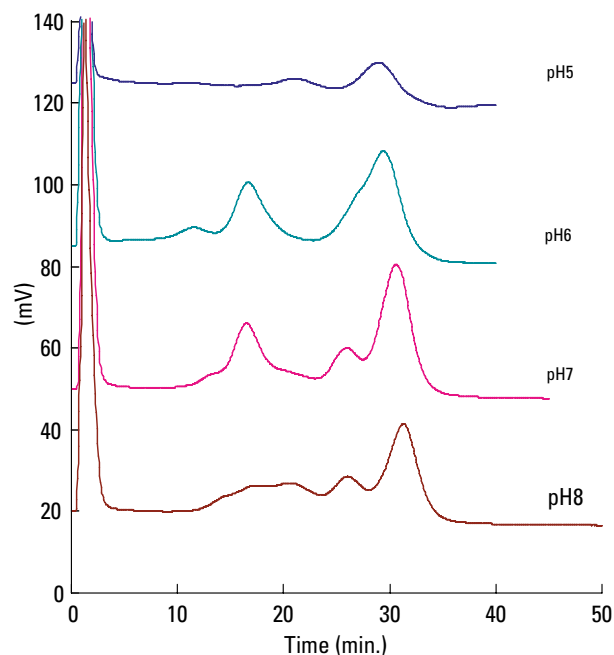
AF-Chelate-650M can be converted to either the Ni⁺⁺, Ca⁺⁺ or Zn⁺⁺ form. When converted to the Ni⁺⁺ form it is an excellent resin for metal ligand affinity for molecules containing His-tags.

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)

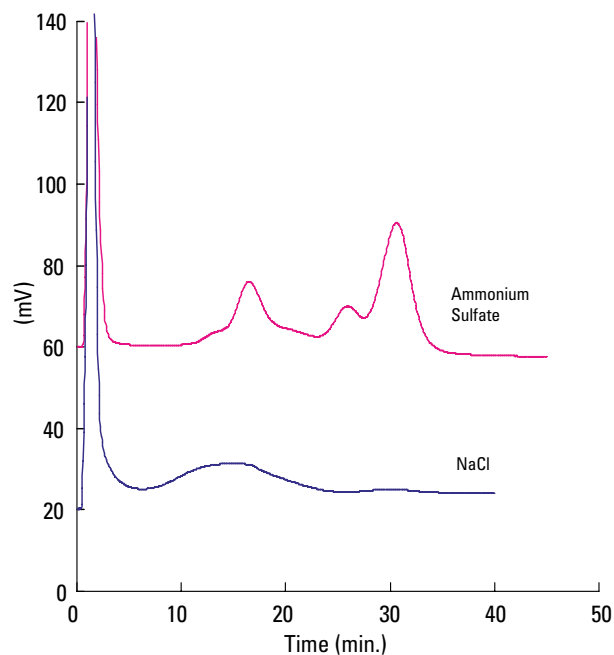


Figure 4 Column: ToyoScreen Phenyl-650M, 1 mL, Mobile phase A: 0.1 mol/L phosphate buffer containing 1.8 mol/L each salt, pH 7.0; B: 0.1 mol/L phosphate buffer, pH 7.0; Flow Rate: 1 mL/min Gradient: 30 min linear, 30 CV; Injection Vol.: 200 L; Samples: Cell culture supernatant (x 4 diluted) (antibody: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

SCALABILITY

Initial results from resin screening and optimization with ToyoScreen columns accurately predict the separation behavior at larger scales. Figure 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

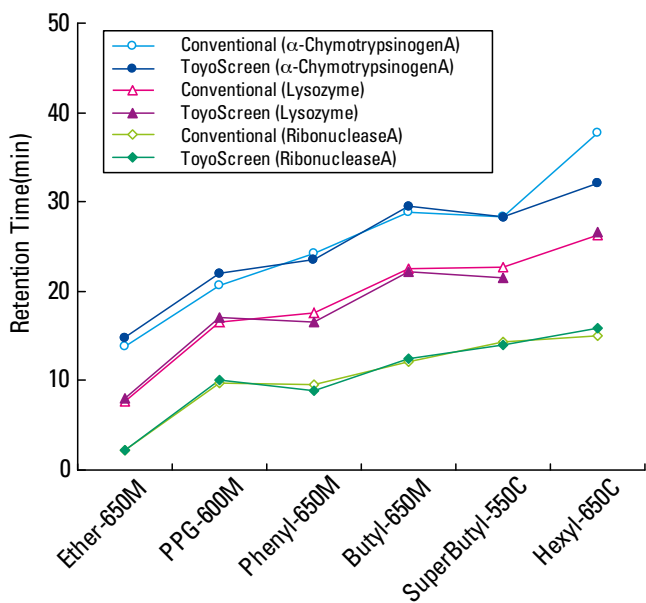


Figure 5

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

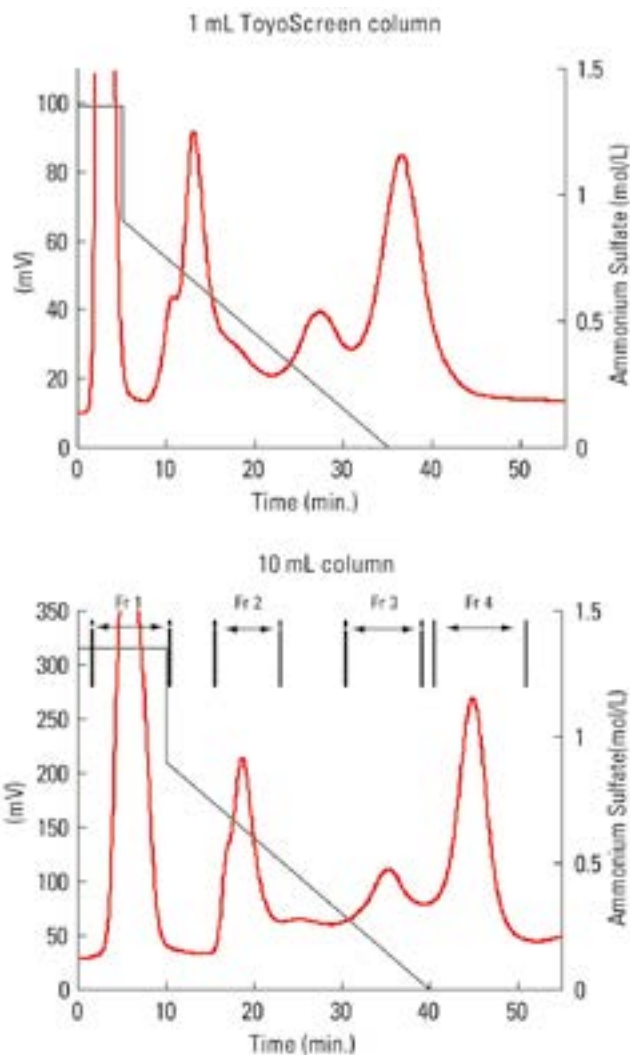


Figure 6

Packing: TOYOPEARL Phenyl-650M; Mobile phase: (A) 0.1 mol/L phosphate buffer containing 1.8 mol/L (NH₄)₂SO₄, 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.

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

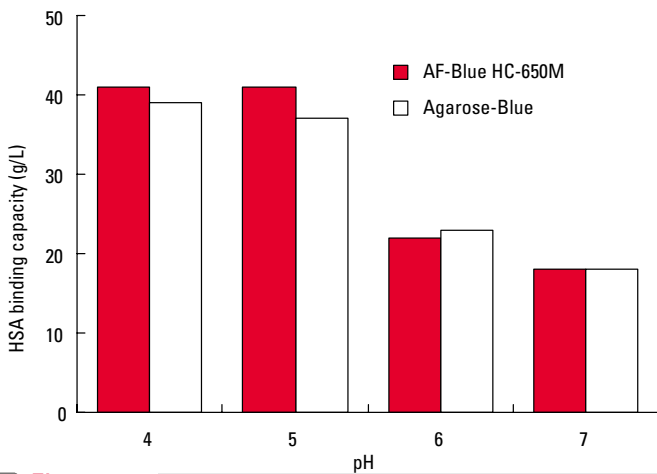


Figure 7

PURIFICATION OF 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-r-PROTEIN A-650F LIGAND STRUCTURE

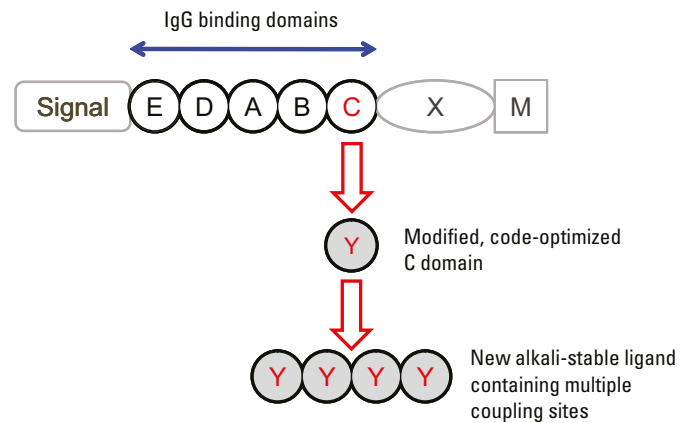


Figure 9

PURIFICATION OF HIS-TAG NusG FUSION PROTEIN

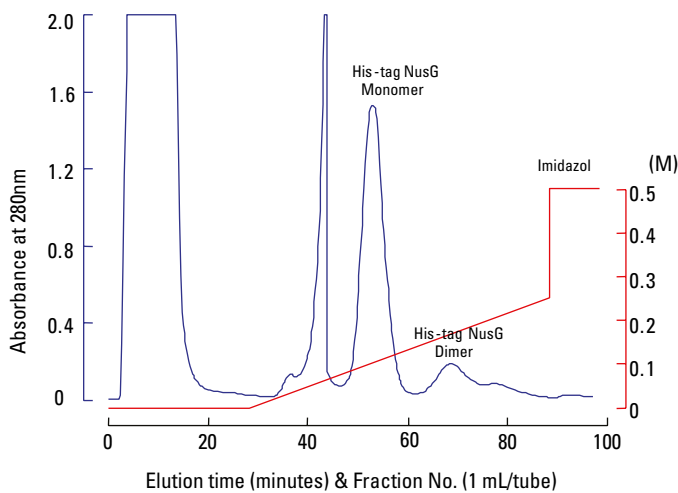


Figure 8

Column: ToyoScreen Chelate-650M (Ni-chelate), 5 mL; Starting Buffer: 20 mmol/L NaPi, pH 8.2, 0.01%NaN₃; Buffer B: 0.5 mol/L Imidazol, 20 mmol/L NaPi, pH 7.4, 0.01% NaN₃; Flow rate: 1 mL/min, at RT, Gradient: 0-25 min=0% B, 25-85 min=0-50% B, 85-90 min=100%, 90-100 min=0% B; Sample: crude cell extract (5 mL, 10 mg, 20 mmol/L NaPi, pH 8.2, 0.01%NaN₃)

PURIFICATION OF HUMANIZED IgG1

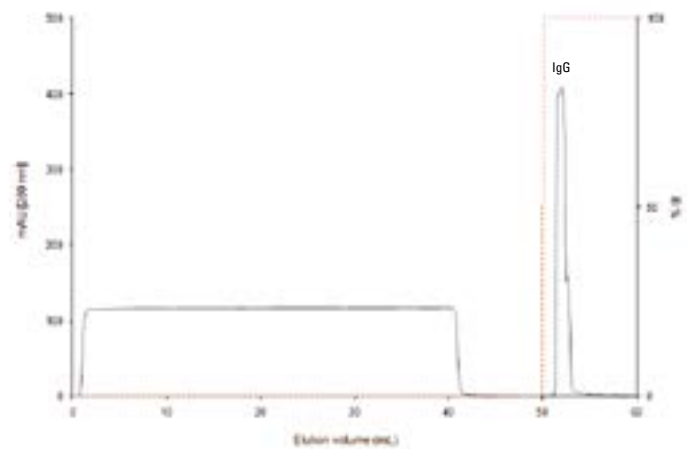


Figure 10

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

Ordering information

ToyoScreen®

Part-No	Description	Comment	Part-No	Description	Comment
ION EXCHANGE			AFFINITY		
21360	ToyoScreen DEAE-650M, 1 mL	1 mL x 6 each	21384	ToyoScreen AF-Chelate-650M, 1 mL	1 mL x 6 each
21361	ToyoScreen DEAE-650M, 5 mL	5 mL x 6 each	21385	ToyoScreen AF-Chelate-650M, 5 mL	5 mL x 6 each
21362	ToyoScreen SuperQ-650M, 1 mL	1 mL x 6 each	21386	ToyoScreen AF-Blue HC-650M, 1 mL	1 mL x 6 each
21363	ToyoScreen SuperQ-650M, 5 mL	5 mL x 6 each	21387	ToyoScreen AF-Blue HC-650M, 5 mL	5 mL x 6 each
21364	ToyoScreen QAE-550C, 1 mL	1 mL x 6 each	21388	ToyoScreen AF-Red-650M, 1 mL	1 mL x 6 each
21365	ToyoScreen QAE-550C, 5 mL	5 mL x 6 each	21389	ToyoScreen AF-Red-650M, 5 mL	5 mL x 6 each
21859	ToyoScreen GigaCap Q-650M, 1 mL	1 mL x 6 each	22809	ToyoScreen AF-rProtein A-650F, 5 mL	1 mL x 5 each
21860	ToyoScreen GigaCap Q-650M, 5 mL	5 mL x 6 each	22810	ToyoScreen AF-rProtein A-650F, 1 mL	5 mL x 1 each
21366	ToyoScreen CM-650M, 1 mL	1 mL x 6 each	22811	ToyoScreen AF-rProtein A-650F, 5 mL	5 mL x 5 each
21367	ToyoScreen CM-650M, 5 mL	5 mL x 6 each	21390	ToyoScreen AF-HeparinHC-650M	1 mL x 6 each
21368	ToyoScreen SP-650M, 1 mL	1 mL x 6 each	21391	ToyoScreen AF-HeparinHC-650M	5 mL x 6 each
21369	ToyoScreen SP-650M, 5 mL	5 mL x 6 each	MIX PACKS		
21370	ToyoScreen SP-550C, 1 mL	1 mL x 6 each	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
21371	ToyoScreen SP-550C, 5 mL	5 mL x 6 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
21868	ToyoScreen GigaCap S-650M, 1 mL	1 mL x 6 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
21869	ToyoScreen GigaCap S-650M, 5 mL	5 mL x 6 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
21870	ToyoScreen MegaCap II SP-550EC, 1 mL	1 mL x 6 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
21871	ToyoScreen MegaCap II SP-550EC, 5 mL	5 mL x 6 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
21992	ToyoScreen Q-600C AR	1 mL x 6 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
21923	ToyoScreen Q-600C AR	5 mL x 6 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
HYDROPHOBIC INTERACTION			TOYOSCREEN COLUMN ACCESSORIES		
21372	ToyoScreen Ether-650M, 1 mL	1 mL x 6 each	21400	ToyoScreen Column Holder	
21373	ToyoScreen Ether-650M, 5 mL	5 mL x 6 each	Please note that all of the ToyoScreen packages shown above require a ToyoScreen column holder either PN 21400 for operation.		
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			

MIXED MODE

22824	ToyoScreen MX-Trp-650M	1 mL x 6 each
22825	ToyoScreen MX-Trp-650M	5 mL x 6 each

TOYOSCREEN COLUMN ACCESSORIES

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





TOYOPEARL® MX-Trp-650M

INTRODUCTION

Multimodal or mixed-mode chromatography expands the range of chromatographic modes applied in biopurification. Mixed-mode media combine ionic and hydrophobic interactions and offer new selectivities and a higher salt tolerance than traditional ion exchange media. Mixed-mode media can be used for direct processing of clarified feedstocks at physiological salt concentrations as well as for intermediate and polishing applications.

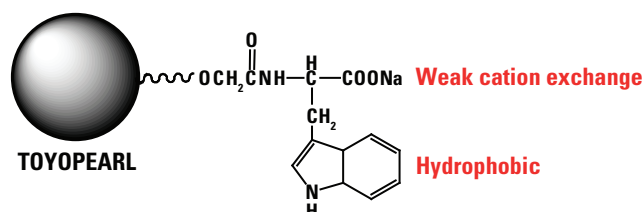
TOYOPEARL MX-Trp-650M is a multimodal cation exchange resin with unique selectivity and high recovery. It provides high protein binding capacities and tolerates high conductivity feedstocks. In addition to ionic groups its ligand also carries hydrophobic regions. Thus, the binding of target molecules is determined by electrostatic and hydrophobic contributions. TOYOPEARL MX-Trp-650M is especially suited for the purification of target molecules that are difficult to purify using common purification platforms.

RESIN STRUCTURE

TOYOPEARL MX-Trp-650M is based on the well proven methacrylic polymer backbone of TOYOPEARL media. The highly rigid base matrix provides excellent pressure/flow properties and allows high flow rates and low back pressure at large scale.

TOYOPEARL MX-Trp-650M uses tryptophan as the active ligand (Figure 1). This amino acid has both weak carboxyl cation exchange and indole hydrophobic functional groups. The selectivity of the resin can be adjusted through control of binding or elution pH, ionic strength, salt type and additives.

TOYOPEARL MX-Trp-650M STRUCTURE



➤ Figure 1

HIGHLIGHTS

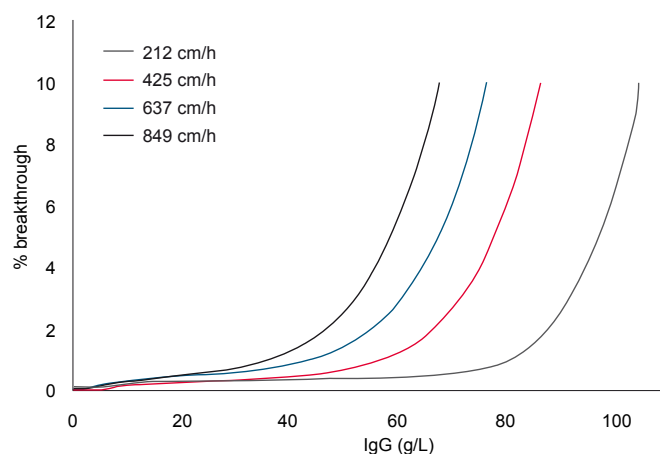
- Multimodal cation exchange resin
- High binding capacity for IgG and other proteins
- Tolerates high conductivity feedstocks
- Sharp elution peaks with mild conditions
- Excellent pressure/flow characteristics

FEATURES

HIGH BINDING CAPACITY AT HIGH CONDUCTIVITIES

TOYOPEARL MX-Trp-650M exhibits dynamic binding capacities (DBC) for immunoglobulin G as high as 90-100 g/L at standard flow rates (Figure 2). At elevated flow rates/shorter residence times the binding capacity still remains high. Table 1 shows the DBC of the new resin at two feedstock conductivities: 12 mS/cm and 17 mS/cm. For comparison purposes, data for another agarose based multimodal cation exchanger (Brand M) is also shown. For both conductivity levels the new TOYOPEARL MX-Trp-650M resin shows much higher DBCs than the agarose based resin.

TOYOPEARL MX-Trp-650M DYNAMIC BINDING CAPACITY



➤ Figure 2

Column: TOYOPEARL MX-Trp-650M (6 mm ID x 4 cm);
 Sample: polyclonal human IgG (1 mg/mL) in 0.05 mol/L NaAc + 0.1 mol/L sodium chloride (pH 4.7); Linear velocity: 212, 425, 637, 849 cm/h; Detection: UV @ 280 nm

HIGH SALT TOLERANCE

Resin	Particle size (µm)	DBC (g/L)	Recovery %
TOYOPEARL MX-Trp-650M (12 mS/cm)	75	95	97
TOYOPEARL MX-Trp-650M (17 mS/cm)	75	48	96
Brand M Agarose (12 mS/cm)	75	14	86
Brand M Agarose (17 mS/cm)	75	11	85

Table 1

Resins: TOYOPEARL MX-Trp-650M, Brand M
 Column size: 6 mm ID x 4 cm; Mobile phase: Buffer (12 mS/cm): 0.05 mol/L acetate (pH 4.3, 4.7, 5.0) + 0.10 mol/L NaCl, Buffer (17 mS/cm): 0.05 mol/L acetate (pH 4.3, 4.7, 5.0) + 0.15 mol/L NaCl; Flow rate: 1.0 mL/min (212 cm/h); Detection: UV @ 280 nm; Sample: polyclonal human IgG (1 mg/mL);
 Dynamic binding capacity (DBC) calculated at 10 % breakthrough.

RIGID POLYMER MATRIX ALLOWS HIGH VELOCITIES

Applying high linear velocities can increase throughput when processing large volumes of feedstock in process scale operations. TOYOPEARL MX-Trp-650M is based on the well proven rigid polymethacrylate matrix used for all TOYOPEARL media. This matrix exhibits high mechanical stability and creates less than half the backpressure of agarose based media of the same particle size (Figure 3).

MASS TRANSFER PARAMETERS

The mass transfer properties of a resin influence the economics of loading and elution and the degree of resolution. In keeping with the exceptional target binding and elution properties of the TOYOPEARL GigaCap® resins, the new TOYOPEARL MX-Trp-650M also shows a narrow elution peak width to complement its higher capacity.

TOYOPEARL MX-Trp-650M PRESSURE / FLOW CURVE

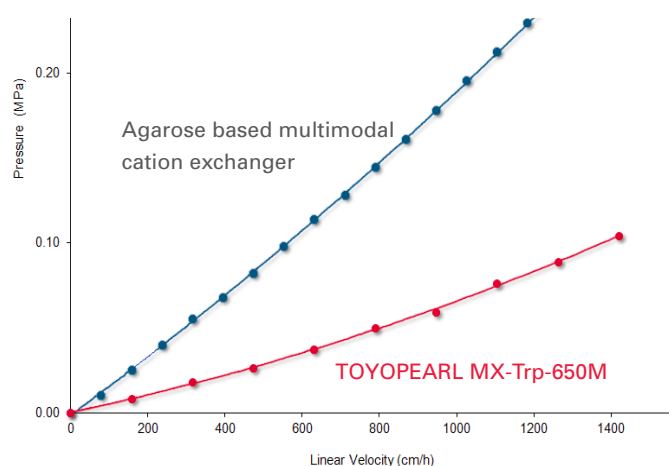


Figure 3

Column size: 22 mm ID x 20 cm; Eluent: distilled water

GOOD PEAK SHAPE AND HIGH RESOLUTION

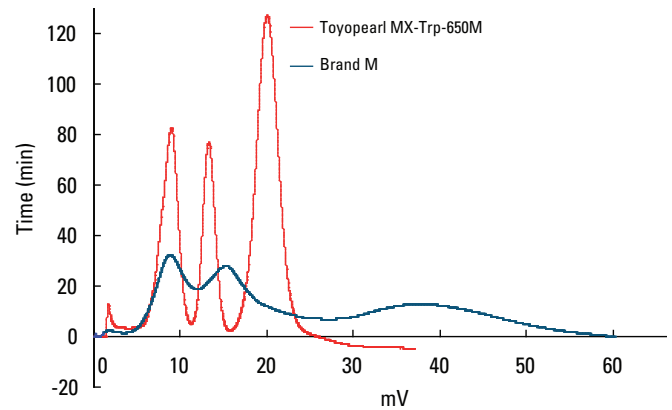


Figure 4

Resins: TOYOPEARL MX-Trp-650M, Brand M
 Column size: 7.5 mm ID x 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: 1.0 mL/min; Detection: UV @ 280nm; Sample: trypsinogen (6.6 mg/mL) cytochrome C (3.6 mg/mL) lysozyme (6.6 mg/mL); Sample volume: 25 µL

The mass transfer properties also minimize peak broadening and contribute to the excellent peak shapes observed when comparing a separation of standard proteins on TOYOPEARL MX-Trp-650M versus the agarose based multimodal cation exchange material (Figure 4).

OPERATION

The ionic and hydrophobic properties of the multimodal ligand vary with salt concentration and pH. Thus optimization of the eluents for adsorption, wash steps and elution is crucial. To facilitate resin screening and method optimization the resin is also available in 1 mL and 5 mL ToyoScreen® columns and in 200 µL and 600 µL ToyoScreen RoboColumns® for automated processing.

INFLUENCE OF pH VALUE ON IgG DYNAMIC BINDING CAPACITY

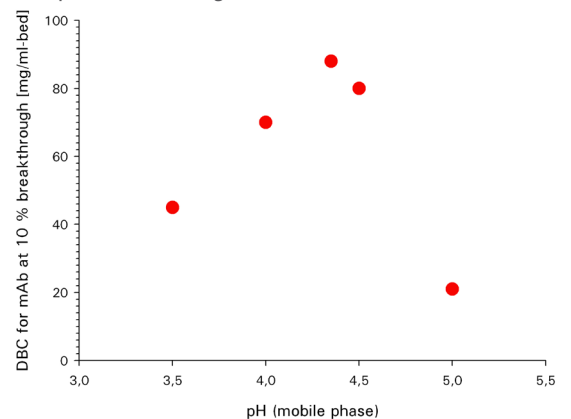


Figure 5

Column size: 6.6 mm ID x 2.2 cm; Binding buffer: 0.1 mol/L acetate buffer (pH 3.5 - 5.0) + 0.2 mol/L NaCl; Linear velocity: 150 cm/h
 Detection: UV @ 280 nm; Sample: humanized monoclonal IgG
 Dynamic binding capacity (DBC) calculated at 10 % breakthrough.

LOADING

The binding capacity of Toyopearl MX-Trp-650M greatly depends on pH (Figure 5). Buffer solutions with a pH approximately two pH units beneath the isoelectric point of the target molecule may serve as a first starting point for screening binding conditions. However it is not recommended to use a loading buffer pH below pH 3.0, as the capacity does not inversely correlate to pH but achieves a maximum at a specific pH, depending on the target protein. Further, very low pH values may accelerate oxidation of the resin.

Besides the pH, the applied salt concentration has a major impact on resin capacity. In a first approach, the overall salt concentrations may range from 0.1 mol/L to 0.3 mol/L. We suggest applying a concentration of 0.1 mol/L of the buffer salt with an addition of sodium chloride. However, the salt dependency of DBC is varying depending on the target molecule. For some proteins the salt dependency is less distinctive than for the mAb data presented in Figure 6.

RESOLUTION & ELUTION

The resin benefits from a selectivity, which is in many respects similar to hydrophobic interaction chromatography. Possible applications include mAb polishing, as the resin allows favorable resolution of closely related protein species, such as aggregates and monomers. This can be achieved by applying a pH gradient.

Antibodies captured by protein A affinity chromatography might be loaded at pH 4.5 and eluted with increased salt concentration and pH. Elution of various IgG monomers and their well separated aggregates was successful using an overall salt concentration of 0.5 mol/L and pH 6 (Figure 7). Monomer purities of up to 98.5 % can be achieved.

STORAGE & CLEANING

Cleaning-in-place (CIP) conditions depend on the type and composition of the feedstock. TOYOPEARL MX-Trp-650M withstands most of the standard CIP procedures.

INFLUENCE OF SALT CONCENTRATION ON IgG DBC

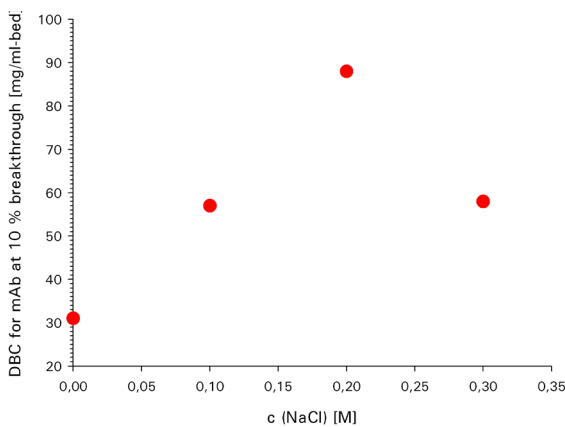


Figure 6

Column size: 6.6 mm ID x 2.2 cm;
 Binding buffer: 0.1 mol/L acetate buffer (pH 4.25) + 0 - 0.30 mol/L NaCl; Linear velocity: 150 cm/h; Detection: UV @ 280 nm;
 Sample: humanized monoclonal IgG
 Dynamic binding capacity (DBC) calculated at 10 % breakthrough.

PURITY CHECK FOR mAb FRACTION

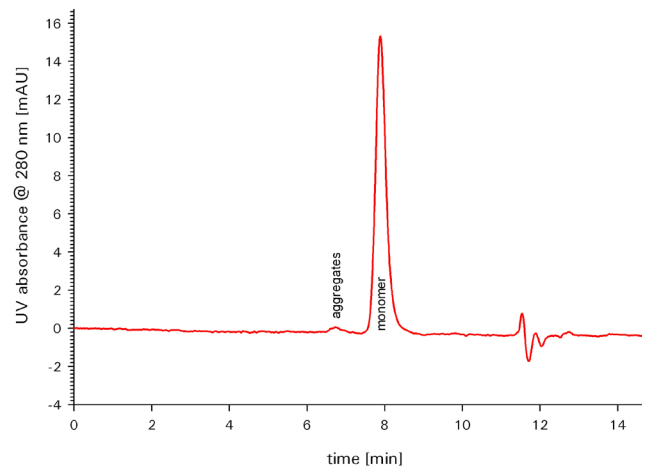


Figure 7

Column: TSKgel G3000SWxL (7.8 mm ID X 30 cm);
 Mobile phase: 0.1 mol/L phosphate buffer (pH 6.7) + 0.1 mol/L Na₂SO₄; Flow rate: 1.0 mL/min; Detection: UV @ 280 nm
 Sample: pooled monoclonal antibody fractions eluted from TOYOPEARL MX-Trp-650M @ 100 mM acetate buffer pH 5.5 + 0.4 mol/L NaCl; Injection Vol.: 20 µL

Figure 8 shows that the dynamic binding capacity for IgG is not changed even after 200 CIP cycles with 3 column volumes of 0.5 mol/L NaOH each.

Tryptophan the ligand of TOYOPEARL MX-Trp-650M is prone to oxidation by strong day light, UV light or oxidizing reagents. The resin is shipped in shaded containers. Avoid oxidizing reagents and exposure to UV light. It is recommended to wrap glass or plastic columns filled with the resin with UV tight material such as silver foil. Oxidation would lead to a discoloration to pale beige.

CIP STUDY WITH 0.5 M NaOH

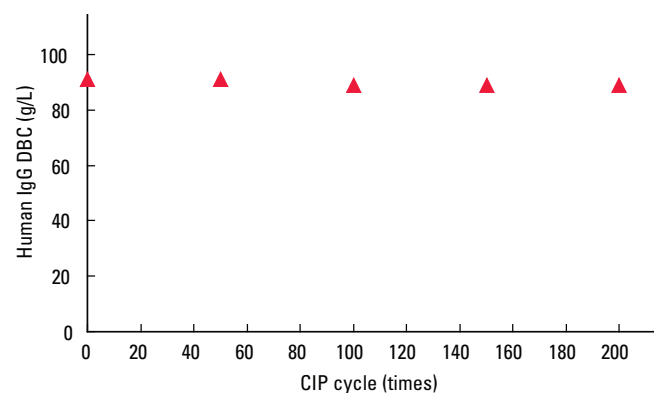


Figure 8

Alkaline cleaning conditions: 0.5 mol/L NaOH (3CV); 0.1 mol/L Tris-HCl pH 8.5 + 0.3 mol/L NaCl (5CV); Flow rate: 1 mL/min
 DBC measurement: Column size: 6 mm ID x 4 cm;
 Loading buffer: 50 mmol/L acetate (pH 4.7) + 0.1 mol/L NaCl; Flow rate: 1 mL/min; Detection: UV@280 nm;
 Sample: polyclonal human IgG; Sample Load: 1 mg/mL

Ordering Information

TOYOPEARL MX-Trp-650M

Part-No	Description	Resin volume	Pore size	Particle size
TOYOPEARL				
22817	MX-Trp-650M	25 mL	1000 Å	75 µm
22818	MX-Trp-650M	100 mL	1000 Å	75 µm
22819	MX-Trp-650M	1 L	1000 Å	75 µm
22820	MX-Trp-650M	5 L	1000 Å	75 µm
ToyoScreen				
22824	MX-Trp-650M	1 mL x 6	1000 Å	75 µm
22825	MX-Trp-650M	5 mL x 6	1000 Å	75 µm
45051	RoboColumn MX-Trp-650M	200 µL x 8	1000 Å	75 µm
45052	RoboColumn MX-Trp-650M	600 µL x 8	1000 Å	75 µm



ToyoScreen® RoboColumns®

INTRODUCTION

ToyoScreen RoboColumns are miniaturized chromatographic columns pre-packed with TOYOPEARL ion exchange, mixed-mode, hydrophobic interaction or affinity media. These columns are available in different volumes and can be operated 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 stand-alone chromatography system. ToyoScreen RoboColumns are packed with TOYOPEARL media by Atoll GmbH, they are identical to the RoboColumns supplied by Atoll GmbH. A 96-well array plate is available to arrange the up to 96 RoboColumn units.

HIGHLIGHTS

- Miniaturized column format
- High throughput parallel chromatography
- Automated screening of resins and parameters
- Better understanding of the design space

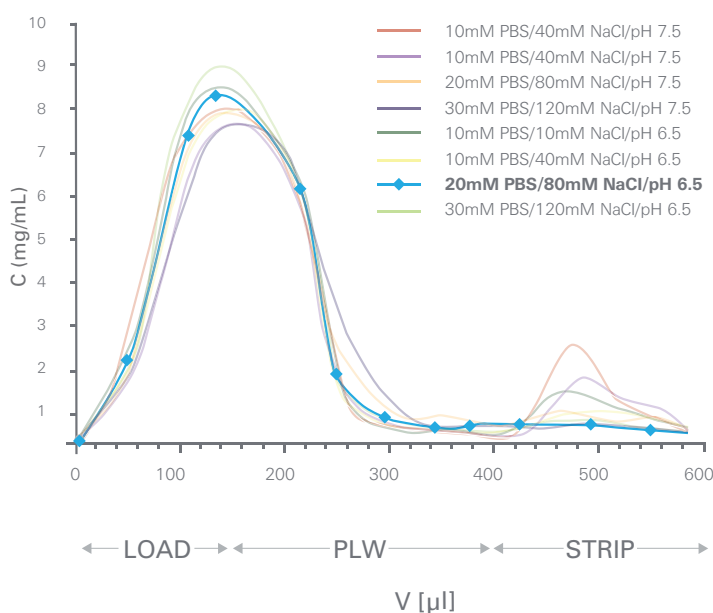
APPLICATIONS

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

SCREENING

Parallel screening of chromatographic media as well as screening of conditions for binding, washing, and elution e.g. buffer pH, ionic strength, type of salt. When screening chromatographic conditions in order to optimize the separation and explore the design space the experimental set-up should simultaneously test many different conditions. 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.

OPTIMIZATION OF ANION EXCHANGE CONDITIONS



➤ **Figure 1**

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

Figure 1 shows a screening experiment to optimize the chromatographic parameters for the intermediate flow-through anion exchange step in a purification platform for monoclonals. Protein binding of a Protein A capture eluate on RoboColumns packed with TOYOPEARL SuperQ-650M 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.

SEPARATION

ToyoScreen RoboColumns can be applied to perform small scale purifications/separations either isocratic or by applying a 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

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® RoboColumn®

Part-No	Description	Volume (µL)	Particle Size (µm)	Pore Size (Å)
45001	GigaCap S-650M	200 x 8	75	1,000
45002	GigaCap S-650M	600 x 8	75	1,000
45003	GigaCap Q-650M	200 x 8	75	1,000
45004	GigaCap Q-650M	600 x 8	75	1,000
45005	GigaCap CM-650M	200 x 8	75	1,000
45006	GigaCap CM-650M	600 x 8	75	1,000
45011	Q-600C AR	200 x 8	65	750
45012	Q-600C AR	600 x 8	65	750
45031	Phenyl-600M	200 x 8	65	750
45032	Phenyl-600M	600 x 8	65	750
45033	Butyl-600M	200 x 8	65	750
45034	Butyl-600M	600 x 8	65	750
45035	PPG-600M	200 x 8	65	750
45036	PPG-600M	600 x 8	65	750
45037	Phenyl-650M	200 x 8	65	1,000
45038	Phenyl-650M	600 x 8	65	1,000
45051	MX-Trp-650M	200 x 8	75	1,000
45052	MX-Trp-650M	600 x 8	75	1,000
45061	AF-rProtein A-650F	200 x 8	45	1,000
45062	AF-rProtein A-650F	600 x 8	45	1,000
45099	Array Plate			



RoboColumn® is a registered trademark of ATOLL GmbH.

Freedom EVO® and Freedom EVOware® are registered trademarks of Tecan Group Ltd.

Te-Chrom™ and Te-Stack™ are trademarks of Tecan Group Ltd.



TOSOH



TOYOPEARL GigaCap[®] Series

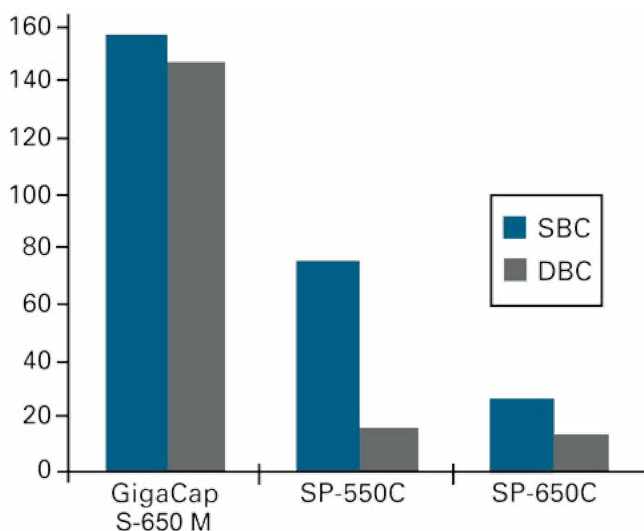
INTRODUCTION

Ion Exchange Chromatography (IEC) is one of the most frequently used chromatographic modes for the separation and purification of biomolecules. Compared with other chromatographic modes, modern ion exchange media offer high dynamic binding capacities and a straightforward method development. IEC is used at all stages and scales of purification of therapeutic proteins: from laboratory scale purification to industrial scale downstream processing (DSP).

The development of cell lines with high expression levels has triggered the demand for high throughput DSP. This requires rigid resins that offer high binding capacities and recoveries at high flow rates and withstand harsh purification procedures. TOYOPEARL GigaCap ion exchange resins were developed to meet the actual needs in high throughput purification of monoclonal antibodies and other proteins by packed bed chromatography.

The TOYOPEARL GigaCap family consists of two cation exchange resins - GigaCap S-650 and GigaCap CM-650 - and the GigaCap Q-650 and DEAE-650 anion exchange resins. While all GigaCap media are available in M-grade particle size (75 µm) for capture and intermediate process steps, GigaCap S and Q are also available in smaller particle size (S-grade; 35 µm) for high resolution intermediate and polishing purification.

STATIC VS DYNAMIC BINDING CAPACITIES FOR HUMAN IgG



➤ **Figure 1**

DBC for GigaCap S-650M calculated at 10% breakthrough
 Column size: 6 mm ID x 4 cm L; Sample: polyclonal human IgG;
 Linear velocity: 212 cm/h

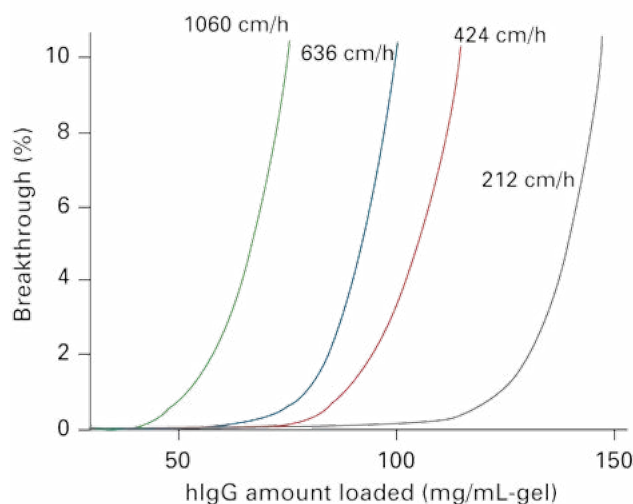
HIGHLIGHTS

- High binding capacities at short residence times
- Fast elution kinetics generating low elution pool volumes
- Excellent mechanical and chemical stability
- Superior pressure flow properties

TOYOPEARL GigaCap S-650

TOYOPEARL GigaCap S-650 is a strong cation exchange resin designed for high throughput process chromatography. Based on the well proven methacrylic polymer backbone of TOYOPEARL and TSKgel media TOYOPEARL GigaCap S combines excellent pressure flow characteristics (Figure 5) with unmatched dynamic binding capacities (DBC) and high recoveries for a wide range of biomolecules. Latest surface modification technology was applied to increase the content of functional groups at the particle surface thus reaching binding capacities up to 150 mg IgG per mL resin (Figure 1, 2). Similar binding capacities can be achieved for smaller proteins such as insulin (Figure 3).

TOYOPEARL GigaCap S-650M BREAKTHROUGH CURVES AT DIFFERENT LINEAR VELOCITIES



➤ **Figure 2**

DBC for GigaCap S-650M calculated at 10% breakthrough
 Column size: 6 mm ID x 4 cm L bed height;
 Sample: Polyclonal human IgG (1 mg/mL); Buffer: 0.1 mol/L acetate buffer (pH 4.7); Linear velocity: 212, 424, 636, 1060 cm/h; Detection: UV@280nm

TOYOPEARL DYNAMIC BINDING CAPACITIES FOR INSULIN

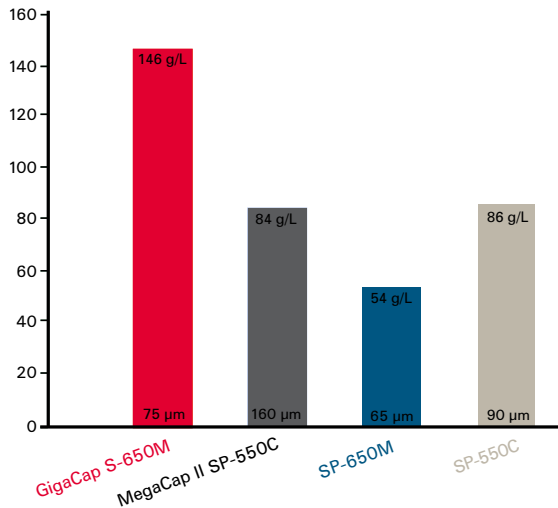


Figure 3

Column size: 6.6 mm ID x 22 mm L; 100 mM citrate buffer (ph 3.0)

The applied ligand chemistry enables a better access of proteins to the charged groups. Besides binding capacity this improves also mass transfer and thereby reduces the target molecule elution volume.

Figure 4 shows the breakthrough curve for TOYOPEARL GigaCap S compared with the most equivalent competitive resin. Each trace shows the dynamic binding capacity of the resin up to 10% breakthrough plus the elution profile. Elution pool volume of TOYOPEARL GigaCap S is remarkably reduced when compared to the competitive resin. The concentration of the eluted protein is proportionally increased as well. It is possible to achieve reductions in elution pool volumes of over 75% (Jackewitz A. BioProcess Int. 6 (7) 2008: 108-110). This has potentially very significant impact on reducing the cost of further downstream process steps. Similar reductions of elution pool volumes can be reached with all GigaCap resins.

TOYOPEARL GigaCap S shows an excellent chemical stability resulting in a minimal loss of IgG binding capacity

REDUCED POOL VOLUME

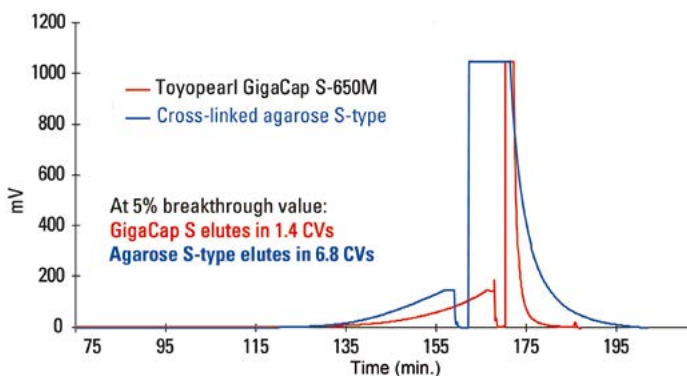


Figure 4

Column size: TOYOPEARL GigaCap S-650M, 6 mm ID x 4 cm L; Sample: polyclonal human IgG (1 mg/mL); Loading buffer: 0.1 mol/L acetate buffer pH 4.7; Elution buffer: 0.1 mol/L acetate buffer pH 4.7 + 1.0 mol/L NaCl; Linear velocity: 212 cm/h; Detection: UV@280 nm

PRESSURE FLOW CURVES OF CATION EXCHANGE RESINS

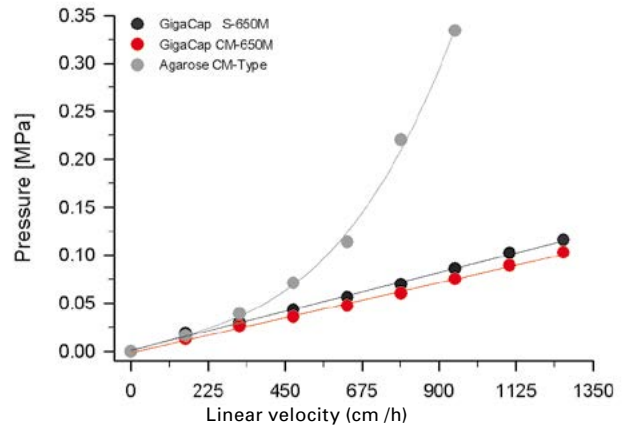


Figure 5

Column size: 22 mm ID x 20 cm L; Mobile phase: Water; Temperature: 25° C

after exposure to 1 M NaOH (Table 1) for 5 weeks. It is therefore ideally suited for process scale protein purification in large industrial process columns.

The small particle size TOYOPEARL GigaCap S-650S offers a higher binding capacity and resolution than the M grade. It is ideally suited for high purity separations needed in final polishing steps. Figure 6 shows the high resolution achieved with TOYOPEARL GigaCap S-650S and its advantageous selectivity, when compared with agarose based cation exchange media with similar particle size.

TOYOPEARL GigaCap S ALKALINE STABILITY

Time (week)		0	1	3	5
IEC capacity	meq/mL	0.159	0.157	0.158	0.156
hIgG-DBC (10% breakthr.)	mg/mL-gel	143	144	140	135
hIgG-recovery	%	99	101	100	99

Table 1

TOYOPEARL GigaCap S-650M stored in 1 M NaOH solution at RT

SELECTIVITY OF SMALL PARTICLE SIZE MEDIA

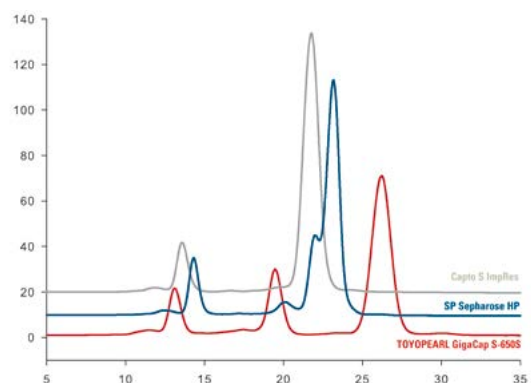


Figure 6

Column: 7.5 mm ID x 7.5 cm; Buffer A: 0.02 mol/L phosphate buffer (pH 7.0), Buffer B: 0.02 mol/L phosphate buffer + 1.0 mol/L NaCl (pH 7.0); Flow rate: 1.0 mL/min; Gradient: 60 min linear from 100% buffer A to 100% buffer B; Detection: UV @ 280nm; Sample: 1. ribonuclease A (9.2 g/L), 2. cytochrome C (4.7 g/L), 3. lysozyme (9.5 g/L); Injection volume: 20 µL

TOYOPEARL GigaCap CM-650M

TOYOPEARL GigaCap CM-650M is a weak cation exchange resin that combines excellent pressure flow characteristics (Figure 5) and high alkaline stability (Table 2) with high dynamic binding capacities and recoveries for both, IgGs and smaller proteins. Latest surface modification technology was applied to increase the content of functional groups at the particle surface thus reaching binding capacities up to 100 mg IgG per mL resin (Figure 7).

Depending on the specific characteristics of the target molecule, GigaCap S-650M and CM-650M exhibit different selectivity as shown in Figure 8 for some standard proteins. Therefore it is strongly recommended to evaluate both, GigaCap CM-650M and S-650M when developing a cation exchange purification step. For fast resin screening all GigaCap resins are available in 1 or 5 mL pre-packed ToyoScreen columns.

TOYOPEARL GigaCap CM ALKALINE STABILITY

Time (week)		0	1	3	5
IEX capacity	meq/mL	0.24	0.23	0.24	0.23
hIgG-DBC (10% breakthr.)	mg/mL-gel	99	101	100	99

Table 2

TOYOPEARL GigaCap CM-650M stored in 0.5 M NaOH solution at RT

BREAKTHROUGH CURVES FOR IgG ON CES RESINS

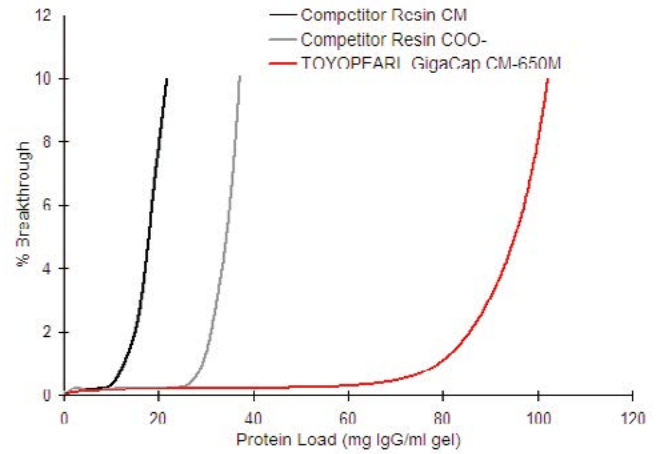


Figure 7

Column size: 6 mm ID x 4 cm L; Sample: polyclonal human IgG (1 mg/mL); Loading buffer: 50 mM sodium acetate buffer (pH 4.7); Elution buffer: 50 mM sodium acetate buffer (pH 4.7) + 0.5 mM NaCl; Linear velocity: 212 cm/h; Detection: UV@280 nm

SELECTIVITY OF TOYOPEARL GigaCap S-650M AND TOYOPEARL GigaCap CM-650M

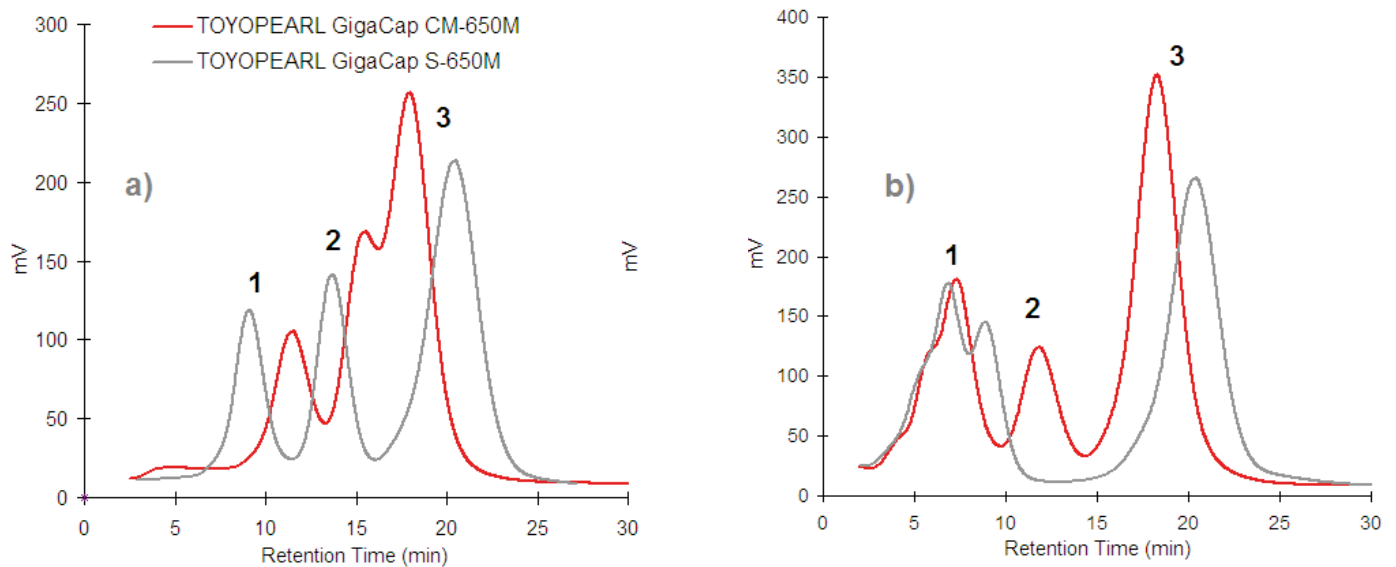


Figure 8

Column size: 6 mm ID x 4 cm L; Flow rate; 1.0 ml/min; Buffer A: 20 mM Phosphate (pH 7.0); Buffer B: 20 mM Phosphate + 1.0 M NaCl (pH 7.0); Gradient: 60 min linear gradient from 100% A to 100% B; Injection Volume: 25 µL; Detection: UV@280nm;

Sample: (a) 1. ribonuclease A (5.0 mg/mL), 2. cytochrome C (1.9 mg/mL), 3. lysozyme (3.8 mg/mL); (b) 1. trypsinogen (3.8 mg/mL), 2. ribonuclease A (5.0 mg/mL), 3. lysozyme (3.8 mg/mL)

TOYOPEARL GigaCap Q-650

TOYOPEARL GigaCap Q-650 is a strong anion exchange resin. It combines excellent pressure flow characteristics (Figure 9) with high dynamic binding capacities and high recoveries for a broad range of proteins. It binds up to 175 mg BSA per mL resin at 212 cm/h and still 164 mg BSA per mL resin at a linear flow of 800 cm/h (Figure 10).

High dynamic binding capacities can be achieved for much larger proteins as well. Even for a very large protein like thyroglobulin a dynamic binding capacity of 49 mg/mL gel can be achieved. As usual the difference between static (SBC) and dynamic binding capacity (DBC) is increasing with the increase in molecular weight (Table 3).

TOYOPEARL GigaCap Q-650M is the ideal anion exchange resin for large scale DSP unit operations. Examples range from flow-through steps in antibody purification to bind-elute steps in plasma protein purification. In flow through steps, TOYOPEARL GigaCap Q-650M can provide a viral clearance of 4 logs for MVM virus and X-MuLV virus.

The small particle size TOYOPEARL GigaCap Q-650S (35 µm) offers a higher binding capacity (190g BSA/L) and higher resolution than the respective M grade. The purification of oligonucleotides using anion exchange chromatography has traditionally fallen to resins such as TSKgel® SuperQ-5PW (20) that offer high resolution and selectivity in conjunction with excellent mechanical stability at very high column pressures. TOYOPEARL GigaCap Q-650S resin offers a low pressure alternative to oligonucleotide purification while preserving the selectivity, resolution and yields of those higher pressure processes. Figure 11 shows that the N-1 peak was slightly better resolved with the TSKgel SuperQ-5PW (20) than with the TOYOPEARL GigaCap Q-650S, perhaps due to the smaller particle size of the TSKgel resin. HPLC analysis of fractions taken across the peaks (data not shown) revealed that both resins were able to adequately resolve the full length oligonucleotide.

PRESSURE FLOW CURVES FOR ANION EXCHANGE RESINS

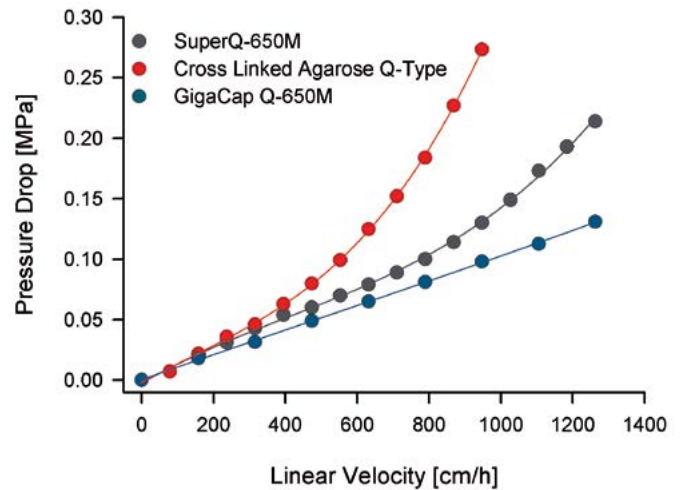


Figure 9

GigaCap Q BSA BINDING CAPACITY AT DIFFERENT FLOW RATES

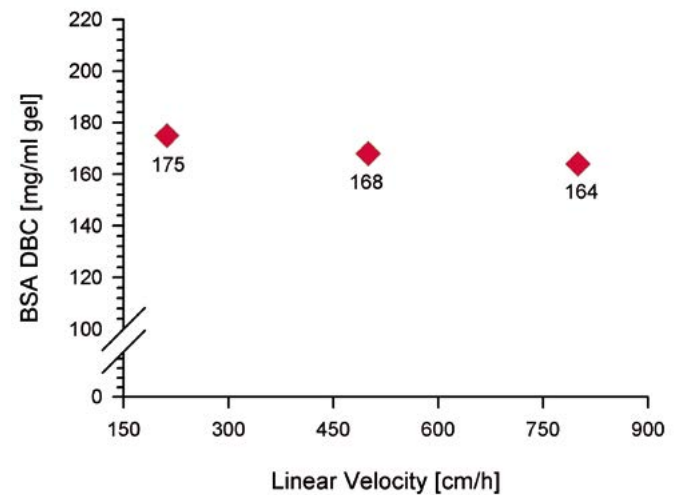


Figure 10

DBC measured at 10% breakthrough; Column size: 6 mm ID x 4 cm L; Sample: Bovine serum albumin (BSA 1 mg/mL) in Tris-HCL pH 8.5

PURIFICATION OF OLIGONUCLEOTIDES

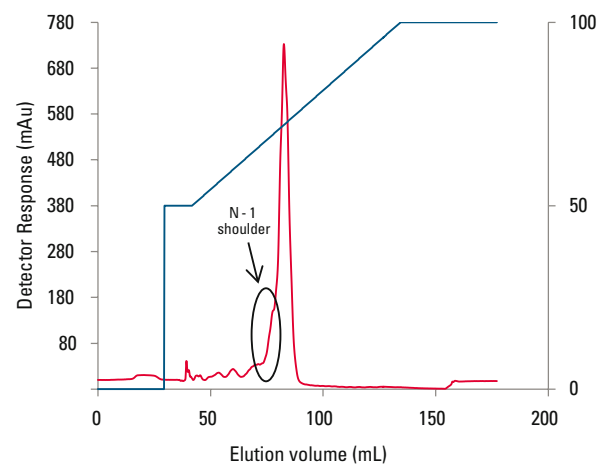
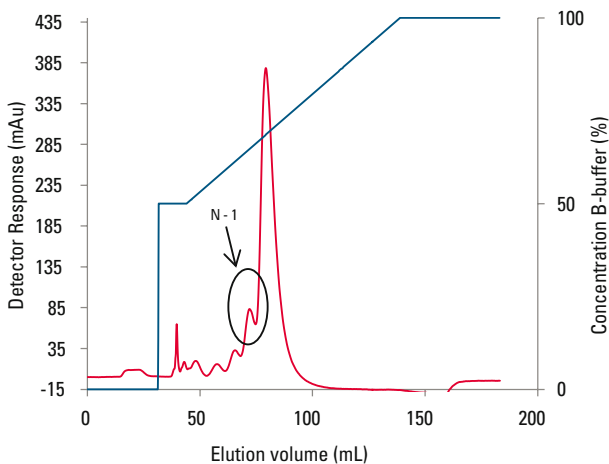


Figure 11

Resin: TSKgel SuperQ-5PW (20); Column size: 6.6 mm ID x 18.5 cm (6.3 mL); Mobile phase: A: 20 mmol/L NaOH; 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 load: 1.0 mg; Sample: crude phosphorothioate deoxyoligonucleotide

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

TOYOPEARL GigaCap DEAE-650

TOYOPEARL GigaCap DEAE-650M is a weak anion exchange resin for process scale applications. The polymeric base bead was chemically modified to provide a greater number of anionic binding sites, resulting in increased binding capacity. TOYOPEARL GigaCap DEAE-650M exhibits typical dynamic binding capacities (DBC) approaching 170 g/L for bovine serum albumin (BSA) and 100 g/L for IgG. It maintains high capacity across a range of linear velocities and exhibits excellent pressure-flow characteristics. The high capacity and low back pressure creates opportunities for increased throughput in various anion exchange purification steps and is ideally suited for efficient plasma protein purification.

Good mass transfer kinetics enable the resin to maintain DBC at faster linear velocities (Figure 12). This fast uptake ability, when coupled with the narrow elution peak typical of TOYOPEARL GigaCap resins, results in smaller and more concentrated in-process pool volumes, thus reducing the amount of water for injection needed and increasing process throughput downstream.

TOYOPEARL GigaCap DEAE-650M is base stable for at least 100 cleaning-in-place (CIP) cycles with 0.5 mol/L NaOH, making multiple uses of the resin possible (Figure 13).

DBC OF TOYOPEARL GigaCap DEAE-650M AT DIFFERENT FLOW-RATES

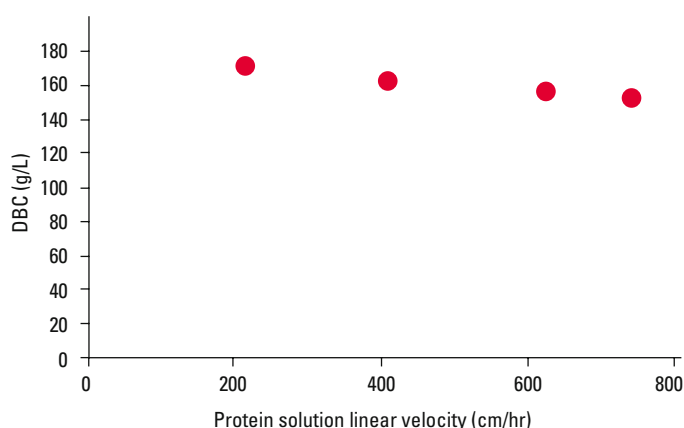


Figure 12
 DBC was determined at 10% breakthrough ; Column size: 6.0 mm ID x 4.0 cm; Sample: BSA (1.0 g/L) in 0.05 mol/L Tris, pH 8.5; Flow rate: 212 cm/h; Detection: UV @ 280 nm

CIP STABILITY OF TOYOPEARL GigaCap DEAE-650M

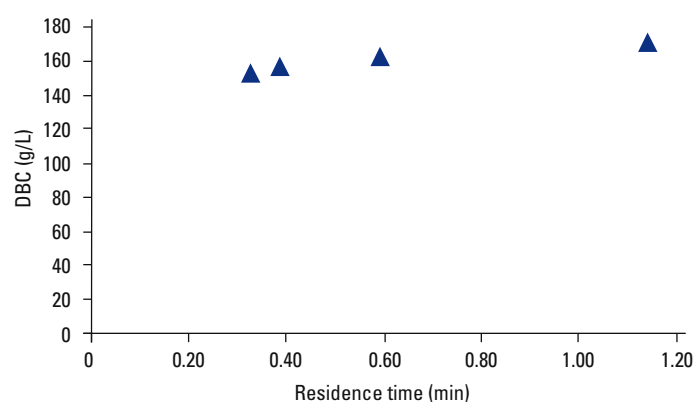


Figure 13
 Alkaline washing solution: 0.5 mol/L NaOH;
 Buffer washing solution: 0.5 mol/L Tris, 0.5 mol/L NaCl, pH 8.5; Flow rate: 106 cm/hr (0.5 mL/min); Alkaline wash volume: 27 CV/cycle; Alkaline contact time: 1 hr; Buffer wash volume: 10 CV/cycle

COMPARISON OF TOYOPEARL GigaCap RESINS

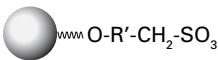

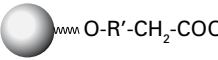
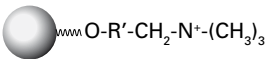

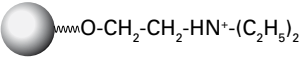
TOYOPEARL	Structure	Particle Size	DBC	IEX Capacity
CATION EXCHANGE:				
GigaCap S-650M	 $\text{O-R}'\text{-CH}_2\text{-SO}_3$	75 μm	150g hIgG/L	0.15 eq/L
GigaCap S-650S	 $\text{O-R}'\text{-CH}_2\text{-SO}_3$	35 μm	145g hIgG/L	0.24 eq/L
GigaCap CM-650M	 $\text{O-R}'\text{-CH}_2\text{-COO}^-$	75 μm	100g hIgG/L	0.225 eq/L
ANION EXCHANGE				
GigaCap Q-650M	 $\text{O-R}'\text{-CH}_2\text{-N}^+(\text{CH}_3)_3$	75 μm	175 g BSA/L	0.15 eq/L
GigaCap Q-650S	 $\text{O-R}'\text{-CH}_2\text{-N}^+(\text{CH}_3)_3$	35 μm	190 g BSA/L	0.20 eq/L
GigaCap DEAE-650M	 $\text{O-CH}_2\text{-CH}_2\text{-NH}^+(\text{C}_2\text{H}_5)_2$	75 μm	170 g BSA/L	0.23 eq/L

Table 3

ORDERING INFORMATION

TOYOPEARL GigaCap®

Cation Exchange Resins

Part-No.	Description	Resin volume
0021833	TOYOPEARL GigaCap® S-650M	100 mL
0021834	TOYOPEARL GigaCap® S-650M	250 mL
0021835	TOYOPEARL GigaCap® S-650M	1 L
0021836	TOYOPEARL GigaCap® S-650M	5 L
0021837	TOYOPEARL GigaCap® S-650M	50 L
0021868	ToyoScreen GigaCap S-650M	1 mL x 6 ea.
0021869	ToyoScreen GigaCap S-650M	5 mL x 6 ea.
0045001	ToyoScreen RoboColumn S-650M	200µl x 8 ea.
0045002	ToyoScreen RoboColumn S-650M	600µl x 8 ea.

0022875	TOYOPEARL GigaCap® S-650S	25 mL
0022876	TOYOPEARL GigaCap® S-650S	250mL
0022877	TOYOPEARL GigaCap® S-650S	1 L
0022878	TOYOPEARL GigaCap® S-650S	5 L
0022879	TOYOPEARL GigaCap® S-650S	50 L

0021946	TOYOPEARL GigaCap® CM-650M	100 mL
0021947	TOYOPEARL GigaCap® CM-650M	250 mL
0021948	TOYOPEARL GigaCap® CM-650M	1 L
0021949	TOYOPEARL GigaCap® CM-650M	5 L
0021950	TOYOPEARL GigaCap® CM-650M	50 L
0021951	ToyoScreen GigaCap CM-650M	1 mL x 6 ea.
0021952	ToyoScreen GigaCap CM-650M	5 mL x 6 ea.
0045005	ToyoScreen RoboColumn CM-650M	200µl x 8 ea.
0045006	ToyoScreen RoboColumn CM-650M	600µl x 8 ea.

Anion Exchange Resins

Part-No.	Description	Resin volume
0021854	TOYOPEARL GigaCap® Q-650M	100 mL
0021855	TOYOPEARL GigaCap® Q-650M	250 mL
0021856	TOYOPEARL GigaCap® Q-650M	1 L
0021857	TOYOPEARL GigaCap® Q-650M	5 L
0021858	TOYOPEARL GigaCap® Q-650M	50 L
0021859	ToyoScreen GigaCap Q-650M	1 mL x 6 ea.
0021860	ToyoScreen GigaCap Q-650M	5 mL x 6 ea.
0045003	ToyoScreen RoboColumn Q-650M	200µl x 8 ea.
0045004	ToyoScreen RoboColumn Q-650M	600µl x 8 ea.

0022881	TOYOPEARL GigaCap® Q-650S	25 mL
0022882	TOYOPEARL GigaCap® Q-650S	250 mL
0022883	TOYOPEARL GigaCap® Q-650S	1 L
0022884	TOYOPEARL GigaCap® Q-650S	5 L
0022885	TOYOPEARL GigaCap® Q-650S	50 L

0022865	TOYOPEARL GigaCap® DEAE-650M	100 mL
0022866	TOYOPEARL GigaCap® DEAE-650M	250 mL
0022867	TOYOPEARL GigaCap® DEAE-650M	1 L
0022868	TOYOPEARL GigaCap® DEAE-650M	5 L
0022869	TOYOPEARL GigaCap® DEAE-650M	50 L
0022872	ToyoScreen GigaCap® DEAE-650M	1 mL x 6 ea.
0022873	ToyoScreen GigaCap® DEAE-650M	5 mL x 6 ea.
0045007	ToyoScreen RoboColumn DEAE-650M	200µl x 8 ea.
0045008	ToyoScreen RoboColumn DEAE-650M	600µl x 8 ea.



TOYOPEARL® AF-rProtein A HC-650F

INTRODUCTION

Protein A based affinity chromatography is the industry standard for the capture of monoclonal antibodies (mAbs) in downstream processing. Affinity media offer high selectivity thereby yielding a high purity of the target molecule in one step. The rising fermentation titers in the production of monoclonal antibodies drive the focus on developing resins that increase downstream capacity and efficiency for these processes.

TOYOPEARL AF-rProtein A HC-650F presents the third generation of TOYOPEARL protein A media. It is a rigid, alkaline resistant protein A affinity resin that offers the largest binding capacity for immunoglobulin G of all protein A affinity media currently available on the market. It is based on a recombinant protein A derived ligand expressed in *E. coli*.

The ligand is derived from one of the IgG-binding domains of the *staphylococcus aureus* protein A (Figure 1). It was further optimized in order to provide a higher binding capacity and alkali stability. Multipoint attachment of the ligand to the TOYOPEARL polymer matrix further enhances chemical and thermal stability of the resin. In practice this pays off for a low level of protein A leaching and also for a high resistance to alkaline solutions applied in cleaning-in-place (CIP) procedures.

RECOMBINANT PROTEIN A DERIVED LIGAND

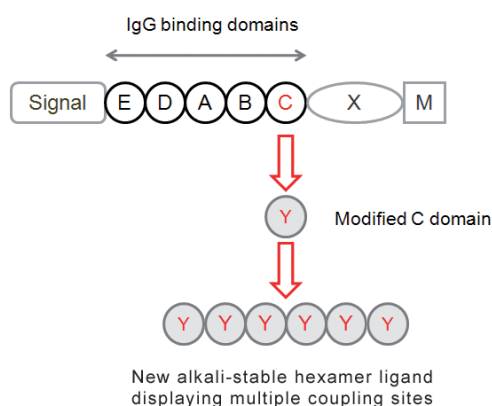


Figure 1

HIGHLIGHTS

- Superior dynamic binding capacity for human IgG
- Capacity increases with feedstock titers up to 10 g/L
- Fast binding kinetics and moderate elution conditions
- Optimized alkali-stable recombinant ligand
- Minimized leaching through multi-point attachment

FEATURES

ULTRA-HIGH BINDING CAPACITY

Besides the ligand itself also the base particle and the ligand density were optimized in order to provide extremely high binding capacities for immunoglobulin G. Static IgG binding capacity (SBC) is > 68 mg/mL resin and dynamic binding capacity (DBC) at 10% breakthrough is > 65 mg/mL resin at 5 minutes residence time. Figure 2 depicts the dynamic binding capacity of TOYOPEARL AF-rProtein A HC-650F at three different linear velocities/residence times compared to the reported DBCs of MabSelect Sure™ and MabSelect Sure™ LX. The excellent lot-to-lot reproducibility is depicted in Figure 3.

DBC OF HIGH CAPACITY PROTEIN A MEDIA

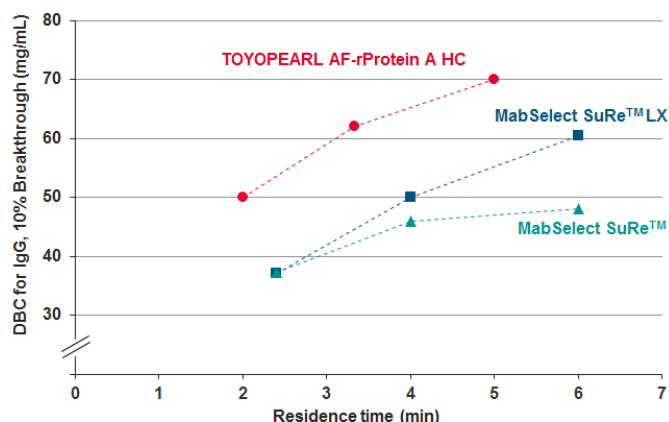


Figure 2

Column: TOYOPEARL AF-rProtein A HC-650F (5 mm ID x 5 cm)
 Mobile phase: 20 mmol/L sodium phosphate, 150 mmol/L NaCl pH 7.4;
 Residence time: 2, 3.3, 5 min; Detection: UV @ 280 nm;
 Sample: polyclonal human IgG @ 1 mg/mL in mobile phase;
 DBC measured at 10 % breakthrough.
 MabSelect Sure™ and MabSelect Sure™ LX DBC data from GE brochure.
 MabSelect Sure™ and MabSelect Sure™ LX are registered trademarks of GE Healthcare Bio-Sciences AB, Uppsala, Sweden.

LOT-TO-LOT VARIATION

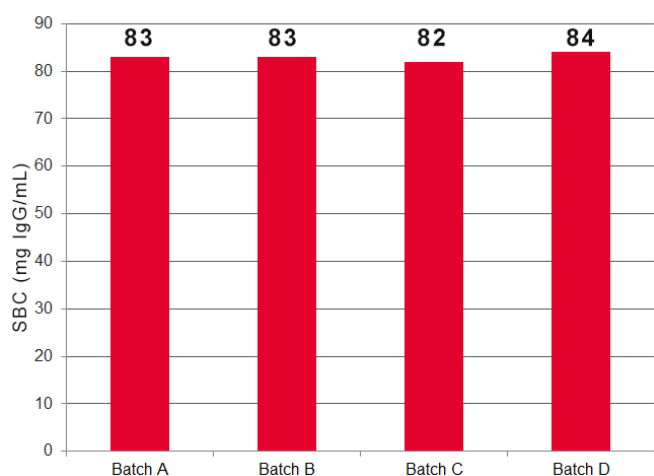


Figure 3
 Sample: polyclonal human IgG
 Lot-to-lot variation of static binding capacities for polyclonal human IgG; Buffer: 0.1 mol/L sodium phosphate, pH 7.0;

FAST BINDING KINETICS

Fast mass transfer kinetics support high binding capacities even when applying high titer feedstocks at high flow rates. IgG binding capacities measured at various linear velocities and various feedstock concentrations (Figure 4) demonstrate the high IgG DBC at high velocities and the superior kinetic performance of TOYOPEARL AF-rProtein A HC-650F. The DBC increases up to a titer of 10 mg/mL cell culture supernatant. In contrast to other high capacity protein A media TOYOPEARL AF-rProtein A HC-650F reaches more than 70% of the maximum dynamic capacity even at very short residence times.

DBC AT VARIOUS LOADS AND RESIDENCE TIMES

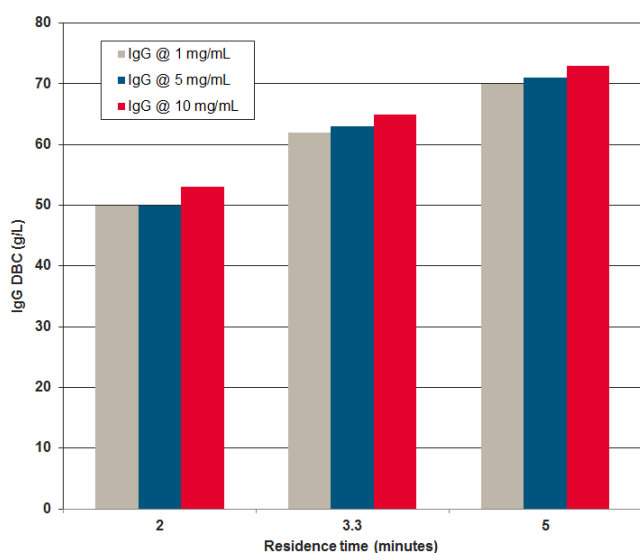


Figure 4
 Column: TOYOPEARL AF-rProtein A HC-650F (5 mm ID x 5 cm)
 Mobile phase: 20 mmol/L sodium phosphate, 150 mmol/L NaCl pH 7.4;
 Residence time: 2, 3.3, 5 min; Detection: UV @ 280 nm
 Sample: polyclonal human IgG @ 1, 5, 10 g/L in mobile phase
 DBC measured at 10% breakthrough

HIGH CIP AND SANITIZATION STABILITY

The structure of the recombinant ligand and its multipoint attachment to the base matrix enhance the stability of TOYOPEARL AF-rProtein A HC-650F in 0.1 - 0.5 M NaOH. The dynamic binding capacity remains high after repeated CIP cycles. After 200 CIP cycles with 0.1 M NaOH at 15 min contact time per cycle more than 95 percent of initial dynamic binding capacity was retained (Figure 5a). When performing cleaning-in-place with 0.5 M NaOH the resin maintains about 80 percent of initial IgG binding capacity after 40 cycles with 15 min contact time (Figure 5b).

Static binding capacity of the resin is not impaired when stored at room temperature at production site. Recommended conditions for long term storage are a storage solution of 20 % ethanol and a temperature of 4 - 8 °C.

CIP STUDY WITH 0.1 AND 0.5 M NaOH

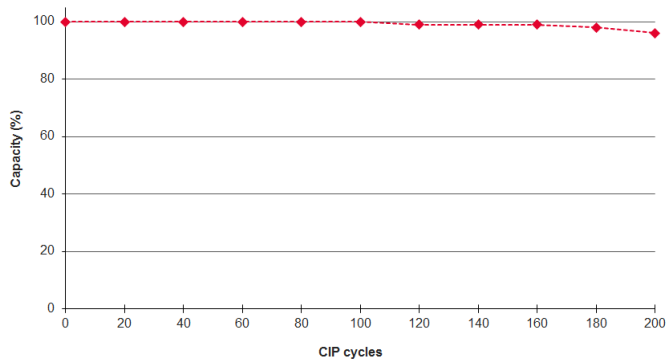


Figure 5a
 Column size: 5 mm ID x 5 cm; Wash procedure: A: 20 mmol/L Na₂HPO₄, 0.15 mol/L NaCl, pH 7.4 (10 CV)
 B: 0.1 mol/L citrate, pH 3.0 (5 CV)
 C: 20 mmol/L Na₂HPO₄, 0.15 mol/L NaCl, pH 7.4 (7 CV)
 D: 0.1 mol/L NaOH (3 CV - 15 min contact time)
 E: 20 mmol/L Na₂HPO₄, 0.15 mol/L NaCl, pH 7.4 (5 CV)
 Capacity: DBC was determined at 10% breakthrough after every 20 cycles

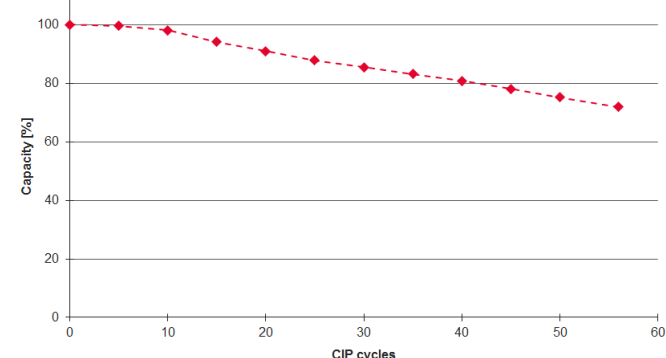


Figure 5b
 Column size: 5 mm ID x 5 cm; Wash procedure: A: 20 mmol/L Na₂HPO₄, 0.15 mol/L NaCl, pH 7.4 (10 CV)
 B: 0.1 mol/L citrate, pH 3.0 (5 CV)
 C: 20 mmol/L Na₂HPO₄, 0.15 mol/L NaCl, pH 7.4 (7 CV)
 D: 0.5 mol/L NaOH (3 CV - 15 min contact time)
 E: 20 mmol/L Na₂HPO₄, 0.15 mol/L NaCl, pH 7.4 (5 CV)
 Capacity: DBC was determined at 10% breakthrough after every 5 cycles

LOW LIGAND LEACHING

The binding of the advanced rProtein A ligand to the TOYOPEARL HW-65F base bead via multipoint attachment is not only resulting in high alkaline stability but also the reason for low ligand leakage (Table 1).

PROTEIN A LIGAND LEAKAGE

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

Table 1

RIGID POLYMER MATRIX ALLOWS HIGH VELOCITIES

TOYOPEARL AF-rProtein A HC-650F is based on the well-proven polymethacrylate matrix used for all TOYOPEARL resins. The unique pressure flow characteristics allow the use of large production scale columns. Figure 6 shows the pressure/flow curve for TOYOPEARL AF-rProtein A HC-650F packed in various column diameters normalized to a bed height of 20 cm.

PRESSURE/FLOW CURVE

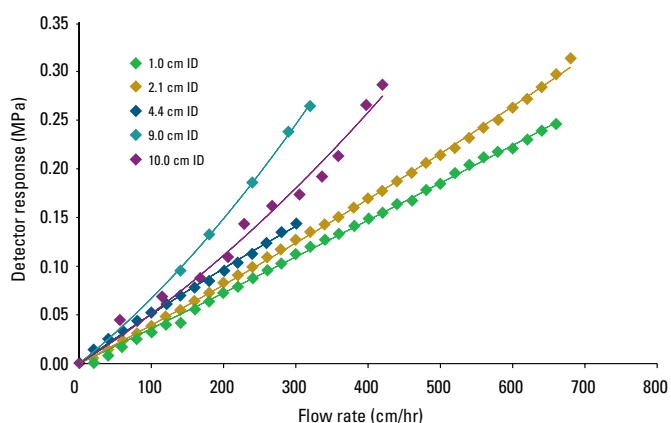


Figure 6

Column size: 1.0 cm ID, 2.1 cm ID, 4.4 cm ID, 9.0 cm ID, 10.0 cm ID; 20 cm normalized bed height; Mobile phase: DI H₂O

APPLICATION

Figure 7 shows the results of the capturing of a therapeutic monoclonal IgG1 diluted at different concentrations into CHO cell culture fluid. The binding capacity of the TOYOPEARL AF-rProtein A HC-650F for this specific antibody is increasing dramatically with increasing feed concentrations. Furthermore, when applying a feed concentration of 10 mg mAb/mL an extremely high capacity of more than 100 mg mAb/mL resin was even reached at 1 min residence time.

DBC FOR A SPECIFIC mAb AT VARIOUS LOADS AND VELOCITIES

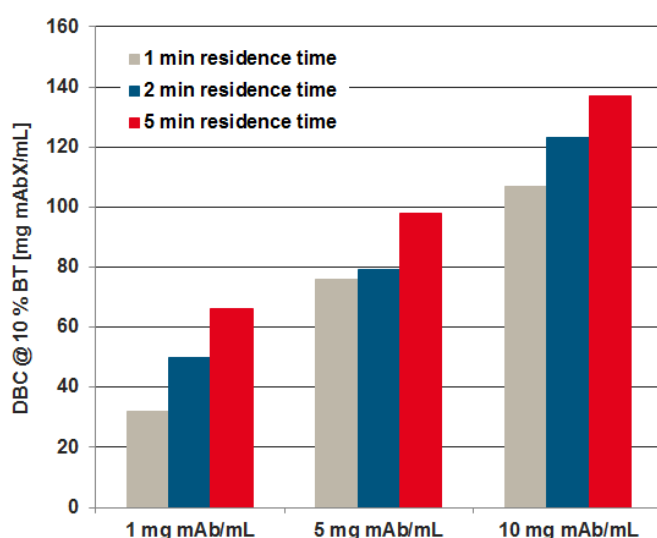


Figure 7

Column: TOYOPEARL AF-rProtein A HC-650F (6.6 mm ID × 2 cm)
Mobile phase: 100 mmol/L sodium phosphate pH 6.5;
Residence time: 1, 2, 5 min; Detection: UV @ 280 nm
Sample: monoclonal antibody mAbX @ 1, 5, 10 g/L in mobile phase
DBC measured at 10% breakthrough

OPERATION

Typically antibodies are captured at a pH close to 7.0 and eluted using acidic conditions. The clarified feedstock is loaded onto the column at a neutral pH. After sufficient washing with the loading buffer, the antibody is eluted at pH 3-4. However, the physicochemical properties of different mAbs are varying depending on the expression system and antibody subclass. Therefore a generic method needs to be optimized for each individual target in order to establish conditions that will bind the highest amount of the target molecule in the shortest time and elute it with the highest purity. For initial scouting of method parameters we recommend using pre-packed ToyoScreen columns or robotic high throughput screening devices with ToyoScreen RoboColumns.

Suitable load/wash buffers are 20-100 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 6.5 - 7.5 or 100 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.2 - 7.5. Washing at reduced pH (e.g. pH 6) might further improve host cell protein reduction. Suitable elution buffer systems are 50-100 mmol/L acetate, citrate or glycine-HCl. The pH shift required for mAb elution depends on a particular mAb and ranges from pH 3.0 to 4.5. For cleaning and sanitization the use of 0.1 to 0.5 molar NaOH is recommended. Depending on the origin and subclass of the antibody, contact time, concentration, and frequency of CIP cycles the conditions should be optimized.

Ordering Information

TOYOPEARL AF-rProtein A HC-650F

Part-No	Description	Resin volume	Pore size	Particle size
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TOYOPEARL

0023425	TOYOPEARL AF-rProtein A HC-650F	10 mL	1000 Å	45 µm
0023426	TOYOPEARL AF-rProtein A HC-650F	25 mL	1000 Å	45 µm
0023427	TOYOPEARL AF-rProtein A HC-650F	100 mL	1000 Å	45 µm
0023428	TOYOPEARL AF-rProtein A HC-650F	1 L	1000 Å	45 µm
0023429	TOYOPEARL AF-rProtein A HC-650F	5 L	1000 Å	45 µm
0023434	TOYOPEARL AF-rProtein A HC-650F	50 L	1000 Å	45 µm

ToyoScreen

0023430	ToyoScreen AF-rProtein A HC-650F	1 mL x 5	1000 Å	45 µm
0023431	ToyoScreen AF-rProtein A HC-650F	5 mL x 1	1000 Å	45 µm
0023432	ToyoScreen AF-rProtein A HC-650F	5 mL x 5	1000 Å	45 µm
0045063	ToyoScreen RoboColumns AF-rProtein A HC-650F	200 µL x 8	1000 Å	45 µm
0045064	ToyoScreen RoboColumns AF-rProtein A HC-650F	600 µL x 8	1000 Å	45 µm

Protein A ELISA

0023433	Protein A-R40 ELISA Kit	Kit		
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