TOSOH

CHROMATOGRAPHIC PROCESS MEDIA CATALOG

TOYOPEARL AND TSKGEL PROCESS RESINS

TOSOH BIOSCIENCE

1
TOSOH BIOSCIENCE GMBH
IM LEUSCHNERPARK 4
64347 GRIESHEIM
GERMANY

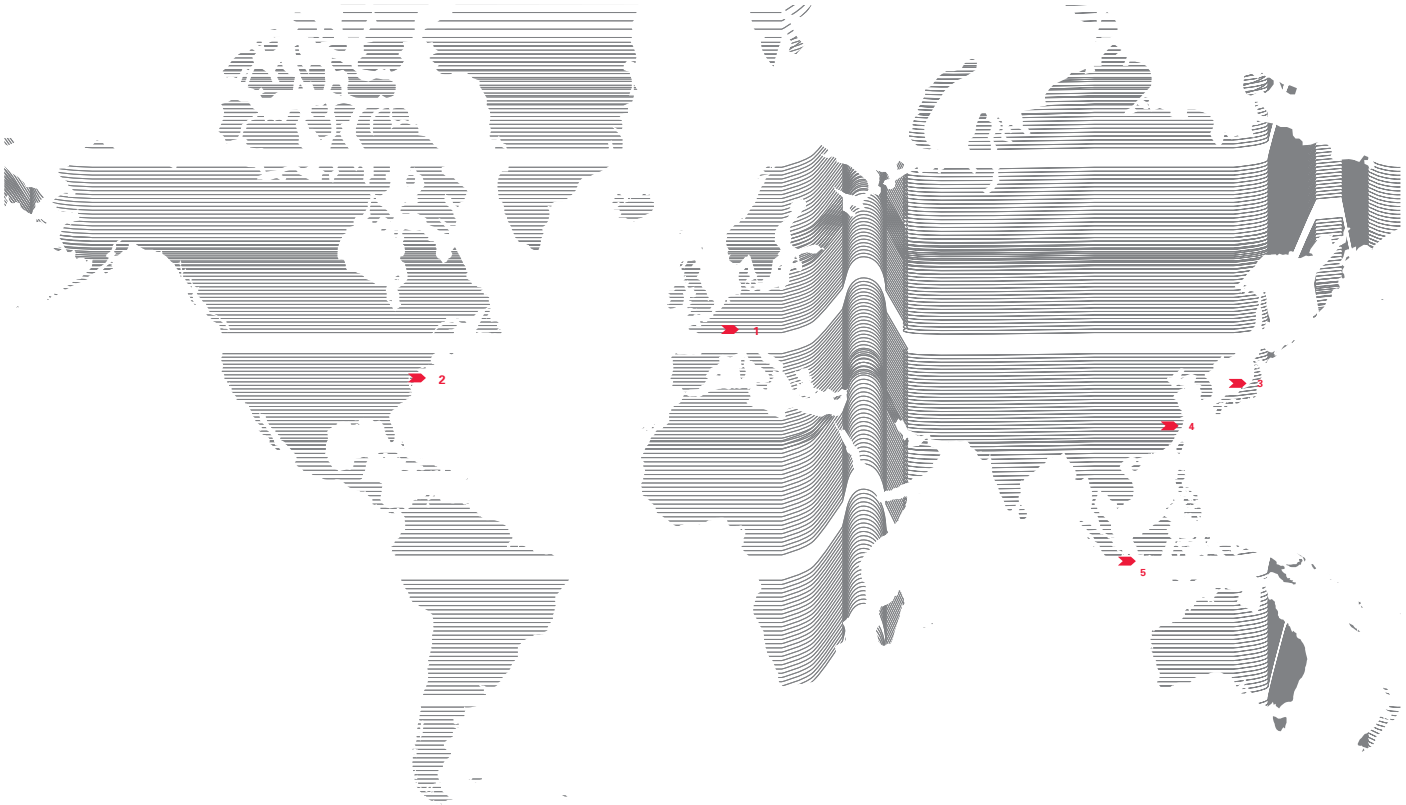
T + 49 (0) 6155 70437 00
INFO.TBG@TOSOH.COM
WWW.TOSOHBIOSCIENCE.DE

2
TOSOH BIOSCIENCE LLC
3604 HORIZON DRIVE,
SUITE 100
KING OF PRUSSIA, PA 19406, USA

T +1 484 805 1219
INFO.TBL@TOSOH.COM
WWW.SEPARATIONS.US.TOSOHBIOSCIENCE.COM

3
TOSOH CORPORATION
3-8-2 SHIBA, MINATO-KU
TOKYO 105-8623
JAPAN

T +81 3 5427 5118
INFO@TOSOH.CO.JP
WWW.TOSOHBIOSCIENCE.COM



4
TOSOH BIOSCIENCE SHANGHAI CO. LTD.
ROOM 1001, INNOV TOWER, BLOCK A,
1801 HONG MEI ROAD
XU HUI DISTRICT
SHANGHAI, 200233, CHINA
T +86 21 3461 0856
INFO@TOSOH.COM.CN
WWW.SEPARATIONS.ASIA.TOSOHBIOSCIENCE.COM

5
TOSOH ASIA PTE. LTD.
63 MARKET STREET #10-03
BANK OF SINGAPORE CENTRE
SINGAPORE 048942, SINGAPORE

T +65 6226 5106
INFO.TSAS@TOSOH.COM
WWW.SEPARATIONS.ASIA.TOSOHBIOSCIENCE.COM

TOSOH HISTORY

1935	Founding of Toyo Soda Manufacturing Co., Ltd.
1936	Operation of Nanyo Manufacturing Complex begins
1979	Tosoh develops TOYOPEARL media
1983	First TOYOPEARL hydrophobic interaction (HIC) resin
1995	Tosoh Nanyo gel factory receives ISO9001
2007	TOYOPEARL GigaCap high capacity ion exchange series starts
2012	A second TOYOPEARL production site doubles manufacturing capacity
2012	First TOYOPEARL multimodal resin
2013	High capacity TOYOPEARL Protein A resin for antibody purification introduced
2014	TOSOH Bioscience GmbH celebrates its 25th anniversary
2016	First salt-tolerant TOYOPEARL ion exchanger
2016	TOYOPEARL® Sulfate-650F receives the TMM Innovation Award 2016
2016	Construction of a third TOYOPEARL factory announced
2017	High capacity TOYOPEARL Protein L resin for antibody purification introduced
2017	Construction of a new R&D laboratory center announced
2017	Tosoh Bioscience Gmbh is awarded Best Global Chromatographic Solutions Supplier

NOMENCLATURE

What's in our names?

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

1. Resin Type

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

5. Additional Abbreviations

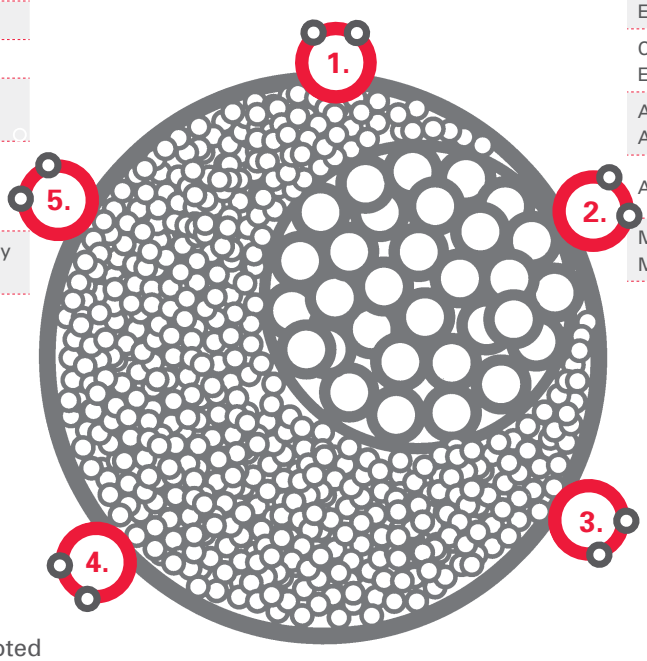
Some of our products have additional features or need clarification about what type of product they are.

We use the following abbreviations to highlight these features:	
HC	High Capacity
AR	Alkaline Resistant
MX	Mixed-Mode
AF	Affinity
Super	High Capacity Ion Exchanger
MegaCap	High Capacity Ion Exchanger for Capturing
GigaCap	Ultra High Capacity Ion Exchanger

2. Ligand

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

Mode	Ligand
HIC	Ether, PPG, Phenyl, Butyl, Hexyl
Anion Exchange	DEAE, QAE, Q, NH_2
Cation Exchange	CM, SP, Sulfate
Antibody Affinity	rProtein A, rProtein L
Affinity	Tresyl, Epoxy, Formyl, Amino, Chelate, Red, Heparin, Carboxy
Mixed-Mode	Tryptophan (Trp)



4. Particle Size

Particle size is typically denoted in the product name as letters or numbers denoting the grade.

Particle size of TOYOPEARL and TSKgel resins (µm)			
Grade	TOYOPEARL	TOYOPEARL GigaCap	TSKgel
EC	200		
C	100 (SEC resins are 75)		
M	65 (MX-Trp is 75)	75	
F	45		
S	35 (SEC resins are 30)	35	
(30)			30
(20)			20

3. Pore Size

TOYOPEARL or TSKgel resins are available in the following pore sizes:

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

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INTRODUCTION
ABOUT US

WITH A GLOBAL PERSPECTIVE.

We, the Separations team of Tosoh Bioscience GmbH, have been acknowledged as a global leader in the field of Bioseparations.

Tosoh Bioscience GmbH, a member of the Tosoh Group, markets and supports liquid chromatography solutions. Our product portfolio encompasses a comprehensive line of process media and prepacked HPLC columns for all modes of liquid chromatography and GPC instruments. We are the only supplier of consumable chromatography solutions in the biopharmaceutical market to offer expertise for all liquid chromatography solutions, from early stage discovery through clinical trials to large-scale production.

With a long history and extensive experience in chromatography, Tosoh Bioscience is more than a provider of analytical (U)HPLC columns and process resins – we have a proven track record of sound scientific knowledge and technical support to our customers.

INTRODUCTION

PRODUCTION

Tosoh's state of the art manufacturing sites in Japan provide products to the sales and support network across the world. The instruments, columns, media are manufactured at Tosoh's Nanyo Complex in the Yamaguchi prefecture at the south-western tip of the mainland of Japan. All chromatography products are shipped from this ISO 9001 registered facility. The Nanyo manufacturing complex is a self-contained city with its own power generation plants and port. It is a model of environmental responsibility and has earned ISO 14001 certification for environmental management.

SUPPLY CHAIN

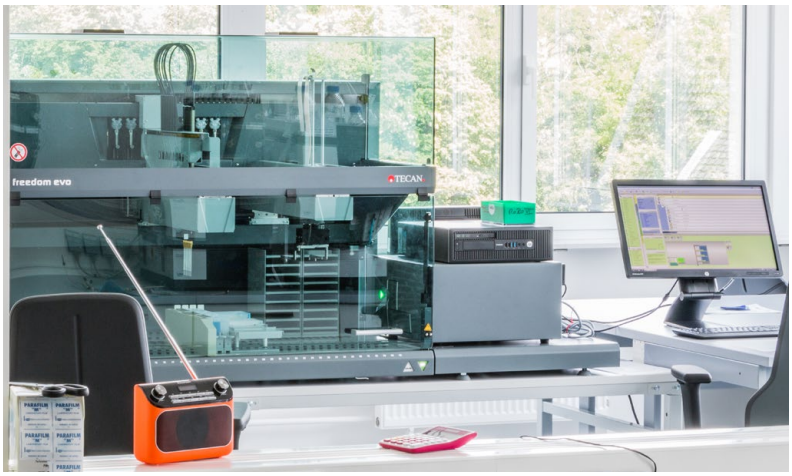
The Bioscience Division of Tosoh Corporation is headquartered in Tokyo, Japan. Tosoh Bioscience Separations in Griesheim, Germany houses all sales and marketing activities for the separation products. The Tosoh Bioscience customer service center is located in Tessenderlo, Belgium. In Tessenderlo, we inventory an extensive line of TSKgel® (U)HPLC columns and Process development columns. TOYOPEARL® and TSKgel PW bulk resin products are also inventoried at Tessenderlo in quantities suitable for resin screening or early GMP production. Larger volumes of our process resins are inventoried at the Tosoh Bioscience manufacturing site in Japan. In many cases, larger customer purchases are made to order. Please be sure to contact us in advance to obtain product availability and pricing for our process resins.

REGULATORY SUPPORT

In preparation for a filing of a new drug with the regulatory agencies it may be advisable to initiate a more detailed discussion about Tosoh Bioscience's products. Tosoh Bioscience recommends establishing a Confidential Information Disclosure Agreement (CIDA).
Tosoh Bioscience maintains Regulatory Support Files (RSF) on most of our process scale media. The file contains detailed information that describes the synthesis and quality control of our manufacturing process. In order to support your application for a new drug, please contact us through your Business Development Manager or at techsupport.tbg@tosoh.com if you would like a copy of the sections of the RSF relevant to the resin being used. Tosoh Bioscience does not disclose this information unless a valid CIDA is established first.

INTRODUCTION

TECHNICAL SUPPORT



Tosoh Bioscience offers a range of Technical Support services to our TSKgel, ToyoScreen, and TOYOPEARL chromatography products and EcoSEC® GPC instruments. We are committed to providing prompt and skilled service for these and other requests: to provide you with the right advice to select the best column, resin, or instrument for your application, to help you with product installation, method development, and troubleshooting, to guide you with packing TOYOPEARL and TSKgel resins into large production columns, to support you with regulatory files for a submission to the FDA.

One of the services that stand out in the industry is the Tosoh Chromatography Workshop Series, providing a comprehensive background to the chromatographic purification of biomolecules. These courses provide a balance of effective presentations and practical hands-on experience under the guidance of qualified tutors.

TOSOH'S TECHNOLOGY

For over forty years, our parent, Tosoh Corporation, has been a world leader in the analysis and purification of proteins. A thorough understanding of the role played by pore diameter and molecular size in chromatographic separations allows Tosoh to design higher performance resins for ion exchange, hydrophobic interaction, mixed mode and affinity applications.

From the research laboratory to full scale manufacturing, we offer the same polymer chemistries in our TSKgel and TOYOPEARL products. Whether you are scaling up from a TSKgel column HPLC method to TOYOPEARL resin for manufacturing, or are scaling down from TOYOPEARL resin based purification to the corresponding TSKgel column for the QC of your target, we make it easy to develop methods to do both.

INTRODUCTION PRODUCT LINES

TSKgel COLUMNS

Our TSKgel prepacked columns for (U)HPLC are used for the analysis and purification of proteins, peptides, biopolymers and low molecular weight compounds. For example, TSKgel SW-type silica-based columns are the biopharmaceutical industry's standard in gel filtration chromatography of biomolecules. Please see our website for more information.

TSKgel RESINS

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

TOYOPEARL RESINS

TOYOPEARL resins are hydrophilic macroporous methacrylic resins. Their rigid polymeric backbone has better pressure-flow properties than most other stationary phases. Therefore, higher linear velocities can be used for faster process throughput and decreased cycling times.

Most TOYOPEARL resins are stable over the pH 2-12 range for normal operating conditions and pH 1-13 for cleaning conditions. The resins are available in average particle sizes of 35 µm, 45 µm, 65 µm, 75 µm, and 100 µm for high resolution, intermediate purification, or capture chromatography. TOYOPEARL resins are also offered in many different pore diameters for size exclusion, ion exchange, hydrophobic interaction, multimodal, and affinity chromatography.

PREPACKED PROCESS DEVELOPMENT PRODUCTS

MiniChrom® Columns with 5 mL bed volume (8mm ID x 10 cm) are the most convenient tools for method development. They are available for most TOYOPEARL and some TSKgel resins.

ToyoScreen® process development columns are easy to use. They are available as 1 mL and 5 mL prepacked cartridges. Placed in the ToyoScreen holder they can be connected to most laboratory chromatographic systems for early development resin screening.

The most popular TOYOPEARL resins are also available in RoboColumn® format. RoboColumns are miniaturized chromatographic columns for operation with a robotic liquid handling system, such as the Freedom EVO® from TECAN. This approach allows automated high throughput, biochromatographic separations by running up to eight individual columns simultaneously.

Resin Seeker 96-well plates are disposable filter plates packed with TOYOPEARL resins available in several configurations for antibody affinity, ion exchange, HIC, and mixed-mode chromatography. Resin Seeker 96-well plates can be used to screen multiple steps of the purification process including binding, wash, and elution conditions in addition to resin selectivity, binding kinetics, purity, and recovery of your target molecule. Resin Seeker plates can be operated manually using a multi-channel pipette or in an automated system designed for high throughput screening.

INTRODUCTION CHROMATOGRAPHY IN DSP

The purification and recovery of a biological target molecule out of a tissue, cell or fermentation broth - the so-called downstream processing - usually requires a combination of separation technologies and involves more than one chromatographic unit operation.

Downstream processing operations are often divided into three groups describing the continuous improvements in purity and concentration of the product: Capture, Intermediate Purification and Polishing.

PRODUCT CAPTURE

The capture step is the first process step just downstream of the harvested feedstock. Its primary function is to *capture* the target and separate it from the most dominant impurities. The target is then eluted into a significantly smaller volume of buffer for further downstream processing. A resin is selected which has the best combination of dynamic binding capacity, mass recovery, and retention of the target's biological activity.

Column dimensions and resin particle sizes tend to be larger in capture steps. The feedstock viscosity, the amount of particulate, and the operational pressure limits of the chromatographic hardware used determine the most appropriate particle size for a capture step. However, modern resins combined with optimized processes allow for better results through capturing on smaller beads.

INTERMEDIATE PURIFICATION

The partially purified feedstock from the capture step will have a higher target concentration and relatively less complex impurity profile (also at higher concentrations). The intermediate purification step uses chromatography's resolving power to separate the target away from other impurities of the feedstock. Because of this, and the improved fluid flow properties, resin particles with smaller sizes are used. In many cases, more than one intermediate purification column may be necessary to achieve the desired degree of product purity.

POLISHING

The final column in a production train is often referred to as the polishing step. At this point, the feedstock contains a relatively high concentration of the target molecule with some select impurities or aggregates. The residual impurities, however, may be very closely related to the target and may be dimer, trimer, or isoforms of the target. Resins with high selectivity and highly efficient smaller particle sizes are typically used.

ARE YOU INTERESTED IN LEARNING MORE ABOUT THE BASICS OF CHROMATOGRAPHY? VISIT US ON YOUTUBE.
TOSOH BASICS - WHAT IS CHROMATOGRAPHY?
www.youtube.com/watch?v=2QVCxK0QPeg

INTRODUCTION BASE MATRIX

TSKgel RESINS

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

TOYOPEARL RESINS

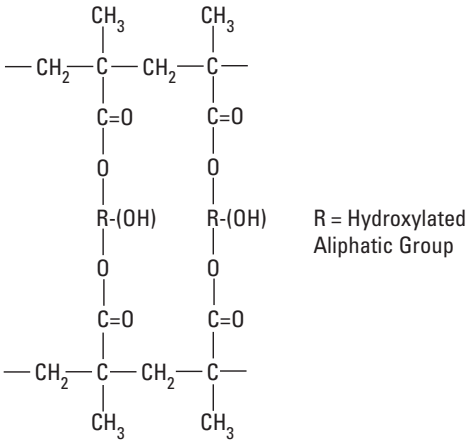
TOYOPEARL resins are macroporous resins specially suited for large-scale chromatographic applications. Their rigid polymeric backbone has better pressure-flow properties than most other commercially made materials. Therefore, higher linear operating velocities can be used for faster process throughput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

TOYOPEARL bare resins are stable over the pH 2-12 range for normal operating conditions and pH 1-13 for cleaning conditions. The resins are available in average particle sizes of 35 µm, 65 µm, 75 µm, and 100 µm for high resolution, intermediate purification, or capture chromatography. TOYOPEARL resins are also offered in many different pore diameters for size exclusion, ion exchange, hydrophobic interaction, and affinity chromatography. Pore diameter and surface area can be optimized to ensure excellent kinetic access and binding capacity of your target regardless of molecular size.

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

FIGURE 1

RESIN CHEMISTRY OF TOYOPEARL SEC RESINS (HYDROXYLATED ACRYLIC)



RESIN CHEMISTRY

TOYOPEARL base resins are highly hydroxylated polymethacrylic polymer beads (Figure 1). Their surface hydroxyl groups render them very hydrophilic and useful for protein separations. TOYOPEARL products including the functionalized materials seen in later catalog sections, have the least non-specific binding of any chromatographic resin. This is of particular note for separations such as blood factors where backbone interactions with the feedstock may result in decreased recovery of the targets. Their semi-rigid polymeric nature also gives them better pressure-flow characteristics than softer materials such as agarose.

PORE SIZE

Commercial TOYOPEARL HW-type size exclusion materials are available in 5 pore sizes (see page 3).

FEATURES	BENEFITS
Large range of particle sizes available	Solutions for all DSP needs
Hydrophilic porous polymer structure	Minimal non-specific adsorption effects
Narrow particle size distribution	High performance chromatography – more efficient separations better pressure-flow characteristics
Good mechanical stability	Excellent flow characteristics in large industrial size columns
Chemically stable (pH 2 – 14)	Constant packing volume over a wide range of salt concentrations Compatible with organic solvents, can be cleaned in place (CIP) with acid or base Stable polymer may be run at elevated temperature (4 - 60 °C) autoclavable at 121 °C
Identical resin structure to TSKgel HPLC resins	Direct scale-up from TSKgel HPLC columns

INTRODUCTION BASE MATRIX

MECHANICAL & CHEMICAL STABILITY

TOYOPEARL resins remain dimensionally stable within wide extremes of pH and ionic strength.

Moreover, the semi-rigid polymeric backbone of TOYOPEARL and TSKgel resins permits high flow rates for maximum throughput and productivity. TOYOPEARL resins may be operated up to 6 bar and TSKgel PW-type resins may be operated up to 20 bar. These properties of the resins combined with the narrow particle size distributions result in superior pressure-flow characteristics for the packed TOYOPEARL bed. Linear velocities of 300 - 500 cm/h generate a pressure of between 1 and 2 bar in a 20 cm length bed. Achievement of high linear velocities at relatively low pressure enables high throughput production scale chromatography using equipment with moderate pressure limitations.

Sanitization or cleaning may be conducted with up to 1.0 mol/L NaOH or 1.0 mol/L HCl depending upon the ligand.

PARTICLE SIZE OPTIMIZATION

TOYOPEARL and TSKgel PW-type methacrylic base beads incorporating the same polymer chemistry are available in a variety of particle sizes:

200 µm	TOYOPEARL EC-grade	Capture
100 µm	TOYOPEARL C-grade	Capture
65 µm	TOYOPEARL M-grade	Intermediate Purification
35 µm	TOYOPEARL S-grade	Intermediate Purification/Polishing
30 & 20 µm	TSKgel PW-type	High Resolution

NOMENCLATURE OF THE DIFFERENT RESINS SUCH AS TYPE OF BASE MATRIX, LIGAND, PORE SIZE, PARTICLE SIZE AND ABBREVIATIONS CAN BE FOUND AS A READING COMPANION ON PAGE 3.

WHAT'S NEW

TOYOPEARL AF-rProtein L-650F - PAGE 18

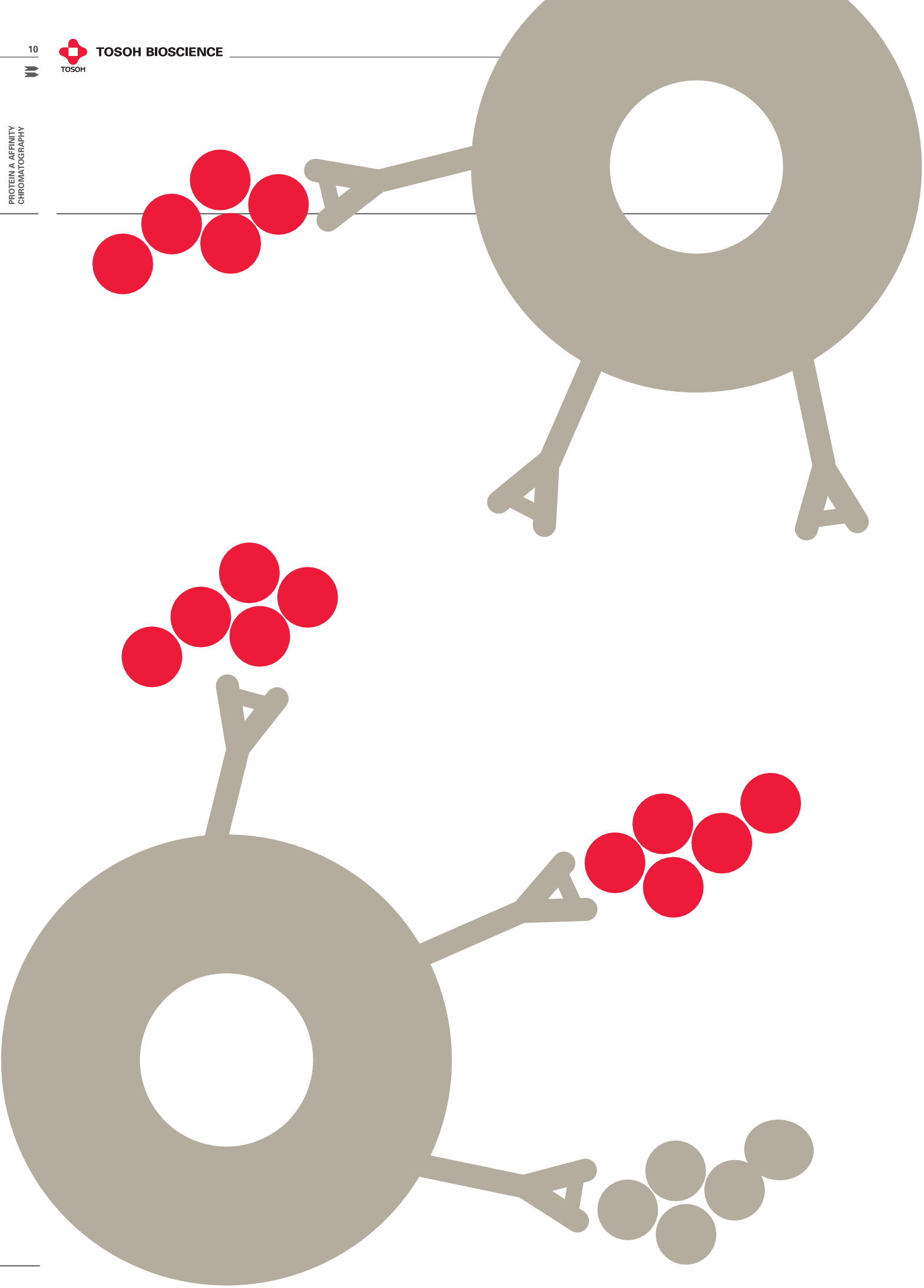
- Capturing of targets that do not bind to Protein A
- Recombinant ligand with high chemical stability
- Improves economics of Protein L unit operations
- Binds a broad range of antibody related targets

TOYOPEARL NH_2-750F - PAGE 35

- High protein binding capacity at elevated ion strength
- Unique selectivity, differing from conventional AEX media
- Binds targets at pH close to isoelectric point
- Aggregate removal in flow-through or bind/elute mode

TOYOPEARL Sulfate-650F - PAGE 33

- High protein binding capacity at elevated ion strength
- Unique selectivity, differing from conventional CEX media
- MAb aggregate removal in bind-elute mode
- Affinity capturing of selected targets



AFC ANTIBODY AFFINITY CHROMATOGRAPHY

ANTIBODY AFFINITY PRODUCTS

- PROTEIN A AFFINITY
  TOYOPEARL AF-rProtein A HC-650F
  TOYOPEARL AF-rProtein A-650F

- PROTEIN L AFFINITY
  TOYOPEARL AF-rProtein L-650F NEW

> TOYOPEARL AF-rProtein L-650F is an innovative and very useful chromatographic resin in my purification toolbox, as it allows capture of multiple antibody types. It is the resin I've been expecting for many years.
>
> Dr. Michael Davids
> Davids Biotechnologie

ANTIBODY AFC HIGHLIGHTS

HIGHLIGHTS PROTEIN A HC

- 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

HIGHLIGHTS PROTEIN L

- Capturing of targets that do not bind to Protein A
- Highest binding capacity available on the market
- Binds a broad range of antibody related targets
- Recombinant ligand with high chemical stability
- Improves economics of Protein L unit operations

FEATURES	BENEFITS
High binding capacity	Increased productivity of antibody purification Lower buffer consumption
Recombinant Protein A/L ligand	Alkaline stable Low Protein A/L leakage
TOYOPEARL polymer matrix	High mechanical stability High chemical stability

ANTIBODY AFC HOW DOES IT WORK?

PROTEIN A CHROMATOGRAPHY – HOW DOES IT WORK?

Protein A affinity chromatography is the most commonly used capture step in antibody purification processes. Its high specificity for the binding of human immunoglobulin allows highly selective capturing of the target protein out of cell culture supernatant. The Protein A capture step is most often followed by ion exchange, HIC and/or mixed-mode polishing steps in order to remove nucleic acids, aggregates, viruses, and leached Protein A.

Protein A is a 40-60 kDa surface protein originally found in the cell wall of the bacteria Staphylococcus aureus. Protein A and its recombinant derivatives bind the Fc region of immunoglobulins through interaction with the heavy chain. The first Protein A affinity resins were introduced in the 1970s based on native Protein A ligands. These media suffered from insufficient alkaline stability, which limited the cleaning in place options for process use. State-of-the-art Protein A resins carry recombinant Protein A variants genetically engineered to provide maximum IgG affinity and base stability.

The binding strength of Protein A for IgG depends on the source species of the immunoglobulin as well as the subclass of IgG. The standard protocol for antibody purification by Protein A chromatography involves loading of the feedstock at physiological pH and ionic strength, washing unbound substances of the column with loading buffer and elution of the bound immunoglobulins by lowering the pH. The change in pH alters the degree of ionization of charged groups on the ligand and the bound antibody thus reducing the affinity. The fractions can be collected into neutralization buffer to return to a neutral pH.

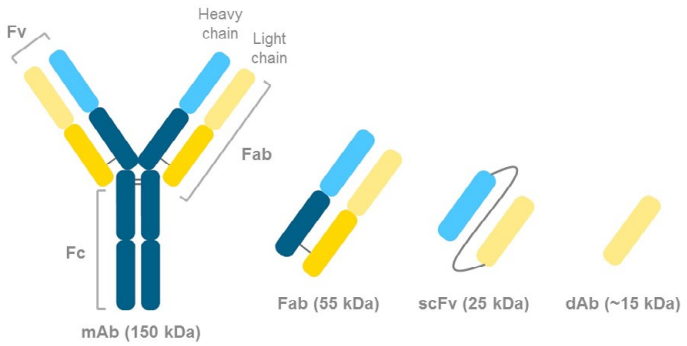
PROTEIN L CHROMATOGRAPHY – HOW DOES IT WORK?

Protein L based affinity chromatography is used for the capture of antibodies and antibody fragments that do not bind to Protein A. Unlike Protein A and G, which bind to the Fc region of immunoglobulins (IgGs), Protein L binds through interactions with the variable region of an antibody's kappa light chain. Therefore Protein L binds a wider range of antibody classes than Protein A. Figure 1 shows typical targets, such as antigen binding fragments (Fabs), single-chain variable fragments (scFvs) and domain antibodies (dAbs).

Native Protein L is expressed from Peptostreptococcus magnus. Similarly to Protein A affinity resins, modern Protein L affinity resins are based on recombinant proteins, allowing for higher binding capacity and stability.

FIGURE 1

TYPICAL TARGETS FOR PROTEIN L AFFINITY CHROMATOGRAPHY



Protein L binds to the variable region of the kappa light chain

FIGURE 2

AFFINITY CHROMATOGRAPHY ILLUSTRATION

ANTIBODY AFC
OVERVIEW OF ANTIBODY AFFINITY LIGANDS

TOYOPEARL PROTEIN A RESINS

The ligands of all TOYOPEARL Protein A resins are recombinant Protein A variants expressed in E. coli. They are derived from one of the IgG binding domains of Protein A. The amino acid sequence is optimized in order to increase the protein's stability towards alkaline solutions and to introduce additional lysine residues that can be utilized for multipoint attachment of the ligand to the TOYOPEARL matrix. The ligand of TOYOPEARL AF-rProtein A-650F consists of a tetramer of these modified Protein A C domains. For the ultra-high capacity TOYOPEARL AF-rProtein A HC-650F this domain was further optimized and expressed as a hexamer in order to further increase IgG binding capacity (Figure 3).

Multipoint attachment of the ligand to the TOYOPEARL matrix enhances the chemical and thermal stability of the resin. In practice this pays off for a low level of Protein A leaching and for a high resistance to alkaline solutions.

TOYOPEARL PROTEIN L RESINS

TOYOPEARL AF-rProtein L-650F is an affinity chromatography resin that combines a rigid polymer matrix with a recombinant ligand, which is derived from the B4 domain of native Protein L from Peptostreptococcus magnus and is expressed in E.coli (Figure 4). Code optimization of the domain results in higher binding capacity and an improved stability of the ligand compared to the native molecule.

FIGURE 3

RECOMBINANT PROTEIN A DERIVED LIGANDS

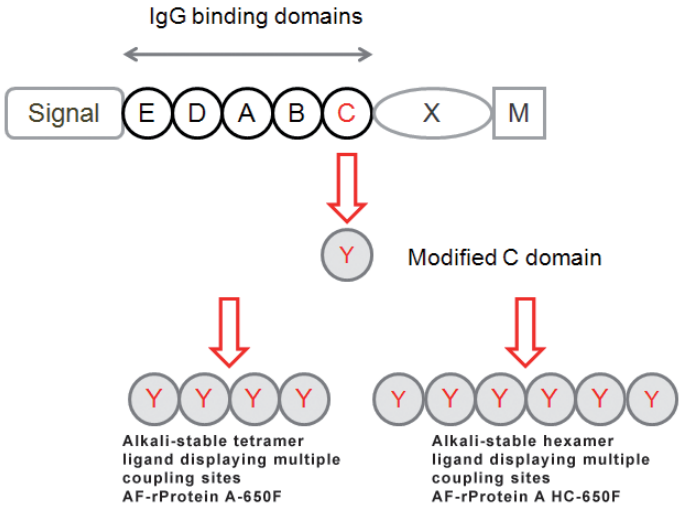
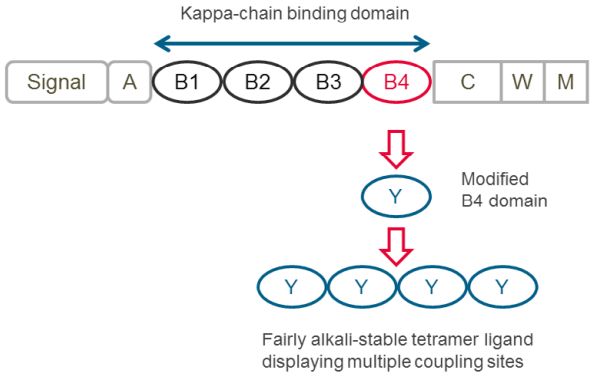


FIGURE 4

KAPPA CHAIN BINDING DOMAINS



ULTRA-HIGH CAPACITY TOYOPEARL AF-rProtein A HC-650F

TOYOPEARL AF-rProtein A HC-650F allows to increase productivity and to reduce production costs:

- It excels over other commerically available Protein A media. IgG DBCs over 100 g/L can even be observed at higher titers. This prevents DSP bottlenecks even at short residence times.
- Its alkali stability allows at least 300 Cleaning-in-Place (CIP) cycles (at 0,2 M NaOH) without significant reduction in dynamic binding capacity.

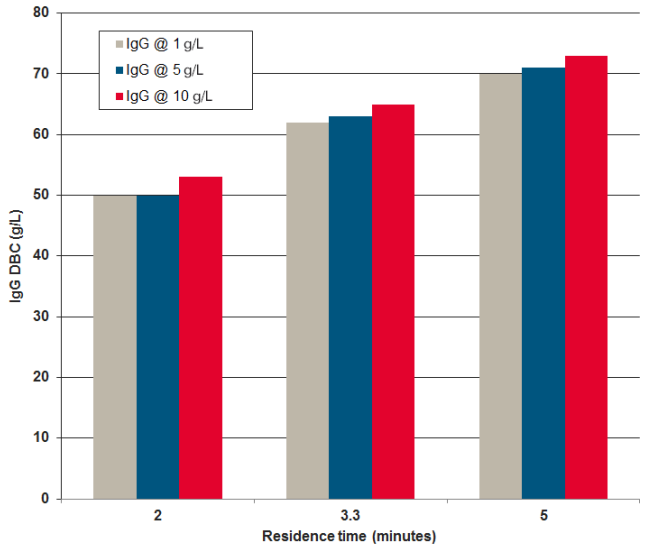
TOYOPEARL AF-rProtein A HC-650F is the newest Protein A affinity resin introduced by Tosoh Bioscience. It exhibits dynamic binding capacities of greater than 65 g/L at residence times of 5 minutes and greater than 50 g/L at 2 minutes residence time with feed stock concentrations from 1.0 g/L to 10.0 g/L (Figure 5).

Improved mass transfer characteristics allow it to maintain a larger percent of its capacity at lower residence times relative to agarose based, caustic stable resins (Figure 6).

The multipoint attachment of the enhanced recombinant Protein A ligand to the TOYOPEARL matrix is resulting in excellent base stability for up to 200 CIP cycles with 0.1 mol/L NaOH at 15 min contact time (Figure 7). It maintains 80% of initial dynamic binding capacity after 40 CIP cycles with 0.5 mol/L NaOH (Figure 8).

FIGURE 5

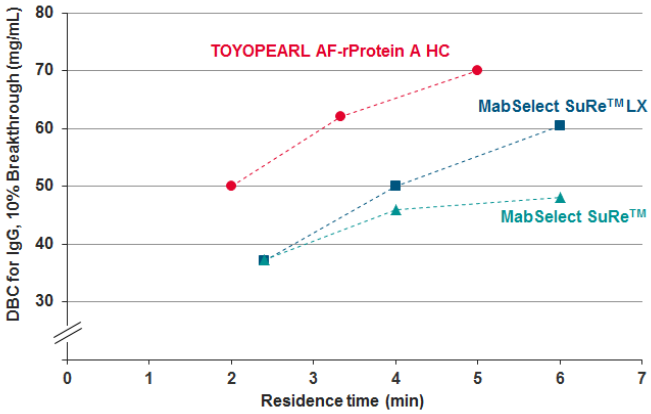
DBC AT VARIOUS LOADS AND RESIDENCE TIMES



Column: TOYOPEARL AF-rProtein A HC-650F (5 mm ID x 5 cm L)
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

FIGURE 6

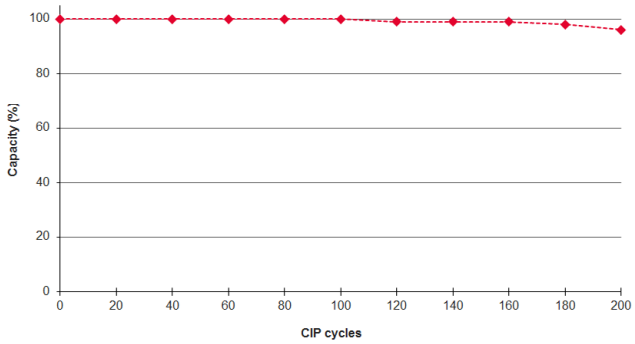
DBC OF HIGH CAPACITY PROTEIN A MEDIA



Column: TOYOPEARL AF-rProtein A HC-650F (5 mm ID x 5 cm L)
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 g/L 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.

FIGURE 7

CIP STUDY WITH 0.1 M NaOH



Column size: 5 mm ID x 5 cm L; Wash procedure: A: 20 mmol/L Na_2HPO_4 0.15 mol/L NaCl, pH 7.4 (10 CV)
B: 0.1 mol/L citrate, pH 3.0 (5 CV)
C: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (7 CV)
D: 0.1 mol/L NaOH (3 CV – 15 min contact time)
E: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (5 CV)

ULTRA-HIGH CAPACITY TOYOPEARL AF-rProtein A HC-650F

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

Achievement of high linear velocities at relatively low pressure enables high throughput at production scale using equipment with moderate pressure limitations (Figure 9).

TABLE I

PROTEIN A LIGAND LEAKAGE

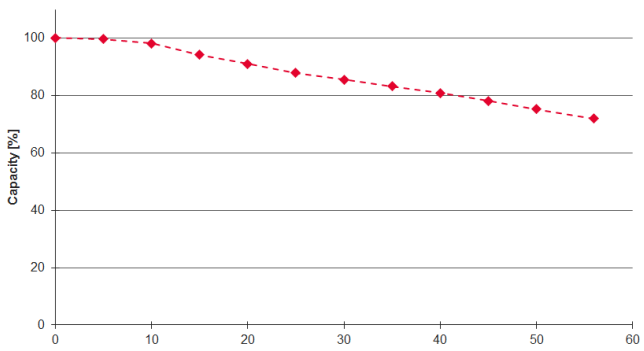
Amount of ligand leakage	Before CIP		After 200 CIP cycles	
	Elution Buffer		Elution Buffer	
	citrate (pH 3.0)	glycine-HCl (pH 3.0)	citrate (pH 3.0)	glycine-HCl (pH 3.0)
(ppm)	1.7	1.6	0.6	0.5

Amount of ligand leakage was determined with TOYOPEARL AF-rProtein A HC-650F ELISA; ppm=µg/g IgG

MORE INFORMATION

FIGURE 8

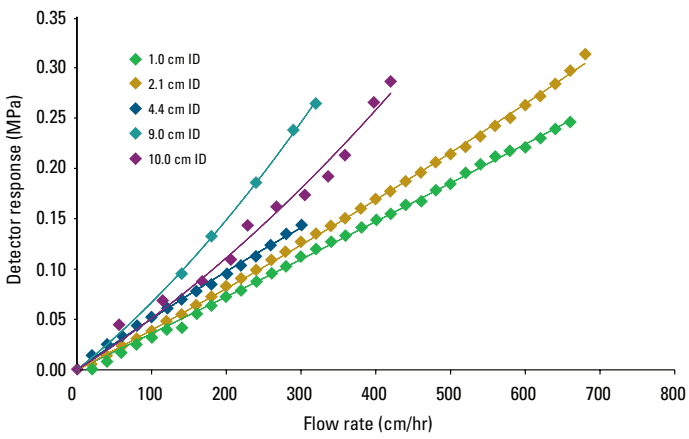
CIP STUDY WITH 0.5 M NaOH



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

FIGURE 9

PRESSURE/FLOW CURVE



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 H_2O

TOYOPEARL AF-rProtein A-650F

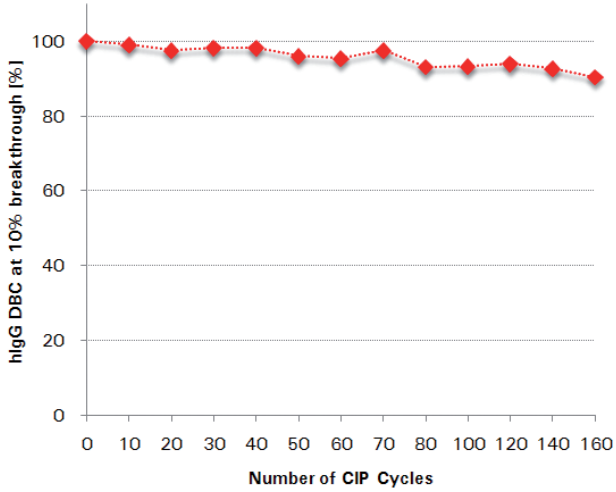
The standard TOYOPEARL AF-rProtein A-650F resin binds human and mouse immunoglobulin G, IgM, and Fab fragments. Typical static IgG binding capacity is > 45 mg/mL resin and typical dynamic IgG binding capacity at 10 % breakthrough is > 30 mg/mL resin at 2 minutes residence time (1 mg/mL protein load). Fast mass transfer kinetics support high binding capacities at high flow rates. IgG breakthrough curves (Figure 10) at various linear velocities demonstrate the superior kinetic performance of TOYOPEARL AF-rProtein A-650F.

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 11). When performing cleaning-in-place with 0.5 M NaOH the resin maintains about 80 % of IgG binding capacity after 50 cycles.

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. Figure 12 shows the thermal stability of the resin. 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.

FIGURE 11

CLEANING-IN-PLACE STUDY WITH 0.1 M NaOH

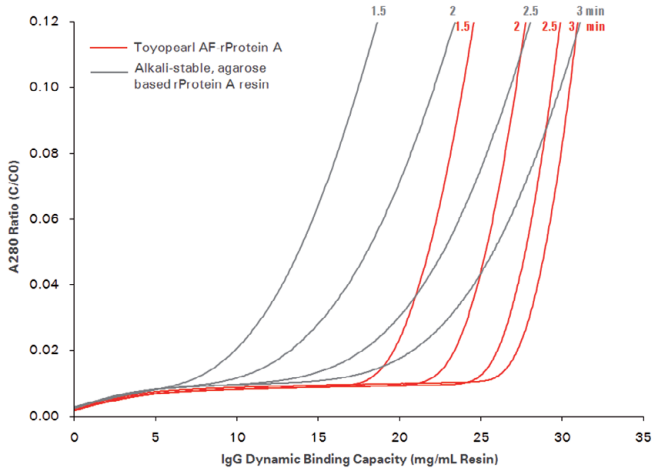


Column: 5 mm ID x 5 cm L
10 column volumes binding buffer pH 7.4
5 column volumes elution buffer pH 3.0
3 column volumes, 0.1 mol/L NaOH,
16 min contact time
3 column volumes binding buffer pH 7.4

MORE INFORMATION

FIGURE 10

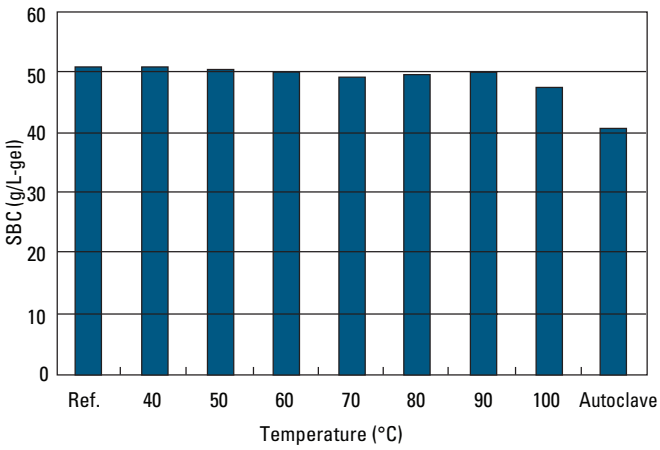
DYNAMIC BINDING CAPACITY



Breakthrough curves for h-IgG loading (polyclonal, 10 g/L) Typical DBC at 10 % breakthrough: 30,5 g/L @ 100 cm/h (3 min residence time) - 24 g/L @ 200 cm/h (1.5 min residence time); Column: 5 mm ID x 5 cm L; Mobile phase: 20 mmol/L sodium phosphate buffer pH 7.2 containing 150 mmol/L NaCl; Residence time: 1.5, 2.0, 2.5, 3.0 min

FIGURE 12

TEMPERATURE STABILITY



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

TOYOPEARL AF-rProtein L-650F

TOYOPEARL AF-rProtein L-650F can considerably improve process economics of Protein L capturing steps:

- It excels all other commercially available protein L media with regards to binding capacity and robustness.
- It is especially suited for the purification of new antibody formats such as antibody fragments, single chain variable fragments, domain antibodies and immunoglobulin types that cannot be purified with Protein A media.

TOYOPEARL AF-rProtein L-650F has high affinity for a large number of antibody formats. Table II shows the typical Protein L binding affinities to antibody classes and fragments of various species.

TOSOH BIOSCIENCE

TABLE II

PROTEIN L SELECTIVITY

Species	Class	Affinity
GENERAL	Kappa light chain	++
	Lambda light chain	-
	Heavy chain	-
	Fab	++
	ScFv	++
	Dab	++
HUMAN	IgG (1-4)	+
	IgA	+
	IgD	+
	IgE	+
	IgM	+
MOUSE	IgG1	+
	IgG2a	+
	IgG2b	+
	IgA	+
	IgM	+
RAT	IgG1	+
	IgG2a, b, c	+
	IgA	+
HEN*	IgM	+
	IgY	+

Valid if appropriate kappa light chains are available for binding.
*The results for hen immunoglobulins have been obtained through customer collaboration.

HIGHEST BINDING CAPACITY

The combination of an optimized recombinant ligand and the proven TOYOPEARL base matrix results in a resin that provides the highest binding capacity available on the market for Fab molecules (Figure 13). Due to the excellent mass transfer characteristics of TOYOPEARL AF-rProtein L-650F, especially dynamic binding capacities at 1 to 3 minutes residence time excel capacities obtained with the agarose-based resin.

As the molecular weight of fragments is much smaller compared to full length IgGs, a dynamic binding capacity of about 50 mg/mL for a Fab with a typical molecular weight of 55 kDa equals a DBC of >130 mg/L for a ~150 kDa IgG when considering molar binding capacities.

IMPROVED PROCESS ECONOMICS

Resin costs represent a considerable part of the overall production costs. The high binding capacity of the new protein L resin can remarkably improve process economics in the production of antibody related recombinant molecules.

RIGID MATRIX ALLOWS HIGH FLOW RATES

TOYOPEARL AF-rProtein L-650F is based on the well proven polymethacrylate matrix used for all TOYOPEARL resins. Figure 14 shows the pressure/flow curve for TOYOPEARL AF-rProtein L-650F packed in a 4.4 cm column with a bed height of 28 cm. Linear velocities up to 600 cm /h can easily be applied to TOYOPEARL Protein L columns.

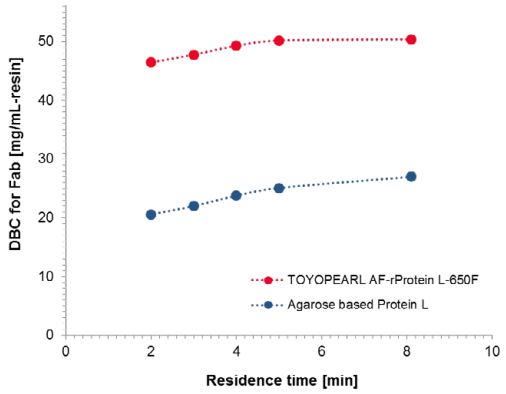
CHEMICAL STABILITY

The multipoint attachment of the ligand results in a fairly high chemical stability. Figure 15 proves the robustness of the resin towards moderate alkaline solution (0.1 M NaOH) in comparison to a competitor Protein L resin.

TOYOPEARL AF-rProtein L-650F

FIGURE 13

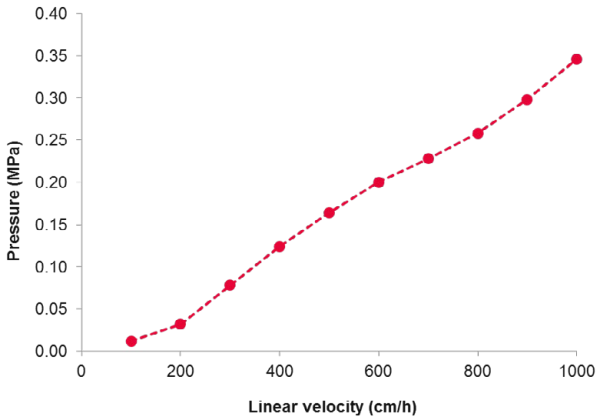
DYNAMIC BINDING CAPACITY OF PROTEIN L MEDIA FOR Fab



Column: TOYOPEARL AF-rProtein L-650F/competitor resin
4.6 mm ID × 50 mm (0.83 mL)
Detection: UV@280 nm
Sample: 2 g/L human Fab in 0.1 mol/L Na-Phosphate (pH 6.5)
DBC measured at 10 % Breakthrough

FIGURE 14

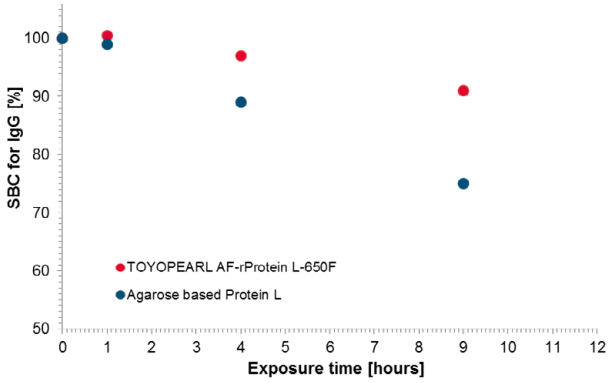
PRESSURE/FLOW CURVE OF TOYOPEARL AF-rProtein L-650F



Column size: 4.4 cm ID × 28 cm; Mobile phase: H_2O

FIGURE 15

ALKALI-STABILITY OF TOYOPEARL PROTEIN L IN 0.1 M NaOH



Static binding capacity for IgG in relation to initial binding capacity (100%) after exposure to 0.1 M NaOH.

MORE INFORMATION

ANTIBODY AFC - APPLICATIONS
PURIFY MONOCLONAL ANTIBODIES

Typically antibodies are captured at near neutral pH 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 low pH. 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 one of our screening tools: ResinSeeker plates, robotic high-throughput screening ToyoScreen RoboColumns, or pre-packed ToyoScreen or MiniChrom columns.

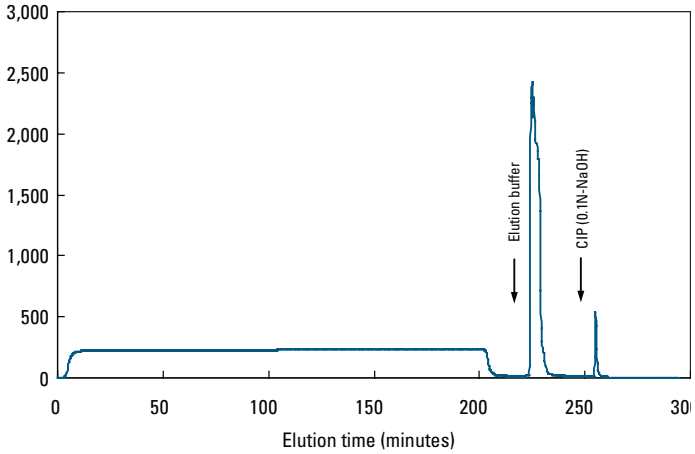
Suitable load/wash buffers are 20-100 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.2 - 7.5 or 100 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.2 - 7.5. Washing at reduced pH (e.g. pH 6) might further improve host cell protein reduction. Suitable elution buffers are 100 mmol/L citrate, 100 mmol/L acetate, or 100 mmol/L glycine-HCl. The pH shift required for mAb elution depends on the 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.

TOYOPEARL AF-rProtein A HC-650F was used for the purification of a monoclonal antibody from CHO cell culture supernatant with a concentration of 1.0 g/L (Figure 16) at 5 minutes residence time in a 5 cm bed height column. As can be seen from the chromatogram, tailing is minimal on the elution peak and the eluted mAb is > 95% pure by SEC.

Figure 17 shows the binding capacities for the capturing of a therapeutic monoclonal IgG1 spiked at different concentrations into CHO cell culture fluid. The binding capacity of 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 a capacity of more than 100 mg mAb/mL resin was reached even at 1 minute residence time.

FIGURE 16

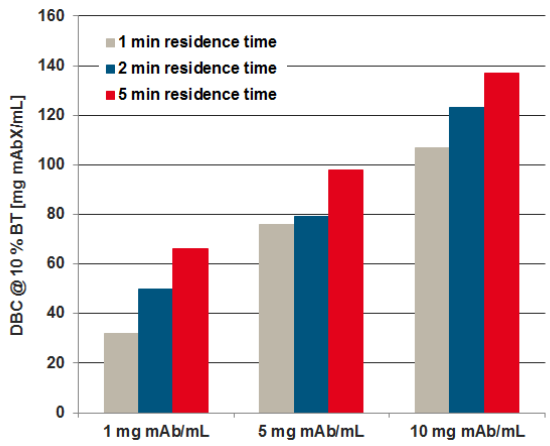
PURIFICATION OF MONOCLONAL ANTIBODY



Resin: TOYOPEARL Protein A HC-650F; Column size: 5 mm ID × 5.0 cm L; Mobile phase: Binding buffer: 20 mmol/L sodium phosphate containing 0.15 mol/L NaCl, pH 7.4, Elution buffer: 0.1 mol/L citrate, pH 3.0; Flow rate: 61 cm/h (0.2 mL/min); Residence time: 5 min; Sample: 40 mL of CHO cell culture, containing 1.0 g/L humanized IgG1

FIGURE 17

DBC FOR A SPECIFIC mAb AT VARIOUS LOADS AND VELOCITIES



Column: TOYOPEARL AF-rProtein A HC-650F (6.6 mm ID × 2 cm L)
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

ANTIBODY AFC - APPLICATIONS REDUCE PRODUCTION COST

With increasing pressures on producers of biopharmaceutical drugs to reduce productions costs, the use of the high capacity TOYOPEARL AF-rProtein A HC resin is a superior way to achieve this goal without making any sacrifice to product quality or process time.

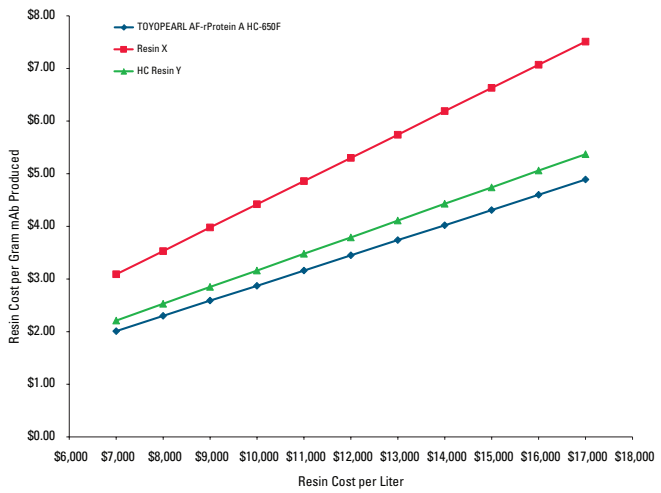
With pressures mounting to reduce production costs at many companies, and Protein A being the most expensive resin used in mAb purification, the use of a high capacity Protein A resin can significantly impact the overall cost of doing business. This report details how using a high capacity Protein A resin will reduce production costs, on a per-gram produced basis, for companies that implement its use in their chromatography platform.

TOYOPEARL AF-rProtein A HC-650F and two other commercially available Protein A resins, one of them also a "high capacity" resin, were compared on a cost-per-gram of mAb produced basis (Figure 18).

For in-depth comparison, the median resin price of $12,000 per liter was used as a basis to determine comparative production costs between the three resins tested.

FIGURE 18

COLUMNS OF EQUAL VOLUME



At a column capacity of approximately 735 grams per load, the TOYOPEARL AF-rProtein A HC-650F had the lowest cost per gram of antibody purified at every similar price point.

Three configurations were examined to model what the resin costs would be in columns that were packed to have equal capacity, equal resin volume, and equal column dimensions. These three configurations were chosen to reflect the ways high capacity Protein A resins could be instituted by individual companies, and also allows for a more complete look at the effects of resin capacity than just a single column configuration.

In this excerpt, the configuration with columns of equal volume is depicted (Table III). The following values were held constant:

Residence time:	3 minutes
DBC:	3 minute residence time (from product literature)
Column load:	80% of stated dynamic binding capacity
Harvest titer:	3 g/L
Harvest volume:	2000 L
Column lifetime:	100 cycles
Column yield:	95%

CONCLUSION

As can be seen from the comparisons, making use of a high capacity Protein A resin in your purification process is an excellent way to save on production costs. At the median resin price of $12,000 per liter, the TOYOPEARL AF-rProtein A HC-650F resin would save customers almost $2.00 per gram of antibody produced over a resin with a capacity of 35 g/L and almost $0.50 per gram over a competitive high capacity resin (Table III and IV).

ANTIBODY AFC - APPLICATIONS REDUCE PRODUCTION COST

TABLE III

RESIN	FLOW RATE (cm/h)	HARVEST TITER (g/L)	HARVEST VOLUME (L)	COLUMN CYCLES PER HARVEST	COLUMN LIFETIME (CYCLES)	COLUMN LIFETIME (HARVESTS)	YIELD	COLUMN LIFETIME PRODUCTIVITY (g)	RESIN COST PER GRAM
TOYOPEARL AF-rProtein A HC-650F	300	3	2000	9	100	11	95%	69,587	$3.45
Resin X	420	3	2000	9	100	11	95%	70,160	$5.30
High capacity resin Y	360	3	2000	9	100	11	95%	69,587	$3.79

TABLE IV

RESIN	RESIN COST PER GRAM AT MEDIAN RESIN PRICE ($12,000)	COMPETITOR RESIN PRICE PER LITER NEEDED TO EQUAL TOSOH
TOYOPEARL AF-rProtein A HC-650F	$3.45	
Resin X	$5.30	$7,500
High capacity resin Y	$3.79	$11,000

MORE INFORMATION

ANTIBODY AFC - APPLICATIONS IMPROVE PERFORMANCE OF CAPTURING STEP

TOYOPEARL AF-rProtein A HC-650F is highly beneficial with regards to process economics. Potential drawbacks with regards to CHOP clearance depend on a particular feedstream and can be compensated by using post-load wash steps. Different post-load wash solutions increase host cell protein removal of TOYOPEARL AF-rProtein A HC-650F.

One drawback of modern Protein A resins is that host cell protein (HCP) clearance can be - depending on the feedstream - comparatively lower than for standard Protein A affinity resins. When aiming for a 2-step platform process, no compromises can be made with regards to HCP removal of the capturing step. The remaining impurity burden would have to be covered by just one subsequent chromatography step. Hence, development of strategies or procedures to reduce the HCP content of the Protein A elution pool are key for the development of such purification processes. Post load washing steps during Protein A chromatography may improve HCP clearance of the capturing step. However, the employed wash buffers must not affect other product related quality criteria, such as aggregate levels and mAb activity. Besides, the impact of different wash steps on the parameters determining process economics, that is product recovery, dynamic binding capacity and resin lifetime, should be evaluated.

HCP CLEARANCE

CHOP log reduction values have been calculated and are shown in Figure 19. The employed reference protocol using 20 mM sodium phosphate, pH 7.4 + 150 mM sodium chloride has a log reduction value of 1.85. Addition of 500 mM arginine, which is also well-known for its positive effects on recovery and mAb stability against aggregation, increases the log reduction value to 2.15. Further increase of the log reduction value for CHOP removal can be achieved using guanidinium hydrochloride for post-load washing. A log reduction value of 2.80 can be reached. The use of arginine or chaotropic wash solutions is documented in literature.

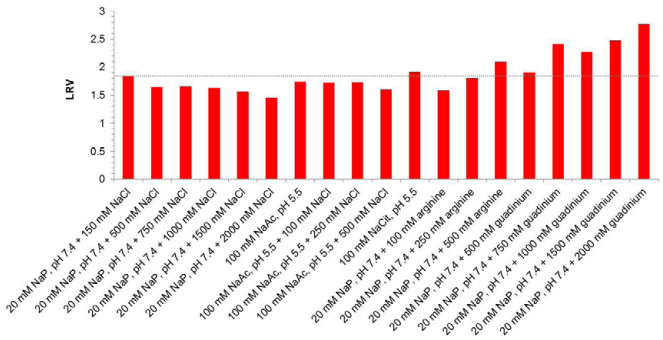
RECOVERY

Recoveries were calculated from the UV absorbance @280 nm. A graph with the corresponding data is shown in Figure 20. Roughly, mAb concentrations of the elute pools range from 9.6 mg/mL to 11.0 mg/mL. Recovery slightly decreases in case of 100 mM sodium acetate, pH 5.5 and phosphate buffer, pH 7.4, containing high concentrations of arginine and guanidinium hydrochloride. However, recoveries exceed 90 % in all cases. Hence, these wash solutions can still be considered useful.

MORE INFORMATION

FIGURE 19

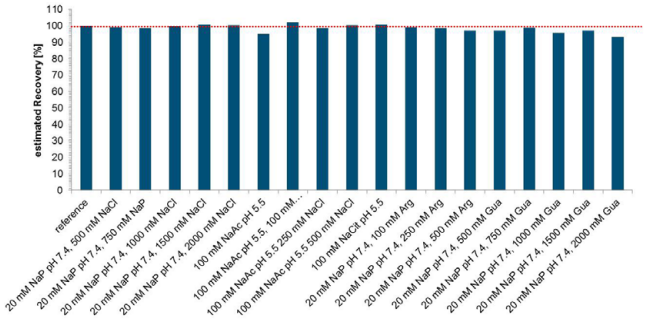
CHOP LOG REDUCTION VALUES



CHOP log reduction values of the parallel chromatography Protein A experiments using different buffers for wash 2. Comparatively higher log reduction values can be achieved using arginine and guanidinium hydrochloride for wash 2

FIGURE 20

RECOVERY



Recovery from Protein A after application of different wash buffers. Recovery is slightly affected by post-load washing with 100 mM sodium acetate, pH 5.5 and high contents of arginine or guanidinium hydrochloride. Recovery is greater than 90 % in all cases.

ANTIBODY AFC
ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

ToyoScreen

PART #	PRODUCT DESCRIPTION	PACKAGE
0023430	ToyoScreen AF-rProtein A HC-650F	1 mL x 5
0023431		5 mL x 1
0023432		5 mL x 5
0022809	ToyoScreen AF-rProtein A-650F	1 mL x 5
0022810		5 mL x 1
0022811		5 mL x 5
0023494	ToyoScreen AF-rProtein L-650F NEW	1 mL x 5
0023495		5 mL x 1
0023496		5 mL x 5

RoboColumns

PART #	PRODUCT DESCRIPTION	PACKAGE
0045061	RoboColumn AF-rProtein A-650F	200 µL x 8
0045062		600 µL x 8
0045063	RoboColumn AF-rProtein A HC-650F	200 µL x 8
0045064		600 µL x 8
0045065	RoboColumn AF-rProtein L-650F NEW	200 µL x 8
0045066		600 µL x 8

MiniChrom

PART #	PRODUCT DESCRIPTION	DIMENSION
0045161	MiniChrom AF-rProtein A HC-650F, 5 mL	8 mm ID x 10 cm L
0045162	MiniChrom AF-rProtein L-650F, 5 mL NEW	8 mm ID x 10 cm L

Resin Seeker

PART #	PRODUCT DESCRIPTION	PACKAGE
0045520	Resin Seeker AF-rProtein A HC-650F NEW	20 µL x 96
0045509	Resin Seeker AF-rProtein L-650F NEW	20 µL x 96

ACCESSORIES

PART #	PRODUCT DESCRIPTION
0021400	ToyoScreen Column Holder
0045099	RoboColumn Array Plate

ANTIBODY AFC
ORDERING INFORMATION AND SPECIFICATIONS

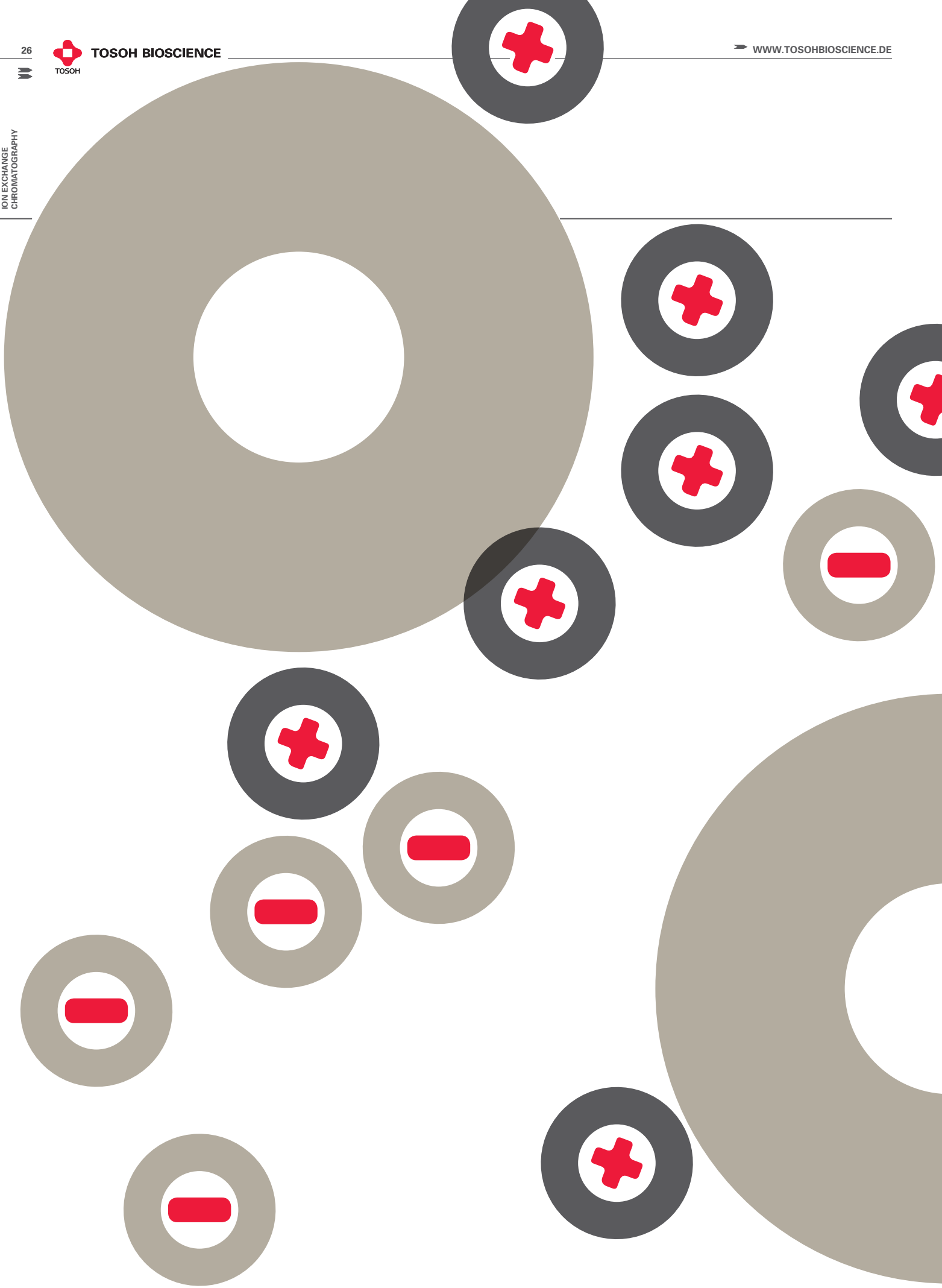
ORDERING INFORMATION

TOYOPEARL PROTEIN A AND L RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE	TYPICAL CAPACITY
0023425	TOYOPEARL AF-rProtein A HC-650F	10	≥68 g/L (IgG)
0023426		25	
0023427		100	
0023428		1,000	
0023429		5,000	
0023434		50,000	
0022803	TOYOPEARL AF-rProtein A-650F	10	≥45 g/L (IgG)
0022804		25	
0022805		100	
0022806		1,000	
0022807		5,000	
0022808		50,000	
0023486	TOYOPEARL AF-rProtein L-650F NEW	10	≥64 g/L (IgG)
0023487		25	
0023488		100	
0023489		1,000	
0023490		5,000	

PROTEIN STANDARDS

PART #	PRODUCT DESCRIPTION
0022836	Protein A-R28 STD 0.5 mL (10 mg/L) for TOYOPEARL AF-rProtein A-650F
0022899	Protein A-R40 STD 0.5 mL (10 mg/L) for TOYOPEARL AF-rProtein A-650F
NEW	Corresponding ELISA kit is supplied by Cygnus Technologies: www.cygnustechnologies.com/product_detail/tosoh-r40-and-r28-protein-a-mix-n-go-elisa.html



IEC
ION EXCHANGE CHROMATOGRAPHY

IEC PRODUCTS

ANION EXCHANGE

TOYOPEARL NH_2-750F NEW
TOYOPEARL SuperQ-650
TOYOPEARL QAE-550
TOYOPEARL Q-600C AR
TOYOPEARL DEAE-650
TOYOPEARL GigaCap Q-650
TOYOPEARL GigaCap DEAE-650
TSKgel SuperQ-5PW
TSKgel DEAE-5PW

CATION EXCHANGE

TOYOPEARL Sulfate-650F NEW
TOYOPEARL MegaCap II SP-550EC
TOYOPEARL SP-650
TOYOPEARL SP-550
TOYOPEARL CM-650
TOYOPEARL GigaCap S-650
TOYOPEARL GigaCap CM-650
TSKgel SP-3PW
TSKgel SP-5PW

> The biggest issue has always been the binding conditions with other ion-exchange resins. Most of the material we are working with simply can't withstand such low amounts of salt. This problem has since been eliminated and on top of that, we're seeing some great separation results in our testing[1].
>
> Leopold Ulrich
> Max-Planck-Institute of Biochemistry

[1] using the salt tolerant ion exchangers TOYOPEARL Sulfate-650F and NH2-750F

ION EXCHANGE CHROMATOGRAPHY HIGHLIGHTS

HIGHLIGHTS SULFATE-650F

- High protein binding capacity at elevated ion strength
- Unique selectivity, differing from conventional CEX media
- mAb aggregate removal in bind-elute mode
- Affinity capturing of selected targets

HIGHLIGHTS NH2-750F

- High protein binding capacity at elevated ion strength
- Unique selectivity, differing from conventional AEX media
- Binds targets at pH close to isoelectric point
- Aggregate removal in flow-through or bind-elute mode

FEATURES	BENEFITS
Porous, hydrophilic polymer based resin	Suitable for laboratory scale and process chromatography
Typical chemical stability	Autoclavable at 121 °C Temperature range 4 - 60 °C pH range 2-13, can be regenerated with acid or base Compatible with organic sovents
Column bed stability	Constant packing volume over a wide range of salt concentrations
Mechanical stability	Excellent flow characteristics in large industrial columns
Continuous selectivity	Easy scale-up from TSKgel IEC columns High yields of biologically active proteins

ION EXCHANGE CHROMATOGRAPHY HOW DOES IT WORK?

Ion Exchange Chromatography (IEC) is the most common liquid chromatographic method used in manufacturing therapeutic proteins. Due to the high dynamic binding capacities of ion exchange resins relative to those of the other chromatographic modes, it is the chromatographic technique selected by many developers for the capture or intermediate / concentration step.

IEC is based on the binding of proteins to positively or negatively charged groups which are immobilized on a stationary phase and which are in equilibrium with free counter ions in the mobile phase. In the process of adsorption, the mobile phase counter ions are exchanged by the protein solute. The binding of proteins to the ion exchange matrix predominantly occurs via charged amino acid residues located at the surface of the protein molecule.

IEC is further subdivided into cation exchange and anion exchange chromatography, depending on the pKa of the IEC ligand (Figure 1). Anion and cation exchange phases are classified as strong or weak, depending on how much the ionization state of the functional groups vary with pH. A strong ion exchange phase has the same charge density on its surface over a broad pH range, whereas the charge density of a weak ion exchange phase changes with pH, affecting its selectivity, which differs at different pH values.

The development of optimum chromatographic system conditions requires knowledge of both the protein's pI and the pKa of the ion exchange media. A binding buffer pH is selected between the pI of the target and the ion exchanger's pKa. This ensures that the protein is in the opposite charge state compared to the ion exchange media. When possible, the pH is also optimized to effect the highest solubility of the target protein. Higher protein solubilities make more efficient use of the overall ion exchange capacity of the resin. A salt is selected as the source of counter ions in the mobile phase and elution occurs as the salt strength is increased to a higher concentration than the target's binding salt conditions.

FIGURE 1

PK_a VALUES FOR ION EXCHANGE GROUPS

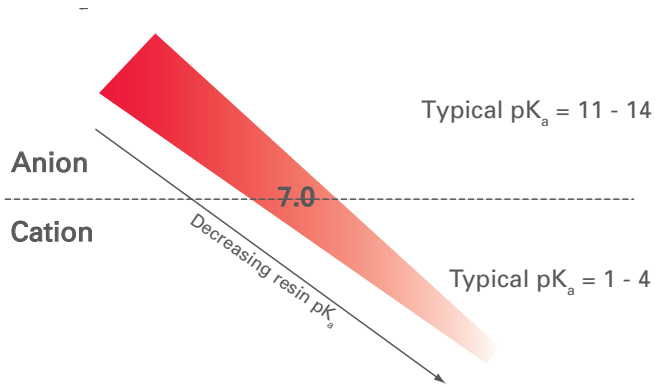
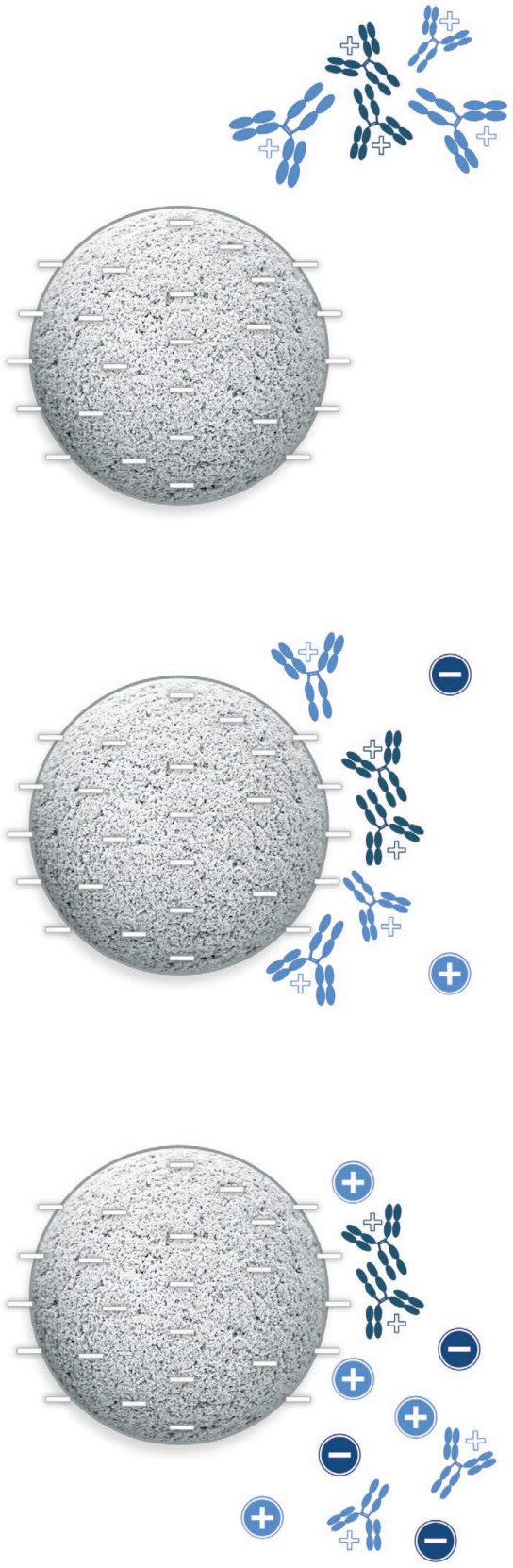


FIGURE 2

ION EXCHANGE ILLUSTRATION



ION EXCHANGE CHROMATOGRAPHY HOW DOES IT WORK?

PORE SIZES

Tosoh Bioscience offers a range of pore sizes for IEC resins originating from our base resins. Four different mean pore diameters are used for the current ion exchange resins: 100 nm, 75 nm, 50 nm, and 25 nm. Depending on the kind of ligand attachment, the effective pore size of the resulting IEC resin is smaller than the pore size of the base bead. When network ligand technology is applied the accessible pore size is varying with pH and salt concentration, therefore all pore sizes mentioned here are those of the respective TOYOPEARL HW or TSKgel base resin.

MULTIPLE PARTICLE SIZES SIMPLIFY SCALE UP / DOWN

Because TOYOPEARL and TSKgel products have similar backbone chemistry and selectivity, scaling up or scaling down for a selected ion exchange method is simple. Practically speaking, the chromatographic conditions that work for one particle size will work for all particle sizes with a given ligand functionality. The elution order of the components will remain the same with increasing resolution as the particle size gets smaller (Figure 3). The availability of smaller bead sizes for greater resolution while maintaining the same selectivity is particularly useful in the areas of oligonucleotide and peptide purification.

RESIN PHYSICAL PROPERTY SELECTION

For resins available in different pore sizes with the same ligand and ligand attachment chemistry.

For bind/elute (B/E) chromatography:

- Select the smallest pore size resin appropriate for the size of the target molecule.
- Select a larger particle size for a capture step, a smaller one for intermediate or polishing steps.

For flow-through (FT) chromatography:

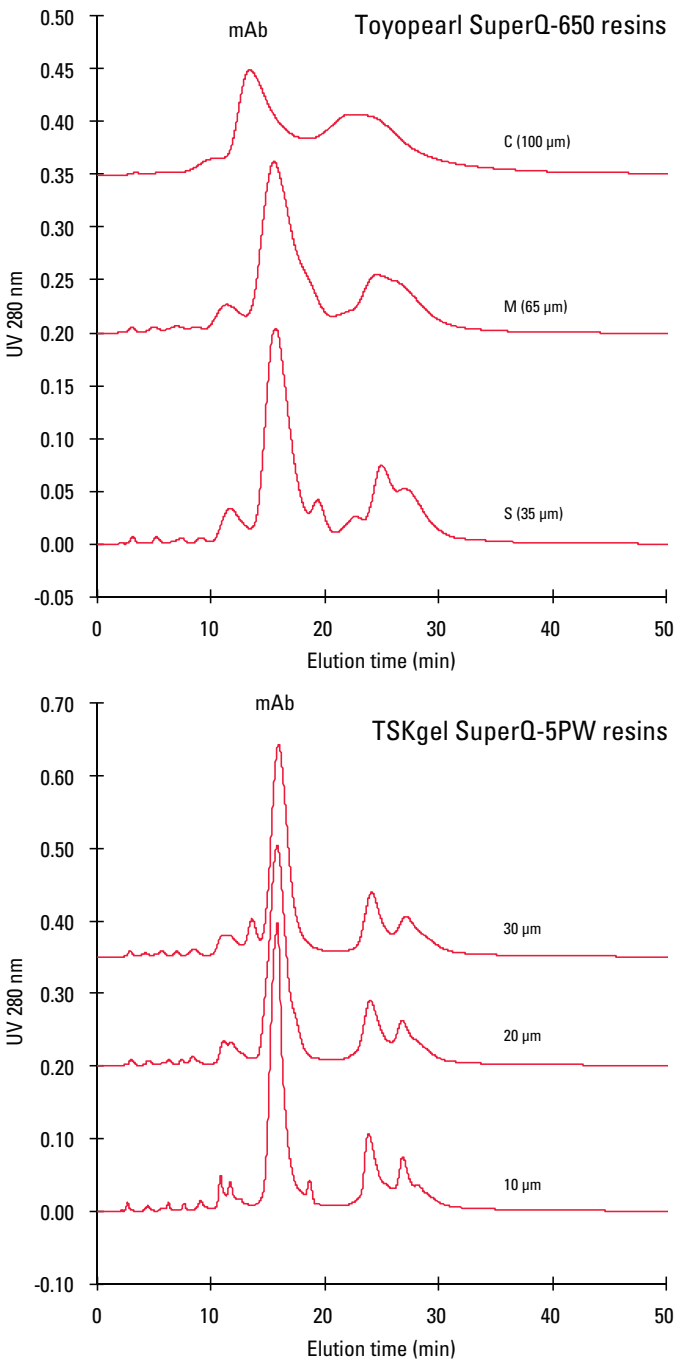
- If the target molecule's size is larger than most components of the feedstream, select a pore size which will tend to exclude it (known as kinetic exclusion, this technique can also be used under binding conditions as the excluded molecule only sees 1% of the resin surface area and the capacity/recovery loss is minimal).

For large molecule impurity clearance:

- Select a pore size which includes the target molecule, but excludes the impurity (see the calibration curves of the TOYOPEARL base beads in the SEC section of the catalog).

FIGURE 3

SCALE UP OR DOWN USING THE SAME LIGAND



Resins: A) TOYOPEARL SuperQ-650C (100 µm); B) TOYOPEARL SuperQ-650M (65 µm); C) TOYOPEARL SuperQ-650S (35 µm); D) TSKgel SuperQ-5PW(30) (30 µm); E) TSKgel SuperQ-5PW(20) (20 µm); F) TSKgel SuperQ-5PW (10 µm)
Column size: 7.5 mm ID x 7.5 cm; Mobile phase: Buffer A: 0.02 mol/L Tris-HCl, pH 8.5; Buffer B: 0.5 mol/L NaCl in Buffer A;
Gradient: 60 min linear gradient from Buffer A to Buffer B; Flow rate: 136 cm/h (1.0 mL/min); Detection: UV @ 280 nm;
Sample: mAb in mouse ascites (dilution, x 5); Sample vol.: 100 µL

ION EXCHANGE CHROMATOGRAPHY LIGAND TECHNOLOGY

Tosoh Bioscience offers four generations of ligand attachment technology. The "traditional" method, or first generation of ion exchange ligand, relies on direct attachment of the ligand to the resin surface through a proprietary spacer arm. TOYOPEARL and TSKgel PW type ion exchange resins using this traditional bead functionalization method are:

- TOYOPEARL SP-650 and SP-550
- TSKgel SP-3PW and SP-5PW
- TOYOPEARL CM-650
- TOYOPEARL Q-600C AR
- TOYOPEARL QAE-550C
- TOYOPEARL DEAE-650
- TSKgel DEAE-5PW
- TOYOPEARL MegaCap II SP-550EC

A second generation has been developed for increasing protein binding within the accessible surface area. The principle is to add a carbon spacer network between the bead surface and the ligand. It is also possible to attach ligand groups along the length of the spacer network thus improving capacity. There are two resins which incorporates this type of ligand attachment chemistry:

- TOYOPEARL SuperQ-650
- TSKgel SuperQ-5PW

A third generation ligand chemistry improves the accessible location of the ligand groups. The result of this modification is significantly increased capacity and improved mass transfer. Improved mass transfer also reduces the target molecule elution volume. All TOYOPEARL GigaCap resins use this type ligand attachment chemistry:

- TOYOPEARL GigaCap S-650
- TOYOPEARL GigaCap CM-650
- TOYOPEARL GigaCap Q-650
- TOYOPEARL GigaCap DEAE-650

A fourth generation ligand technology has been developed to provide an increased salt tolerance. This accounts for more flexibility in process development and for more gentle conditions for the purification of biomolecules. The two newest TOYOPEARL IEC resins are based on this approach:

- TOYOPEARL NH2-750F
- TOYOPEARL Sulfate-650F

WHICH RESIN SHOULD YOU EVALUATE IN PRIORITY?

- **Top-performers with salt tolerance - TOYOPEARL Sulfate-650F (CEX) and TOYOPEARL NH2-750F (AEX)**
- **All-rounders with ultra high binding capacity - TOYOPEARL GigaCap Series**
- **Highly selective AEX resin for oligonucleotide purification - TSKgel SuperQ-5PW**

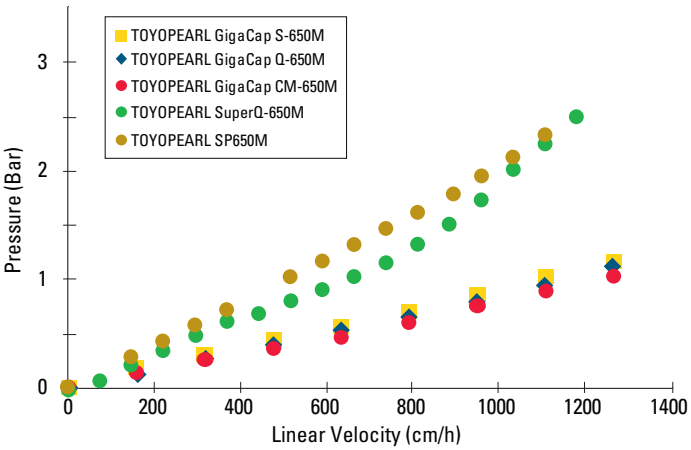
ION EXCHANGE CHROMATOGRAPHY TECHNICAL CHARACTERISTICS

IMPACT OF PORE SIZE AND LIGAND ATTACHMENT ON DBC

Table I shows DBC data for five of our TOYOPEARL resins using three proteins with different sized. There are three different pore sizes and three different ligand attachment methods represented. TOYOPEARL GigaCap Q-650M has the highest capacity for all combinations of pore size and attachment chemistries. Please note the decrease in capacity for the larger proteins on the TOYOPEARL SuperQ-650M resin indicating that the accessible pore volume has diminished by the ligand attachment chemistry used.

FIGURE 4

PRESSURE-FLOW CURVE COMPARISON



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

RESIN PRESSURE FLOW PROPERTIES

All TOYOPEARL resins are designed to withstand pressures up to at least 6 bar. TOYOPEARL GigaCap resins have a particle size of 50-100 microns which is slightly larger than our normal M-grade 40-90 micron beads. This particle size difference generates a lower back pressure (Figure 4) than our more traditional M-grade ion exchange products. The TSKgel 5PW type resins can be operated at pressures up to 20 bar.

If recommended packing procedures are followed, TOYOPEARL and TSKgel IEC resins maintain stable bed volumes during the pH and ionic strength changes that occur during normal ion exchange chromatography (Consult Section: *How to pack a column* on page 98 for some recommended packing conditions).

Multi-cycle gradient operation and re-equilibration are accomplished without volume changes in the TOYOPEARL column bed. The mechanical stability of the resins allows the use of longer column beds with more efficiency or higher operational flow rates.

TABLE I

DYNAMIC BINDING CAPACITY VARIES WITH PROTEIN SIZE

RESIN	PORE SIZE (nm)	BINDING CAPACITY (g/L gel) BSA 66 kDa	HUMAN IgG 160 kDa	THYROGLOBULIN 660 kDa
TOYOPEARL GigaCap Q-650M	100	173	108	71
TOYOPEARL SuperQ-650M	100	145	13	3
TOYOPEARL Q-600C AR	75	108	90	26
TOYOPEARL QAE-550C	50	29	32	6
TOYOPEARL DEAE-650M	100	25	31	3

Column size: 6 mm ID x 4 cm L; Sample concentration: 1 mg/mL; Loading buffers: BSA 0.05 mol/L Tris-HCl (pH = 8.5); Human IgG 0.05 mol/L Tris-HCl (pH = 8.7); Thyroglobulin 0.05 mol/L Tris-HCl (pH = 8.7) + 0.15 mol/L NaCl
Elution buffers: loading buffer + 1.0 mol/L NaCl; Flow rate: 212 cm/h; Detection: UV @ 280 nm

ION EXCHANGE CHROMATOGRAPHY TOYOPEARL Sulfate-650F

TOYOPEARL Sulfate-650F stands out by its various modes of interaction for a broad range of applications and its high binding capacity for IgG for efficient aggregate removal. All in all, this resin is a highly selective, salt tolerant and high capacity cation exchange resin for the capture and intermediate polishing of biomolecules. It offers chromatographers the ability to use mobile phases at physiological conditions without any loss of capacity or selectivity. It received the 2016 Innovation Awards from the Medicine Maker.

TOYOPEARL Sulfate-650F is based on the proven polymeric TOYOPEARL bead, which is functionalized with sulfate groups. The resulting cation exchange resin offers a high binding capacity for immunoglobulin G (IgG) across a range of pH values and conductivities. It provides an increased salt tolerance and its selectivity is different from most sulfate type cation exchange media currently available.

TOYOPEARL Sulfate-650F is ideal for a range of process scale applications including the capture of proteins from biological feedstock (mammalian cell culture or bacterial feedstock) without dilution and the intermediate or final purification of monoclonal antibodies (mAbs). It is especially suitable for post-Protein A removal of aggregates in bind-elute mode. In addition, it provides a affinity-like binding to other target molecules such as blood factors.

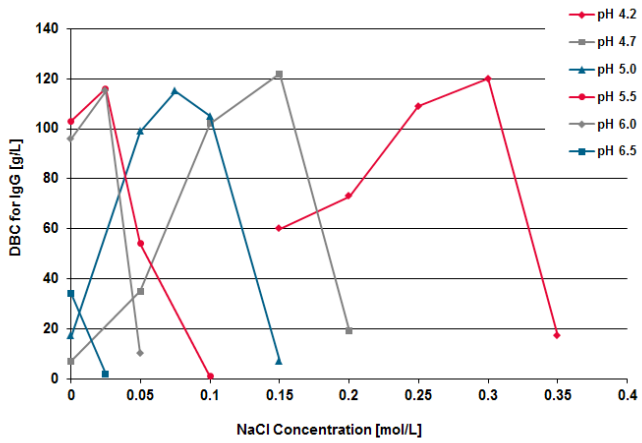
the Medicine Maker
Innovation Awards
2016

SALT TOLERANCE

TOYOPEARL Sulfate-650F shows a high binding capacity for IgG of typically more than 95 g IgG/L at a broad range of salt concentrations. Figure 5 shows the effect of buffer pH and sodium chloride concentration on IgG binding capacity. At a pH of 4.2 the highest binding capacity for polyclonal IgG is reached at a sodium chloride concentration of 300 mmol/L.

FIGURE 5

EFFECT OF pH AND CONDUCTIVITY ON IgG DBC



Column: TOYOPEARL Sulfate-650F 6.0 mm ID.x 4 cm L
Sample: 1 g/L polyclonal hIgG (Kakatsuken) in x mol/L NaCl + 0.054 mol/L acetate (pH 4.2-5.5) or MES buffer (pH 6.0, 6.5)
Residence time: 4 min

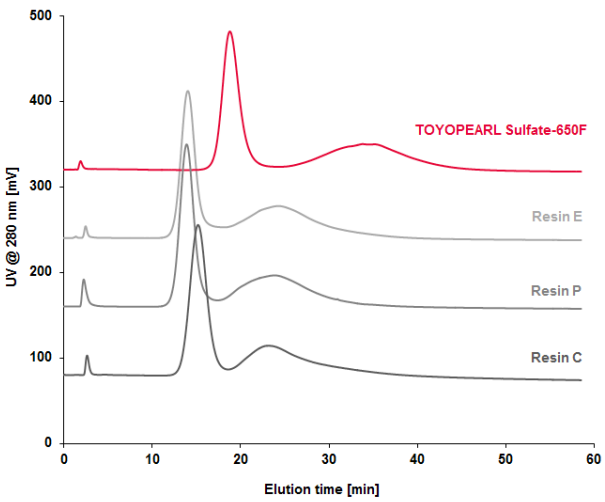
ION EXCHANGE CHROMATOGRAPHY
TOYOPEARL Sulfate-650F

AGGREGATE REMOVAL

Post-Protein A aggregate removal is a demanding step in downstream processing of monoclonal antibodies and cation exchange chromatography is the most popular mode applied for this purpose. TOYOPEARL Sulfate-650F was developed to provide a good resolution between mAb monomers and aggregates. Figure 6 proves the superior separation power of the new resin compared to other cation exchange media on the market.

FIGURE 6

AGGREGATE REMOVAL ON VARIOUS CATION EXCHANGE MEDIA



Column: Cation exchanger, 7.5 mm ID X 7.5 cm L
Eluents: A: 0.054 mol/L acetate buffer (pH 5.5), B: 1.0 mol/L NaCl in A
Gradient: 58.5 min linear 0% -100% B (NaCl conc. + 0.0167 M/min)
Flow rate: 1.0 mL/min
Sample: 3 g/L monoclonal humanized IgG acid/heat treatment, Injection vol.: 90 µL (Aggregate content ~1.9%)

MORE INFORMATION

ION EXCHANGE CHROMATOGRAPHY TOYOPEARL NH2-750F

TOYOPEARL NH2-750F is a salt tolerant anion exchange resin capable of aggregate removal in both, flow-through and bind/elute modes.

TOYOPEARL NH2-750F is ideal for process scale applications ranging from the capture of proteins from biological feedstock without dilution to the intermediate or final purification of monoclonal antibodies (mAbs) where aggregates and other impurities are removed from the target of interest.

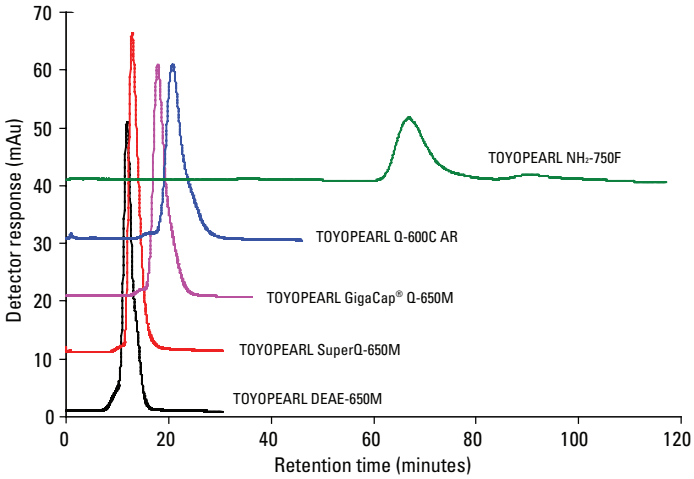
Tosoh Bioscience has developed the salt tolerant anion exchange resin, TOYOPEARL NH2-750F, offering high binding capacity across a range of pH values and conductivities. A TOYOPEARL HW-75 polymeric bead has been functionalized with a primary amine-containing ligand resulting in a resin with increased salt tolerance and selectivity different than that of quaternary amine anion exchange resins currently available. This resin is ideal for process scale applications from the capture of proteins from biological feedstock (mammalian cell culture, plasma, bacterial feedstock, etc.) without dilution to the intermediate or final purification of monoclonal antibodies (mAbs) where aggregates and other impurities are removed from the target of interest. TOYOPEARL NH2-750F is capable of post-Protein A removal of aggregates in both, flow-through and bind/elute modes.

TOYOPEARL NH2-750F offers static binding capacities approaching 70 g/L for bovine serum albumin across a range of pH values and conductivities. Increased salt tolerance of TOYOPEARL NH2-750F as compared to other TOYOPEARL anion exchange resins can be seen in Figure 7. While BSA is starting to elute at 0.14 mol/L NaCl for most conventional ion exchangers, the BSA peak begins to elute from the TOYOPEARL NH2-750F column at a concentration of approximately 1 mol/L NaCl.

Retention can be affected by mobile phase pH (Figure 8) without greatly changing the selectivity of the resin. BSA binding occurs even at pH values similar to the isoelectric point, indicating a multimodal binding mechanism of the resin. This allows for a large design space in which to develop a separation method.

FIGURE 7

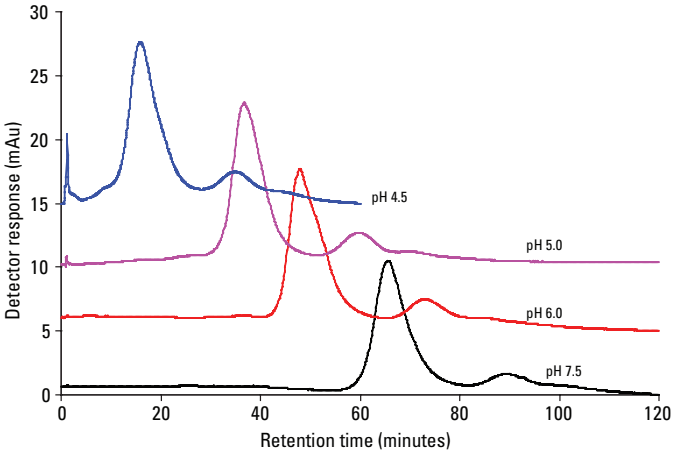
COMPARISON OF SALT TOLERANCE OF AEX RESINS



Resins: various; Column size: 5 mm ID × 5 cm L; Mobile phase: A: 20 mmol/L Tris-HCl, pH 8.0, B: mobile phase A + 2.0 mol/L NaCl; Gradient: 0 -100% B (120 min); Flow rate: 300 cm/h (1.0 mL/min); Detection: UV @ 280 nm; Temperature: ambient; Sample: BSA (1.0 g/L)

FIGURE 8

INFLUENCE OF pH ON BSA ELUTION



Resins: TOYOPEARL NH2-750F; Column size: 5 mm ID × 5 cm L; Mobile phase: A: 20 mmol/L N-methyl piperazine, pH 4.5 and 5.0; 20 mmol/L Bis-Tris, pH 6.0; 20 mmol/L Tris-HCl, pH 7.5 B: mobile phase A + 2.0 mol/L NaCl; Gradient: 0 -100% B (120 min); Flow rate: 300 cm/hr (1.0 mL/min); Detection: UV @ 280 nm; Temperature: ambient; Sample: BSA (1 mg, pI 4.7-4.9)

ION EXCHANGE CHROMATOGRAPHY TOYOPEARL NH2-750F

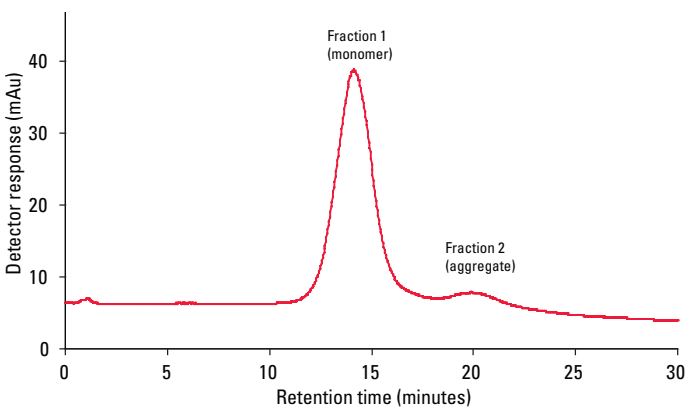
TOYOPEARL NH2-750F is effective at removing aggregates from mAbs, as demonstrated in Figure 9. SEC analysis of the peaks demonstrated that fraction 1 contains pure monomer. High molecular weight aggregates are completely removed from the mAb peak.

TOYOPEARL NH2-750F offers good caustic stability and exhibits excellent pressure-flow characteristics (Figure 10).

MORE INFORMATION

FIGURE 9

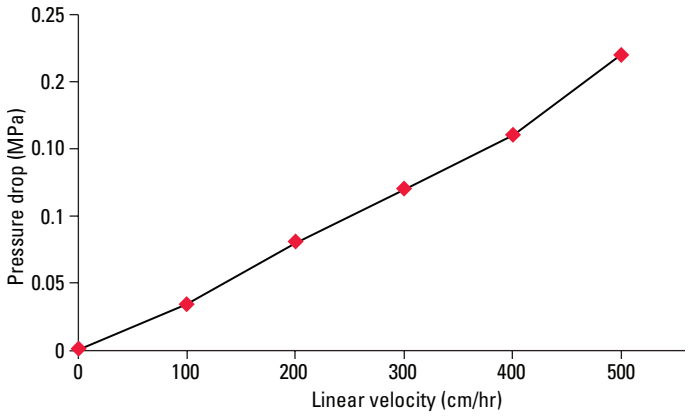
SEPARATION OF AGGREGATES FROM IgG1 MONOMER



Resin: TOYOPEARL NH2-750F; Column size: 5 mm ID × 5 cm L;
Mobile phase: A: 20 mmol/L Tris-HCl, pH 8.0; B: mobile phase A + 1.0 mol/L NaCl; Gradient: 0 -100% B (60 min);
Flow rate: 300 cm/h (1.0 mL/min); Detection: UV @ 280 nm;
Temperature: ambient; Sample: mAb (IgG1) 0.5 g/L

FIGURE 10

PRESSURE – FLOW CHARACTERISTICS OF TOYOPEARL NH2-750F



Resin: TOYOPEARL NH2-750F; Column size: 4.4 cm ID × 29 cm L;
Mobile phase: 0.1 mol/L NaCl; Flow rate: multiple

IEC - APPLICATIONS
OPTIMIZE THE POST-PROTEIN A STEP

TOYOPEARL Sulfate-650F improves purity of target antibody with an acceptable amount of HMW proteins and HCP while nearly no Protein A ligand is detected in the collected IgG1 peak.

A crude sample containing IgG1 was passed through the Protein A column and fractions of IgG1 were collected for further work. Figure 11 demonstrates that the IgG1 was purified by Protein A chromatography. The eluate peak was collected and further analyzed by size exclusion chromatography using a TSKgel G3000SWXL SEC column for monomer and aggregate yield, host cell protein (HCP) content and Protein A ligand leaching (see Table II on next page).

DYNAMIC BINDING CAPACITY (DBC) OPTIMIZATION

DBC was optimized by DoE with a three-level, full-factorial method at pH 4.8 – 5.6 and 100 – 200 mmol/L NaCl (results as shown in Figure 12). A maximum DBC of >120 mg/mL-resin was observed between pH 4.8, 150 mmol/L NaCl, and pH 5.2, 100 mmol/L NaCl. Conditions of pH 5.2, 12.1 mS/cm were used for the elution optimization experiments for maximum binding.

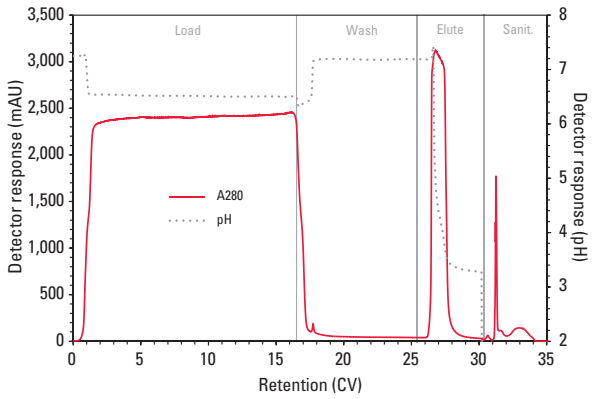
OPTIMIZE CONDITIONS FOR SEPARATION AND ELUTION OF MONOMER AND HIGH MOLECULAR WEIGHT PEAKS

To optimize elution conditions, a gradient elution was performed at pH 5.2. A peak conductivity of 30.1 mS/cm was noted (ca. 288 mmol/L NaCl). Experiment was repeated as a step gradient at 260, 290, or 320 mmol/L NaCl (see Figure 13). Due to peak tailing during elution, 1-CV fractions were collected throughout elution. Fractions were analyzed for IgG1 concentration, aggregate, HCP and Protein A, and results were analyzed to determine optimum NaCl concentration and peak volume.

Data analysis suggests the optimum aggregate and HCP removal are obtained at 260 mmol/L NaCl in elution buffer and maximum (9 CV) elution volume. Protein A ligand content at these conditions (40 ppb) is significantly lower than that found in the load material (1200 ppb).

FIGURE 11

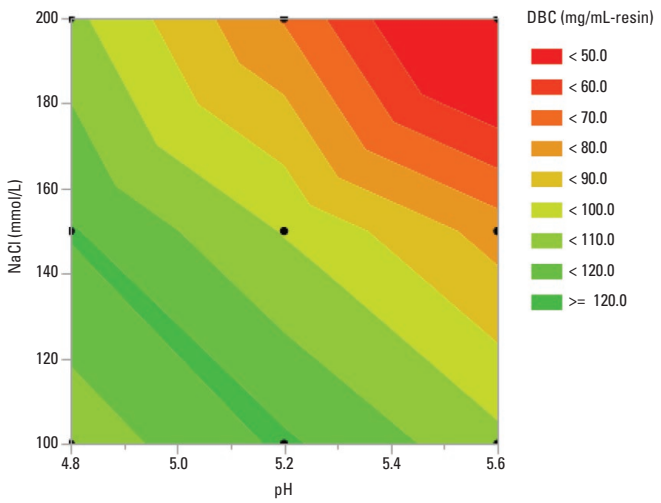
PURIFICATION OF IgG1 FROM CHO SUPERNATANT



Resin: TOYOPEARL AF-rProtein A HC-650F
Column: 25 mm ID × 15 cm (74 mL)
Mobile phase: A: 20 mmol/L Tris-acetate, 150 mmol/L NaCl, pH 7.4
B: 50 mmol/L acetic acid
C: 0.1 mol/L NaOH
Flow rate: 225 cm/h (4 min residence time)
Detection: UV @ 280 nm (mAU), pH
Temperature: ambient
Injection vol.: 1200 mL (48 mg/mL-resin load ratio)
Sample: TBL-mAb-01 CSS @ 2.95 g/L

FIGURE 12

DYNAMIC BINDING CAPACITY OPTIMIZATION FOR TOYOPEARL Sulfate-650F USING THE COLLECTED IgG1



IEC - APPLICATIONS
OPTIMIZE THE POST-PROTEIN A STEP

Figure 14 shows data from the SEC analysis of the eluate pool at 260 mmol/L NaCl, 9 CV volume. Data shows there is a reduction in aggregate content (in particular HMW impurities), relative to the collected IgG1 eluate peak material from the Protein A resin eluate peak.

The peaks from the SEC column were analyzed for high molecular weight, HCP and Protein A ligand content. Table II shows that after passing through the TOYOPEARL Sulfate-650F resin, the collected IgG1 peak has significantly reduced amounts of HMW, HCP and Protein A ligand. This suggests that TOYOPEARL Sulfate-650F resin can effectively remove and reduce impurities of IgG1.

CONCLUSION

The TOYOPEARL Sulfate-650F resin offers a high dynamic binding capacity (>120 mg/mL-resin) with DBC maxima at pH 4.8, 150 mmol/L NaCl and pH 5.2, 100 mmol/L NaCl. With elution at pH 5.2, recovery and impurity removal (aggregate, HCP, leached Protein A) is optimal. In fact, analyzed data of the collected IgG1 monomer peak from TOYOPEARL Sulfate-650F showed that its purity was significantly improved with an acceptable amount of HMW proteins and HCP while nearly no Protein A ligand was detected in the collected IgG1 peak. By selecting this strong cation exchange resin as a step after mAb post-Protein A purification, only a minimal adjustment to pH or salt concentration to the sample is needed.

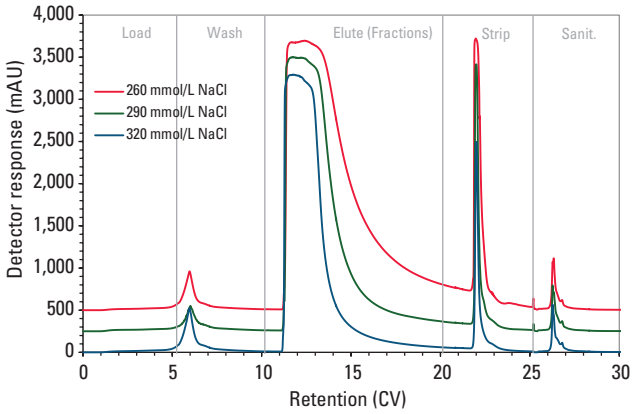
TABLE II

INTEGRATED PEAK DATA FROM SEC COLUMN

Impurity	ProA eluate	Sulfate eluate
Dimer %	3.9	2.4
HMW %	0.54	0.07
HCP (ppm)	1260	134
ProA (ppm)	1.2	0.6040

FIGURE 13

PROFILING O THE COLLECTED IgG1 ELUATE PEAK SEPARATED BY TOYOPEARL Sulfate-650F AT VARIOUS CONDITIONS

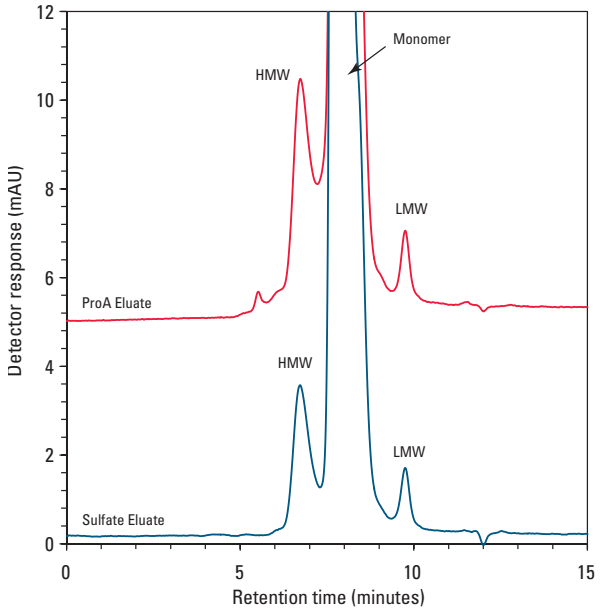


Resin: TOYOPEARL Sulfate-650F Column; 6.6 mm ID × 3.0 cm (1.0 mL)
Mobile phase: A: 50 mmol/L acetate-Tris, 100 mmol/L NaCl, pH 5.2
B: 50 mmol/L acetate-Tris, pH 5.2, NaCl as indicated
C: 50 mmol/L acetate-Tris, 1.0 mol/L NaCl, pH 5.2
D: 0.5 mol/L NaOH
Flow rate: 45 cm/h (4 min residence time)
Detection: UV @ 280 nm (mAU)
Temperature: ambient
Injection vol.: 5.3 mL (97 mg/mL-resin load ratio)
Sample: TBL-mAb-01, 19.1 mg/mL

MORE INFORMATION

FIGURE 14

COLLECTED MONOMER PEAKS ANALYZED BY SEC



Column: TSKgel G3000SWXL (7.8 mm ID x 30 cm)
Mobile phase: 0.1 mol/L phosphate, 0.1 mol/L sodium sulfate, 0.1% sodium azide, pH 6.7
Gradient: isocratic
Flow rate: 1.00 mL/min
Detection: UV @ 280 nm (mAU)
Temperature: 25° C
Injection vol.: 10 µL (100 µg total IgG)
Sample: ProA or Sulfate eluate as indicated

IEC - APPLICATIONS
REMOVE AGGREGATES IN BIND/ELUTE & FLOW-THROUGH

TOYOPEARL NH_2-750F is a salt tolerant anion exchange resin for downstream processing with high binding capacity for immunoglobulin. The resin is ideally suited to develop a polishing step for monoclonal antibodies by either using the resin in bind/elute mode or in flow-through mode.

Purification schemes for monoclonal antibodies typically consist of three chromatographic steps accompanied by filtration steps. The common Protein A capturing is typically followed by ion exchange, hydrophobic interaction or mixed-mode polishing steps. Residual DNA, viruses, and host cell proteins are usually removed by flow-through anion exchange chromatography while aggregates can be reduced through a cation exchange, mixed-mode, or HIC step.

The salt tolerant anion exchange resin TOYOPEARL NH_2-750F provides a unique selectivity compared to other anion exchange resins and was found to be suited for aggregate removal, too. Herein we describe the development of an anion exchange polishing step for the purification of a monoclonal antibody by using TOYOPEARL NH_2-750F. In general, anion exchange resins can be used in bind and elute (B/E) mode as well as in flow-through (FT) mode. Both options were evaluated. To increase the amount of aggregates of the test sample, a monoclonal antibody was aggregated by acidic incubation and subsequently diluted to 1 g/L in loading buffer.

FIGURE 15

ELUTION PROFILE OF AGGREGATED ANTIBODY ON TOYOPEARL NH_2-750F IN BIND AND ELUTE MODE

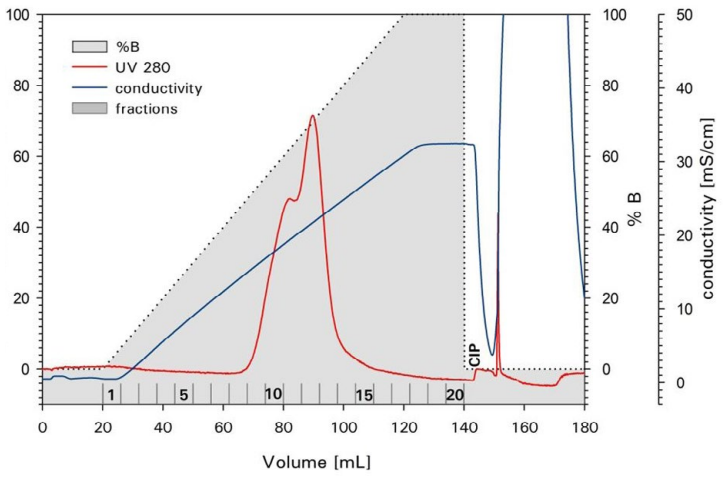
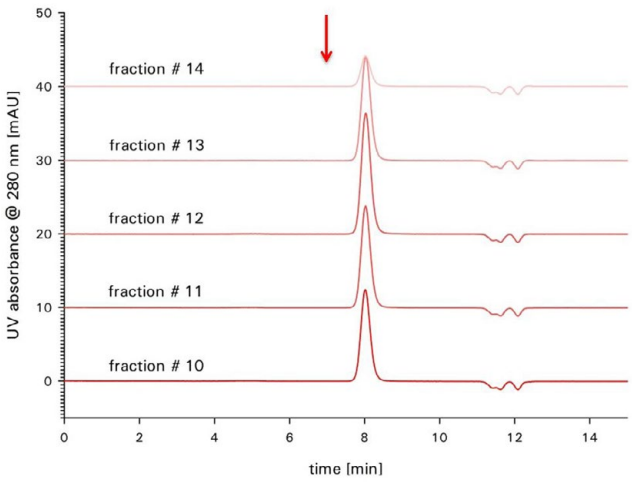


FIGURE 16

SEC ANALYSIS OF THE BIND/ELUTE OF FRACTIONS OF THE AGGREGATED mAb SAMPLE



IEC - APPLICATIONS REMOVE AGGREGATES

RESULTS

The dynamic binding capacity of TOYOPEARL NH_2-750F for the mAb used in this study was evaluated and a value of 95 mg/mL could be reached with 10 mM Tris/HCl, pH 8.0. Figure 15 shows the elution profile of the aggregated antibody on TOYOPEARL NH_2-750F in B/E mode. Fractions were collected and analyzed by SEC (Figure 16). The results prove that fractions 10 to 14 have an aggregate content below the limit of detection of SEC. The aggregates did not elute in the salt gradient and remained bound until the column was CIPed with sodium hydroxide.

In order to establish a FT polishing step, buffer conditions were evaluated to optimize non-binding conditions for the monomer by varying pH (pH 7 to 8) and salt content (250 - 500 mM NaCl). Best results were obtained with 10 mM Tris/HCl, pH 7.0 at a sodium chloride concentration of 250 mM (Figure 17). To analyze the aggregate removal, 100 mg aggregated antibody were loaded on a 2 mL column and fractions of the flow through were analyzed by SEC. All FT fractions are essentially aggregate free (Figure 18).

CONCLUSION

TOYOPEARL NH_2-750F is a salt tolerant anion exchange resin for downstream processing with high binding capacity for immunoglobulin. The resin is ideally suited to develop a polishing step for monoclonal antibodies by either using the resin in B/E mode or in FT mode. For both modes ideal conditions for aggregate removal could be established. An additional benefit when using this resin in FT mode is the delivered excellent viral clearance. Typical virus log reduction exceeds five for enveloped and non-enveloped DNA and RNA viruses.

FIGURE 17

ELUTION PROFILE OF AGGREGATED ANTIBODY ON TOYOPEARL NH_2-750F IN FT MODE

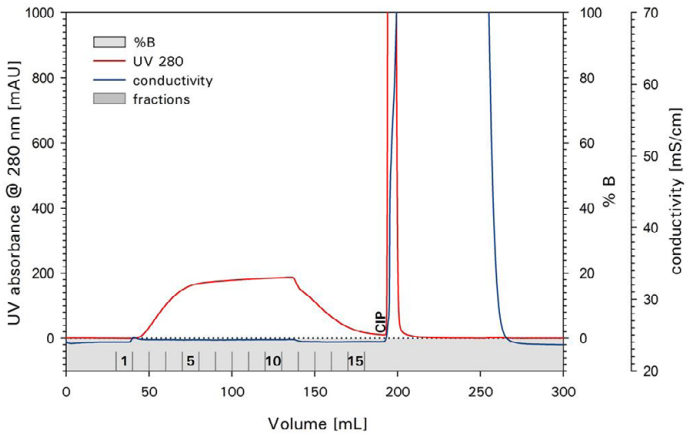
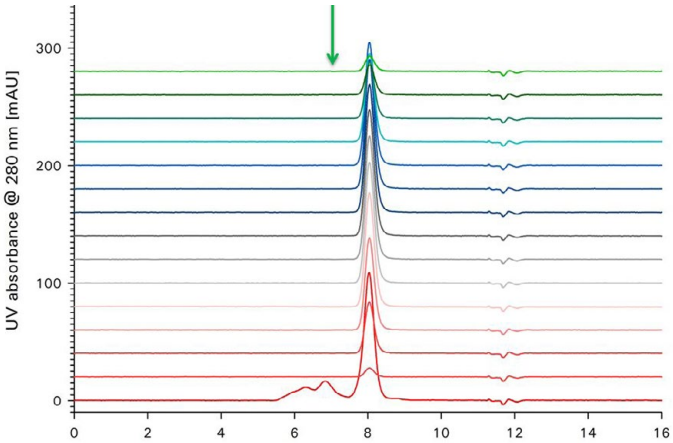


FIGURE 18

SEC ANALYSIS OF THE AGGREGATED mAb SAMPLE (RED LINE AT 0 mAU) AND FLOW-THROUGH FRACTIONS IN INCREASING FRACTION ORDER FROM BOTTOM TO TOP



MORE INFORMATION

IEC - APPLICATIONS
REDUCE ELUTION POOL VOLUMES

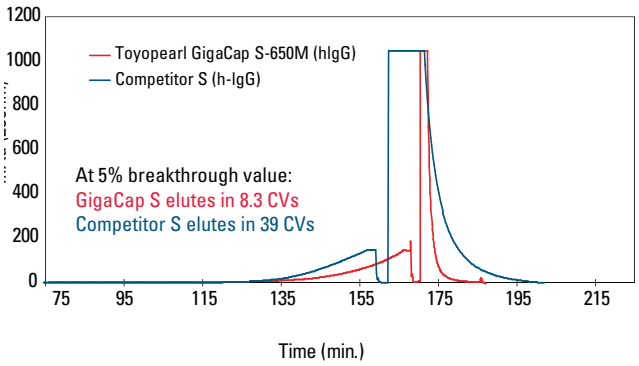
Figures 19, 20 and 21 show the breakthrough curves for three TOYOPEARL GigaCap resins. They are compared where possible with the most current equivalent competitive resin. Each trace shows the dynamic binding capacity of the resin up to 10% breakthrough plus the elution profile for the target molecule. Please note the significant reduction in elution pool volumes of the TOYOPEARL GigaCap resins when compared to other products.

The concentration of the eluted peak is proportionally increased as well. It is possible to achieve reductions in elution pool volumes of over 75%. This can reduce the cost of further downstream process steps.

The strong ion exchange resins TOYOPEARL GigaCap S and GigaCap Q are now also available with smaller particle size (S-grade, 35 µm), providing higher resolution for better separation of process impurities.

FIGURE 19

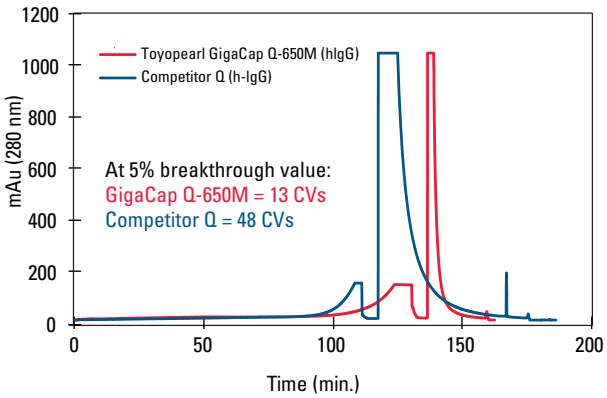
TOYOPEARL GigaCap S-650M VS. COMPETITOR S ELUTION POOL VOLUME COMPARISON



Column size: 6 mm ID x 40 mm bed; 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

FIGURE 20

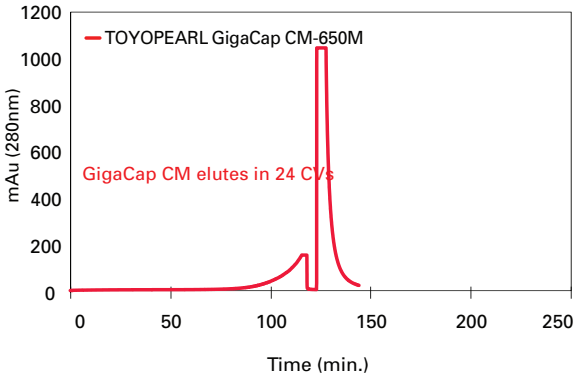
TOYOPEARL GigaCap Q-650M VS. COMPETITOR Q ELUTION POOL VOLUME COMPARISON



Column size: 6 mm ID x 40 mm L; Sample: polyclonal human IgG (1 mg/mL); Loading buffer: 15 mmol/LTris-HCl (pH= 8.7);
Elution buffer: 15 mmol/L Tris-HCl (pH= 8.7) + 1.0 mol/L NaCl;
Linear velocity: 212 cm/h; Detection: UV @ 280 nm

FIGURE 21

TOYOPEARL GigaCap CM-650M ELUTION POOL VOLUME



Column size: 6 mm ID x 40 mm L; Sample: polyclonal human IgG (1 mg/mL); Loading buffer: 50 mmol/L sodium acetate buffer (pH= 4.7); Elution buffer: 15 mmol/L Tris-HCl (pH= 8.7) + 1.0 mol/L NaCl
Linear velocity: 212 cm/h; Detection: UV @ 280 nm

MORE INFORMATION

IEC - APPLICATIONS ALKALINE STABILITY

Tosoh has focused on improving the alkaline stability of its newer ion exchange resins. Higher capacity resins can bind not only more of the target molecule, but the impurities and isoforms as well. In some cases more rigorous cleaning agents like 0.5 mol/L NaOH and even 1.0 mol/L NaOH are needed to insure proper resin regeneration. Naturally, the resins need to tolerate these more stringent conditions. As seen in Table III, TOYOPEARL GigaCap series ion exchange resins have excellent alkaline stability.

TOYOPEARL Q-600C AR (alkaline resistant)

A high capacity, alkaline resistant, Q anion exchange media, TOYOPEARL Q-600C AR resin (using first generation ligand attachment chemistry) was developed by Tosoh for CIP of difficult to remove impurities. This new resin has a slightly smaller pore size than TOYOPEARL GigaCap Q-650M resin and has a typical BSA binding capacity of 100 mg/mL. As shown in Figure 22, after 100 days of exposure to 1 mol/L NaOH, the DBC of TOYOPEARL Q-600C AR resin remains unchanged.

Figure 23 shows the preservation of selectivity after extensive exposure to caustic.

TABLE III

TOYOPEARL GigaCap RESIN BASE STABILITY

RESIN	STORAGE SOLUTION	TEST MOLECULE	CAPACITY	STARTING CAPACITY	WEEK 1	WEEK 2	WEEK 3
TOYOPEARL GigaCap S-650M	1.0 mol/L NaOH	h-IgG	Dynamic	143 (g/L)	144	140	135
TOYOPEARL GigaCap CM-650M	0.5 mol/L NaOH	h-IgG	Dynamic	99 (g/L)	88	90	91
TOYOPEARL GigaCap Q-650M	0.5 mol/L NaOH	BSA	Static	166 (g/L)	NA	153*	136

* 12 days

FIGURE 22

TOYOPEARL Q-600C AR RESIN DBC AS A FUNCTION OF SODIUM HYDROXIDE EXPOSURE

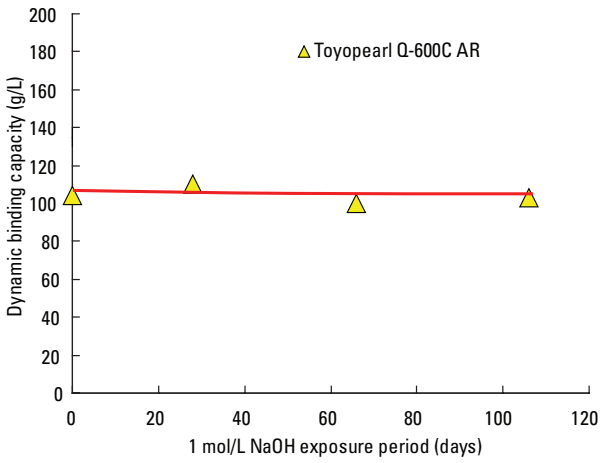
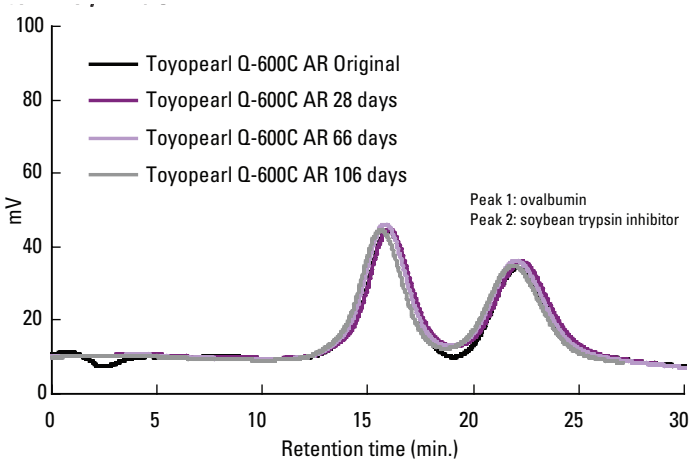


FIGURE 23

STABILITY OF TOYOPEARL Q-600C AR RESIN AFTER EXPOSURE TO 1 MOL/L NaOH



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

IEC - APPLICATIONS OLIGONUCLEOTIDE PURIFICATION

Resins with SuperQ functionalities are typically made for oligonucleotide purification. Table IV shows the different particle sizes available with the anion exchange SuperQ functionality, which is typically used for oligonucleotide purifications. The relative binding capacities and predicted resolution of the five particle sizes are depicted by a series of "+" characters.

The more "+" characters listed in the table the better one resin is relative to another for that parameter. If a process is developed using one of the five resins and more resolution is needed, select an appropriate smaller particle size product. Similarly if more capacity is needed, and resolution is not a critical issue, a larger particle size resin can be selected.

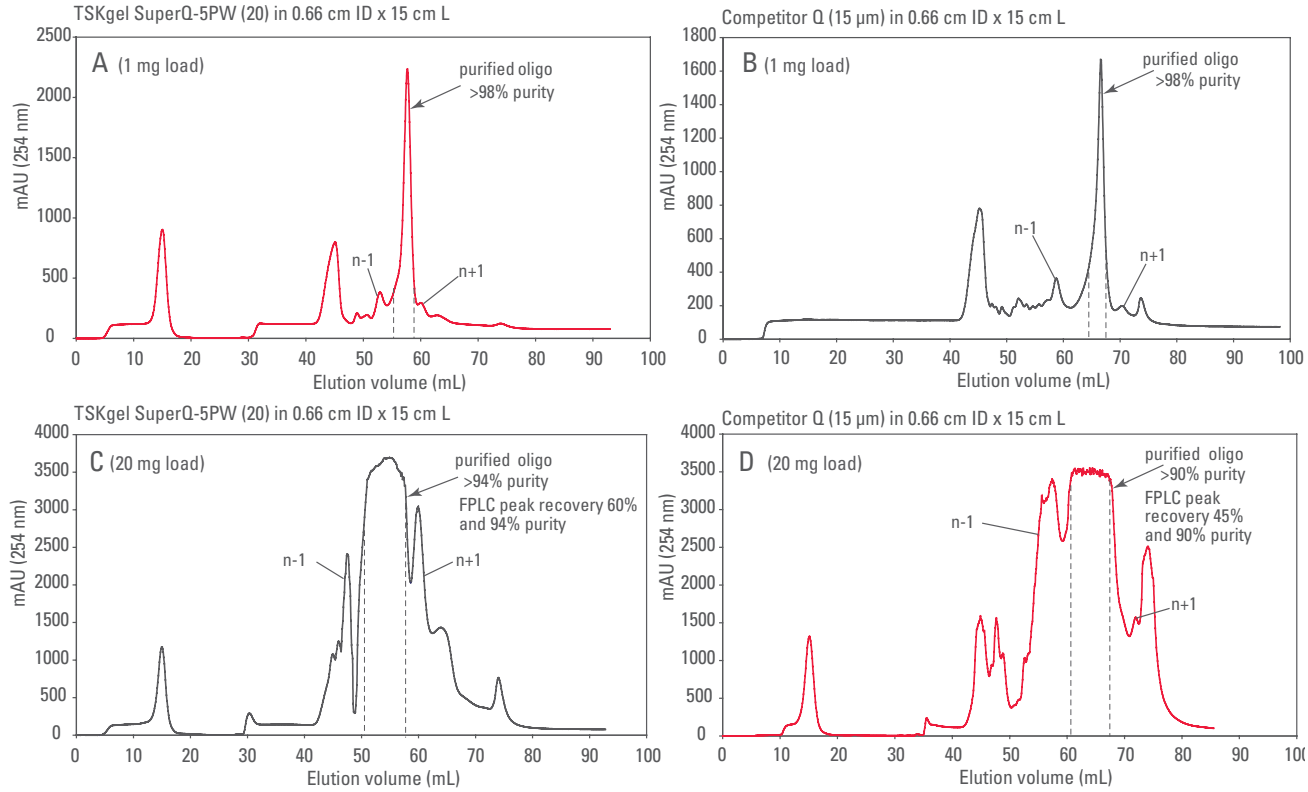
The larger particle TOYOPEARL resins are less crosslinked than the corresponding TSKgel 5PW resins and have more active sites for ligand attachment. Thus they have higher capacities than the TSKgel 5PW-type resins. In some cases, TOYOPEARL GigaCap Q-650M (also in Table IV) and its high capacity can be used, though its selectivity is slightly different.

TSKgel SuperQ-5PW products typically have 2-4 times the binding capacity of other small particle anion exchange resins available on the market. This has significant bearing in the area of difficult to resolve "n-1" DNA and RNAi purifications as loading amounts are increased. Under higher loading conditions, the TSKgel SuperQ resins maintain their resolution much better than resins with smaller particle and/or lower capacity. The smaller particle products may start out with a slight separation advantage under low oligonucleotide loading conditions, but this vanishes as the feedstock load is increased.

Figure 24 shows a comparison of one smaller particle size, competitive product, which starts out having better resolution than TSKgel SuperQ-5PW (20) resin at oligonucleotide binding of 1 mg/mL of resin. At 20 mg/mL of resin, however, the resolution of peaks on the competitive product deteriorates significantly. The TSKgel SuperQ-5PW (20) retains excellent resolution even at this higher oligonucleotide level.

FIGURE 24

TSKgel SuperQ-5PW (20) MAINTAINS RESOLUTION AT HIGH OLIGONUCLEOTIDE LOAD



Column: 0.66 cm x 15 cm L (5.1 mL) (resin as noted in figure); Flow rate: 1.43 mL/min (250 cm/h)

Buffer A: 20 mmol/L Tris-HCl + 10 mmol/L EDTA (pH= 9.0); Buffer B: 20 mmol/L Tris-HCl + 10 mmol/L EDTA + 1.0 mol/L NaCl (pH= 9.0)

Sample loaded: DNA based oligonucleotides were loaded as followed: 1 mg/column panels A & B, 20 mg/column panels C & D

Separation conditions: Column was washed with 5 CV 100 % buffer A followed by 11 mL injection. Column was then washed with 3 CV 100 % buffer A followed by 6 CV of linear gradient 35-53 buffer B. Finally, column was washed with 5 CV 100 % buffer B.

Detection: UV @ 254 nm; Fractions: 0.5 mL fractions were taken from peaks of interest and analyzed on a TSKgel DNA-NPR column

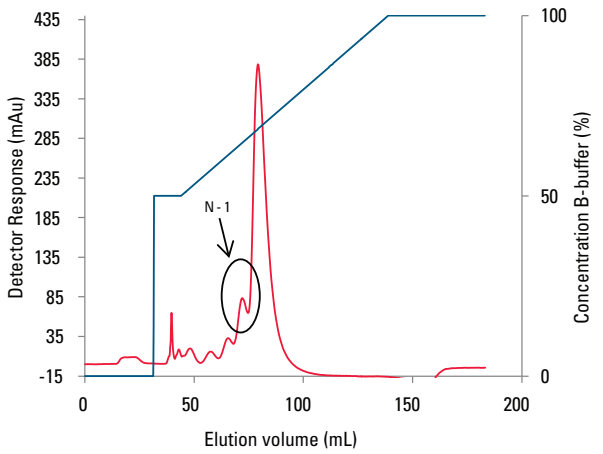
IEC - APPLICATIONS
OLIGONUCLEOTIDE PURIFICATION

TOYOPEARL GigaCap Q-650S resin offers a low pressure alternative to oligonucleotide purification with TSKgel resins while preserving similar selectivity, resolution and yields of those higher pressure processes.

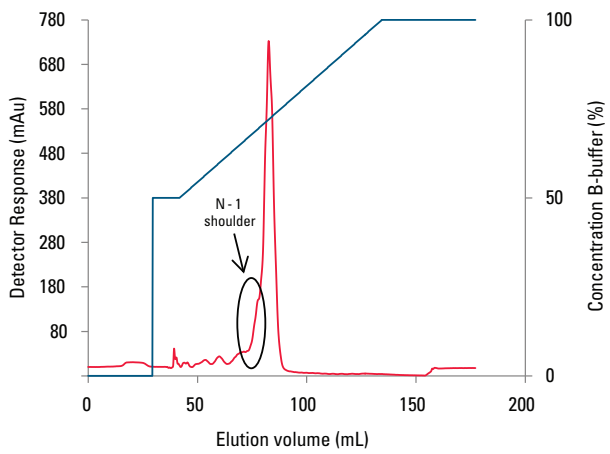
Figure 25 shows that the N-1 peak was slightly better resolved with the TSKgel SuperQ-5PW (20) than with the TOYOPEARL GigaCap Q-650S, due to the smaller particle size of the TSKgel resin. HPLC analysis of fractions taken across the peaks revealed that both resins were able to adequately resolve the full length oligonucleotide.

FIGURE 25

PURIFICATION OF OLIGONUCLEOTIDES



Resin: TSKgel SuperQ-5PW (20); Column size: 6.6 mm ID × 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/h (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 × 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/h (1.14 mL/min); Detection: UV @ 254 nm; Sample load: 1.0 mg; Sample: crude phosphorothioate deoxyoligonucleotide

TABLE IV

Oligonucleotide Purification Products:

	Bead size (mean µm)	Binding capacity	Resolution	Bead type	Ligand attachment
TSKgel SuperQ-5PW (20)	20	++	+++++	methacrylic	Type A
TSKgel SuperQ-5PW (30)	30	++	++++	methacrylic	Type A
TOYOPEARL SuperQ-650S	35	++++	+++	methacrylic	Type A
TOYOPEARL SuperQ-650M	65	++++	++	methacrylic	Type A
TOYOPEARL SuperQ-650C	100	++++	+	methacrylic	Type A
TOYOPEARL GigaCap Q-650M	75	+++++	++	methacrylic	Type B
TOYOPEARL GigaCap Q-650S	35	+++++	++++	methacrylic	Type B

Peptide Purification Products:

	Bead size (mean µm)	Binding capacity	Resolution	Bead type	Ligand attachment
TSKgel SP-5PW (20)	20	++	+++++	methacrylic	Traditional
TSKgel SP-5PW (30)	30	++	++++	methacrylic	Traditional
TSKgel SP-3PW (30)	30	+++	++++	methacrylic	Traditional
TOYOPEARL SP-650S	35	++++	+++	methacrylic	Traditional
TOYOPEARL SP-650M	65	++++	++	methacrylic	Traditional
TOYOPEARL SP-650C	100	++++	+	methacrylic	Traditional
TOYOPEARL GigaCap S-650M	75	+++++	++	methacrylic	Type B
TOYOPEARL GigaCap S-650S	35	+++++	++++	methacrylic	Type B

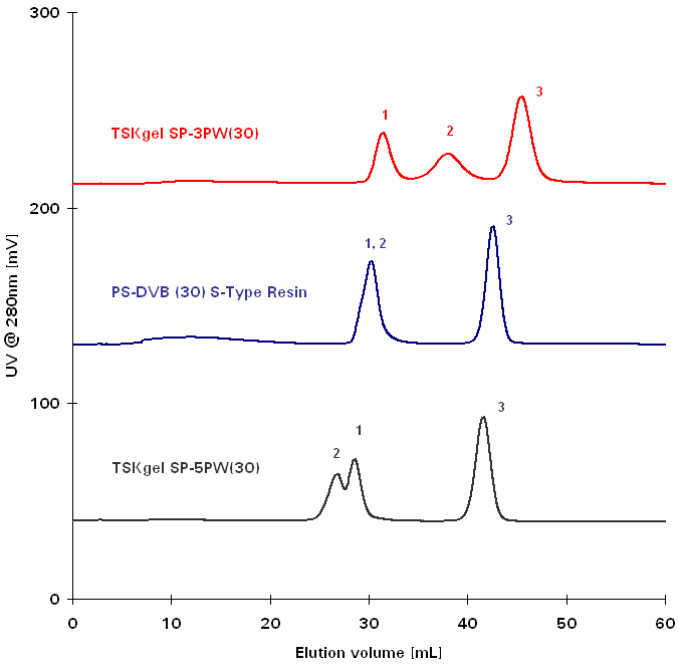
IEC - APPLICATIONS PEPTIDE PURIFICATION

Cation exchange chromatography is commonly used for peptide purification. Table V shows the same particle size availability of TOYOPEARL and TSKgel PW strong cation exchange resins. Based on the needs for capacity and resolution, an appropriate S or SP resin is selected for a particular peptide application. TSKgel SP-3PW (30) is based on a 25 nm pore resin. It was developed to provide high binding capacities for peptides and small proteins. It also has a different selectivitiy than TSKgel SP-5PW (30). It is especially suited for insulin purification.

Table V compares the insulin binding capacity of TSKgel SP-3PW (30) to TSKgel SP-5PW (30) and Source 30S resin. The improved resolving power of TSKgel SP-3PW (30) resin is demonstrated in Figure 26.

FIGURE 26

SELECTIVITY COMPARISON



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 mol/L 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)

TABLE V

INSULIN DYNAMIC BINDING CAPACITIES

Matrix	TSKgel SP-3PW (30) polymethacrylate	TSKgel SP-5PW (30) polymethacrylate	PS-DVB (30) S Type Resin 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	25 nm	100 nm	NR

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

ION EXCHANGE CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

ToyoScreen

PART #	PRODUCT DESCRIPTION	PACKAGE
ANION EXCHANGE		
0023443	ToyoScreen NH_2-750F NEW	1 mL x 6
0023444		5 mL x 6
0021364	ToyoScreen QAE-550C	1 mL x 6
0021365		5 mL x 6
0021992	ToyoScreen Q-600C AR	1 mL x 6
0021993		5 mL x 6
0021859	ToyoScreen GigaCap Q-650M	1 mL x 6
0021860		5 mL x 6
0022872	ToyoScreen GigaCap DEAE-650M	1 mL x 6
0022873		5 mL x 6
0021362	ToyoScreen SuperQ-650M	1 mL x 6
0021363		5 mL x 6
0021360	ToyoScreen DEAE-650M	1 mL x 6
0021361		5 mL x 6
CATION EXCHANGE		
0023472	ToyoScreen Sulfate-650F NEW	1 mL x 6
0023473		5 mL x 6
0021868	ToyoScreen GigaCap S-650M	1 mL x 6
0021869		5 mL x 6
0021951	ToyoScreen GigaCap CM-650M	1 mL x 6
0021952		5 mL x 6
0021370	ToyoScreen SP-550C	1 mL x 6
0021371		5 mL x 6
0021368	ToyoScreen SP-650M	1 mL x 6
0021369		5 mL x 6
0021366	ToyoScreen CM-650M	1 mL x 6
0021367		5 mL x 6
0021870	ToyoScreen MegaCap II SP-550EC	1 mL x 6
0021871		5 mL x 6
0021392	ToyoScreen IEC Anion Mix Pack DEAE-650M, SuperQ-650M,QAE-550C, GigaCap Q-650M, Q-600C AR)	1 mL x 5 Grades x 1 each
0021393		5 mL x 5 Grades x 1 each
0021394	ToyoScreen IEC Cation Mix Pack (CM-650M, SP-650M, SP-550C, GigaCap CM-650M, GigaCap S-650M)	1 mL x 5 Grades x 1 each
0021395		5 mL x 5 Grades x 1 each
0021396	ToyoScreen IEC Mix Pack (GigaCap Q-650M, GigaCap S-650M, GigaCap CM-650M, Q-600C AR, SuperQ-650M, SP-550C)	1 mL x 6 Grades x 1 each
0021397		5 mL x 6 Grades x 1 each

ION EXCHANGE CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

RoboColumns

PART #	PRODUCT DESCRIPTION	PACKAGE
ANION EXCHANGE		
0045021	RoboColumn NH_2-750F NEW	200 µL x 8
0045022		600 µL x 8
0045011	RoboColumn Q-600C AR, 200 µL	200 µL x 8
0045012		600 µL x 8
0045003	RoboColumn GigaCap Q-650M, 200 µL	200 µL x 8
0045004		600 µL x 8
0045007	RoboColumn GigaCap DEAE-650M, 200 µL	200 µL x 8
0045008		600 µL x 8
CATION EXCHANGE		
0045027	RoboColumn Sulfate-650F NEW	200 µL x 8
0045028		600 µL x 8
0045001	RoboColumn GigaCap S-650M, 200 µL	200 µL x 8
0045002		600 µL x 8
0045005	RoboColumn GigaCap CM-650M, 200 µL	200 µL x 8
0045006		600 µL x 8

ToyoScreen COLUMN ACCESSORIES

PART #	PRODUCT DESCRIPTION
0021400	ToyoScreen Column Holder
0045099	RoboColumn Array Plate

MiniChrom

PART #	PRODUCT DESCRIPTION	DIMENSION
ANION EXCHANGE		
0045108	MiniChrom TOYOPEARL NH_2-750F, 5 mL NEW	8 mm ID x 10 cm L
0045119	MiniChrom TOYOPEARL QAE-550C, 5mL	8 mm ID x 10 cm L
0045115	MiniChrom TOYOPEARL Q-600C AR, 5mL	8 mm ID x 10 cm L
0045105	MiniChrom TOYOPEARL GigaCap Q-650S, 5mL	8 mm ID x 10 cm L
0045104	MiniChrom TOYOPEARL GigaCap Q-650M, 5mL	8 mm ID x 10 cm L
0045106	MiniChrom TOYOPEARL GigaCap DEAE-650M, 5mL	8 mm ID x 10 cm L
0045114	MiniChrom TOYOPEARL Super Q-650S, 5mL	8 mm ID x 10 cm L
0045109	MiniChrom TOYOPEARL Super Q-650M, 5mL	8 mm ID x 10 cm L
0045113	MiniChrom TOYOPEARL DEAE-650S	8 mm ID x 10 cm L
0045112	MiniChrom TOYOPEARL DEAE-650M	8 mm ID x 10 cm L

ION EXCHANGE CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

MiniChrom - TOYOPEARL

PART #	PRODUCT DESCRIPTION	DIMENSION
CATION EXCHANGE		
0045117	MiniChrom TOYOPEARL Sulfate-650F, 5 mL NEW	8 mm ID x 10 cm L
0045102	MiniChrom TOYOPEARL GigaCap S-650S, 5 mL	8 mm ID x 10 cm L
0045101	MiniChrom TOYOPEARL GigaCap S-650M, 5mL	8 mm ID x 10 cm L
0045103	MiniChrom TOYOPEARL GigaCap CM-650M, 5mL	8 mm ID x 10 cm L
0045185	MiniChrom TOYOPEARL SP-550C, 5mL	8 mm ID x 10 cm L
0045111	MiniChrom TOYOPEARL SP-650S, 5mL	8 mm ID x 10 cm L
0045110	MiniChrom TOYOPEARL SP-650M, 5mL	8 mm ID x 10 cm L
0045182	MiniChrom TOYOPEARL CM-650S, 5mL	8 mm ID x 10 cm L
0045181	MiniChrom TOYOPEARL CM-650M, 5mL	8 mm ID x 10 cm L
0045186	MiniChrom TOYOPEARL MegaCap II SP-550EC, 5mL	8 mm ID x 10 cm L

MiniChrom - TSKgel

PART #	PRODUCT DESCRIPTION	DIMENSION
ANION EXCHANGE		
0045107	MiniChrom TSKgel SuperQ-5PW (20), 5 mL	8 mm ID x 10 cm L
0045184	MiniChrom TSKgel DEAE-5PW (20), 5mL	8 mm ID x 10 cm L
CATION EXCHANGE		
0045116	MiniChrom TSKgel SP-5PW (20), 5 mL	8 mm ID x 10 cm L
0045183	MiniChrom TSKgel SP-3PW (30), 5mL	8 mm ID x 10 cm L

Resin Seeker

PART #	PRODUCT DESCRIPTION	PACKAGE
ANION EXCHANGE		
0045501	Resin Seeker AIEX	20 µL x 96
0045507	Resin Seeker NH_2-750F	20 µL x 96
0045503	Resin Seeker GigaCap Q-650M	20 µL x 96
0045504	Resin Seeker GigaCap DEAE-650M	20 µL x 96
CATION EXCHANGE		
0045501	Resin Seeker CIEX	20 µL x 96
0045508	Resin Seeker Sulfate-650F	20 µL x 96
0045505	Resin Seeker GigaCap S-650M	20 µL x 96
0045506	Resin Seeker GigaCap CM-650M	20 µL x 96

TSKgel LABPAK

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)
0043380	IEXPAK PW (20) (SP-5PW, DEAE-5PW, SuperQ-5PW)	3 x 25 mL	20
0043280	IEXPAK PW (30) (SP-5PW, DEAE-5PW, SuperQ-5PW)	3 x 25 mL	30

ION EXCHANGE CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

TOYOPEARL LABPAK

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)
0019817	IEXPAK HP (CM-650S, SP-650S, DEAE-650S, SuperQ-650S)	4 x 25 mL	20-50
0043210	AIEXPAK (GigaCap Q-650M, SuperQ-650M, Q-600C AR)	3 x 100 mL	40-90 and 50-150
0043220	CIEXPAK (GigaCap CM-650M, GigaCap S-650M, SP-650M, SP-550C)	3 x 100 mL	40-90 and 50-150

ANION EXCHANGE RESINS

TOYOPEARL BULK MEDIA

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	ION EXCHANGE CAPACITY (eq/L resin)	TYPICAL CAPACITY (g/L)	
					BSA	IgG
0023438	TOYOPEARL NH_2-750F NEW	100	45	0.07-0.13		≥ 70
0023439		250				
0023440		1,000				
0023441		5,000				
0023442		50,000				
0043271	TOYOPEARL QAE-550C	100	100	0.28-0.38	60-80	32
0014026		250				
0014704		1,000				
0014027		5,000				
0018365		50,000				
0021985	TOYOPEARL Q-600C AR	100	100	0.15-0.20	>120	90
0021986		250				
0021987		1,000				
0021988		5,000				
0021989		50,000				
0022881	TOYOPEARL GigaCap Q-650S	25	35	0.10-0.20	>170	
0022882		250				
0022883		1,000				
0022884		5,000				
0022885		50,000				
0021854	TOYOPEARL GigaCap Q-650M	100	75	0.10-0.20	>162	108
0021855		250				
0021856		1,000				
0021857		5,000				
0021858		50,000				

ION EXCHANGE CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

TOYOPEARL BULK MEDIA

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	ION EXCHANGE CAPACITY (eq/L resin)	TYPICAL CAPACITY (g/L)	
					BSA	IgG
0022865	TOYOPEARL GigaCap DEAE-650M	100	75	0.17-0.28	>156	
0022866		250				
0022867		1,000				
0022868		5,000				
0022869		50,000				
0019823	TOYOPEARL SuperQ-650S	25	35	0.20-0.30	105-155	
0017223		250				
0017224		1,000				
0017225		5,000				
0019679		50,000				
0043205	TOYOPEARL SuperQ-650M	100	65	0.20-0.30	105-155	13
0017227		250				
0017228		1,000				
0017229		5,000				
0021311		50,000				
0043275	TOYOPEARL SuperQ-650C	100	100	0.20-0.30	105-155	
0017231		250				
0017232		1,000				
0017233		5,000				
0019804	TOYOPEARL DEAE-650S	25	35	0.08-0.12	25-35	
0007472		250				
0014692		1,000				
0007973		5,000				
0021483		50,000				
0043201	TOYOPEARL DEAE-650M	100	65	0.08-0.12	25-35	31
0007473		250				
0014693		1,000				
0007974		5,000				
0018367		50,000				
0007988	TOYOPEARL DEAE-650C	250	100	0.05-0.11	25-35	
0014694		1,000				
0007989		5,000				

ION EXCHANGE CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

TSKgel BULK RESIN

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	ION EXCHANGE CAPACITY (eq/L resin)	TYPICAL CAPACITY (g BSA/L resin)
0043383	TSKgel SuperQ-5PW (20)	25	20	0.12-0.18	52-88
0018535		250			
0018546		1,000			
0018547		5,000			
0043283	TSKgel SuperQ-5PW (30)	25	30	0.12-0.18	52-88
0018536		250			
0018548		1,000			
0018549		5,000			
0043381	TSKgel DEAE-5PW (20)	25	20	0.05-0.11	25-45
0014710		250			
0014711		1,000			
0018436		5,000			
0043281	TSKgel DEAE-5PW (30)	25	30	0.05-0.11	20-40
0014712		250			
0014713		1,000			
0018370		5,000			

ION EXCHANGE CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

CATION EXCHANGE RESINS

TOYOPEARL BULK MEDIA

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	ION EXCHANGE CAPACITY (eq/L resin)	TYPICAL CAPACITY (g/L resin)
0023467	TOYOPEARL Sulfate-650F NEW	100	45	≥ 0.53	≥ 114 (γ-globulin)
0023468		250			
0023469		1,000			
0023470		5,000			
0023471		50,000			
0022875	TOYOPEARL GigaCap S-650S	25	35	0.14-0.18	>150 (γ-globulin)
0022876		250			
0022877		1,000			
0022878		5,000			
0022879		50,000			
0021833	TOYOPEARL GigaCap S-650M	100	75	0.14-0.18	136-176 (γ-globulin)
0021834		250			
0021835		1,000			
0021836		5,000			
0021837		50,000			
0021946	TOYOPEARL GigaCap CM-650M	100	75	0.17-0.28	>110 (γ-globulin)
0021947		250			
0021948		1,000			
0021949		5,000			
0021950		50,000			
0043272	TOYOPEARL SP-550C	100	100	0.14-0.18	80-120 (lysozyme)
0014028		250			
0014705		1,000			
0014029		5,000			
0018366		50,000			
0019822	TOYOPEARL SP-650S	25	35	0.13-0.17	40-60 (lysozyme)
0008437		250			
0014698		1,000			
0008438		5,000			
0021477		50,000			
0043202	TOYOPEARL SP-650M	100	65	0.13-0.17	40-60 (lysozyme)
0007997		250			
0014699		1,000			
0007998		5,000			
0018369		50,000			
0007994	TOYOPEARL SP-650C	250	100	0.12-0.18	35-55 (lysozyme)
0014700		1,000			
0007995		5,000			

ION EXCHANGE CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

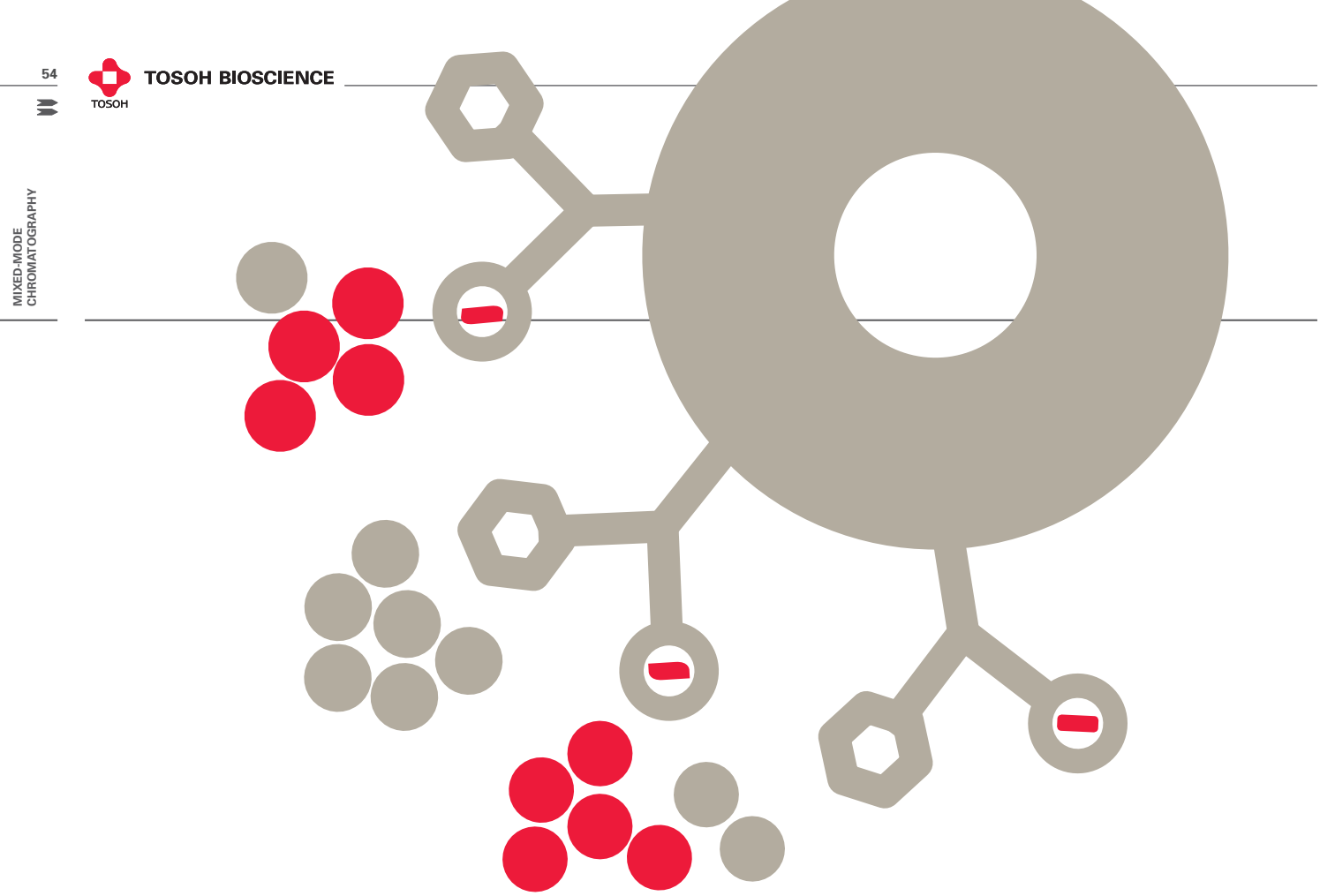
ORDERING INFORMATION

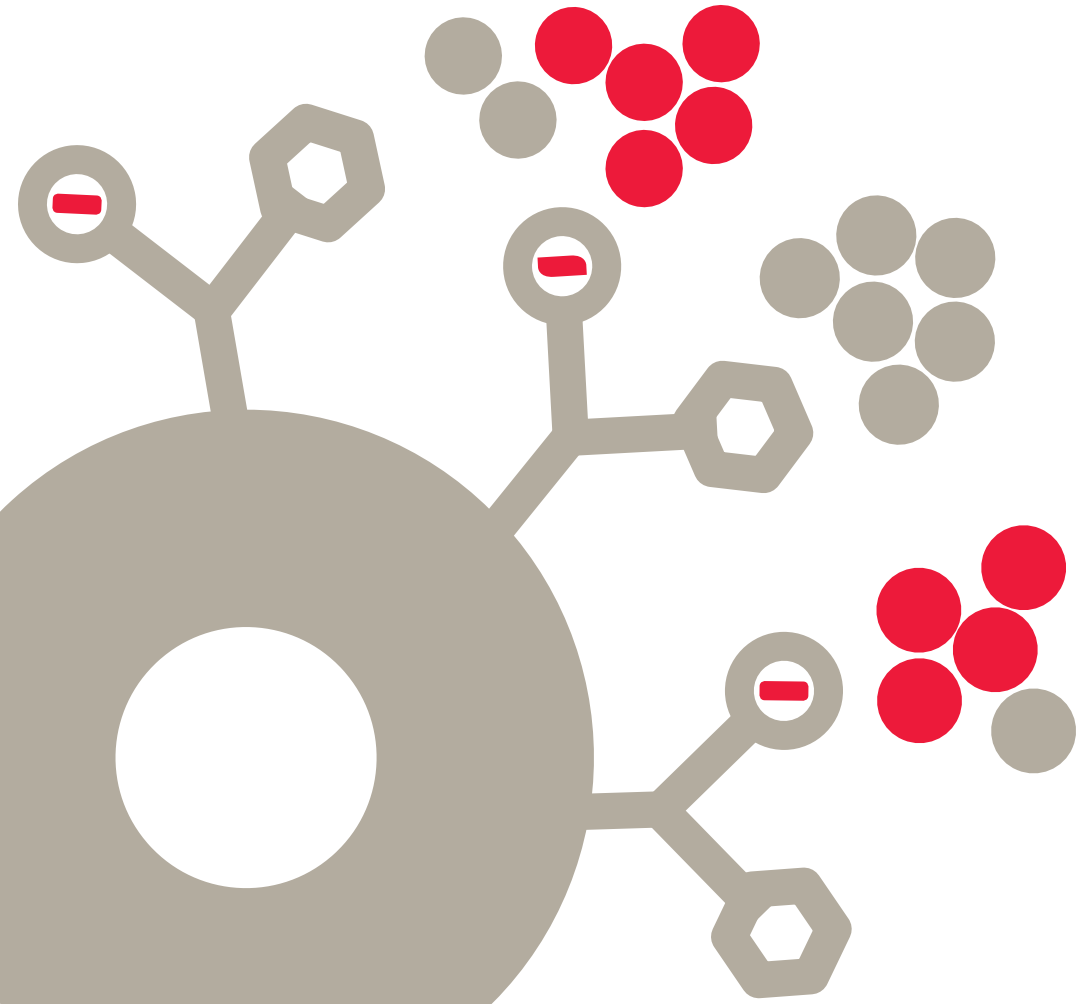
TOYOPEARL BULK MEDIA

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	ION EXCHANGE CAPACITY (eq/L resin)	TYPICAL CAPACITY (g/L resin)
0019803	TOYOPEARL CM-650S	25	35	0.08-0.12	30-50 (lysozyme)
0007474		250			
0014695		1,000			
0007971		5,000			
0043203	TOYOPEARL CM-650M	100	65	0.08-0.12	30-50 (lysozyme)
0007475		250			
0014696		1,000			
0007972		5,000			
0019839		50,000			
0007991	TOYOPEARL CM-650C	250	100	0.05-0.11	25-45 (lysozyme)
0014697		1,000			
0007992		5,000			
0019329		50,000			
0021804	TOYOPEARL MegaCap II SP-550EC	100	200	0.14-0.18	60-90 (lysozyme)
0021805		250			90-120 (insulin)
0021806		1,000			
0021807		5,000			
0021808		50,000			

TSKgel BULK MEDIA

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	ION EXCHANGE CAPACITY (eq/L resin)	TYPICAL CAPACITY (g Insulin/L resin)
0021976	TSKgel SP-3PW (30)	25	30	0.07- 0.22	≥ 65
0021977		250			
0021978		1,000			
0021979		5,000			
0043382	TSKgel SP-5PW (20)	25	20	0.06-0.12	20-40
0014714		250			
0014715		1,000			
0018435		5,000			
0043282	TSKgel SP-5PW (30)	25	30	0.06-0.12	20-40
0014716		250			
0014717		1,000			
0018384		5,000			





MIXED MODE CHROMATOGRAPHY

MIXED-MODE PRODUCTS

- TOYOPEARL MX-Trp-650M

> There is a real need of mixed-mode chromatography resin to simplify the purification process of complex biopharmaceuticals. This need was even increased by the FDA requirements, which calls for the use of more orthogonal chromatography methods.

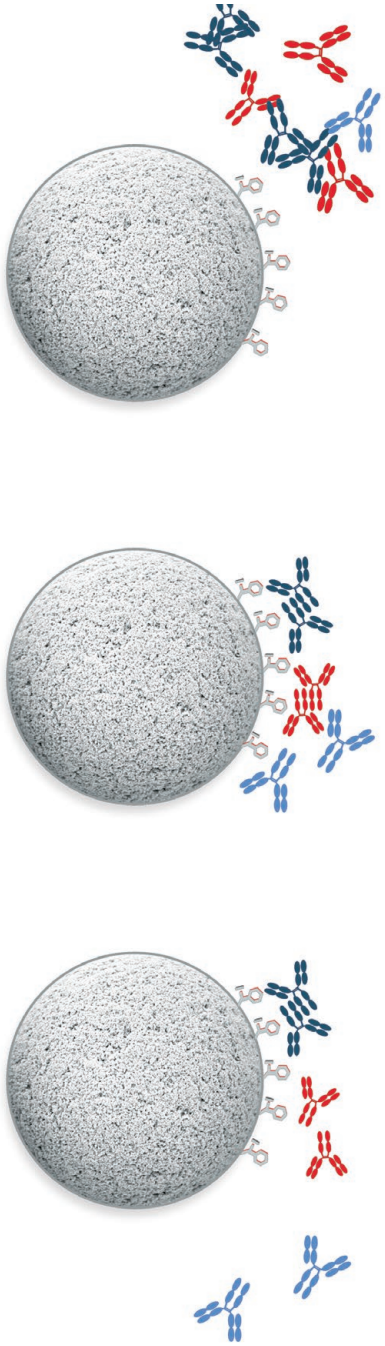
MIXED MODE CHROMATOGRAPHY HIGHLIGHTS

HIGHLIGHTS MX-Trp-650M

- 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

FIGURE 1

ILLUSTRATION OF MIXED-MODE CHROMATOGRAPHY



FEATURES

- Multimodal cation exchange resin
- Fast mass transfer kinetics
- High mechanical stability

BENEFITS

- Selectivity adjustable by pH, salt type and ionic strength
- Tolerates high conductivity feedstocks
- High binding capacities for IgG and other proteins
- Can be used for processing of clarified feedstocks at physiological salt concentrations as well as for intermediate and polishing applications
- Sharp elution peaks with mild conditions
- Excellent flow characteristics in large columns

MIXED MODE CHROMATOGRAPHY HOW DOES IT WORK?

Multimodal or mixed-mode chromatography (MXC) denotes a quite heterogeneous segment of liquid chromatography. In general this term is used when a stationary phase offers different modes of chromatography, modulated by the properties of the mobile phase. In bioprocess chromatography this term is most often used when ion exchange (IEX) and hydrophobic interaction (HIC) are combined in one resin. Depending on the nature of IEX ligand – anion exchange versus cation exchange – there are currently two versions of mixed mode process resins on the market: multimodal hydrophobic cation exchanger and hydrophobic anion exchanger. Multimodal or mixed-mode chromatography expands the range of chromatographic modes applied in biopurification and offers new selectivity options and a higher salt tolerance than traditional ion exchange media.

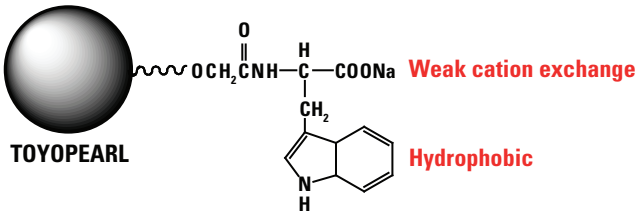
TOYOPEARL MX-Trp-650M belongs to the category of multi-modal cation exchangers. It uses tryptophan as the active ligand (Figure 2). This amino acid has both weak carboxyl cation exchange and indole hydrophobic functional groups. The selectivity of the resin can be adjusted through control of binding or elution pH, ionic strength, salt type and additives.

Depending of the nature of the mobile phase the interaction of the proteins and the stationary phase is dominated by either hydrophobic interactions (e.g. at high salt concentrations) or by ionic interactions. 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.

The binding capacity of mixed-mode resin greatly depends on pH (Figure 3). 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.

FIGURE 2

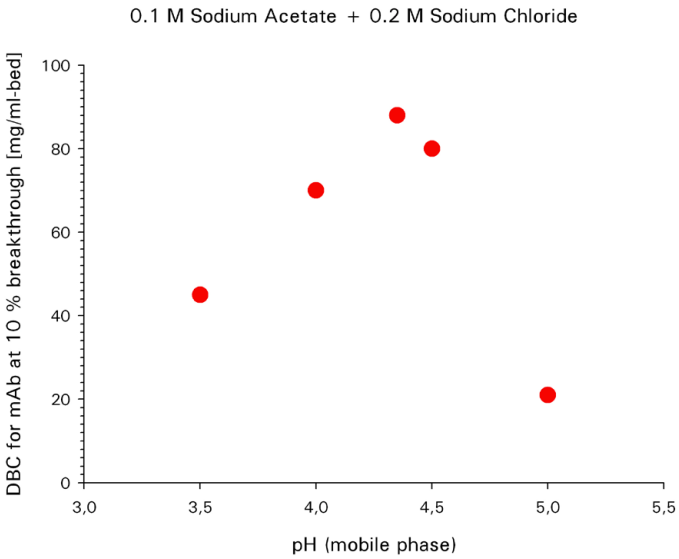
TOYOPEARL MX-Trp-650M STRUCTURE



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

FIGURE 3

INFLUENCE OF pH VALUE ON IgG DYNAMIC BINDING CAPACITY



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.

MIXED MODE CHROMATOGRAPHY TOYOPEARL MX-Trp-650M

TOYOPEARL MX-Trp-650M offers higher binding capacity at high conductivities, higher mechanical stabilities, and better mass transfer parameters versus agarose based multi-modal cation exchange materials.

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 4). At elevated flow rates/ shorter residence times the binding capacity still remains high. Table I 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 multi-modal cation exchanger (Brand M) is also shown. For both conductivity levels the TOYOPEARL MX-Trp-650M resin shows much higher DBCs than the agarose based resin.

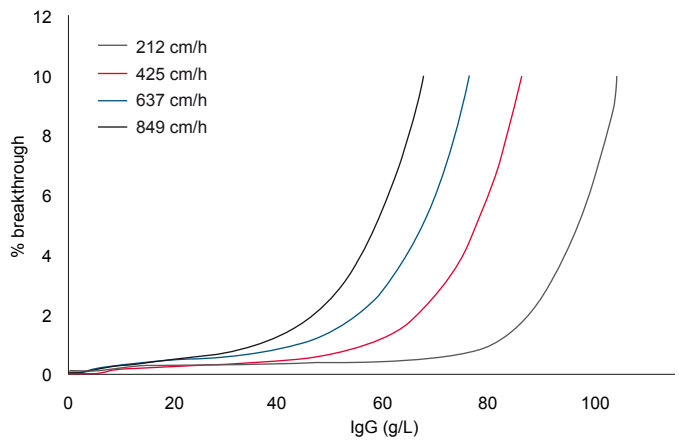
MECHANICAL STABILITY

The rigid polymer matrix allows high velocities. This can considerably 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 5).

FIGURE 4

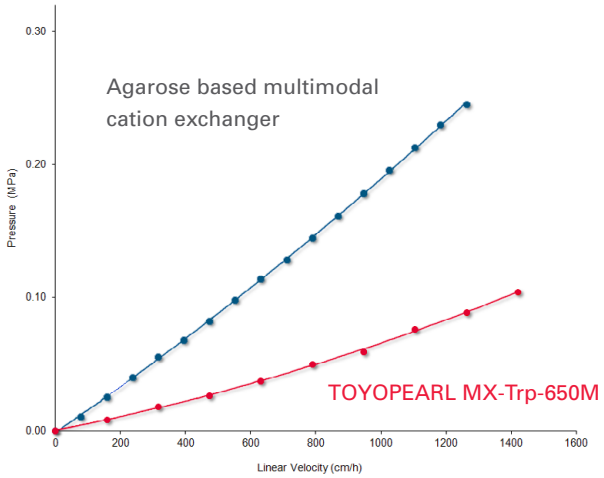
TOYOPEARL MX-Trp-650M DYNAMIC BINDING CAPACITY



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

FIGURE 5

TOYOPEARL MX-Trp-650M PRESSURE / FLOW CURVE



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

MIXED MODE CHROMATOGRAPHY TOYOPEARL MX-Trp-650M

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. 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 6).

TABLE I

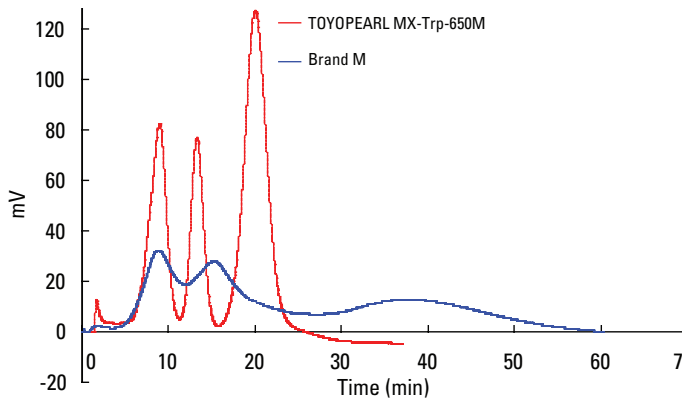
HIGH SALT TOLERANCE

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

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 g/L);

FIGURE 6

GOOD PEAK SHAPE AND HIGH RESOLUTION



Resins: TOYOPEARL MX-Trp-650M, Brand M
Column size: 7.5 mm ID × 7.5 cm; Mobile phase: Buffer A: 20 mmol/L phosphate (pH 7.0); Buffer B: 20 mmol/L phosphate + 1.0 mol/L NaCl (pH 7.0); Gradient: 30 min linear gradient from buffer A to buffer B; Flow rate: 1.0 mL/min; Detection: UV @ 280 nm;
Sample: trypsinogen (6.6 g/L) cytochrome C (3.6 g/L) lysozyme (6.6 g/L); Sample volume: 25 µL

MIXED-MODE CHROMATOGRAPHY
TOYOPEARL MX-Trp-650M

MXC - APPLICATIONS
AGGREGATE REMOVAL BY SALT/pH GRADIENTS

TOYOPEARL MX-Trp-650M combined with traditional method development and advanced systematic robotic screening is a perfect tool for the polishing of mAbs.

The importance of proper aggregate removal during polishing of a monoclonal antibody (mAb) for therapeutic use is beyond controversy. Severe anaphylactic reactions have been described in the literature for the application of aggregated proteins as a drug byproduct. Traditionally, ion exchange chromatography or hydrophobic interaction chromatography are utilized to purify a structurally homogeneous product. In case these platforms do not satisfy the requirements for mAb polishing, advanced chromatography resins need to be considered. For instance, mixed-mode stationary phases like TOYOPEARL MX-Trp-650M combining the advantages of hydrophobic interaction chromatography (HIC) and ion exchange chromatography (IEX) may pave the way for more challenging polishing applications.

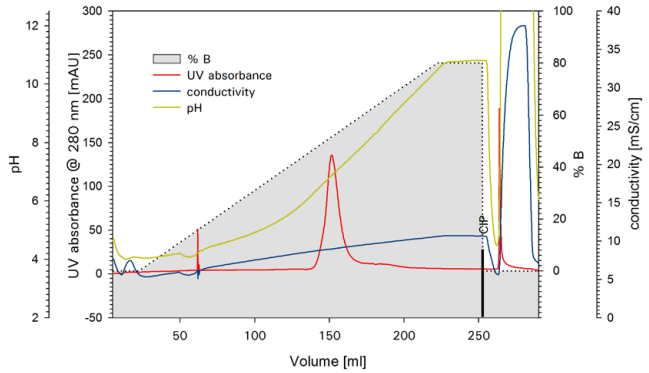
SCREENING FOR THE APPROPRIATE CONDITIONS FOR mAb AGGREGATE REMOVAL

The preferred environment for an antibody restricts the conditions. Binding and elution with moderate salt and pH conditions, as well as capacities comparable to IEX are in focus of ligand selection. Moreover, the need for an appropriate selectivity sets tight bounds.

In accordance with this, method development becomes more complex, as well. In case no robotic system is at hand, a straight-forward approach how to handle the increased number of parameters affecting the process of binding and elution is described in following example, the polishing of a humanized, monoclonal IgG. The major factors influencing binding and separation of proteins on TOYOPEARL MX-Trp-650M are the pH and the salt concentration.

FIGURE 8

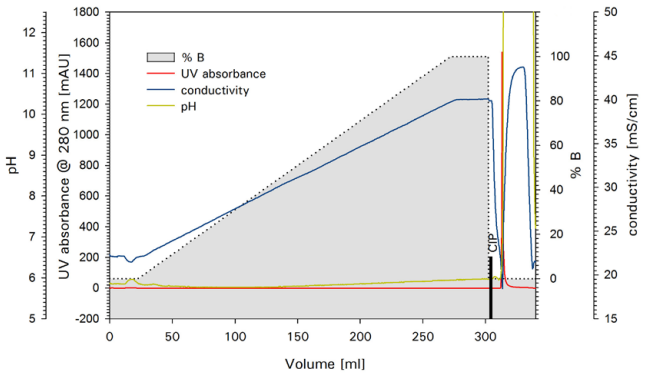
ELUTION BY pH GRADIENT



Column: 6.6 mm ID x 2 cm; Mobile phase A: buffer pH 4.0 + 0.2 mol/L NaCl; Mobile phase B: buffer pH 12.0 + 0.2 mol/L NaCL; Linear flow: 150 cm/h; Sample: 10 mg mAb + mAb aggregates (conc. 1 g/L)

FIGURE 7

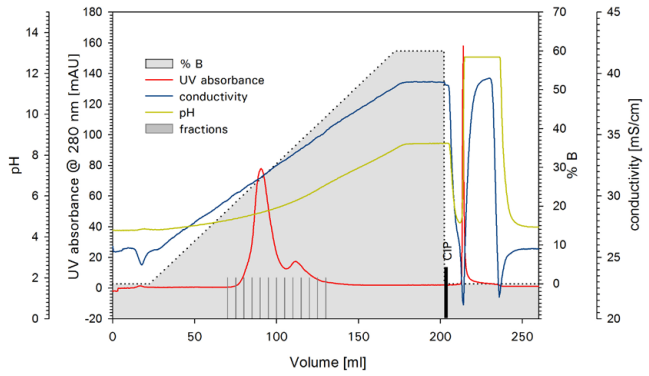
ELUTION BY SALT GRADIENT



Column: 6.6 mm ID x 2 cm; Mobile phase A: buffer pH 4.0 + 0.2 mol/L NaCl; Mobile phase B: buffer pH 4.0 + 0.5 mol/L NaCl; Linear flow: 150 cm/h; Sample: 10 mg mAb + mAb aggregates (conc. 1 g/L)

FIGURE 9

ELUTION BY COMBINED pH AND SALT GRADIENT



Column: 6.6 mm ID x 2 cm; Mobile phase A: buffer pH 4.0 + 0.2 mol/L NaCl; Mobile phase B: buffer pH 12 + 0.4 mol/L NaCl; Linear flow: 150 cm/h; Sample: 10 mg mAb + mAb aggregates (conc. 1 g/L)

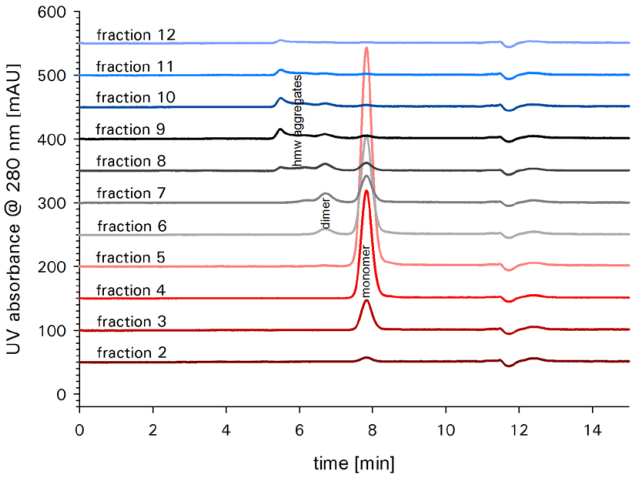
MXC - APPLICATIONS
AGGREGATE REMOVAL BY SALT/pH GRADIENTS

For a start, three linear gradient runs will provide hints on the actual working frame for a certain molecule. Figure 7, 8 & 9 show three chromatograms of the mAb sample containing approximately 17% aggregates. The three runs illustrate a salt gradient (constant pH), a pH gradient (constant salt concentration) and a combined salt and pH gradient. The pH span of the applied chromatofocusing buffer system depends, of course, on the stability of the sample. These buffer systems are either commercially available as ready to use buffer systems or can be prepared by arranging various (zwitter-) ionic buffer salts with pKs values covering the desired pH span.

While the salt gradient does not allow protein recovery, the pH gradient leads to the elution of one protein peak. In contrast, the combined pH and salt gradient recovers the protein in two peaks, a monomer peak in the front, followed by the aggregates. Quantitative and qualitative analysis of the collected protein peaks was performed by size exclusion chromatography (SEC) using TSKgel G3000SWXL. The corresponding results are presented in Figure 10.

FIGURE 10

SEC CHROMATOGRAMS OF THE COLLECTED FRACTIONS OF COMBINED SALT & pH GRADIENT ON TOYOPEARL MX-Trp-650M (SEE FIG. 1C).



Column: TSKgel G3000SWXL 7.8 mm ID x 30 cm; Mobile phase: 0.1 mol/L sodium phosphate + 0.1 mol/L sodium sulfate, pH 6.7; Flow rate: 1 mL/min; Detection: UV @ 280 nm; Sample: 100 µl of each fraction

MORE INFORMATION

MIXED MODE CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

ToyoScreen

PART#	PRODUCT DESCRIPTION	PACKAGE
0022824	ToyoScreen MX-Trp-650M	1 mL x 6
0022825		5 mL x 6

RoboColumns

PART#	PRODUCT DESCRIPTION	PACKAGE
0045051	RoboColumn MX-Trp-650M	200 µL x 8
0045052		600 µL x 8

MiniChrom

PART #	PRODUCT DESCRIPTION	DIMENSION
0045151	MiniChrom TOYOPEARL MX-Trp-650M, 5 mL	8 mm ID x 10 cm L

Resin Seeker

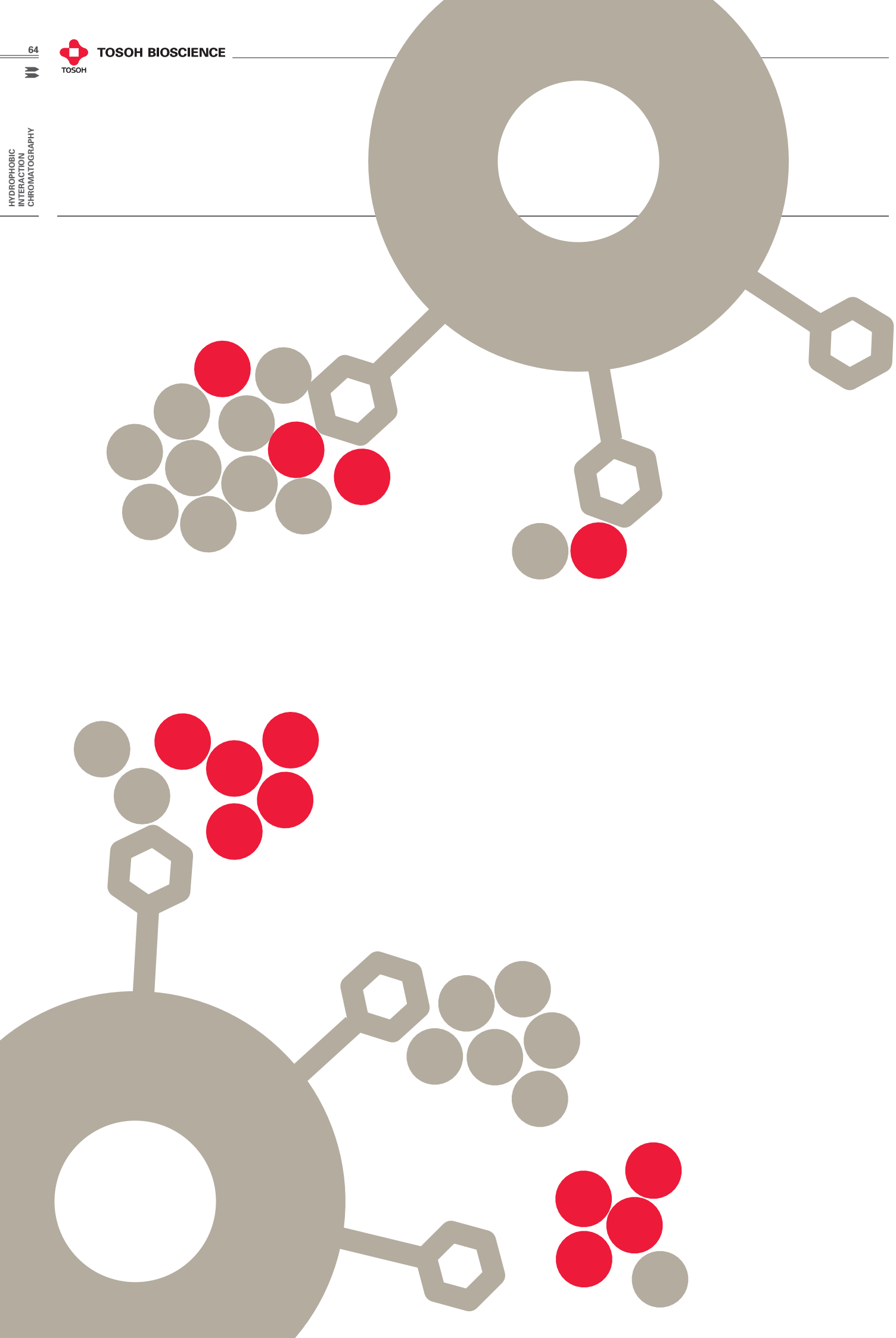
PART #	PRODUCT DESCRIPTION	PACKAGE
0045510	Resin Seeker MMC	20 µL x 96

ToyoScreen COLUMN ACCESSORIES

PART#	PRODUCT DESCRIPTION
0021400	ToyoScreen column holder
0045099	RoboColumn Array Plate

TOYOPEARL MIXED-MODE RESINS

PART#	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	TYPICAL CAPACITY (g IgG /L resin)
0022817	TOYOPEARL MX-Trp-650M	25	75	>75
0022818		100		
0022819		1,000		
0022820		5,000		



HIC
HYDROPHOBIC INTERACTION CHROMATOGRAPHY

HIC PRODUCTS

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

- TSKgel Ether-5PW
  TSKgel Phenyl-5PW

Tosoh Bioscience is the sole sponsor of the HIC-DSP conference.

"The intimate character of the conference offers an unparalleled opportunity to network and exchange scientific ideas. Better than other conferences I attended."

The 11th edition will take place on February 18-21, 2019 in Interlaken (Switzerland). More information: www.hic-dsp.org

HYDROPHOBIC INTERACTION CHROMATOGRAPHY HOW DOES IT WORK?

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

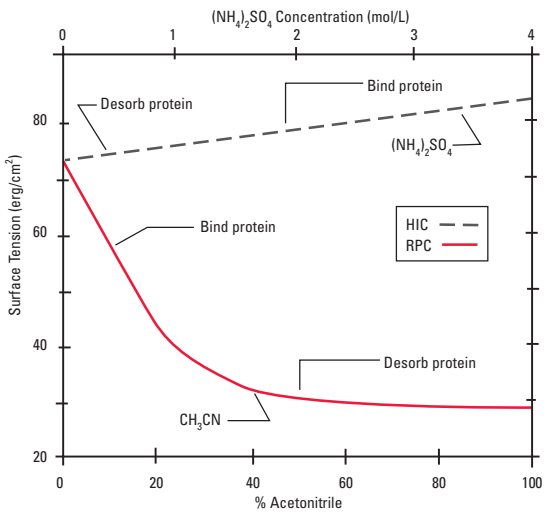
Proteins and other molecules with hydrophobic surfaces are attracted to the hydrophobic ligands of both reversed phase (RPC) and HIC resins. RPC resins have higher surface coverage and/or more hydrophobic ligand compared to HIC resins. Because of this, in a RPC separation the target binding readily occurs in an aqueous solution, and desorption is promoted by the addition of an increasing amount of organic solvent.

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

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

FIGURE 1

SURFACE TENSION OF AQUEOUS SOLUTIONS USED IN HIC & RPC

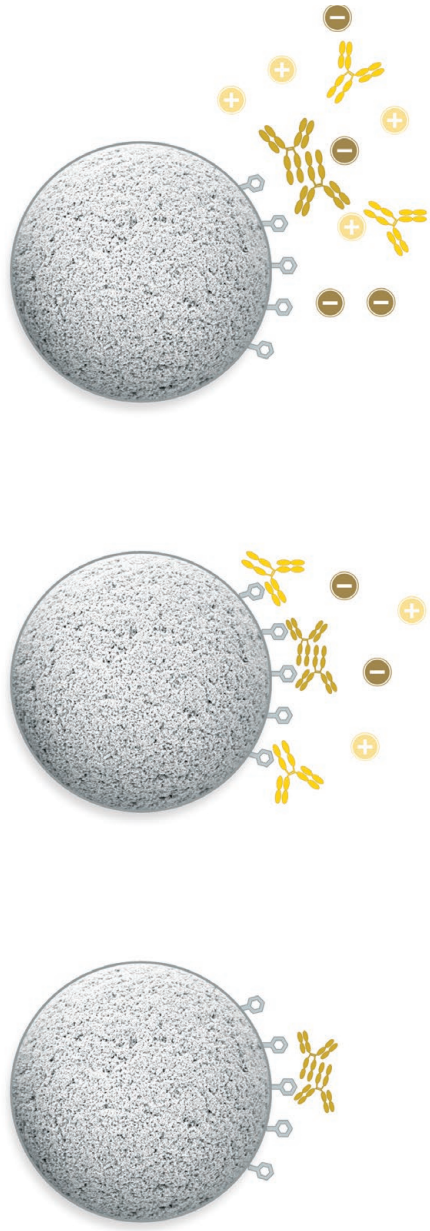


Mode	Gradient (typical)	Δ Surface tension (erg/cm²)
HIC	1.8 to 0 mol/L $(NH_4)_2SO_4$/ aqueous buffer	4
RPC	10 to 50 % ACN/ 0.1 % TFA	23

C. Horvath et. al., Separation Processes in Biotechnology, (J. Asenjo, Ed.) 9, 447 (1990) Marcel Dekker

FIGURE 2

HYDROPHOBIC INTERACTION CHROMATOGRAPHY ILLLUSTRATION



HYDROPHOBIC INTERACTION CHROMATOGRAPHY FEATURED PRODUCTS

FIVE DIFFERENT HYDROPHOBIC SURFACES AND SELECTIVITIES

Tosoh Bioscience offers five HIC ligands featuring different degrees of hydrophobicity and selectivity. The hydrophobicity of TOYOPEARL HIC resins increases through the ligand series: Ether, PPG (polypropylene glycol), Phenyl, Butyl, and Hexyl (Figure 3).

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

An optimum HIC process step will balance high dynamic binding capacity, adequate selectivity, good mass recovery and retention of biological activity. The wide range of TOYOPEARL selectivities enables a developer to optimize protein separations at the extremes of the hydrophobic spectrum. Highly retentive TOYOPEARL Hexyl-type and TOYOPEARL Butyl-type resins are used to separate hydrophilic proteins. These two resins should also be considered for separations requiring a low salt environment. TOYOPEARL Ether-type resin is used for the purification of very hydrophobic targets such as certain monoclonal antibodies and membrane proteins. These proteins may bind irreversibly to other more hydrophobic resins. TOYOPEARL PPG-type and TOYOPEARL Phenyl-type phases complement the other HIC ligands available in the TOYOPEARL series and offer alternatives for mid-range hydrophobic proteins.

TABLE I

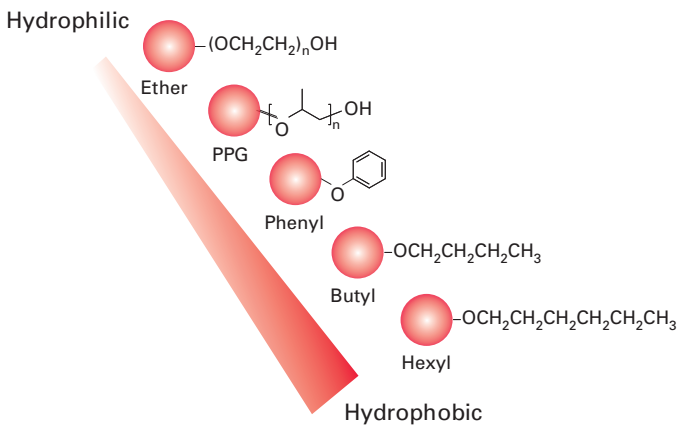
HIGH MASS RECOVERY (%) OF PROTEINS

	TOYOPEARL HIC resin		
	Ether-650M	Phenyl-650M	Butyl-650M
Bovine serum albumin	84	62	76*
α-chymotrypsinogen	96	88*	90
Cytochrome C	-	81*	87*
IgG	91	-	-
α-Lactalbumin	90	-	-
Lysozyme	94	92	85
Ovalbumin	83	88	73
Ribonuclease A	-	72*	82*

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

FIGURE 3

HIC LIGAND CANDIDATES



FEATURES

- Hydrophilic polymer resin matrix
- Good mechanical stability

BENEFITS

- Robust chemical stability between pH 1 - 13
- Temperature range 4 - 60 °C
- Autoclavable at 121 °C
- Compatible with organic solvents
- Constant bed volume over a wide range of salt concentrations
- Low non specific protein binding
- Superior protein recovery
- Excellent flow characteristics in large industrial size columns
- Direct scale-up from TSKgel HIC HPLC columns

HIC - APPLICATIONS
INFLUENCE OF SALT TYPES AND MIXTURES

The retention and selectivity of protein standards on TOYOPEARL HIC resins using the ToyoScreen process development columns are shown in Figure 4.

INFLUENCE OF SALT TYPE

In addition to the hydrophobicity of the ligand, the selectivity in HIC is influenced by the eluent salt type. Figure 5 demonstrates the effect of salt type on the resolution factor of different protein pairs.

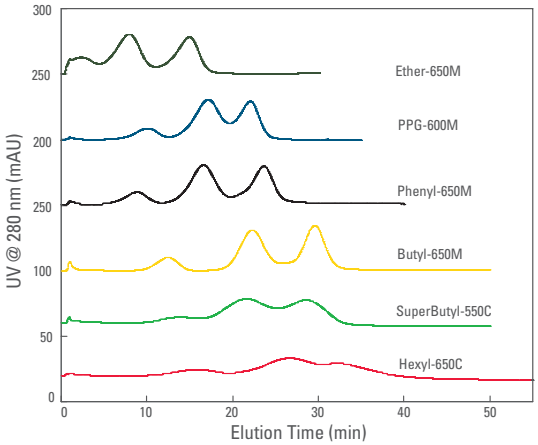
The Hofmeister lyotropic salt series shown in Figure 6 ranks anions and cations by their ability to promote protein precipitation. Ions on the left are referred to as "lyotropic" while the ions on the right are called "chaotropic".

Lyotropic salts will precipitate or "salt out" proteins at high salt concentrations due to increased hydrophobic interaction, while chaotropic salts will promote protein denaturation at high salt concentrations.

Application of mixed electrolytes provides an additional parameter for the optimization of a process step. Dual salt mixtures increase the recovery and purity of mAb process solutions for bind and elute and flowthrough mode. Variation of molar fractions can help to find a compromise between increased recovery and improved purity (Figure 7).

FIGURE 4

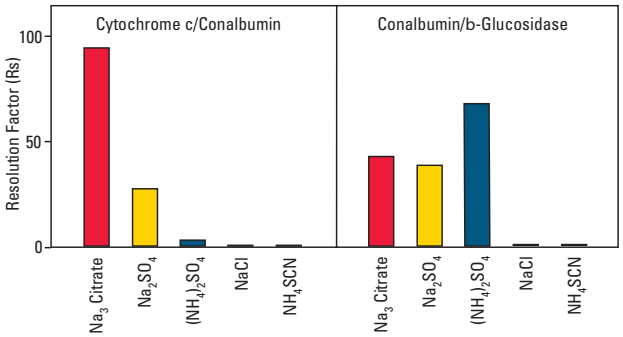
SCREENING OF TOYOPEARL HIC RESINS - STANDARD PROTEINS



Column: ToyoScreen (1 mL)
Mobile phase A: 0.1 mol/L phosphate buffer + 1.8 mol/L sodium sulfate (pH 7.0); Eluent B: 0.1 mol/L phosphate buffer (pH 7.0);
Gradient: 30 min linear gradient from A to B; Flow rate: 1 mL/min; Inj. vol.: 50 µL; Detector: UV @ 280 nm; Samples: Ribonuclease A, Lysozyme, α-Chymotrypsinogen, 1 g/L

FIGURE 5

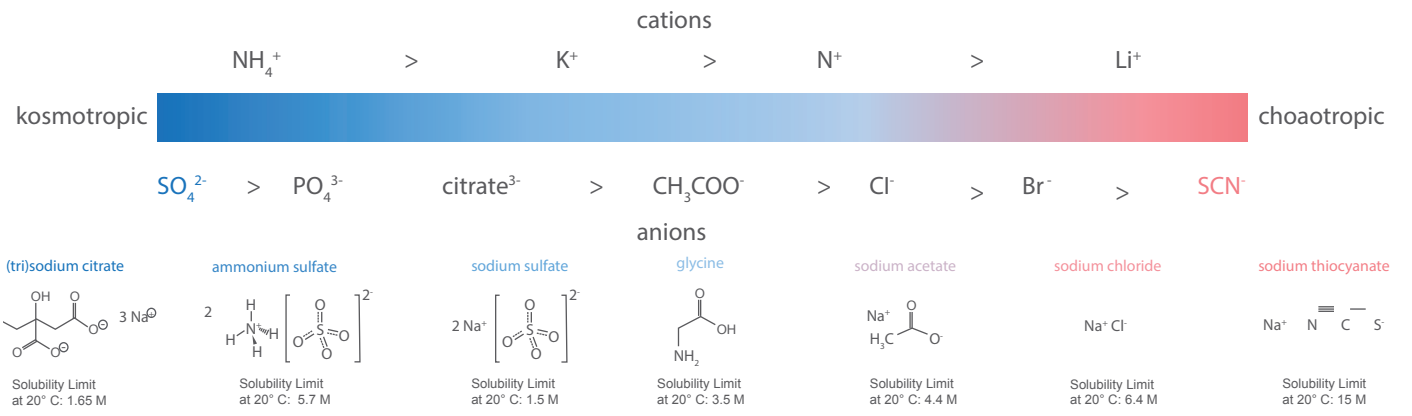
INFLUENCE OF SALT-TYPE ON RESOLUTION



Chromatography on a TOYOPEARL Butyl-substituted support
Column dimensions: 4.1 mm ID x 4 cm L
Mobile phase: Linear gradient, 20 min, 1.0 mol/L to 0 mol/L of indicated salt in 20 mmol/L phosphate buffer (pH 7.0);
Flow rate, 1 mL/min; Detector: UV @ 280 nm

J. Fausnaugh, L. Kennedy and F. Regnier, J. Chromatography 317, 141 (1984)

FIGURE 6



HIC - APPLICATIONS
PARTICLE AND PORE SIZE OPTIMIZATION

PARTICLE SIZE OPTIMIZATION

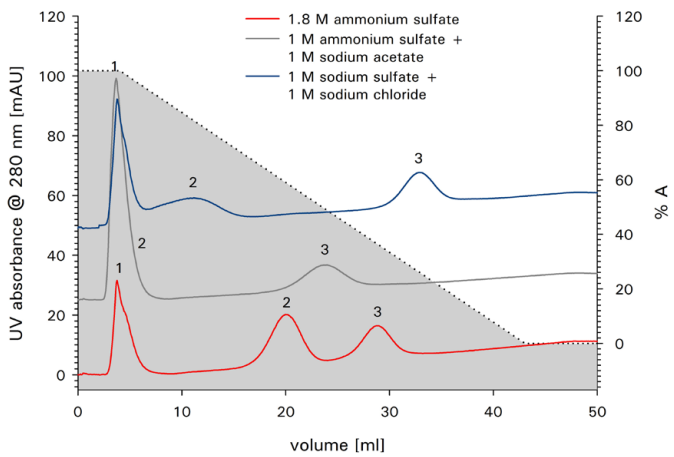
TOYOPEARL and TSKgel PW-type methacrylic base beads incorporate the same polymer chemistry and are available in a variety of particle sizes. This leads to different pressure-flow behaviors (Figure 8).

PORE SIZE OPTIMIZATION

Most TOYOPEARL HIC products are derived from the versatile base resin, TOYOPEARL HW-65 (100 nm mean pore size), as the base bead for the majority of protein separations. But the pore size and accessible surface area of TOYOPEARL resins can be optimized for a given protein. More accessible surface area increases the dynamic binding capacity (DBC) of the bead for a particular therapeutic target. This has led to the development of two specialty lines of HIC materials with higher dynamic binding capacities.

FIGURE 7

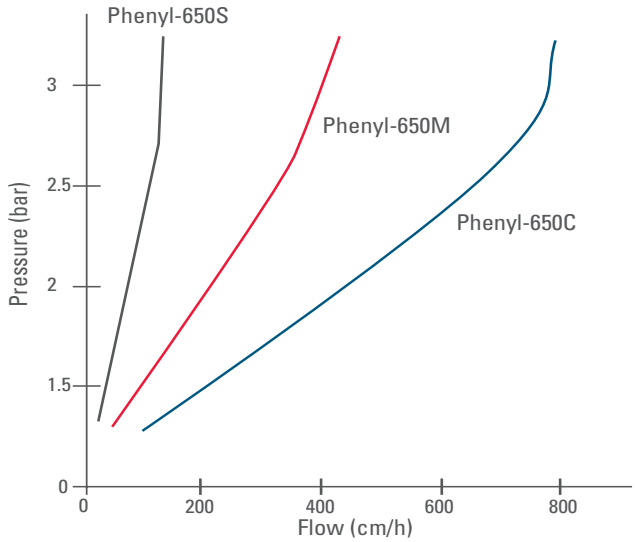
INFLUENCE OF SALT MIXTURES ON RESOLUTION



Separation of cytochrome C (1), ribonuclease A (2) and lysozyme (3) on TOYOPEARL PPG-600M. Ribonuclease A is hardly retained for the mixtures. Lysozyme is further retained for the sodium sulfate + sodium chloride mixture than for ammonium sulfate as a single salt.

FIGURE 8

PRESSURE-FLOW CURVE FOR TOYOPEARL PHENYL-650 RESINS OF VARIOUS PARTICLE SIZE



Column: TOYOPEARL Phenyl-650C, M and S, 25 mm ID x 25 cm L

Mobile phase: 2 mol/L $(NH_4)_2SO_4$

HIC - APPLICATIONS
DYNAMIC BINDING CAPACITY AND SELECTIVITY

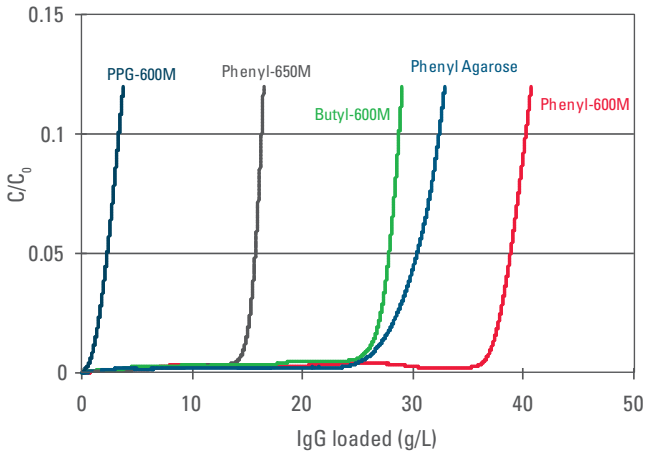
The large choice of pore and particle sizes for TOYOPEARL HIC resins gives the possibility to screen for the resin offering at the same time the highest binding capacity and the highest selectivity.

For monoclonal antibodies a pore size of 75 nm is optimum. A specially made base resin, TOYOPEARL HW-60, has this pore size. Three ligands are available on TOYOPEARL HW-60: polypropylene glycol (PPG), phenyl, and butyl. A comparison of their DBCs with TOYOPEARL Phenyl-650M resin is shown in Figure 9.

The selectivities of TOYOPEARL Butyl-600M, TOYOPEARL PPG-600M and the TOYOPEARL Phenyl-600M resins are shown in Figure 10.

FIGURE 9

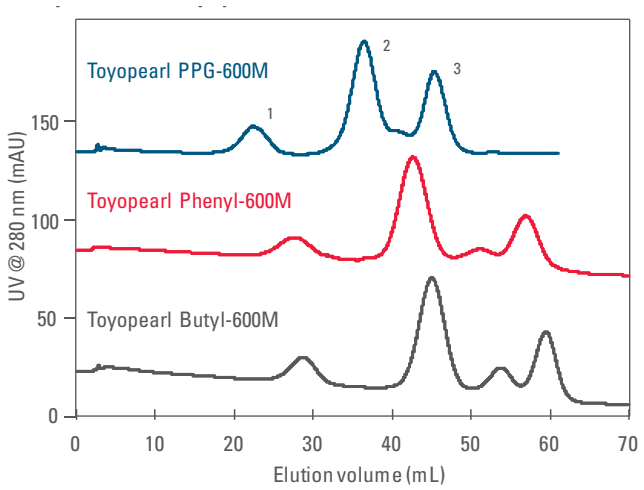
BREAKTHROUGH CURVES OF POLYCLONAL IgG ON VARIOUS HIC RESINS



Column: 7.8 mm ID X 20 cm L; Sample: polyclonal human IgG
Binding buffer: 1 g/L IgG in 0.8 mol/L $(NH_4)_2SO_4$ + 0.1 mol/L sodium phosphate (pH 7.0); Linear velocity: 300 cm/h
Temperature: 25 °C; Detector: UV @ 280 nm
DBC was calculated at 10% of breakthrough.

FIGURE 10

COMPARISON OF TOYOPEARL 600M SERIES RESINS



Column: 7.5 mm ID X 7.5 cm L; Sample: 1 g/L RNase A (1), lysozyme (2) and α-chymotrypsinogen A (3)
Sample load: 100 µL; Gradient: 60 min linear gradient from buffer A to B; Buffer A: 1.8 mol/L $(NH_4)_2SO_4$ + 0.1 mol/L sodium phosphate (pH 7.0); Buffer B: 0.1 mol/L sodium phosphate (pH 7.0)
Linear velocity: 136 cm/h (1.0 mL/min); Temperature: 25 °C
Detector: UV @ 280 nm

HIC - APPLICATIONS
DYNAMIC BINDING CAPACITY AND SELECTIVITY

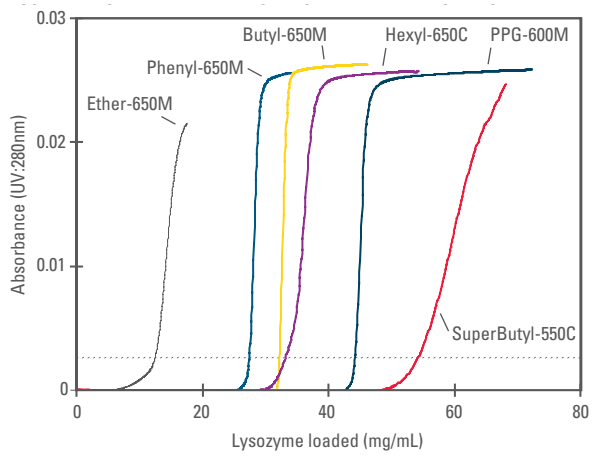
Figure 11 compares the selectivities of the TOYOPEARL Phenyl-600M, TOYOPEARL Phenyl-650M and an Agarose Phenyl resin.

For smaller molecules such as lysozyme (14,300 Da), the even narrower pore diameter TOYOPEARL SuperButyl-550C resin (derived from the 50 nm pore diameter TOYOPEARL HW-55) is recommended. A comparison of the DBC of TOYOPEARL SuperButyl-550C with other TOYOPEARL HIC resins is shown in Figure 12.

The TOYOPEARL Phenyl-600M resin also has a high DBC for lysozyme (Figure 13). The engineered higher dynamic binding capacity of the 600 and 550 series HIC products for their specific targets and the selectivity differences induced by the smaller mean pore size of the respective beads can have a dramatic impact on process economics.

FIGURE 12

TYPICAL DYNAMIC BINDING CAPACITIES FOR LYSOZYME

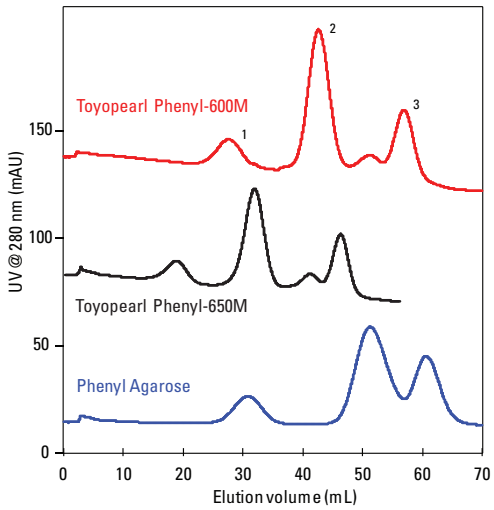


	Binding capacity (mg/mL) (10% Breakthrough)
Ether-650M	12.5
Phenyl-650M	27.5
Butyl-650M	32.2
Hexyl-650C	33.2
PPG-600M	44.2
SuperButyl-550C	54.3

Column size: 7.8 mm ID x 20 cm L; Sample: 1 mg/mL Lysozyme in 0.1 mol/L phosphate buffer + 1.8 mol/L sodium sulfate (pH 7.0); Linear Velocity: 100 cm/h (0.8 mL/min); Detection: UV @ 280 nm

FIGURE 11

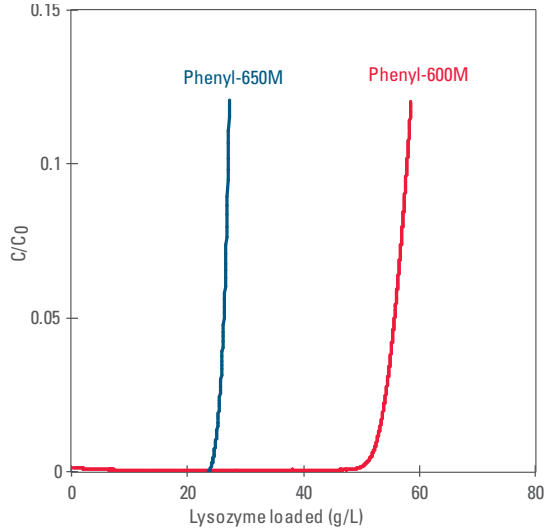
SELECTIVITY COMPARISON OF PHENYL-TYPE RESINS



Column: 7.5 mm ID x 7.5 cm L; Sample: 1 g/L RNase A (1), lysozyme (2) and α-chymotrypsinogen A (3); Sample load: 100 µL;
Gradient: 60 min. linear gradient from buffer A to B;
Buffer A: 1.8 mol/L $(NH_4)_2SO_4$ + 0.1 mol/L sodium phosphate (pH 7.0);
Buffer B: 0.1 mol/L sodium phosphate (pH 7.0);
Linear velocity: 136 cm/h (1.0 mL/min); Temperature: 25 °C;
Detector: UV @ 280 nm

FIGURE 13

TOYOPEARL Phenyl-600M BREAKTHROUGH CURVE (LYSOZYME)



	Binding capacity (g/L) (10% Breakthrough)
Phenyl-600M	58
Phenyl-650M	27

Column: 7.8 mm ID x 20 cm L; Sample: 1 g/L lysozyme in 0.1 mol/L phosphate buffer (pH 7.0) + 1.8 mol/L (NH4)2SO4
Linear velocity: 300 cm/h; Detector: UV @ 280 nm

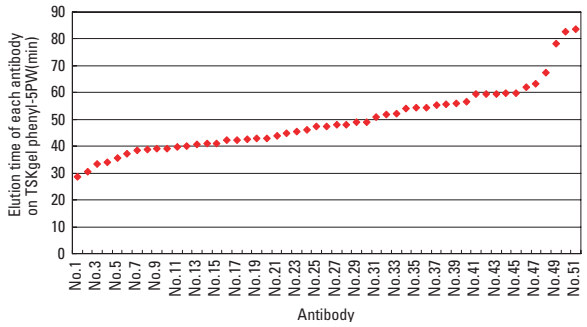
HIC - APPLICATIONS MONOCLONAL ANTIBODIES

Hydrophobic interaction is a very useful technique for the purification of monoclonal antibodies. The diverse hydrophobic nature of mAbs is seen in Figure 14. This figure measures the hydrophobicity (using elution time as a surrogate measurement) of 51 different mouse IgGs on a TSKgel Phenyl-5PW analytical column. Some of the IgGs have elution times 2-3 times longer than others indicating greater hydrophobicity. The TOYOPEARL series of HIC ligands with their different hydrophobicities gives chromatographic developers a range of options for finding the right ligand for their target molecule.

For a very hydrophobic mAb, such as mouse anti-chicken 14 kDa lectin, the less hydrophobic TOYOPEARL Ether ligand works quite well. The purification from ascites fluid (Figure 15) was performed with a 10 µm TSKgel Ether-5PW semi-preparative column. Identical selectivity for scale-up was found with corresponding 65 µm TOYOPEARL Ether-650M resin.

FIGURE 14

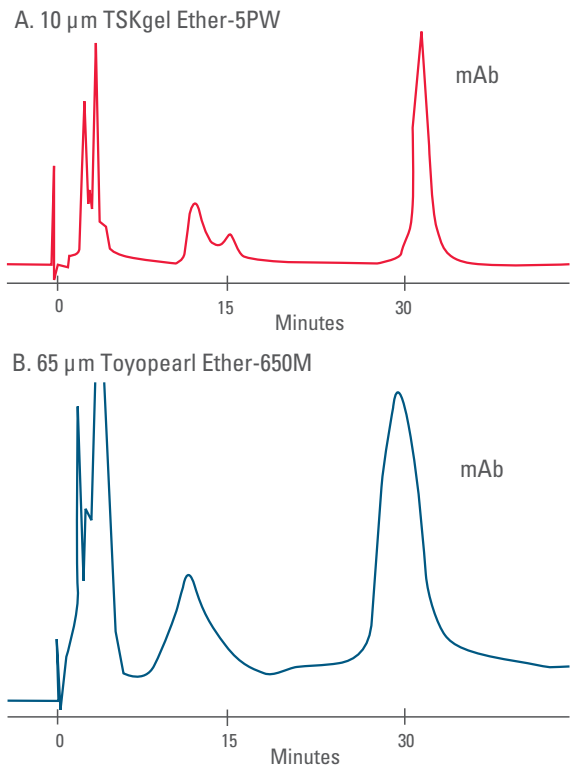
HYDROPHOBIC DIVERSITY OF MOUSE MONOCLONALS



Plot of chromatographic elution times for 51 different mouse mAbs
Column: TSKgel Phenyl-5PW; Mobile phase: (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 mL/min; Gradient: (B) 0 % (0 min)-0 % (5 min)-100 % (65 min) linear; Detector: UV @ 280 nm; Samples: 51 kinds of mouse monoclonal

FIGURE 15

PURIFICATION OF mAbs FROM ASCITES FLUID



Column: A. TSKgel Ether-5PW, 7.5 mm ID x 7.5 cm L
B. TOYOPEARL Ether-650M, 7.5 mm ID x 7.5 cm L
Sample: anti-chicken 14 kDa lectin, diluted ascites fluid,
A. 1.5 mg in 100 µL; B. 0.76 mg in 50 µL
Mobile phase: 60 min linear gradient from 1.5 mol/L to 0 mol/L $(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer (pH 7.0)
Linear velocity: 136 cm/h; Detection: UV @ 280 nm

HIC - APPLICATIONS
AGGREGATES, GLYCOPROTEINS, DNA AND MISFOLDING

PROTEIN AGGREGATE REMOVAL

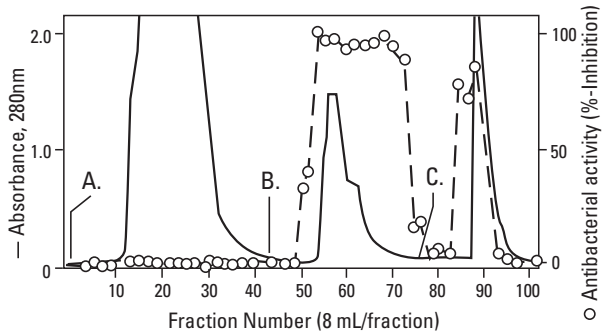
The larger pore TOYOPEARL products such as TOYOPEARL Butyl-650 and TOYOPEARL Phenyl-650 resins are very useful for protein aggregate separation and removal. Early work by Karger et al.[1] in 1989 involving proteins and aggregates larger than 200 kDa demonstrated the effectiveness of HIC for this application.

GLYCOPROTEINS

TOYOPEARL HIC resins can purify glycoproteins, which often bind irreversibly to saccharide-based chromatographic media. Figure 16 shows the purification of a large glycoprotein on TOYOPEARL Butyl-650S resin.

FIGURE 16

LARGE GLYCOPROTEIN PURIFIED ON TOYOPEARL Butyl-650S



Column: TOYOPEARL Butyl-650S, 22 mm ID x 26 cm L
Sample: crude protein from sea hare Aplysia kurodai;
Mobile phase: multi-step $(NH_4)_2SO_4$ in 50 mmol/L phosphate buffer (pH 7.0);
A. load & wash: 40 % saturated $(NH_4)_2SO_4$; B. 20 % saturated $(NH_4)_2SO_4$;
C. 0% saturated $(NH_4)_2SO_4$; Detector: UV @ 280 nm

DNA PLASMID PURIFICATION AND ENDOTOXIN REMOVAL

TOYOPEARL Hexyl-650C resin was successfully used for plasmid DNA purification by Cambrex, Baltimore, MD (US patent 6,953,686). Hexyl-650C was shown to be the most effective among HIC resins for endotoxin removal with capacities exceeding 2 million EU/mL of resin. Additionally, RNA and protein impurities were effectively eliminated. Hexyl-650C was also effective in separating the supercoiled and open circular forms of plasmid DNA.

REMOVAL OF MISFOLDED PROTEINS

Because misfolded proteins will generally be more hydrophobic than the native protein, TOYOPEARL Butyl-650M resin is used frequently for the removal of misfolded proteins. In many cases flow through chromatography can be accomplished under eluent conditions binding the misfolds while allowing the target to flow through the column.

Go to www.tosohbioscience.de, to check our database on the website for additional applications.

[1] Grinberg, N. Blanco, R. Yarmush, D. Karger, B. L. Protein Aggregation in High Performance Liquid Chromatography: Hydrophobic Interaction Chromatography of ß-Lactoglobulin, Anal. Chem. 1989, 61, 514-520.

HYDROPHOBIC INTERACTION CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

ToyoScreen

PART #	PRODUCT DESCRIPTION	PACKAGE
0021372	ToyoScreen Ether-650M	1 mL x 6
0021373		5 mL x 6
0021380	ToyoScreen PPG-600M	1 mL x 6
0021381		5 mL x 6
0021892	ToyoScreen Phenyl-600M	1 mL x 6
0021893		5 mL x 6
0021374	ToyoScreen Phenyl-650M	1 mL x 6
0021375		5 mL x 6
0021494	ToyoScreen Butyl-600M	1 mL x 6
0021495		5 mL x 6
0021376	ToyoScreen Butyl-650M	1 mL x 6
0021377		5 mL x 6
0021382	ToyoScreen SuperButyl-550C	1 mL x 6
0021383		5 mL x 6
0021378	ToyoScreen Hexyl-650C	1 mL x 6
0021379		5 mL x 6
0021398	ToyoScreen HIC Mix Pack, (PPG-600M, Butyl-600M, Phenyl-650M, Butyl-650M, Phenyl-600M, Hexyl-650C)	1 mL x 6 Grades x 1 each
0021399		5 mL x 6 Grades x 1 each

ToyoScreen COLUMN ACCESSORIES

PART #	PRODUCT DESCRIPTION
0021400	ToyoScreen Column Holder

RoboColumns

PART #	PRODUCT DESCRIPTION	PACKAGE
0045035	RoboColumn PPG-600M	200 µL X 8
0045036		600 µL X 8
0045031	RoboColumn Phenyl-600M	200 µL X 8
0045032		600 µL X 8
0045037	RoboColumn Phenyl-650M	200 µL X 8
0045038		600 µL X 8
0045033	RoboColumn Butyl-600M	200 µL X 8
0045034		600 µL X 8

HYDROPHOBIC INTERACTION CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

MiniChrom

PART #	PRODUCT DESCRIPTION	DIMENSION
0045124	MiniChrom TOYOPEARL PPG-600M, 5 mL	8 mm ID x 10 cm L
0045123	MiniChrom TOYOPEARL Phenyl-600M, 5 mL	8 mm ID x 10 cm L
0045122	MiniChrom TOYOPEARL Phenyl-650S, 5 mL	8 mm ID x 10 cm L
0045121	MiniChrom TOYOPEARL Phenyl-650M, 5 mL	8 mm ID x 10 cm L
0045127	MiniChrom TOYOPEARL Butyl-600M, 5 mL	8 mm ID x 10 cm L
0045126	MiniChrom TOYOPEARL Butyl-650S, 5 mL	8 mm ID x 10 cm L
0045125	MiniChrom TOYOPEARL Butyl-650M, 5 mL	8 mm ID x 10 cm L
0045128	MiniChrom TOYOPEARL SuperButyl-550C, 5 mL	8 mm ID x 10 cm L
0045129	MiniChrom TOYOPEARL Hexyl-650C, 5 mL	8 mm ID x 10 cm L

Resin Seeker

PART #	PRODUCT DESCRIPTION	PACKAGE
0045511	Resin Seeker HIC	20 µL x 96

TSKgel LABPAK

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)
0043278	HICPAK PW (20) (Ether-5PW, Phenyl-5PW)	2 x 25 mL	10-30
0043175	HICPAK PW (30) (Ether-5PW, Phenyl-5PW)	2 x 25 mL	20-40

HYDROPHOBIC INTERACTION CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

TOYOPEARL HIC RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	TYPICAL CAPACITY (g LYSOZYME/L RESIN)
0043151	TOYOPEARL Ether-650S	25	35	10-30
0016172		100		
0016174		1,000		
0016176		5,000		
0019805	TOYOPEARL Ether-650M	25	65	10-30
0016173		100		
0016175		1,000		
0016177		5,000		
0021301	TOYOPEARL PPG-600M	25	65	45-55
0021302		100		
0021303		1,000		
0021304		5,000		
0021305		50,000		
0021887	TOYOPEARL Phenyl-600M	25	65	45-65
0021888		100		
0021889		1,000		
0021890		5,000		
0021891		50,000		
0043152	TOYOPEARL Phenyl-650S	25	35	30-50
0014477		100		
0014784		1,000		
0014935		5,000		
0019818	TOYOPEARL Phenyl-650M	25	65	30-50
0014478		100		
0014783		1,000		
0014943		5,000		
0018364		50,000		
0043126	TOYOPEARL Phenyl-650C	25	100	30-50
0014479		100		
0014785		1,000		
0014944		5,000		
0021448	TOYOPEARL Butyl-600M	25	65	40-60 (g/L γ-globulin)
0021449		100		
0021450		1,000		
0021451		5,000		
0021452		50,000		
0043153	TOYOPEARL Butyl-650S	25	35	30-50
0007476		100		
0014701		1,000		
0007975		5,000		
0018826		50,000		

HYDROPHOBIC INTERACTION CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

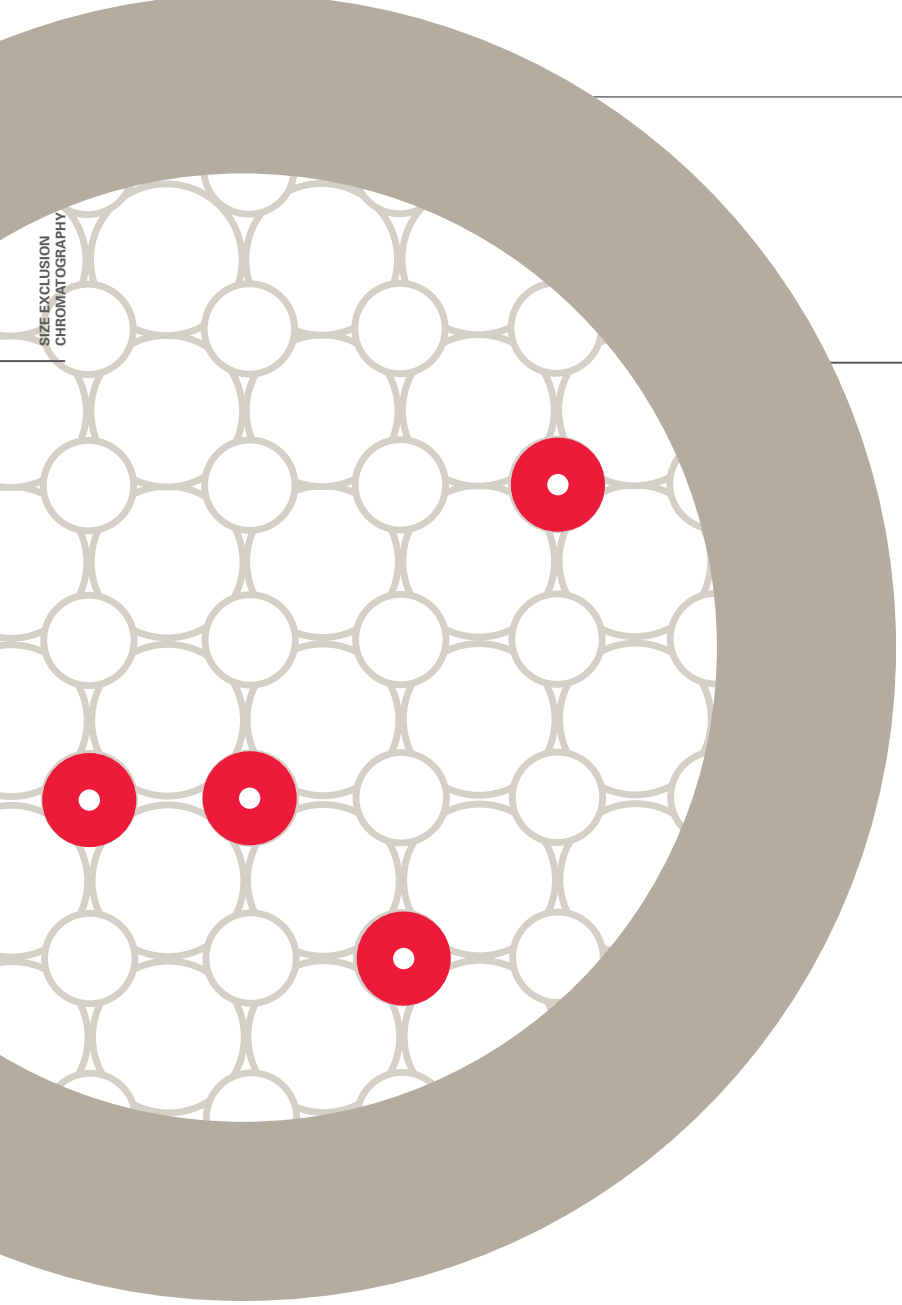
ORDERING INFORMATION

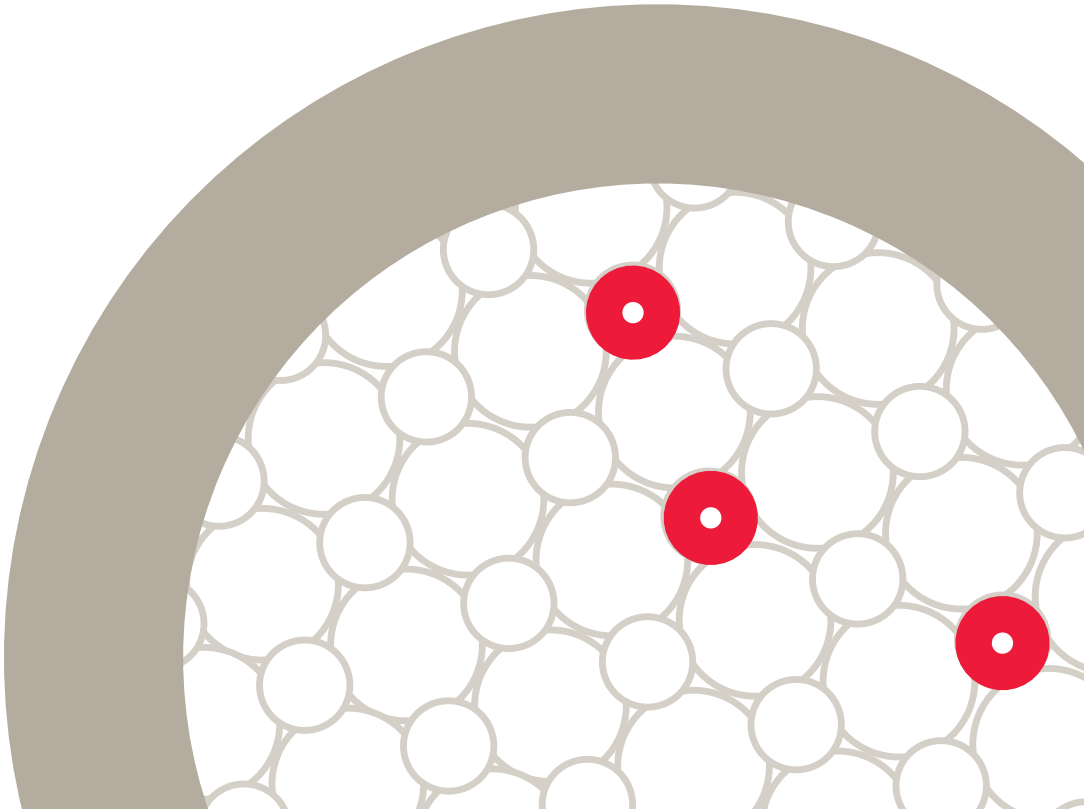
TOYOPEARL HIC RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	TYPICAL CAPACITY (g LYSOZYME/L RESIN)
0019802	TOYOPEARL Butyl-650M	25	65	30-50
0007477		100		
0014702		1,000		
0007976		5,000		
0018355		50,000		
0043127	TOYOPEARL Butyl-650C	25	100	30-50
0007478		100		
0014703		1,000		
0007977		5,000		
0019955	TOYOPEARL SuperButyl-550C	25	100	52-70
0019956		100		
0019957		1,000		
0019958		5,000		
0019959		50,000		
0044465	TOYOPEARL Hexyl-650C	25	100	30-50
0019026		100		
0019027		1,000		
0019028		5,000		

TSKgel 5PW HIC RESINS FOR HIGH RESOLUTION

0043276	TSKgel Ether-5PW (20)	25	10-30	10-30
0016052		250		
0016053		1,000		
0018437		5,000		
0043176	TSKgel Ether-5PW (30)	25	20-40	10-30
0016050		250		
0016051		1,000		
0018439		5,000		
0043277	TSKgel Phenyl-5PW (20)	25	10-30	10-30
0014718		250		
0014719		1,000		
0018438		5,000		
0043177	TSKgel Phenyl-5PW (30)	25	20-40	10-30
0014720		250		
0014721		1,000		
0017210		5,000		





SEC
SIZE EXCLUSION CHROMATOGRAPHY

SEC PRODUCTS

TOYOPEARL Resins for SEC

TOYOPEARL HW-40
TOYOPEARL HW-50
TOYOPEARL HW-55
TOYOPEARL HW-65
TOYOPEARL HW-75

Tosoh is well known for offering not only process resins, but also (U)HPLC columns for the analytical separation of biomolecules in the biopharmaceutical industry.

"Although, several columns showed a comparable resolution, the Tosoh TSKgel UP-SW3000 column (2 µm, 4.6 x 30 mm) convinced us in terms of robustness, especially the high lot-to-lot stability, an absolute requirement for quality control under GMP conditions."

Dr. Raphael Ruppert
Roche Diagnostics

SIZE EXCLUSION CHROMATOGRAPHY HOW DOES IT WORK

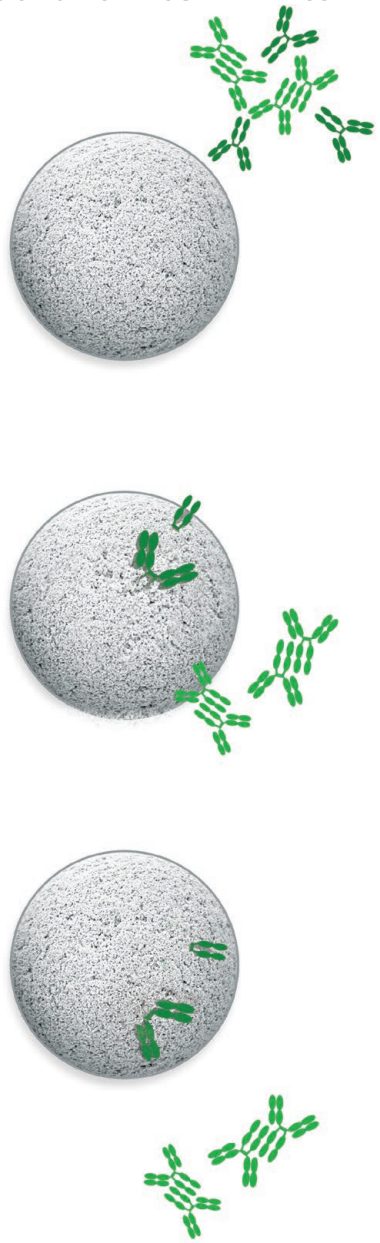
THE ROLE OF SIZE EXCLUSION CHROMATOGRAPHY IN PROCESS PURIFICATION

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

SEC is applicable in final polishing steps where a target protein is being separated from its aggregates or other significantly different molecular weight impurities. Another application area is the desalting of the purified target protein.

FIGURE 1

SIZE EXCLUSION CHROMATOGRAPHY ILLUSTRATION



FEATURES

- Small particles available
- Hydrophilic porous polymer structure
- Narrow particle size distribution
- Good mechanical stability
- Chemically stable (pH 2 – 14)
- Identical resin structure to TSKgel HPLC resins

BENEFITS

- High resolution
- Minimal non-specific adsorption effects
- High performance SEC – more efficient separations
- Better pressure-flow characteristics
- Excellent flow characteristics in large industrial size columns
- Constant packing volume over a wide range of salt concentrations
- Compatible with organic solvents, can be cleaned in place (CIP) with acid or base
- Stable polymer may be run at elevated temperature (4 - 60 °C)
- Autoclavable at 121 °C
- Direct scale-up from TSKgel HPLC columns

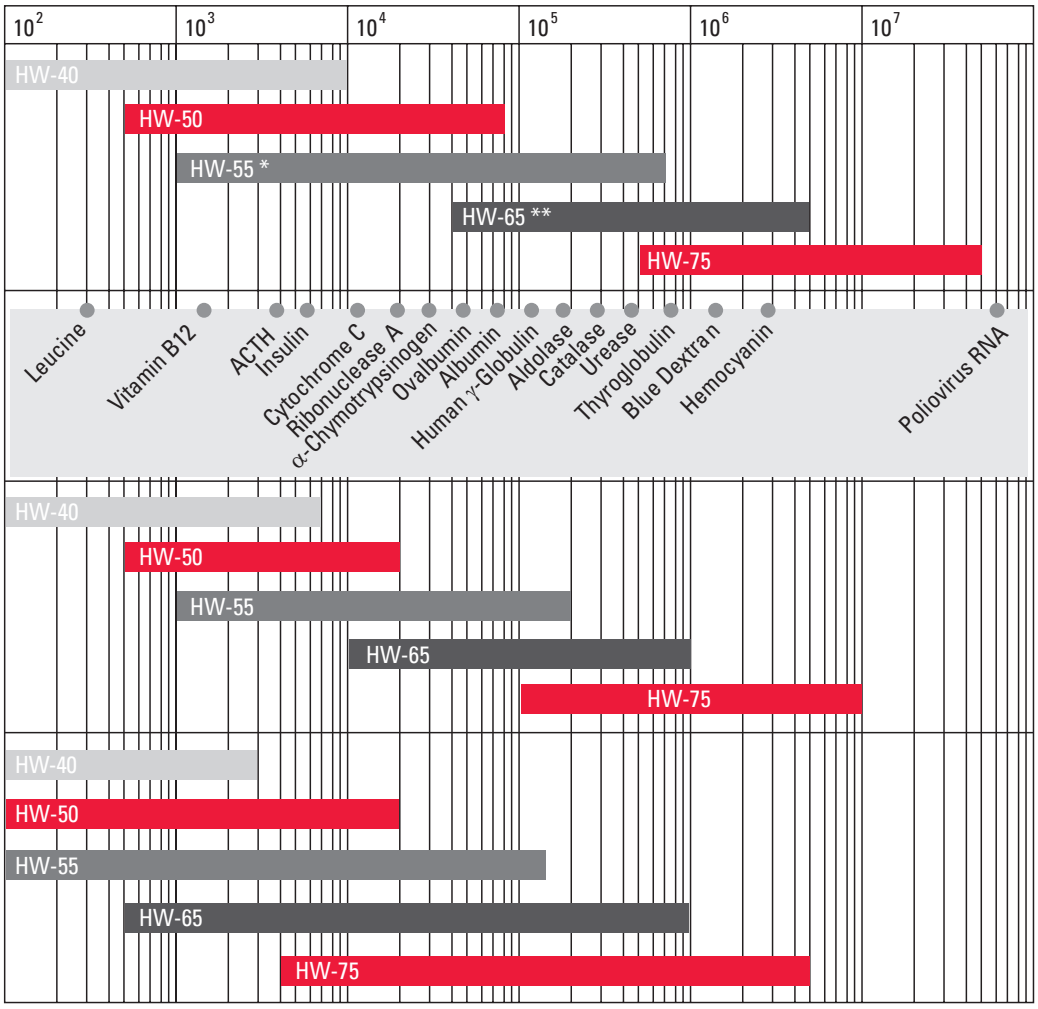
SIZE EXCLUSION CHROMATOGRAPHY PRODUCT OVERVIEW

TABLE I

PROPERTIES AND MOLECULAR WEIGHT SEPARATION RANGES FOR TOYOPEARL HW-TYPE RESINS
(HW = HYDROPHILIC, WATER-COMPATIBLE POLYMERIC BASE RESINS)

			Molecular weight of sample (Da)		
TOYOPEARL RESIN	PARTICLE SIZE (µm)	PORE SIZE (nm)	POLYETHYLENE GLYCOLS AND OXIDES	DEXTRANS	GLOBULAR PROTEINS
HW-40S HW-40F HW-40C	30 45 75	5	100 - 3,000	100 - 7,000	100 - 10,000
HW-50S HW-50F	30 45	12,5	100 - 18,000	500 - 20,000	500 - 80,000
HW-55S HW-55F	30 45	50	100 - 150,000	1,000 - 200,000	1,000 - 700,000
HW-65S HW-65F HW-65C	30 45	100	500 - 1,000,000	10,000 - 1,000,000	40,000 - 5,000,000
HW-75F	45	> 1000	4,000 - 5,000,000	100,000 - 10,000,000	500,000 - 50,000,000

TABLE II



A) GLOBULAR PROTEINS

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

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

CALIBRATION MOLECULES

B) DEXTRANS

C) POLYETHYLENE GLYCOLS

SEC - APPLICATIONS

The TOYOPEARL HW-type resin range spans peptide and protein molecular weights between 100 - 50,000,000 Daltons. Each TOYOPEARL HW-type resin displays a typical calibration curve and exclusion limit for globular proteins (Figure 2).

PARTICLE SIZE

Resolution increases with decreasing particle size (Figure 3). Resin particle size is proportional to HETP and inversely proportional to the column efficiency and resolution of two peaks.

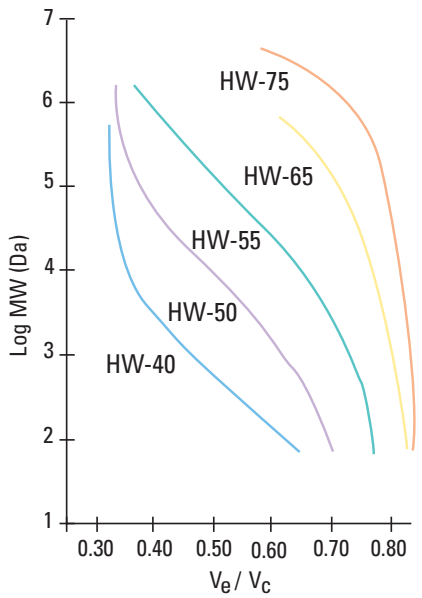
Most TOYOPEARL HW-type resins are available in three particle size ranges:

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

When the highest resolution is needed, the smaller S and F grade beads are preferred for process SEC. For desalting, where the resin is used in a filtration mode to remove the target from a buffer, the C grade is primarily employed because of its better flow dynamics at lower operating pressures.

FIGURE 2

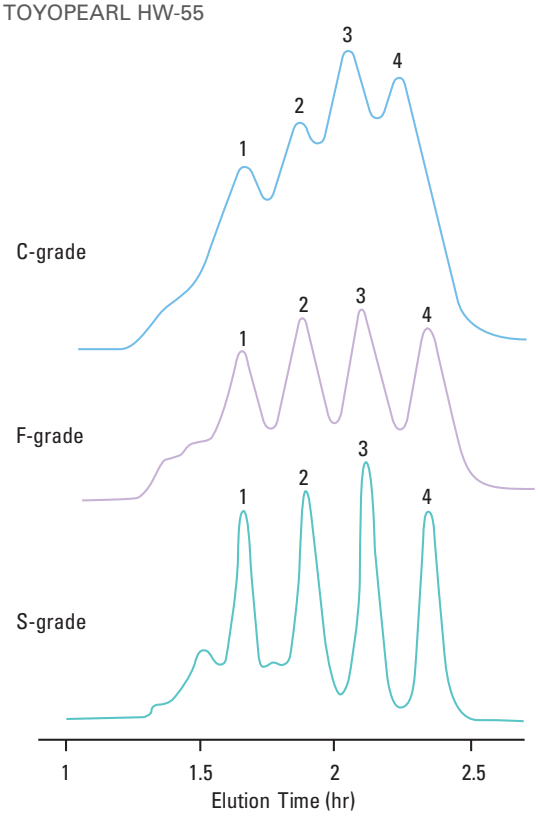
CALIBRATION CURVES FOR GLOBULAR PROTEINS ON TOYOPEARL



Column: 22 mm ID x 30 cm L; Sample: Protein standards; Mobile phase: 0.06 mol/L phosphate buffer (pH 7.0) in 0.06 mol/L KCl; Legend: Ve = elution volume, Vc = column volume; Detection: UV @ 280 nm

FIGURE 3

COMPARISON OF RESOLUTION ON DIFFERENT PARTICLE SIZES OF TOYOPEARL HW-55



Column: TOYOPEARL HW-55, 26 mm ID x 70 cm L
Sample: 1. Thyroglobulin (0.3 %), 2. γ -Globulin (0.3 %), 3. β-Lactoglobulin (0.3 %), 4. Cytochrome C (0.1 %); Mobile phase: 33.3 mmol/L phosphate buffer (pH 7.0), 0.2 mol/L NaCl; Flow rate: 106 mL/h (20 cm/h); Inj. vol.: 1 mL; Temperature: 25 °C; Detection: UV @ 280 nm

SEC - APPLICATIONS

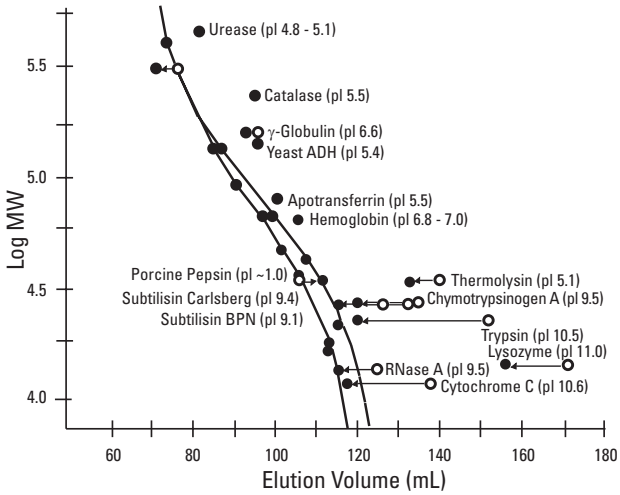
MOBILE PHASE

Mobile phase components, such as salts, can affect SEC separations. The presence or absence of sodium chloride influences the elution volume of proteins. This is demonstrated in Figure 4, in which a mixture of various proteins was separated on a column packed with TOYOPEARL HW-55F. Salt concentrations can change the hydrodynamic radius of proteins and either increase or decrease their molecular size as a function of salt strength. Ideally, in SEC sample components do not interact with the packing material. In practice it is often necessary to select a salt concentration which minimizes secondary interactions of the sample components with the resin. However, there are instances where secondary interactions, particularly hydrophobic interactions at higher salt concentrations, can be exploited.

It is important to note that relatively minor changes in protein structure may affect protein solubility and encourage secondary hydrophobic interactions, causing similarly sized proteins or analogs to elute at different times. In those cases it may be necessary to modify the mobile phase composition to regain a separation based on molecular size alone.

FIGURE 4

COMPARISON OF THE ELUTION VOLUMES OF PROTEINS IN PRESENCE AND ABSENCE OF NaCl



Column: TOYOPEARL HW-55F, 22 mm ID x 50 cm L; Elution: 25 mmol/L Tris-HCl with (•) or without (°) 0.5 mol/L NaCl, (pH 7.5); Flow rate: 16 cm/h; Temperature: 5 - 10 °C; Detection: UV @ 280 nm, 420 nm for heme proteins, 200 nm for proteins without aromatic amino acid

SEC - APPLICATIONS

PROPERTIES IN ORGANIC ELUENTS

TOYOPEARL resins can be used in organic solvents or mixtures of organic solvents and water. Bed volumes may swell or shrink relative to water depending on the solvent as shown in Tables III and IV. DMSO can be used for SEC of oligosaccharides and polyethylene glycols. The compatibility of DMF with TOYOPEARL also permits SEC separation of hydrophobic substances such as polystyrenes.

OTHER APPLICATIONS

The TOYOPEARL HW-type resins are commonly used in size exclusion chromatography and desalting applications. Some other important uses of these materials are:

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

TABLE III

SWELLING PROPERTIES IN VARIOUS SOLVENTS

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

TABLE IV

ADDITIONAL SWELLING DATA FOR TOYOPEARL HW-40

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

SIZE EXCLUSION CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

RoboColumns

PART #	PRODUCT DESCRIPTION	PACKAGE
0045071	ToyoScreen RoboColumn HW-40F	200 µL x 8
0045072		600 µL x 8

MiniChrom

PART #	PRODUCT DESCRIPTION	DIMENSION
0045171	MiniChrom TOYOPEARL HW-40F, 5 mL	8 mm ID x 10 cm L

TOYOPEARL LABPAK

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)
0019821	SECPAK LMW (HW-40F, HW-50F, HW-55F)	3 x 150	45
0019819	SECPAK HMW (HW-55F, HW-65F, HW-75F)	3 x 150	45
0019820	SECPAK HP (HW-40S, HW-50S, HW-55S, HW-65S)	4 x 150	30

TOYOPEARL SEC RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	EXCLUSION LIMIT (Da)
0019809	TOYOPEARL HW-40S	150	30	3×10^3
0007451		250		
0014681		1,000		
0007967		5,000		

SIZE EXCLUSION CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

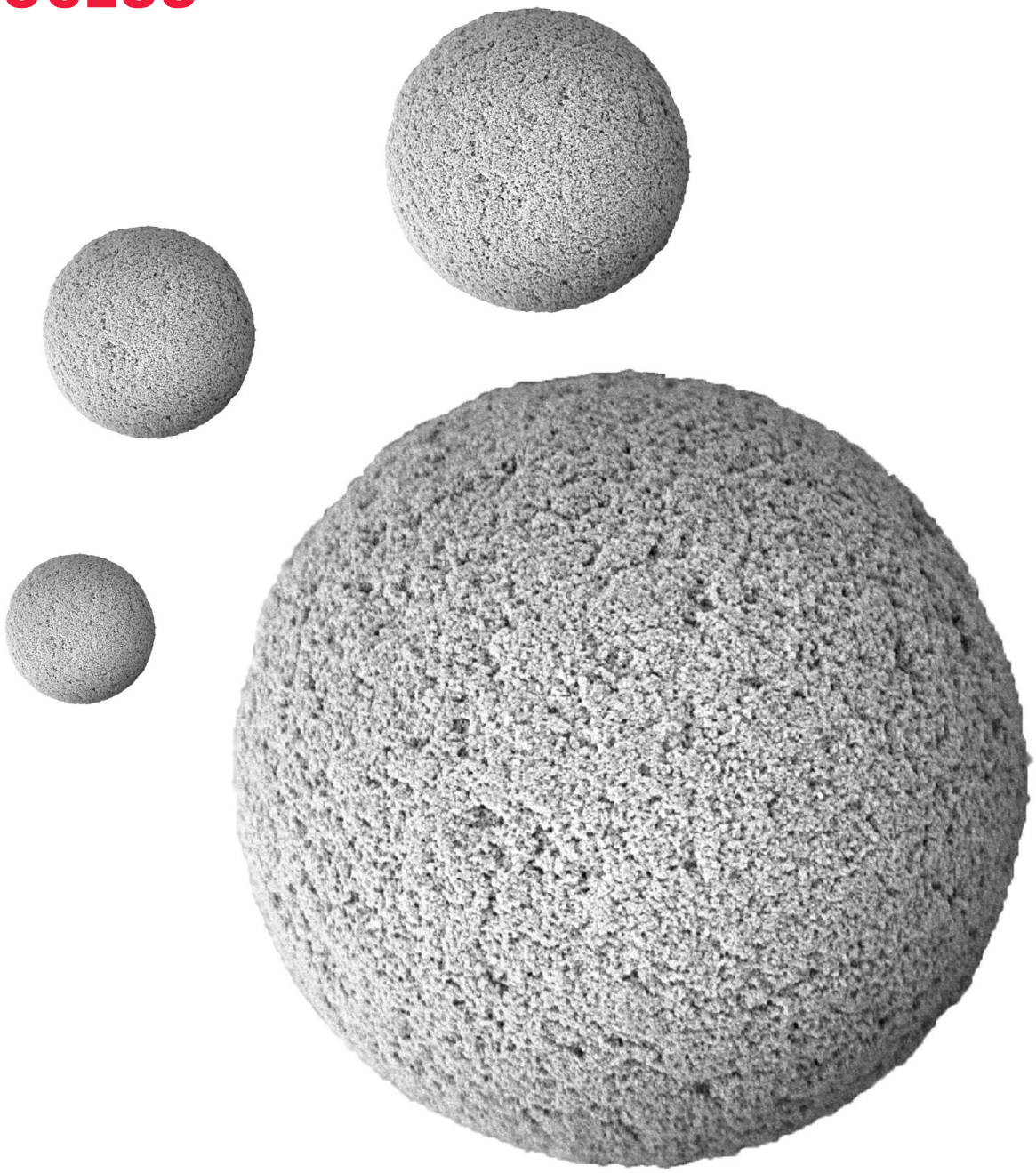
TOYOPEARL SEC RESINS

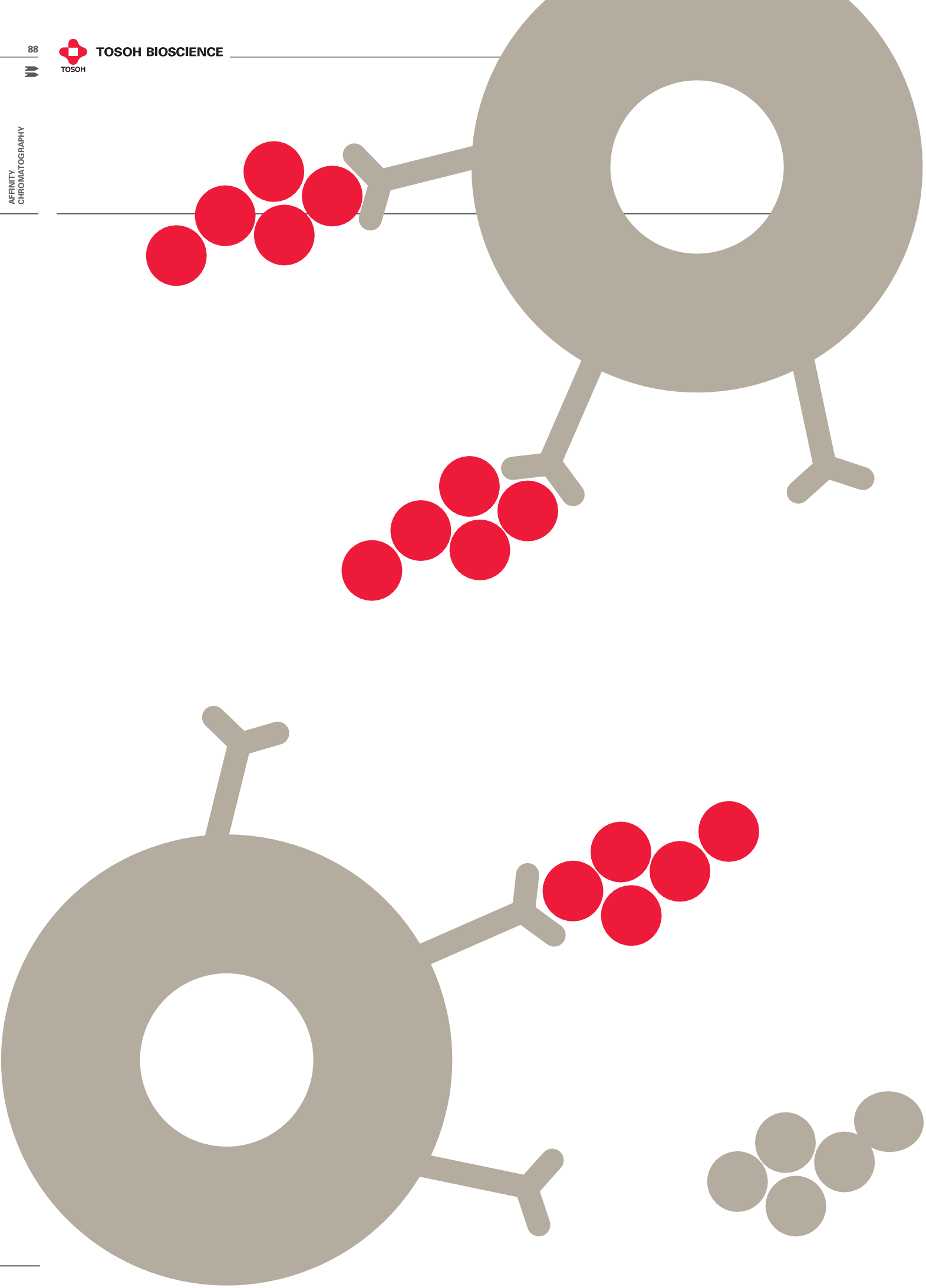
PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	EXCLUSION LIMIT (Da)
0019808	TOYOPEARL HW-40F	150	45	3×10^3
0007448		500		
0014682		1,000		
0007968		5,000		
0019807	TOYOPEARL HW-40C	150	75	3×10^3
0007449		500		
0014683		1,000		
0007969		5,000		
0019811	TOYOPEARL HW-50S	150	30	1.8×10^4
0007455		250		
0014684		1,000		
0008059		5,000		
0019810	TOYOPEARL HW-50F	150	45	1.8×10^4
0007453		500		
0014685		1,000		
0008060		5,000		
0018368		50,000		
0019813	TOYOPEARL HW-55S	150	30	1.5×10^5
0007459		250		
0014686		1,000		
0008062		5,000		
0019812	TOYOPEARL HW-55F	150	45	1.5×10^5
0007457		500		
0014687		1,000		
0008063		5,000		
0019815	TOYOPEARL HW-65S	150	30	1×10^6
0007467		250		
0014688		1,000		
0008068		5,000		
0019814	TOYOPEARL HW-65F	150	45	1×10^5
0007465		500		
0014689		1,000		
0008069		5,000		
0021481	TOYOPEARL HW-65C	150	75	1×10^5
0007466		500		
0014690		1,000		
0008070		5,000		
0021482		50,000		
0019816	TOYOPEARL HW-75F	150	45	8.25×10^5
0007469		500		
0014691		1,000		
0008072		5,000		

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

TOYOPEARL & TSKgel RESINS

THE FIRST CHOICE FOR YOUR PURIFICATION PROCESS





AFC AFFINITY CHROMATOGRAPHY

AFC PRODUCTS

- Activated TOYOPEARL Resins for Affinity Ligand Coupling
  - TOYOPEARL AF-Tresyl-650M
  - TOYOPEARL AF-Epoxy-650M
- Reactive TOYOPEARL Resins for Affinity Ligand Coupling
  - TOYOPEARL AF-Formyl-650M
  - TOYOPEARL AF-Amino-650M
  - TOYOPEARL AF-Carboxy-650M
- Group specific TOYOPEARL Affinity resins
  - TOYOPEARL AF-Chelate-650M
  - TOYOPEARL AF-Red-650M
  - TOYOPEARL AF-Heparin HC-650M

> TOYOPEARL AF-Tresyl-650M is perfect for coupling any kind of biomolecules at physiological conditions. For example, enzymes linked to the resin keep their activities allowing the development of resins for on-column biocatalysis.

AFFINITY CHROMATOGRAPHY HOW DOES IT WORK

In affinity chromatography, the target protein is specifically and reversibly bound by a complementary ligand. The sample is applied under conditions that favor specific binding to the ligand. Unbound material is washed out of the column, and bound target protein is recovered by changing conditions to those favoring elution. Elution is performed specifically, using a competitive ligand, or nonspecifically, by changing, for example, pH, ionic strength, or polarity. The target protein is usually eluted in a purified and concentrated form.

There are many custom designed affinity ligands available to the chromatographer besides antibody affinity resins. TOYOPEARL affinity chromatography resins are functionalized with chemically active groups or group-specific ligands (Table I). Resins with activated functional groups are ready to directly couple a protein or other ligand. Resins with reactive groups require carbodiimide coupling or reductive amination to achieve a stable covalent linkage.

TABLE I

Activated resin	Reactive resin	Group specific
AF-Tresyl	AF-Amino	AF-Red
AF-Epoxy	AF-Carboxy	AF-Chelate
	AF-Formyl	AF-Heparin HC

FEATURES

- High binding capacity
- Recombinant Protein A/L ligand
- TOYOPEARL polymer matrix

BENEFITS

- Increased productivity of antibody purification
- Lower buffer consumption
- Alkaline stable
- Low Protein A/L leakage
- High mechanical stability
- High chemical stability

AFFINITY CHROMATOGRAPHY FEATURED PRODUCTS

TOYOPEARL offers a spectrum of carefully selected affinity resins primed with activated or reactive groups which can be used to covalently attach almost any custom ligand. The structures of TOYOPEARL group-specific, activated and reactive ligands are shown in Figure 1 and 2.

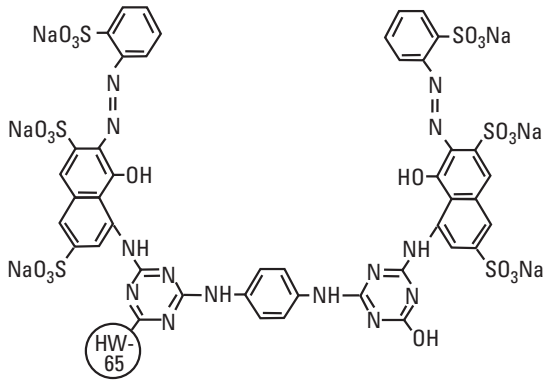
In general, TOYOPEARL AF-Tresyl-650M and TOYOPEARL AF-Formyl-650M resin are recommended for coupling proteins, while TOYOPEARL AF-Epoxy-650M resin is suited for coupling lower molecular weight ligands. TOYOPEARL AF-Amino-650M and TOYOPEARL AF-Carboxy-650M resins may be used for both.

TOYOPEARL affinity resins may be used in combinatorial chemistry or for solid phase synthesis of peptides and oligonucleotides because of their excellent stability in a variety of organic solvents and under extremes of pH.

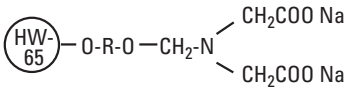
FIGURE 1

GROUP-SPECIFIC TOYOPEARL AFFINITY RESIN

Toyopearl AF-Red

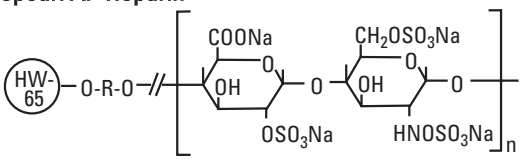


Toyopearl AF-Chelate



Ligand Density: 20 mmol/L

Toyopearl AF-Heparin

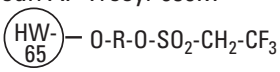


Approximate Ligand Density: 5 g/L

FIGURE 2

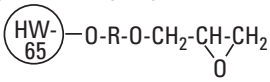
ACTIVATED TOYOPEARL AFFINITY RESIN

Toyopearl AF-Tresyl-650M



Ligand Density: 80 µmol/g (dry)

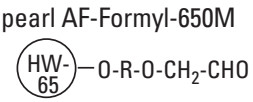
Toyopearl AF-Epoxy-650M



Ligand Density: 800 µmol/g (dry)

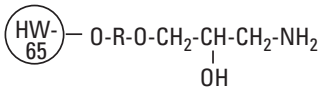
REACTIVE TOYOPEARL AFFINITY RESIN

Toyopearl AF-Formyl-650M



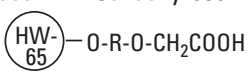
Ligand Density: 60 meq/L

Toyopearl AF-Amino-650M



Ligand Density: 100 mmol/L

Toyopearl AF-Carboxy-650M



Ligand Density: 100 meq/L

AFFINITY CHROMATOGRAPHY FEATURED PRODUCTS

TOYOPEARL AF-Tresyl-650M activated resin is highly reactive toward amine and thiol groups. It is provided in dry form, ready for reaction in buffered solutions containing protein or other ligand. Coupling is accomplished in neutral to slightly alkaline (pH 7 - 8) solution (Figure 3).

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

TOYOPEARL AF-Epoxy-650M activated resin, also packaged in dry form, has a high density of epoxy-functionality (ca. 800 µmol/mL). Under appropriate reaction conditions, this may be used for immobilization of proteins or low molecular weight ligands. It is particularly useful when high densities of low molecular weight ligands must be attached (Figure 4). Glutathione and glycine have, for example, been coupled at densities greater than 100 µmol/mL hydrated resin.

TOYOPEARL AF-Epoxy-650M resin is a highly versatile starting material for conversion to other chemically active functional groups required in special applications. This resin may be readily activated to hydrazide-bearing materials. This is particularly useful for immobilization of carbohydrates or glycoproteins. Using the reaction sequences described, special ligands may be introduced onto this dimensionally stable, macroporous support.

FIGURE 3

TOYOPEARL AF-TRESYL COUPLING PROCEDURE

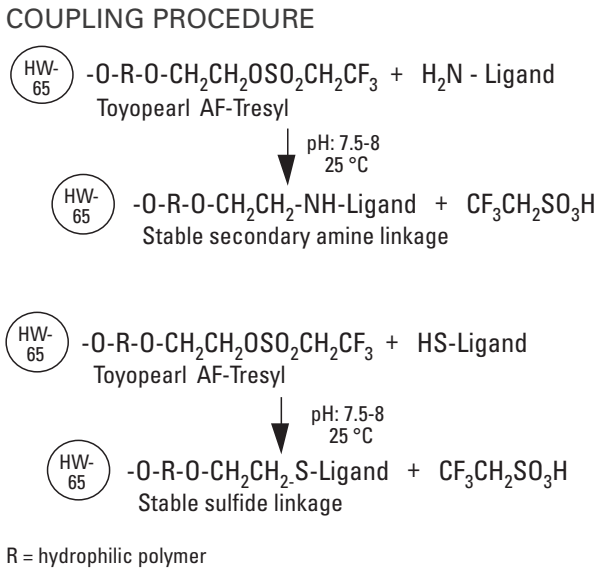
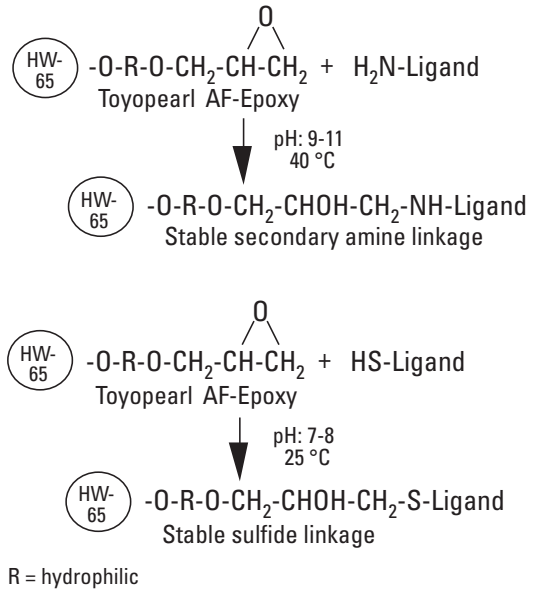


FIGURE 4

TOYOPEARL AF-EPOXY COUPLING PROCEDURE



AFFINITY CHROMATOGRAPHY - APPLICATIONS ACTIVATED RESINS – DIRECT ATTACHMENT

Ligands may be coupled to **TOYOPEARL AF-Formyl-650M** (aldehyde-bearing) resin under mild conditions exclusively using primary amines. The ligand is bound to the resin by a stable secondary amine linkage (Figure 5). Representative coupling capacities are shown in Table II.

A wide variety of industrial enzymes have been immobilized on aldehyde-bearing supports. Typically, these supports have been synthesized by industrial users by partial oxidation of polysaccharide supports (e.g. cellulose and agarose) or partial hydrolysis of polyacetals. In contrast, TOYOPEARL AF-Formyl-650M resin is a ready-to-use aldehyde support formulated from a dimensionally stable, macroporous matrix. Consistent aldehyde content and physical properties are assured from batch to batch.

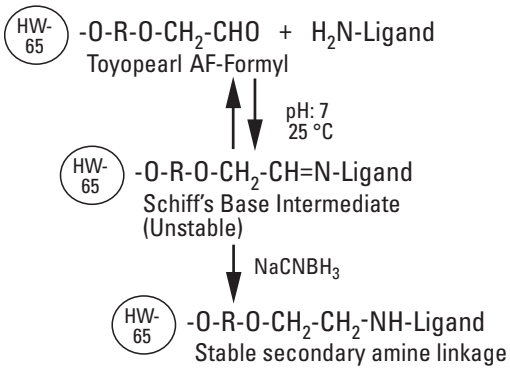
TOYOPEARL AF-Amino-650M resin may be used to couple ligands using their carboxyl groups (peptide bond formation) or aldehyde groups (reductive amination) as shown in Figure 6. Aldehyde groups may be present in a carbohydrate or glycoprotein ligand or may be introduced into the ligand by mild, periodate oxidation.

The optimized functional group density of TOYOPEARL AF-Amino-650M (100 mmol/L) is ideal for coupling of either proteins or low molecular weight ligands. For example, lactose was coupled by reductive alkylation to yield a ligand density of ca. 30 µmol/mL resin. Coupling densities for various proteins are given in Table II.

TOYOPEARL AF-Carboxy-650M resin provides another useful and milder approach for coupling to amino groups of proteins or low molecular weight ligands. The carbodiimide mediated coupling reaction produces an amide bond between ligand and support (Figure 7). Representative coupling densities are given in Table II.

FIGURE 5

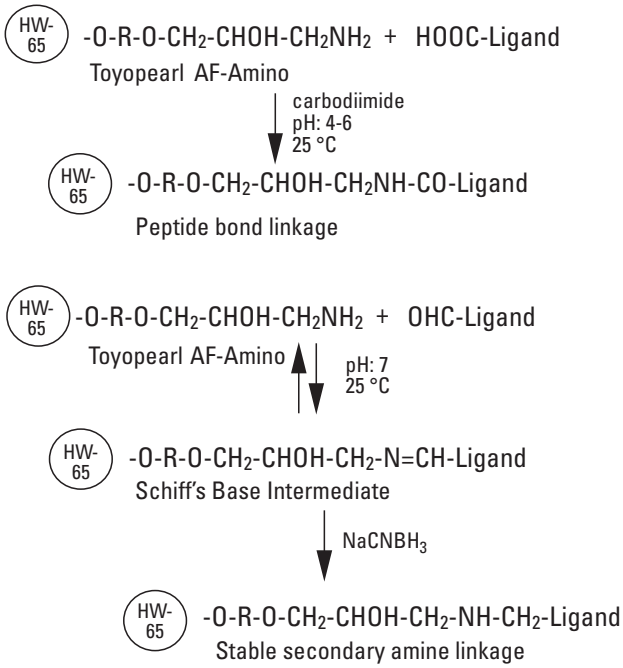
TOYOPEARL AF-FORMYL COUPLING PROCEDURE



R=hydrophilic polymer

FIGURE 6

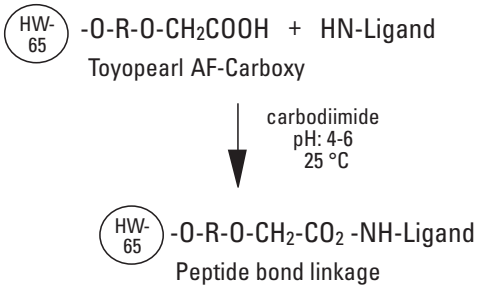
TOYOPEARL AF-AMINO COUPLING PROCEDURE



R = hydrophilic

FIGURE 7

TOYOPEARL AF-CARBOXY COUPLING PROCEDURE



R = hydrophilic polymer

AFFINITY CHROMATOGRAPHY - APPLICATIONS
REACTIVE RESINS - ACTIVATED ATTACHMENT

TABLE II

REPRESENTATIVE COUPLING DENSITIES FOR ACTIVATED AND REACTIVE TOYOPEARL MEDIA

TOYOPEARL resin Protein coupled (mg/mL resin)	AF-Tresyl-650M	AF-Formyl-650M	AF-Amino-650M	AF-Carboxy-650M
Soybean trypsin inhibitor	16	3.5	5.8	15
Protein A	1.9	-	-	-
Concanavalin A	13	-	-	-
α1-Antitrypsin	12.3	-	-	-
α-Chymotrypsin	12.5	-	-	-
Myoglobin	12.4	-	-	-
Ovalbumin	-	2.5	6.7	0.8
Bovine serum albumin	12.4	14	19.2	3.3
Human IgG	10.0	15	6.7	11.7
Cytochrome C	-	5.8	3.3	7.5
Lysozyme	60	20	5.8	17.5
Coupling agent	not required	$NaCNBH_3$	$NaCNBH_3$ or Carbodiiamide	Carbodiimide
Optimal pH	7.0 - 9.0	6.9 - 9.0	4.5 - 6.0	4.5 - 6.0

AFFINITY CHROMATOGRAPHY - APPLICATIONS RESINS WITH GROUP SPECIFIC LIGANDS

TOYOPEARL AF-Chelate-650M

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

TOYOPEARL AF-Red-650ML

Toyoscreen and TOYOPEARL AF-Red-650ML resins are functionalized with Procion Red HE-3B, (also known as Reactive Red 120). This resin is useful for the purification of nucleotide dependent enzymes, lipoproteins, plasminogen, peptides, hormones and cytotoxins.

These two dye-ligand resins are useful in binding/purification of nucleotide-dependent enzymes, albumin, cell growth factors, interferons, transferases, cyclases. and polymerases. Typical binding capacities are shown in Table III.

TOYOPEARL AF-Heparin HC-650M

Heparin is a linear and highly sulfated glycosaminoglycan which has anti-coagulant properties. Due to its polyanionic nature, heparin interacts with a wide range of biomolecules including plasma components, lipoprotein lipase, collagenase, and DNA polymerase.

Immobilized heparin is widely used as an adsorbent in affinity chromatography for the purification of biological substances. TOYOPEARL AF-Heparin HC-650M resin is a high capacity, affinity adsorbent with excellent chemical stability.

TABLE III

REPRESENTATIVE BINDING CAPACITIES FOR TOYOPEARL DYE-LIGAND AFFINITY MEDIA

Protein (mg/mL resin)	AF-Red-650ML
Hexokinase	-
Bovine serum albumin	-
Human serum albumin	3.5 ±1
Lactate dehydrogenase	11

AFFINITY CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

ToyoScreen

PART #	PRODUCT DESCRIPTION	PACKAGE
0021384	ToyoScreen AF-Chelate-650M	1 mL x 6
0021385		5 mL x 6
0021388	ToyoScreen AF-Red-650ML	1 mL x 6
0021389		5 mL x 6
0021390	ToyoScreen AF-Heparin HC-650M	1 mL x 6
0021391		5 mL x 6

ToyoScreen COLUMN ACCESSORIES

PART #	PRODUCT DESCRIPTION
0021400	ToyoScreen Column Holder

TOYOPEARL LABPAK

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)
0043400	AFFIPAK ACT (AF-Epoxy-, AF-Tresyl-650M)	2 x 5 g	65
0043410	AFFIPAK (AF-Amino-, AF-Carboxy-, AF-Formyl-650M)	3 x 10 mL	65

TSKgel RESINS FOR AFC

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)
0016208	Tresyl-5PW (10)	2 g	10

TOYOPEARL AFFINITY CHROMATOGRAPHY RESIN

GROUP SPECIFIC RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	TYPICAL LIGAND DENSITY	TYPICAL CAPACITY
0008651	TOYOPEARL AF-Red-650ML	25	5 mmol/L	2.5 - 4.5 g/L (HSA)
0019801		100		
0042102		1,000		
0014475	TOYOPEARL AF-Chelate-650M	25	25-45 meq/L	–
0019800		100		
0014907		1,000		
0014908		5,000		
0020030	TOYOPEARL AF-Heparin HC-650M	10	–	5 g/L (AT III)
0020031		100		
0020032		1,000		
0020033		5,000		

AFFINITY CHROMATOGRAPHY ORDERING INFORMATION AND SPECIFICATIONS

ORDERING INFORMATION

TOYOPEARL AFFINITY CHROMATOGRAPHY RESIN

REACTIVE RESINS

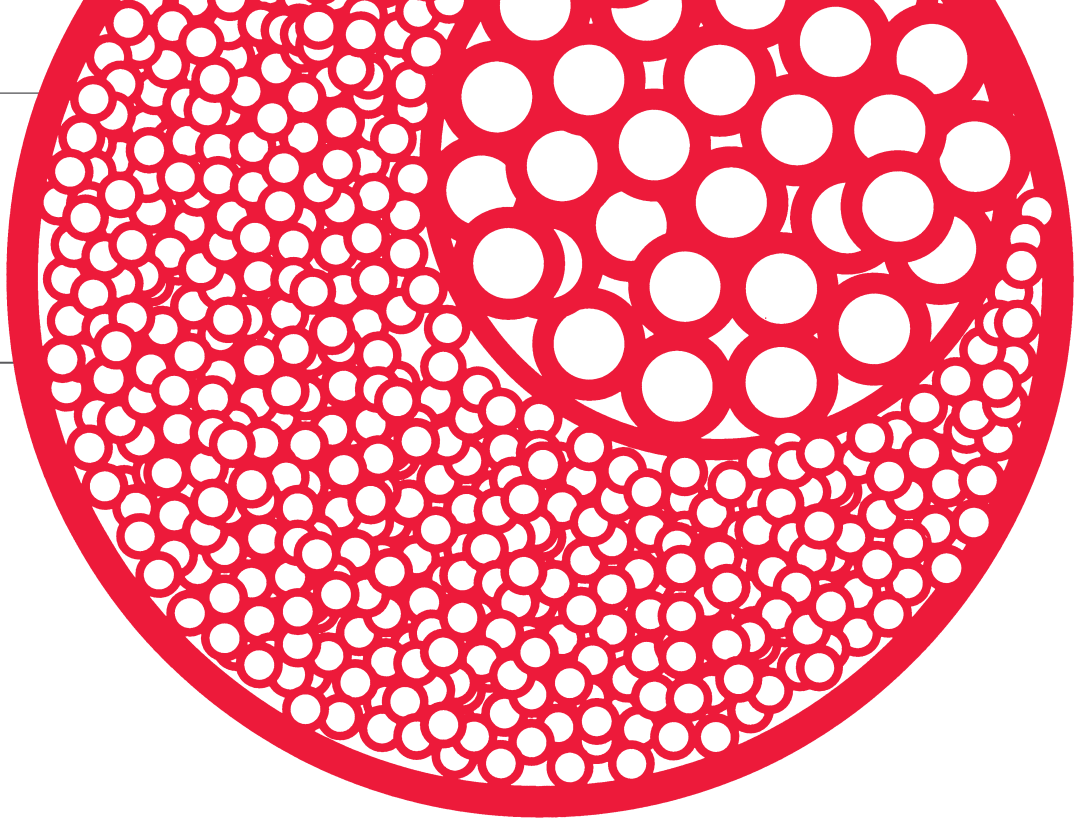
PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	TYPICAL LIGAND DENSITY	TYPICAL CAPACITY
0043411	TOYOPEARL AF-Amino-650M	10	70-130 meq/L	–
0008002		25		
0008039		100		
0018074		1,000		
0018316		5,000		
0043412	TOYOPEARL AF-Carboxy-650M	10	80-120 meq/L	–
0008006		25		
0008041		100		
0018827		1,000		
0018828		5,000		
0043413	TOYOPEARL AF-Formyl-650M	10	40-70 meq/L	–
0008004		25		
0008040		100		
0017396		1,000		
0017397		5,000		

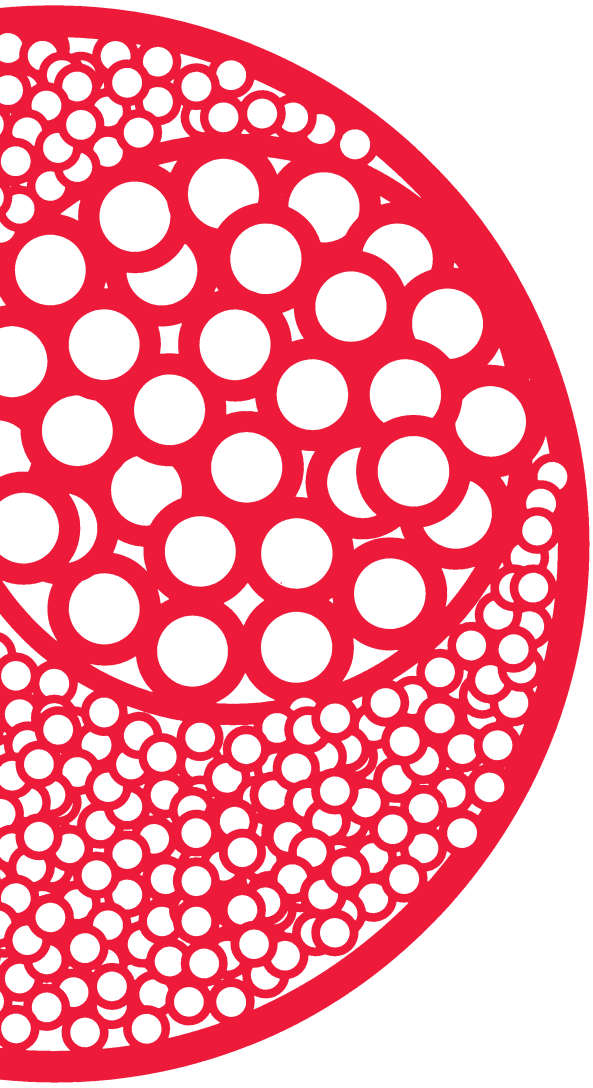
ACTIVATED RESINS

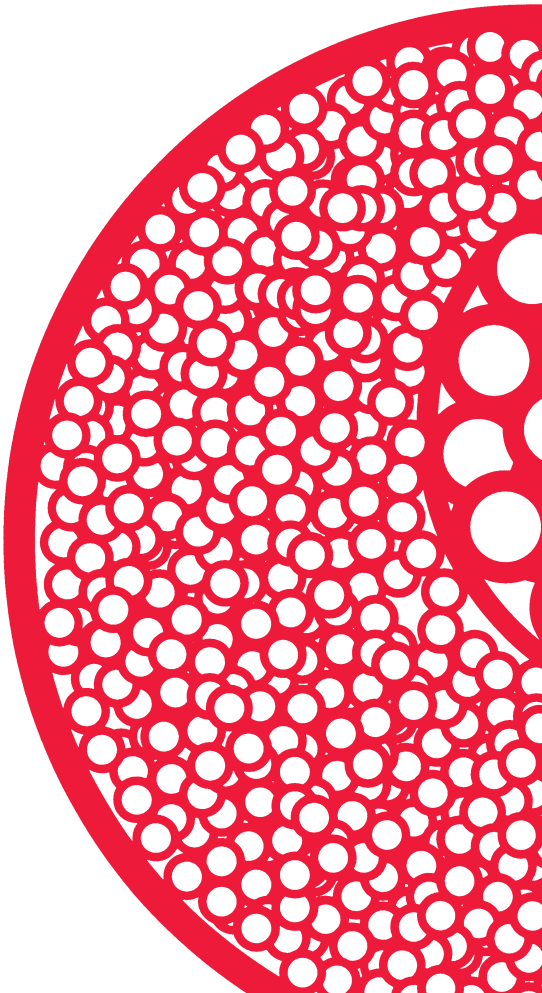
PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	TYPICAL LIGAND DENSITY	TYPICAL CAPACITY
0043402	TOYOPEARL AF-Epoxy-650M	5 g*	600 - 1000 µeq/g	–
0008000		10 g*		
0008038		100 g*		
0018315		1,000 g*		
0014471	TOYOPEARL AF-Tresyl-650M	5 g*	80 mmol/L	–
0014472		100 g*		
0014906		1,000 g*		

Conditions: All TOYOPEARL affinity resins are provided at a particle size of 65 µm. This particle size is ideal for both small and large scale separations.

*1 g yields approximately 3.5 mL of hydrated resin.







HOW TO PACK A COLUMN?

CUSTOM RESINS

- HOW TO PACK A COLUMN

Tosoh Bioscience is offering packing workshops and in-house seminars. Hundreds of biotech experts have been trained in the past 20 years.

"A big 'Thank you' for the training. Great feedback from the colleagues and looking forward for repetition!"

Frank Gündel
Ferring GmbH

HOW TO PACK A COLUMN

The art of packing a column is to distribute all the particles in the column in such a way that you obtain an homogeneous packed bed in a reproducible manner. Let's take packing a lab column as an example.

EQUIPMENT

a) The lab-size columns are all similar: a tubing in polymer or glass, a bottom frit which can be fix or adjustable, and a top frit which can be moved along the column through the adapter.

b) The resin - It will most of the time be delivered as a suspension of chromatographic beads in 20 % ethanol.

c) The packing buffer - it is best to choose empirically, since the optimal buffer will vary with your specific application. In general, the mobile phase with the highest viscosity to be used in the separation (including the cleaning and sanitization steps) is a suitable starting point.

d) A pump system - probably already a part of your chromatography equipment.

SLURRY PREPARATION

After de-fining the resin, the slurry is prepared by re-suspending the resin in the packing buffer.
The slurry concentration should then be adjusted for packing the column. The slurry concentration is calculated as the volume of settled gel divided by the total volume of the slurry, and the slurry concentration is adjusted as follows:

a) Re-suspend the resin slurry in the de-fining vessel and transfer the homogeneous slurry to a graduated cylinder.

b) Allow the slurry to settle overnight (>12 hours) for best results.

c) Determine the settled resin volume, and adjust the slurry concentration to 30 - 50 % by adding or removing packing buffer.

PACKING THE COLUMN

a) Before packing the column, make sure that the total volume of your column is sufficient to contain the whole slurry volume. If not, add a reservoir on top of the column.

b) Ensure that the column is leveled prior to packing. Wet the bottom frit in the column with buffer. Allow the buffer to drain a few seconds to remove any air bubbles. Plug the outlet of the column and leave 3 - 5 mm of buffer in the bottom of the column.

c) Re-suspend the resin slurry to ensure homogeneity. On lab scale, avoid using a vortexer, as it would add air in the slurry. The best way to re-suspend the slurry and to eliminate air bubbles is to place it for a couple of minutes in an ultrasonic bath.

d) Carefully pour the resin slurry down along the inside wall of the column. Pouring along the wall prevents air from being trapped in the resin slurry.

e) After the resin slurry is transferred to the column, rinse the inside walls of the column using a squirt bottle containing packing buffer.

f) Immediately place the flow adapter of the column onto the resin slurry. There should be no trapped air between the flow adapter and the buffer.

g) Open the column outlet, and start the pump. Start slowly to flow packing buffer through the column.

h) Slowly increase to the final flow rate. This prevents hydraulic shock to the forming bed and prevents uneven packing of the column bed. The flow rate can be ramped up in several incremental changes. These increments will be determined by the size of the column and target flow rate. Some examples are listed in Table I.

i) After the bed has fully formed, shut off the pump, and close the column outlet. If you do not use a packing reservoir on top of your column, go directly to (m).

TABLE I

EXAMPLES OF PACKING FLOW RATES, INCREMENTS, AND HOLD TIMES

COLUMN SIZE (ID X L)	TARGET FLOW RATE (mL/min)	INCREMENT (mL/min)	HOLD TIME (min)
2.2 cm x 60 cm	2	0,5	0,5
9.0 cm x 30 cm	300	50	2
25 cm x 30 cm	2.000	400	3

HOW TO PACK A COLUMN

j) The entire bed should reside in the lower column section if using a packing reservoir. Using a pipette or pump, siphon the supernatant from the upper reservoir. Remove the upper reservoir and the coupling ring.

k) Carefully place the flow adapter into the column, approximately 2 - 3 cm away from the consolidated bed. Avoid introduction of air into the column.

l) Secure the flow adapter in place, begin the pump as described in step h, and open the column outlet.

m) The bed will compress further. When compression is complete and pressure is stable, stop the pump and close the column outlet.

n) Carefully lower the adapter near to the resin bed. Take care not to disturb the resin bed when moving the flow adapter.

o) Repeat steps l) - n), until there is no further compression of the resin bed from the flow adapter. It will usually take 2-3 iterations until the bed is stable.

p) In the final step lower the adapter 1 - 5 mm into the bed.

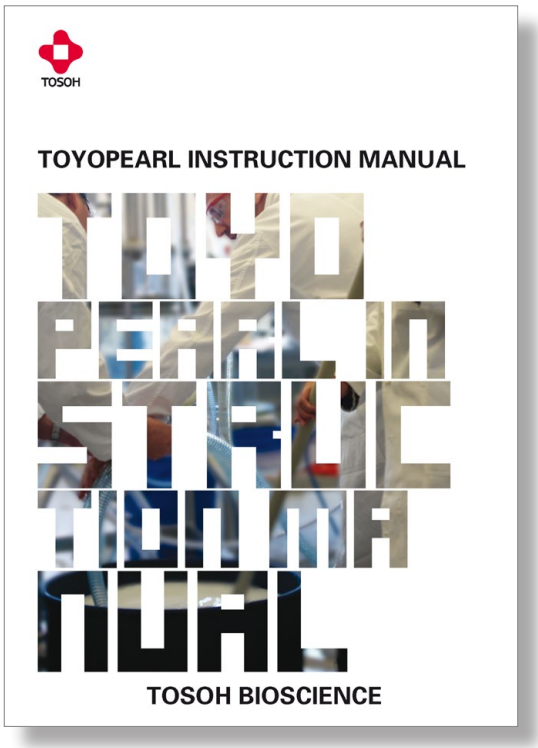
The column is now packed, but not yet ready to use! Before moving on with the real interesting part of the work - performing the chromatographic separation of the target biomolecule - never forget to evaluate the quality of the packing! Only after evaluation of the column, you will be able to ensure reproducible results and to avoid wasting precious compounds by injecting them on a badly packed column.

You can inject acetone or high-salt-buffer and analyze the correspoinding peak. If you packed the column in high-salt-buffer, you can inject a zero-salt buffer and analyze the corresponding negative peak.

Detailed procedure can be found in the TOYOPEARL Instruction Manual.

Detailed procedures for all preparation / packing / testing / using / cleaning of lab and industry columns can be found in the TOYOPEARL Instruction Manual:
bit.ly/TosohTIM

You can also join one of our renowned packing courses or seminars:
bit.ly/TosohCW



TECHNICAL DATA AND TRADEMARKS

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