TOSOH

CHROMATOGRAPHIC PROCESS MEDIA CATALOG

TOSOH BIOSCIENCE

ABOUT US

WITH A GLOBAL PERSPECTIVE.

Tosoh Bioscience GmbH, Separations Business Unit, Stuttgart, is an acknowledged global leader in the field of bioseparations. Established as TosoHaas in 1987, the original joint venture between Tosoh Corporation of Japan and the Rohm and Haas Company, USA, has become synonymous with advanced products and quality support. In the year 2000, Tosoh Corporation acquired a 100% controlling interest changing the name to Tosoh Biosep. In the course of unifying all Tosoh affiliates, the new Brand Name Tosoh Bioscience evolved. Today, the two branches, Bioseparations and Diagnostics operate with the same name Tosoh Bioscience - Separations Business Unit and accordingly Diagnostics Business Unit. Tosoh manufacturing sites in Japan provide products to the sales and support subsidiaries in the U.S. and Europe, ensuring full global coverage. Over the last 30 years, TSKgel SW columns have become the worldwide industry standard for size exclusion chromatography of biomolecules.

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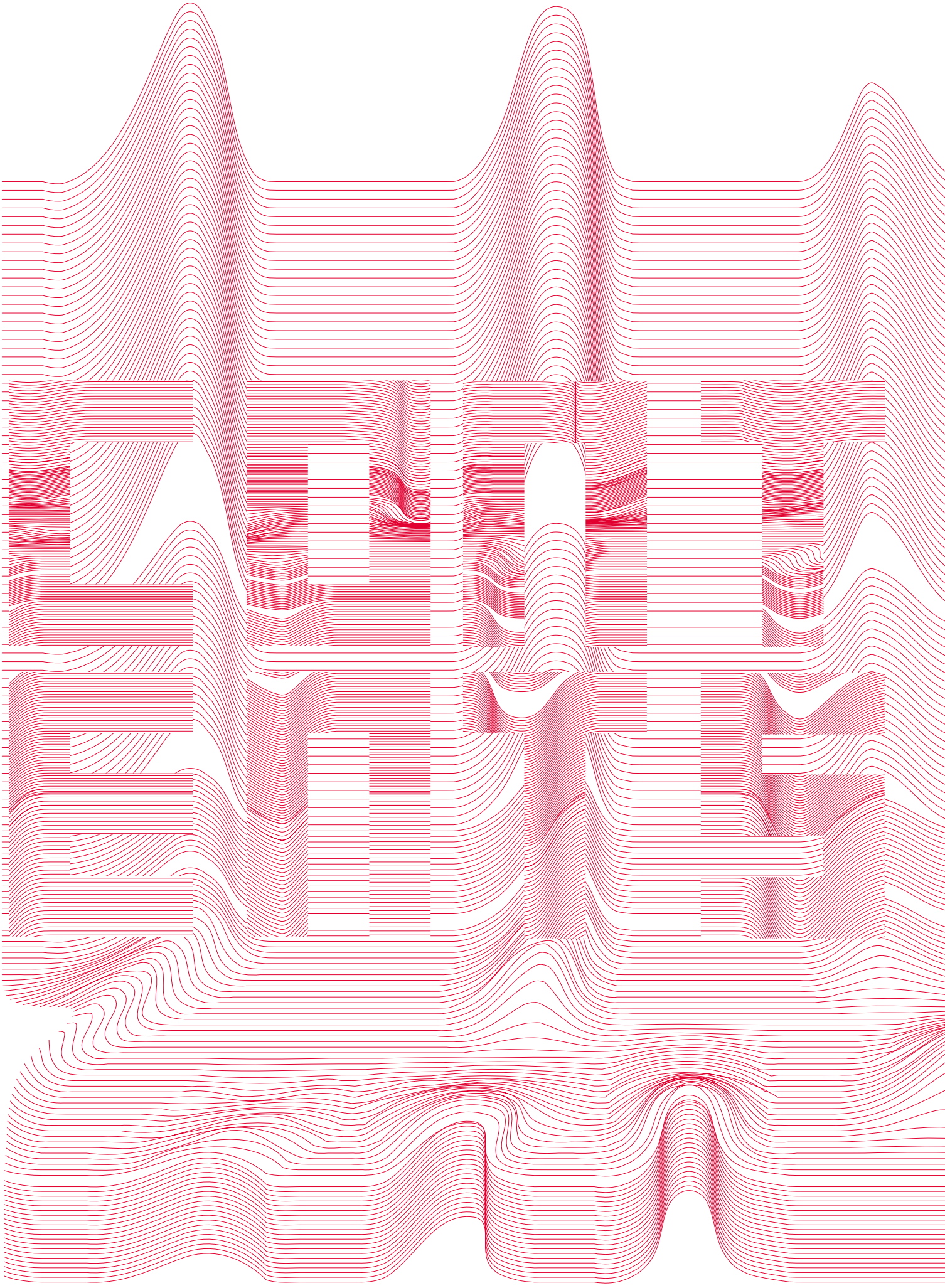
TOSOH HISTORY

1935	FOUNDING OF TOYO SODA MANUFACTURING CO., LTD.
1936	OPERATION OF NANYO MANUFACTURING COMPLEX BEGINS
1971	SCIENTIFIC INSTRUMENTS DIVISION FORMED, FIRST GPC COLUMN USING TSKGEL DEVELOPED BY TOSOH
1974	HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COLUMN PLANT IS COMPLETED
1979	TOSOH DEVELOPS TOYOPEARL MEDIA
1983	TOSOH DEVELOPS HYDROPHOBIC INTERACTION MEDIA
1987	TOSOHAAS US OPERATIONS FORMED IN MONTGOMERYVILLE
1989	TOSOHAAS GMBH OPERATIONS FORMED IN STUTTGART
1995	TOSOH NANYO GEL FACILITY RECEIVES ISO 9001
2000	IN NOVEMBER FORMER TOSOHAAS US OPERATIONS BECOMES TOSOH BIOSEP LLC, A 100% SUBSIDIARY OF TOSOH CORPORATION
2001	IN JANUARY FORMER TOSOHAAS GMBH EUROPEAN OPERATIONS BECOMES TOSOH BIOSEP GMBH, A 100% SUBSIDIARY OF TOSOH CORPORATION
2002/2003	ALL TOSOH AFFILIATED SCIENTIFIC & DIAGNOSTIC SYSTEM RELATED COMPANIES IN EUROPE ARE UNIFIED UNDER THE NAME TOSOH BIOSCIENCE.
2008	EcoSEC, THE 7TH GENERATION GPC SYSTEM IS INTRODUCED GLOBALLY
2009	TOSOH BIOSCIENCE GMBH CELEBRATES ITS 20TH ANNIVERSARY IN STUTTGART
2010	TOSOH CELEBRATES ITS 75TH YEAR IN BUSINESS WITH THE OPENING OF FIVE NEW PLANTS, AND CONTINUED RAPID EXPANSION IN CHINA
2011	TOSOH BIOSCIENCE CELEBRATES 40 YEARS OF OPERATION
2012	TOSOH RELEASES FIRST TOYOPEARL MIXED-MODE RESIN TOYOPEARL MX-Trp-650M
2013	TOSOH RELEASES NEW TSKgel SuperSW mAb COLUMNS


FUTURE

CONTENTS

INTRODUCTION

OUR HISTORY

Tosoh Bioscience is an acknowledged leader in the field of bioseparations. Established in August 1987 as TosoHaas, we were the joint venture of two multi-billion dollar companies: Tosoh Corporation of Japan and Rohm and Haas Company, USA. In the year 2000, Tosoh Corporation purchased Rohm and Haas Company's business interest in TosoHaas and formed Tosoh Biosep subsidiaries with sales, marketing, and technical service offices in the U.S. and Germany. In 2002, Tosoh Biosep was re-organized with Tosoh's clinical diagnostic business units under the name of Tosoh Bioscience. In 2007, Tosoh Scientific Instrument Division changed its name to Tosoh Bioscience Division and Tosoh Bioscience Shanghai Co., Ltd. was also created.

A GLOBAL PERSPECTIVE

Since our beginnings, we have become an effective manufacturing partner with many of the world's most innovative pharmaceutical and biotechnology companies. Our TSKgel® and TOYOPEARL® chromatographic resins are important components in the analysis, isolation, and purification of biomolecules. Tosoh's portfolio of over 500 specialty items encompassing all common modes of liquid chromatography can help purify any protein, peptide, enzyme, nucleic acid, antibiotic, or small molecule that may be encountered.

SEAMLESS SCALE-UP WITH POLYMER RESINS

The polymeric TSKgel PW-type analytical HPLC columns and TOYOPEARL production scale resins are made using the same chemistry. This gives them the same chromatographic selectivity so that an analytical method developed on a TSKgel column can be easily scaled to the corresponding bulk TOYOPEARL resin for manufacturing use. This product synergy saves valuable development time.

TOSOH'S TECHNOLOGY

For over thirty-five years our parent, Tosoh Corporation, has been a world leader in the analysis and purification of proteins. Tosoh's TSKgel SW-type columns are the industry standard for size exclusion chromatography of biomolecules. This has led Tosoh to a fundamental understanding of the role played by pore diameter and molecular size in chromatographic separations. This knowledge allows Tosoh to design higher performance resins for ion exchange, hydrophobic interaction, affinity hydrophilic, and reversed phase applications.

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TOSOH CORPORATION - NANYO COMPLEX
TOYOPEARL RESINS ARE PRODUCED AT THE TOSOH NANYO COMPLEX – A SPRAWLING 3 MILLION SQ. METER FACILITY THAT IS JAPAN'S LARGEST CHEMICAL MANUFACTURING COMPLEX.

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INTRODUCTION

NEW PRODUCT CREATION

Tosoh Bioscience and our parent, Tosoh Corporation, provide solutions for today's biological purification needs. The "Custom Resin" section of this catalog describes how we are ready to solve future separation challenges as well. Whether it is a surface modification of an existing product or the creation of a new resin, we encourage you to contact us to provide you with a customized solution.

THE SKILLS TOOLBOX

The science of biopurification is one of the fastest growing technologies in the world. Companies that accept the pace and are flexible will win. At Tosoh, we engineer: particle size, pore size, surface area, ligand chemistry, and innovation. The "Tosoh Resin Innovation Program" (TRIP) discussed in the "Custom Resin" section of this catalog describes our core technologies for solving customer problems.

CUSTOMER TRUST IS OUR NO.1 PRIORITY

Customer trust is earned, not given. Since 1987, Tosoh Bioscience has helped develop processes from benchtop discovery to multi-thousand liter manufacturing scale. A number of our process customers have been with us continuously for over 20 years. We have earned their trust. From development to new product launch we share in the excitement of our customers' successes, as well as their clinical disappointments. In either case, Tosoh Bioscience uses its technology, innovation, and customer commitment to expedite the development process to build better customer relationships and trust for the future.

THE BEST EXPERIENCE FOR OUR CUSTOMERS

As your manufacturing partner we want our logistical support to be seamless to you. From your initial product evaluation to full scale manufacturing, our technical support, order administration, and final shipment procedures are in place to make your experience with us a thoroughly enjoyable one.

Courteous professionals are available to answer all of your questions. Our customer service processes are also in place to deliver our resins to you as agreed. Journal references, product specifications, samples, quotations, applications and scale up support are all just a telephone call, email, or mouse-click away.

COMPLETE REGULATORY SUPPORT

Controlled production processes and auditable manufacturing facilities are the regulatory requirements of our industry. Well characterized, consistent resin products are as important to us as your therapeutic molecules with their known impurity profiles are to you. For this reason Tosoh Corporation's manufacturing site in Japan and both Tosoh Bioscience subsidiaries in the US and Europe are registered to the ISO 9001 standard.

Our manufacturing facilities are continuously audited by customers and we pass their tests.

We maintain regulatory support information on our chromatographic resins available to our customers for their process registration needs.

COMPREHENSIVE TECHNICAL SUPPORT

Do you need help developing an HPLC assay for the analysis of a new therapeutic target? How about monitoring the therapeutc's metabolites in the human body? Do you need help with a "self-packing" process column or a multi-thousand liter one? Our technical support specialists can provide assistance in all of these areas and more.

We offer on-site training, application specific seminars, and are the sole sponsor of the HIC/RPC Bioseparation Conference series (www.HIC-RPC.org).

THE PRODUCTS TOOLBOX

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 a TOYOPEARL resin purification to the corresponding TSKgel column for the QC of your target, we make it easy to develop methods to do both.

TSKgel COLUMNS

Our TSKgel prepacked columns for high performance liquid chromatography are used for the analysis and purification of proteins, peptides, biopolymers and low molecular weight compounds. Many different chromatographic modes are offered including: size exclusion, ion exchange, hydrophobic interaction, hydrophilic, reversed phase, affinity, and normal phase chromatography.

The column packing materials are either spherical silica or polymer particles, ranging in size from 2 µm to 30 µm. Columns are constructed of stainless steel, glass, or polyetheretherketone (PEEK) and are available from microbore to preparative sizes. Please see our website for more information.

INTRODUCTION

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 hydrophilic macroporous methacrylic resins 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 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 prepacked TSKgel columns. This allows the seamless direct scale-up of methods developed on TSKgel columns to TOYOPEARL resins.

TABLE I

COMPARISON OF PREPACKED COLUMNS WITH RESPECTIVE BULK POLYMERIC MEDIA

MODE	TSKgel COLUMN FOR ANALYSIS	ToyoScreen® PROCESS DEVELOPMENT COLUMNS	BULK POLYMERIC MEDIA FOR SCALE-UP AND PRODUCTION	
SEC	TSKgel G1000PW - G6000PW			TOYOPEARL HW-40 - HW-75
IEC	TSKgel SP-5PW	ToyoScreen SP-650M	TSKgel SP-5PW	TOYOPEARL SP-650S, M or C
		ToyoScreen SP-550C		TOYOPEARL SP-550C
		ToyoScreen MegaCap® II SP-550EC		TOYOPEARL MegaCap II SP-550EC
		ToyoScreen GigaCap® S-650M		TOYOPEARL GigaCap S-650M
		ToyoScreen GigaCap CM-650M		TOYOPEARL GigaCap CM-650M
	TSKgel CM-5PW	ToyoScreen CM-650M		TOYOPEARL CM-650S, M or C
	TSKgel DEAE-5PW	ToyoScreen DEAE-650M	TSKgel DEAE-5PW	TOYOPEARL DEAE-650S, M or C
	TSKgel SuperQ-5PW	ToyoScreen SuperQ-650M	TSKgel SuperQ-5PW	TOYOPEARL SuperQ-650S, M or C
		ToyoScreen GigaCap Q-650M		TOYOPEARL GigaCap Q-650M
		ToyoScreen GigaCap DEAE-650M		TOYOPEARL GigaCap DEAE-650M
		ToyoScreen QAE-550C		TOYOPEARL QAE-550C
		ToyoScreen Q-600C AR		TOYOPEARL Q-600C AR
HIC	TSKgel Ether-5PW	ToyoScreen Ether-650M	TSKgel Ether-5PW	TOYOPEARL Ether-650S, M
	TSKgel Phenyl-5PW	ToyoScreen Phenyl-650M	TSKgel Phenyl-5PW	TOYOPEARL Phenyl-650S, M or C
		ToyoScreen Phenyl-600M		TOYOPEARL Phenyl-600M
		ToyoScreen Butyl-650M		TOYOPEARL Butyl-650S, M or C
		ToyoScreen Butyl-600M		TOYOPEARL Butyl-600M
		ToyoScreen Hexyl-650C		TOYOPEARL Hexyl-650C
		ToyoScreen PPG-600M		TOYOPEARL PPG-600M
		ToyoScreen SuperButyl-550C		TOYOPEARL SuperButyl-550C
MX		ToyoScreen MX-Trp-650M		TOYOPEARL MX-Trp-650M
AFC	TSKgel Chelate-5PW	ToyoScreen AF-Chelate-650M		TOYOPEARL AF-Chelate-650M
	TSKgel Tresyl-5PW		TSKgel Tresyl-5PW	TOYOPEARL AF-Tresyl-650M
		ToyoScreen AF-Blue HC-650M		TOYOPEARL AF-Blue HC-650M
		ToyoScreen AF-Red-650M		TOYOPEARL AF-Red-650M
		ToyoScreen AF-HeparinHC-650M		TOYOPEARL AF-HeparinHC-650M
		ToyoScreen AF-rProtein A-650F		TOYOPEARL AF-rProtein A-650F
		ToyoScreen AF-rProtein A HC-650F		TOYOPEARL AF-rProtein A HC-650F

INTRODUCTION

ToyoScreen PROCESS DEVELOPMENT COLUMNS

ToyoScreen process development columns are easy to use. They are available as 1 mL and 5 mL prepacked columns of your favorite TOYOPEARL hydrophobic interaction, ion exchange or affinity resins. They can be connected to most laboratory chromatographic systems for early development resin screening.

The most popular TOYOPEARL resin are now also availabe as ToyoScreen RoboColumns. ToyoScreen 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, small-scale biochromatographic separations of protein samples by running up to eight individual columns simultaneously. ToyoScreen RoboColumns packed with the most popular TOYOPEARL SEC/IEC/MIXED-MODE/HIC media are available with 200 µL and 600 µL resin volumes.

INTRODUCTION

TECHNICAL DATA AND TRADEMARKS

The technical information and data herein contained (the "Technical Data" are based on information Tosoh Bioscience believes to be reliable and are offered in good faith, but are given without warranty or representation, as the conditions of use and application by you or your customers of our products and the Technical Data are beyond the control of Tosoh Bioscience. You should test our products and Technical Data to determine to your satisfaction whether they will be suitable for the intended use and applications of you or your customers. Suggestions for the uses of our products should not be understood as recommending the use of our products in violation of any patent or other intellectual property right or as permission or license to use any patent or other intellectual property right.

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Triton is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.

Capto and Source are registered trademarks of GE Healthcare Bio-Sciences.

MabSelect SuRe is a trademark of GE Healthcare Life Sciences.

WHAT'S NEW

WHAT'S NEW

TOYOPEARL AF-rPROTEIN A HC-650F

TOYOPEARL AF-rProtein A HC-650F is the latest addition to the TOYOPEARL line of affinity media for immunoglobulin purification. HC standing for high capacity indicates that this resin has a superior dynamic binding capacity for human IgGs. It was designed to provide a considerably higher binding capacity than all other commercially available Protein A resins. Typical dynamic binding capacities for monoclonals reach 70 g/L at 5 minutes residence time. The capacity is not diminished by feedstock titers up to 10 g/L allowing for constant process flow rates. The alkaline stable, recombinant protein A ligand is coupled to the renowned TOYOPEARL polymeric base particle by multipoint-attachment in order to minimize protein A leaching.

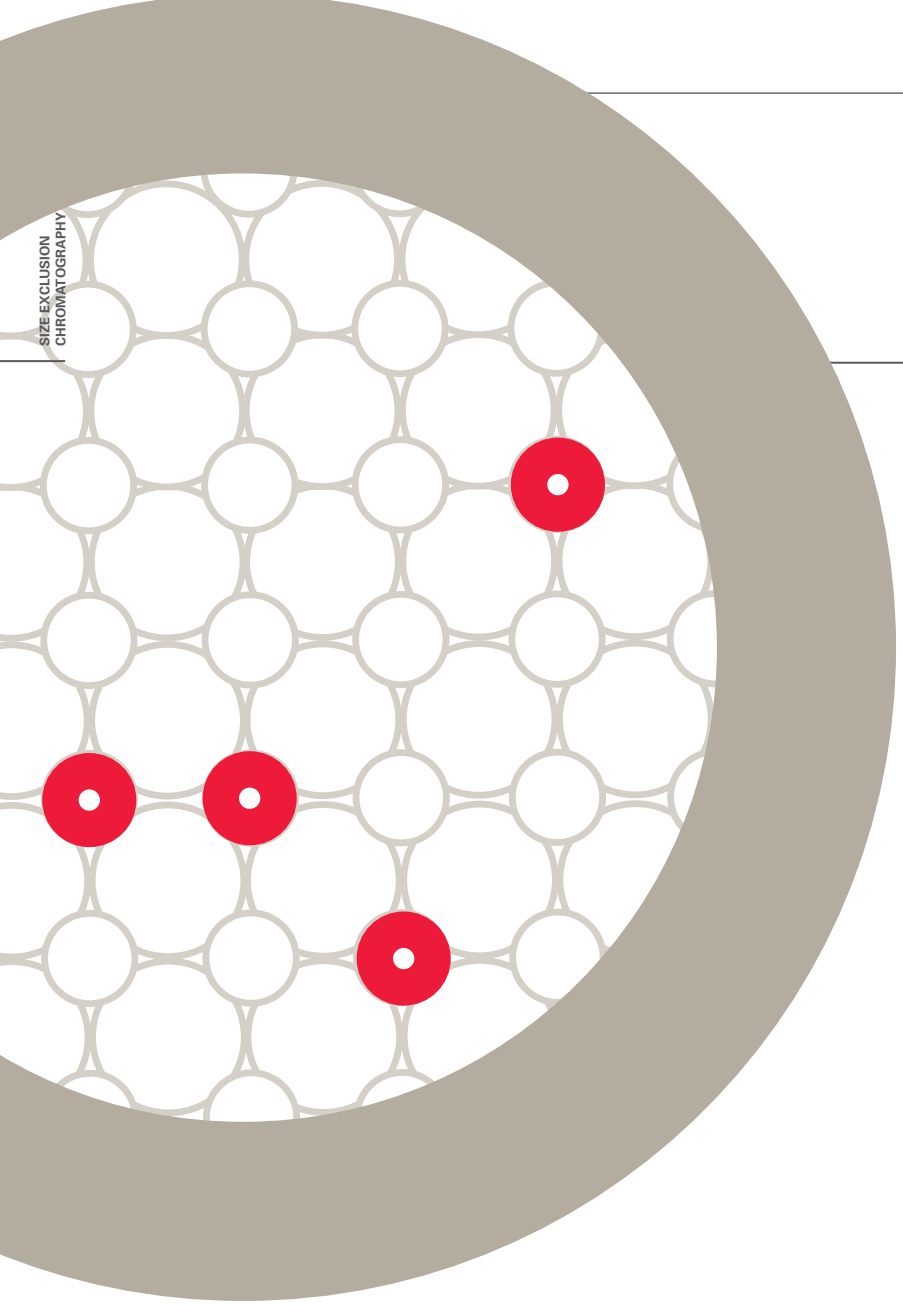
TOYOPEARL MX-Trp-650M

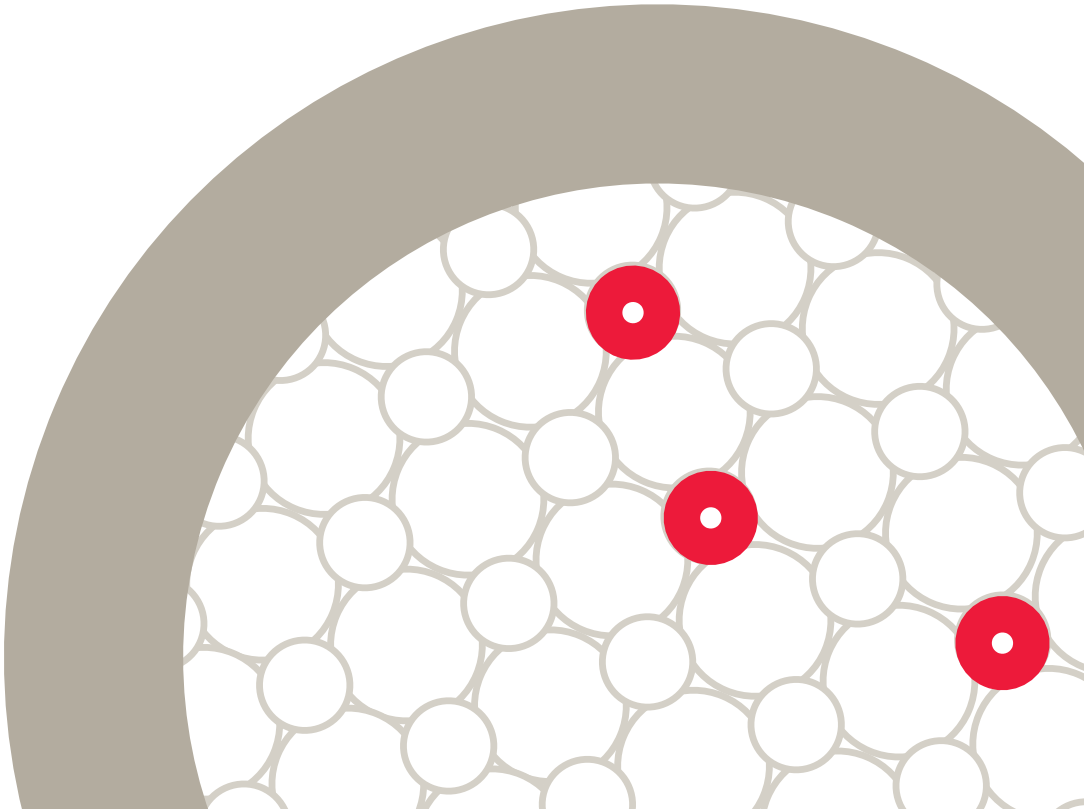
TOYOPEARL MX-Trp-650M is a new multimodal cation exchange resin with unique selectivity and high recovery. It provides high protein binding capacities and tolerates high conductivity feedstocks. TOYOPEARL MX-Trp-650M is based on a rigid methacrylic polymer matrix, which provides excellent pressure/flow properties and allows high flow rates at large scale. Its ligand, the amino acid tryptophan, provides ionic and hydrophobic interactions. This new mixed-mode resin is well-suited for intermediate and polishing steps, such as aggregate removal in antibody purification, and for the purification of targets that are difficult to purify using common purification platforms.

TOYOPEARL GigaCap DEAE-650M

TOYOPEARL GigaCap DEAE-650 is the newest member of the renowned TOYOPEARL GigaCap series of high capacity ion exchange media. The polymeric base bead was chemically modified to provide a high number of anionic binding sites for increased resin capacity. The typical dynamic binding capacity (DBC) of over 150 g/L for bovine serum albumin exceeds the DBC of all other commercial available DEAE chromatography media. The resin was proved to be stable for more than 100 cleaning-in-place (CIP) cycles with 0.5 M NaOH. Good mass transfer kinetics enable the resin to maintain DBC at faster linear velocities and leads to a narrow elution peak. This results in smaller and more concentrated in-process pool volumes, thus reducing the amount of water and buffer needed. The high capacity and low back pressure creates opportunities for increased throughput in various anion exchange purification steps and is ideally suited for efficient plasma protein purification.

In addition to the new DEAE functionality the TOYOPEARL GigaCap series was also extended by small particle (S-grade, 35 µm) versions of the strong ion exchangers TOYOPEARL GigaCap Q and S.





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 FACT

Tosoh has a long history in size exclusion chromatography (SEC). In 1978 Tosoh first introduced porous silica-based SW columns for the isolation of proteins using LC. These first gels were quickly adopted and referred to as the standard for analytical SEC on FPLC and HPLC systems.

The first TOYOPEARL process resins for size exclusion chromatography were developed in 1979. TOYOPEARL HW resins are based on polymethacrylate and are available in various particle and pore sizes. They are used as starting material for the production of all other functionalized TOYOPEARL resins.

SIZE EXCLUSION CHROMATOGRAPHY

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.

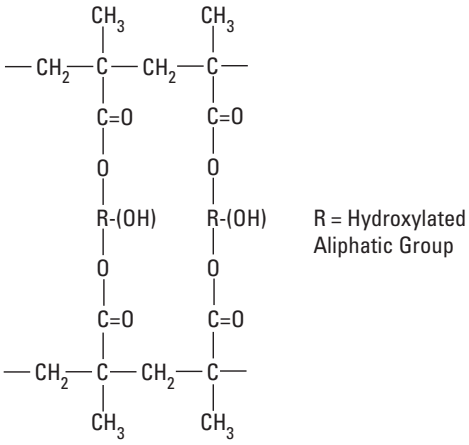
SEC is applicable in final polishing steps where a target protein is being separated from its aggregates or other significantly different molecular weight impurities. Another application area is the desalting of the purified target protein.

Tosoh Bioscience offers a number of TOYOPEARL HW-type products for size exclusion chromatography (Table I). These same SEC TOYOPEARL HW-type products are chemically modified with functional groups to make the TOYOPEARL products shown in the later sections of this catalog.

Much of the information in this catalog section pertains to the available pore sizes, fractionation ranges, and particle sizes of the TOYOPEARL HW-type products, along with their physical and chemical properties. This data not only aids in the evaluation of the best resin for a SEC process step, but also lends insight into understanding the physical nature and the selection process of all TOYOPEARL resins.

FIGURE 1

RESIN CHEMISTRY OF TOYOPEARL SEC RESINS (HYDROXYLATED ACRYLIC)



RESIN CHEMISTRY

TOYOPEARL size exclusion 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 covering 5 different fractionation ranges. The choice of TOYOPEARL HW products depends on the molecular weight of the feedstock components. Tables I and II show this information for proteins, dextrans and PEG polymers.

FEATURES	BENEFITS
small particles available	high resolution
hydrophilic porous polymer structure	minimal non-specific adsorption effects
narrow particle size distribution	high performance SEC – 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

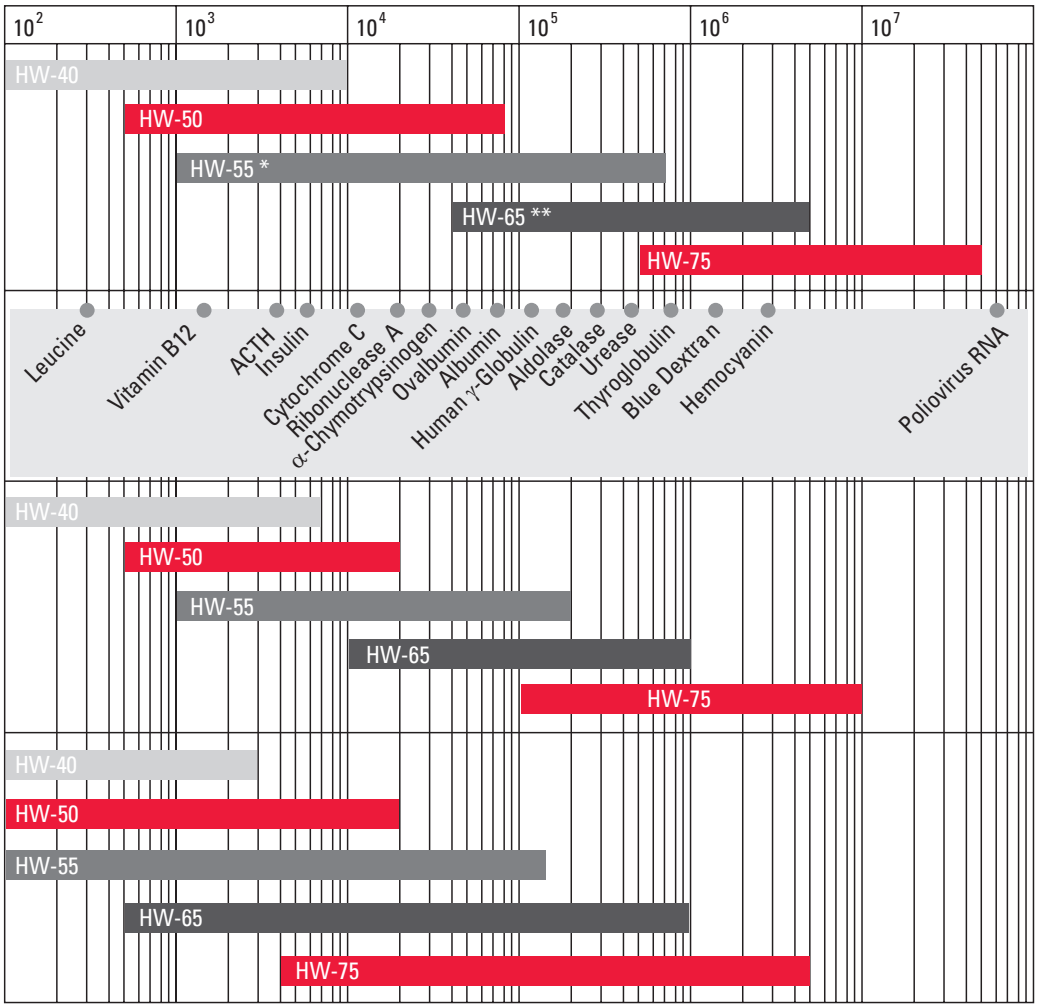
SIZE EXCLUSION CHROMATOGRAPHY

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	20 - 40 30 - 60	5	100 - 3,000	100 - 7,000	100 - 10,000
HW-40C	50 - 100				
HW-50S HW-50F	20 - 40 30 - 60	12,5	100 - 18,000	500 - 20,000	500 - 80,000
HW-55S HW-55F	20 - 40 30 - 60	50	100 - 150,000	1,000 - 200,000	1,000 - 700,000
HW-65S	20 - 40	100	500 - 1,000,000	10,000 - 1,000,000	40,000 - 5,000,000

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

SIZE EXCLUSION CHROMATOGRAPHY

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).

TOYOPEARL HW-65 and HW-55 resins are the base beads for many TOYOPEARL products listed in the later sections of this catalog. A product having "-650" in its name uses the TOYOPEARL HW-65 resin for the base bead. Products having "-550" are derived from the TOYOPEARL HW-55 bead. The impact of pore size and its relationship to dynamic binding capacity and resin selectivity for a particular protein application is discussed in subsequent catalog sections.
See the hydrophobic interaction chromatography section of this catalog for TOYOPEARL PPG-600, TOYOPEARL Phenyl-600 and TOYOPEARL Butyl-600 resins which are functionalized on the TOYOPEARL HW-60 bead (not available for use in SEC).

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:

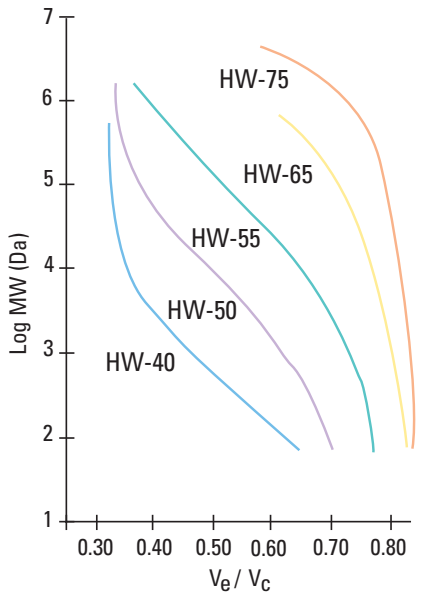
S-grade = 20 - 40 µm (Superfine)
F-grade = 30 - 60 µm (Fine)
C-grade = 50 - 100 µm (Coarse)

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.

Some TOYOPEARL HW-type products are also manufactured in "M-grade" (40-90 µm) and "EC-grade" (100-300 µm) to be used as the base beads for functionalized materials. These special grades are not commercially offered as SEC products.
Please note that for the functionalized base beads in later catalog sections a "C-grade" is specified as a 50-150 µm bead and is not the SEC "C-grade" range of 50-100 µm.

FIGURE 2

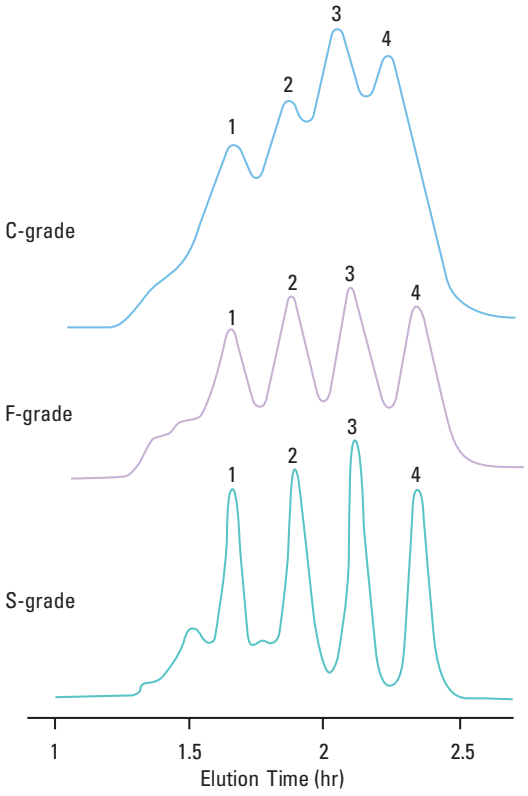
CALIBRATION CURVES FOR GLOBULAR PROTEINS ON TOYOPEARL



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

SIZE EXCLUSION CHROMATOGRAPHY

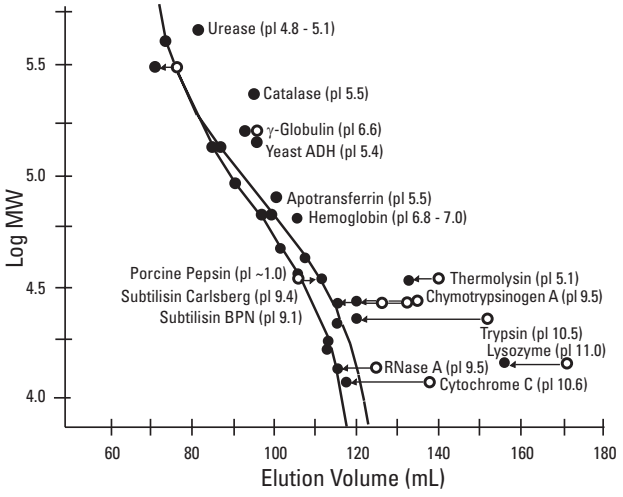
MOBILE PHASE

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

PROPERTIES OF TOYOPEARL SEC RESINS IN AQUEOUS ELUENTS

- HIGH MECHANICAL STABILITY
  TOYOPEARL resins can be operated at pressures up to 3 bar without deformation.

- MINIMUM CHANGE IN GEL BED VOLUME
  Changes in the column bed volume under operational salt conditions are negligible. TOYOPEARL does not shrink or swell even in high concentrations of strong denaturing agents such as urea or guanidine hydrochloride.

- CHEMICAL STABILITY
  TOYOPEARL is stable from pH 2-13, and tolerant to pH 0-14 for short periods. Biomolecules which are only soluble at extreme pH values can be readily separated.

- SHARP CHROMATOGRAPHIC PEAKS
  TOYOPEARL's narrow particle size distribution (min. 80% within declared limits) results in better peak shapes and higher elution target concentrations than other SEC materials.

- TEMPERATURE STABILITY
  TOYOPEARL is thermally stable and does not degrade or denature even in boiling water. TOYOPEARL resins can be sterilized by autoclaving at 121 °C.

- MICROORGANISM RESISTANCE
  TOYOPEARL is an organosynthetic material, and is resistant to degradation by microorganisms.

- SUITABILITY FOR ENZYME IMMOBILIZATION
  TOYOPEARL resins contain numerous hydroxyl groups on the external and internal bead surfaces. These, in combination with the chemical stability of the polymer, make the resin well suited for the covalent bonding of enzymes or other ligands. (Please see the AFC section for more information.)

SIZE EXCLUSION CHROMATOGRAPHY

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

ToyoScreen 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, small-scale biochromatographic separations of protein samples by running up to eight individual columns simultaneously. ToyoScreen RoboColumns packed with the most popular TOYOPEARL SEC/IEC/HIC media are available with 200 µL and 600 µL resin volumes.

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

ORDERING INFORMATION

TOYOPEARL LABPAK

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)
0019821	SECPAK LMW (HW-40F, HW-50F, HW-55F)	3 x 150	30 - 60
0019819	SECPAK HMW (HW-55F, HW-65F, HW-75F)	3 x 150	30 - 60
0019820	SECPAK HP (HW-40S, HW-50S, HW-55S, HW-65S)	4 x 150	20 - 40

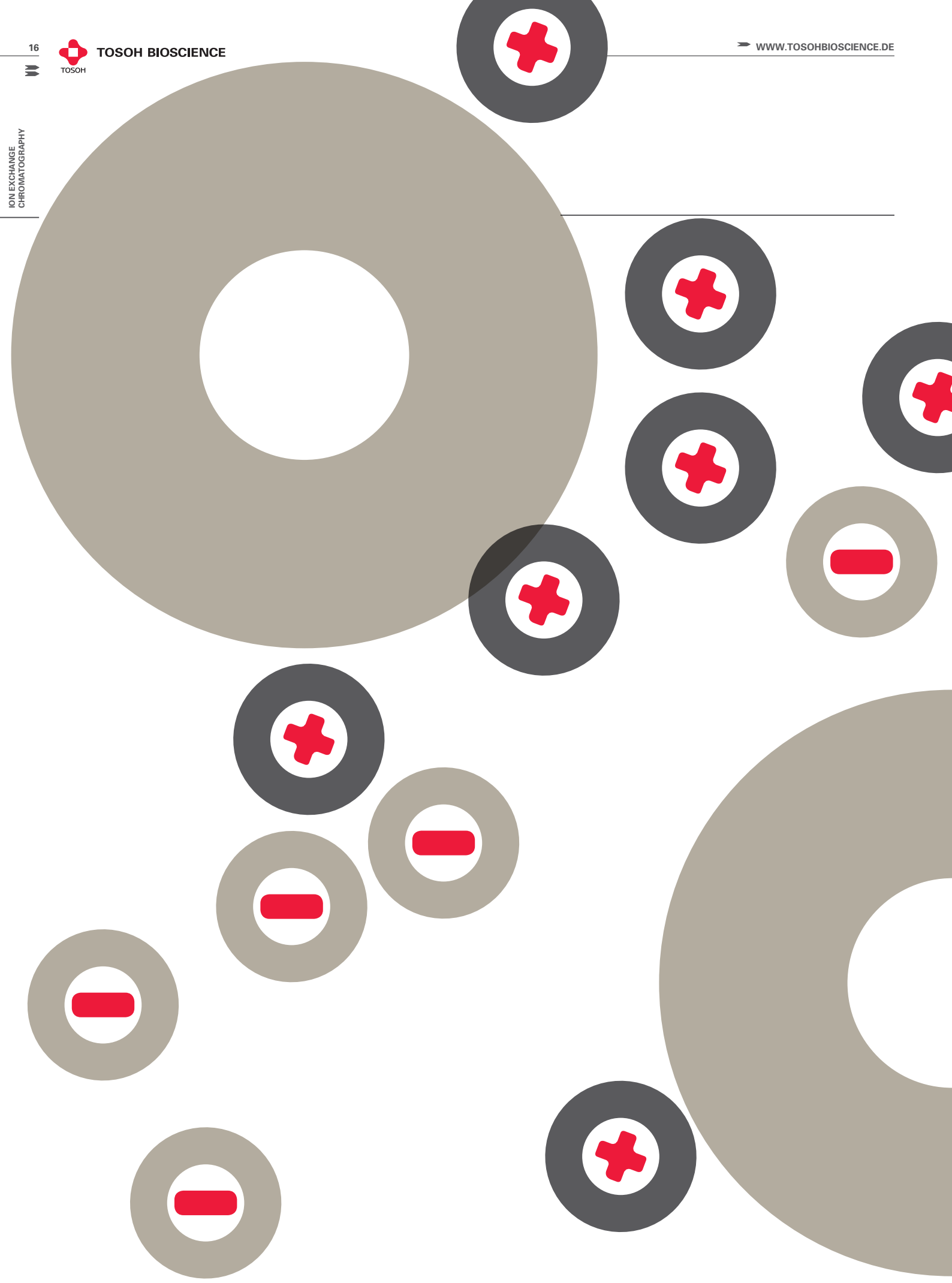
SIZE EXCLUSION CHROMATOGRAPHY

ORDERING INFORMATION

TOYOPEARL SEC RESINS

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

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	EXCLUSION LIMIT (Da)
0019809	TOYOPEARL HW-40S	150	20 - 40	3×10^3
0007451		250		
0014681		1,000		
0007967		5,000		
0019808	TOYOPEARL HW-40F	150	30 - 60	3×10^3
0007448		500		
0014682		1,000		
0007968		5,000		
0019807	TOYOPEARL HW-40C	150	50 - 100	3×10^3
0007449		500		
0014683		1,000		
0007969		5,000		
0019811	TOYOPEARL HW-50S	150	20 - 40	1.8×10^4
0007455		250		
0014684		1,000		
0008059		5,000		
0019810	TOYOPEARL HW-50F	150	30 - 60	1.8×10^4
0007453		500		
0014685		1,000		
0008060		5,000		
0018368		50,000		
0019813	TOYOPEARL HW-55S	150	20 - 40	1.5×10^5
0007459		250		
0014686		1,000		
0008062		5,000		
0019812	TOYOPEARL HW-55F	150	30 - 60	1.5×10^5
0007457		500		
0014687		1,000		
0008063		5,000		
0019815	TOYOPEARL HW-65S	150	20 - 40	1×10^6
0007467		250		
0014688		1,000		
0008068		5,000		
0019814	TOYOPEARL HW-65F	150	30 - 60	1×10^5
0007465		500		
0014689		1,000		
0008069		5,000		
0021481	TOYOPEARL HW-65C	150	50 - 100	1×10^5
0007466		500		
0014690		1,000		
0008070		5,000		
0021482		50,000		
0019816	TOYOPEARL HW-75F	150	30 - 60	8.25×10^5
0007469		500		
0014691		1,000		
0008072		5,000		
0045071	ToyoScreen RoboColumns HW-40F	0.2 x 8	30 - 60	3×10^3
0045072		0.6 x 8		



IEC
ION EXCHANGE CHROMATOGRAPHY

IEC PRODUCTS

ANION EXCHANGE

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 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

TOSOH FACT

The Tosoh logo symbolizes the corporate philosophy of Tosoh's vision of the ideal.

The curved lines represent the realization of happiness, reflecting Tosoh's management philosophy of putting people first. The square in the center expresses the advanced nature of Tosoh's technology and also represents the outstanding quality of Tosoh's products. The right-angle cut at the top portrays an image of contributing to society, Tosoh's stance towards the outside world. The red corporate color symbolizes the Tosoh spirit, which guides the ceaseless efforts to realize the ideal.

ION EXCHANGE CHROMATOGRAPHY

TOYOPEARL ION EXCHANGE CHROMATOGRAPHY RESINS

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 concentration step. Tosoh Bioscience offers a broad range of products for ion exchange applications.

HOW DOES IEC WORK?

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.

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 (Figure 1). 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.

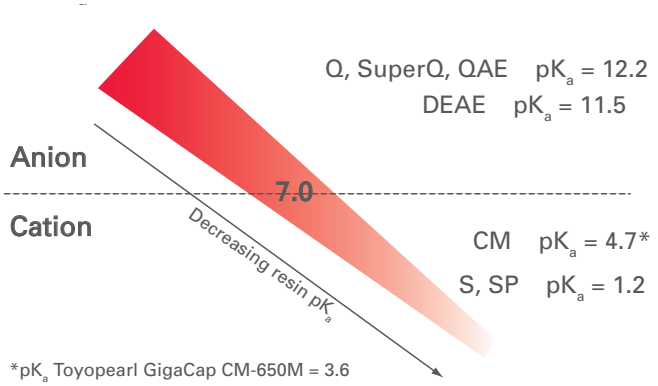
ION EXCHANGE GROUPS AVAILABLE

TOYOPEARL and TSKgel PW-type IEC resins are available with six different ion exchange groups as shown in Table I:

- 3 for anion exchange – Q, QAE, DEAE
- 3 for cation exchange – S, SP, CM

FIGURE 1

PK_a VALUES FOR ION EXCHANGE GROUPS



*pK_a Toyopearl GigaCap CM-650M = 3.6

PORE SIZES

Tosoh Bioscience offers a range of pore sizes for IEC resins originating from our size exclusion chromatography base resins. Four different mean pore diameters are used for the current ion exchange resins: 100 nm, 75 nm, 50 nm, and 25 nm (Table II). 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.

HIGHER ACCESSIBLE SURFACE AREA - MORE CAPACITY

A bead with a small pore size has theoretically more surface area than the same size bead with a larger pore. Figure 2 shows insulin binding capacity on six different pore size beads. As the pore size increases to the point where the insulin has maximal access to the internal surface area the insulin capacity increases.

FEATURES	BENEFITS
porous, hydrophilic polymer based resin	suitable for laboratory scale and process chromatography
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
continous selectivity	easy scale-up from TSKgel IEC columns high yields of biologically active proteins

ION EXCHANGE CHROMATOGRAPHY

TABLE I

ANION EXCHANGE RESIN	Base bead	Pore size (nm)	Particle size (µm)	IEC (eq/L)	DBC (BSA) (g/L-gel)	Recovery (%)
TOYOPEARL GigaCap Q-650S	HW-65	100	50-100	0.17	172	97
TOYOPEARL GigaCap Q-650M	HW-65	100	50-100	0.17	172	97
TOYOPEARL SuperQ-650M	HW-65	100	40-90	0.24	145	98
TOYOPEARL DEAE-650M	HW-65	100	40-90	0.11	25	97
TOYOPEARL Q-600C AR	HW-60	75	50-150	0.18	100	98
TOYOPEARL QAE-550C	HW-55	50	50-150	0.36	30	96
CATION EXCHANGE RESIN	**Base bead**	**Pore size (nm)**	**Particle size (µm)**	**IEC (eq/L)**	**DBC (hIgG) (g/L-gel)**	**Recovery (%)**
TOYOPEARL GigaCap S-650S	HW-65	100	50-100	0.16	145	98
TOYOPEARL GigaCap S-650M	HW-65	100	50-100	0.16	145	98
TOYOPEARL GigaCap CM-650M	HW-65	100	50-100	0.24	100	98
TOYOPEARL SP-650C	HW-65	100	50-150	0.12	12	98
TOYOPEARL CM-650C	HW-65	100	50-150	0.09	35 (lysozyme)	
TOYOPEARL SP-550C	HW-55	50	50-150	0.13	14	98

TABLE II

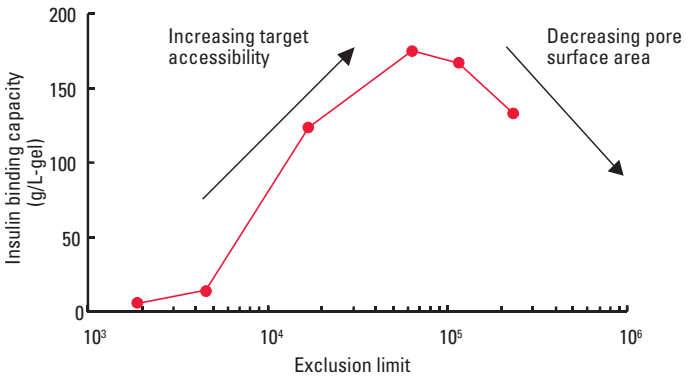
Base bead	TOYOPEARL HW-65 TSKgel G5000PW	TOYOPEARL HW-60	TOYOPEARL HW-55	TSKgel G3000PW
Pore Diameter	100 nm	75 nm	50 nm	25 nm
	TOYOPEARL GigaCap S-650 TOYOPEARL GigaCap CM-650 TOYOPEARL GigaCap Q-650 TOYOPEARL GigaCap DEAE TOYOPEARL SuperQ-650 TOYOPEARL DEAE-650 TOYOPEARL SP-650 TOYOPEARL CM-650 TSKgel SP-5PW TSKgel SuperQ-5PW TSKgel DEAE-5PW	TOYOPEARL Q-600C AR	TOYOPEARL SP-550 TOYOPEARL MegaCap II SP-550 TOYOPEARL QAE-550	TSKgel SP3-PW

However, there is a point of diminishing return. Because the absolute surface area decreases as the pores become larger, the insulin capacity decreases accordingly. This effect is similarly demonstrated by the lysozyme static binding capacity range specifications of TOYOPEARL SP-550C and TOYOPEARL SP-650C which are 80-120 g/L and 35-55 g/L, respectively (data not shown).

For more information on pore size and particle size combinations not contained in our commercial products, please see the Custom Resin section of this catalog.

FIGURE 2

OPTIMIZATION OF INSULIN BINDING CAPACITY AS A FUNCTION OF PORE SIZE OF EXPERIMENTAL TSKgel SP-TYPE RESINS



ION EXCHANGE CHROMATOGRAPHY

MULTIPLE PARTICLE SIZES SIMPLIFY SCALING UP OR DOWN

Because TOYOPEARL HW-65 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). Figure 4 lists the complete range of ion exchange products, particle sizes and suggests how they are typically placed into a manufacturing scheme. 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 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 chromatography:

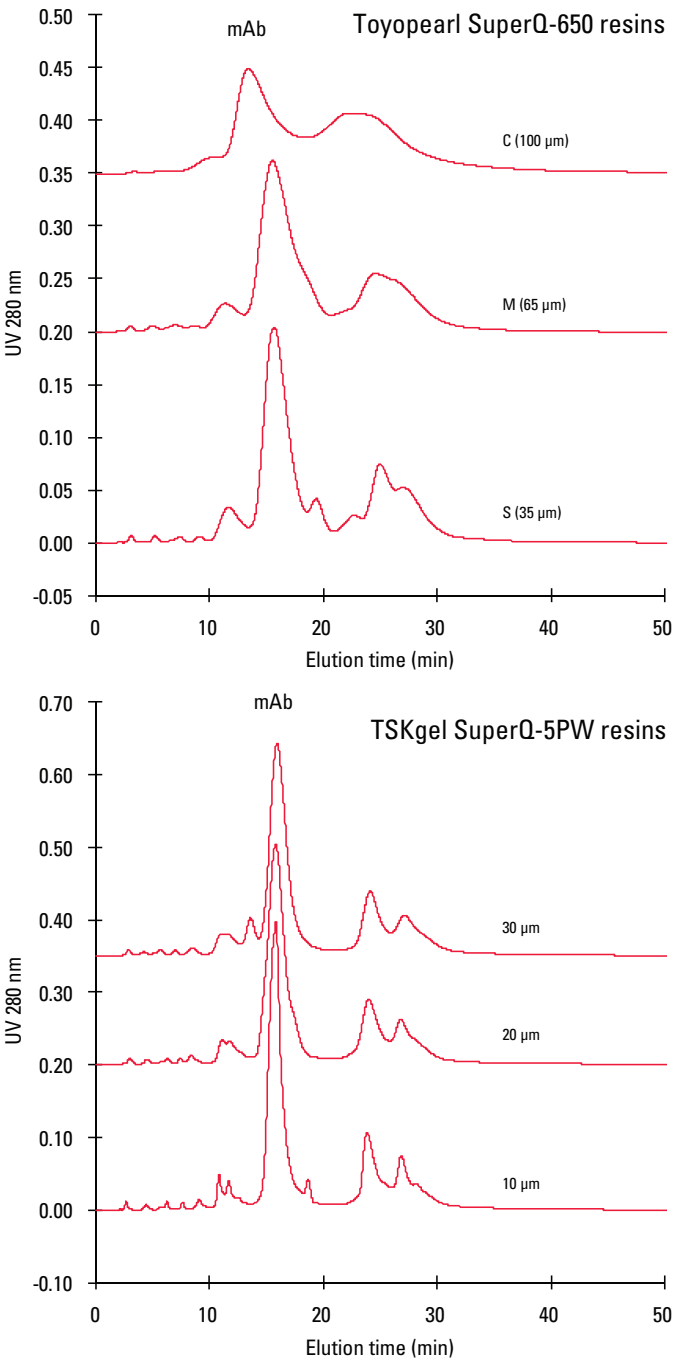
- If the target molecule's size is larger than most components of the feedstream, select a pore size which will tend to exclude it (known as kinetic exclusion, this technique can also be used under binding conditions as the excluded molecule only sees 1% of the resin surface area and the capacity/recovery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

FIGURE 3

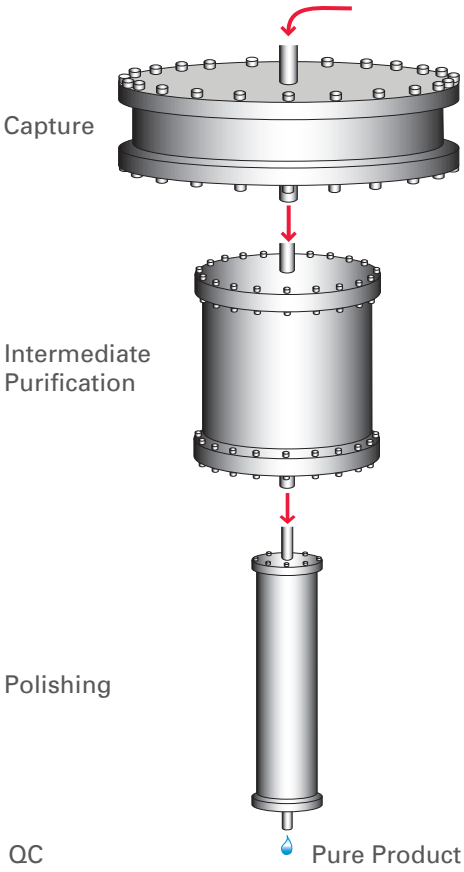
SCALE UP OR DOWN USING THE SAME LIGAND



Resins: 1) TOYOPEARL SuperQ-650C (100 µm); 2) TOYOPEARL SuperQ-650M (65 µm); 3) TOYOPEARL SuperQ-650S (35 µm); 4) TSKgel SuperQ-5PW(30) (30 µm); 5) TSKgel SuperQ-5PW(20) (20 µm); 6) 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

FIGURE 4



	Process media	
	ANION	CATION
200 µm		TOYOPEARL MegaCap II SP-550EC
100 µm	TOYOPEARL SuperQ-650C TOYOPEARL DEAE-650C TOYOPEARL QAE-550C TOYOPEARL Q-600C AR	TOYOPEARL SP-650C TOYOPEARL SP-550C TOYOPEARL CM-650C
75 µm	TOYOPEARL GigaCap Q-650M TOYOPEARL GigaCap DEAE-650M	TOYOPEARL GigaCap S-650M TOYOPEARL GigaCap CM-650M
65 µm	TOYOPEARL SuperQ-650M TOYOPEARL DEAE-650M	TOYOPEARL SP-650M TOYOPEARL CM-650M
35 µm	TOYOPEARL SuperQ-650S TOYOPEARL DEAE-650S TOYOPEARL GigaCap Q-650S	TOYOPEARL SP-650S TOYOPEARL CM-650S TOYOPEARL GigaCap S-650S
30 µm	TSKgel SuperQ-5PW (30) TSKgel DEAE-5PW (30)	TSKgel SP-5PW (30) TSKgel SP-3PW (30)
20 µm	TSKgel SuperQ-5PW (20) TSKgel DEAE-5PW (20)	TSKgel SP-5PW (20)
10 µm	TSKgel SuperQ-5PW 7.5 mm IDx 7.5 cm L TSKgel DEAE-5PW 7.5 mm ID x 7.5 cm L	TSKgel SP-5PW 7.5 mm ID x 7.5 cm L TSKgel CM-5PW 7.5 mm ID x 7.5 cm L

Same selectivity HPLC columns are available for most process media

LIGAND ATTACHMENT TECHNOLOGY

Tosoh Bioscience applies three different ligand attachment chemistries reflecting three generations of ligand attachment technology. The “traditional” method, or first generation of attaching the ion exchange ligand, is directly 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 Q-550
- TOYOPEARL DEAE-650
- TSKgel DEAE-5PW
- TOYOPEARL MegaCap II SP-550EC

A second generation attachment chemistry (Type A noted in Table IV) for increasing protein binding within the accessible surface area, 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 A ligand attachment chemistry:

- TOYOPEARL SuperQ-650
- TSKgel SuperQ-5PW

A third generation ligand attachment chemistry (Type B noted in Table IV) 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 B ligand attachment chemistry:

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

ION EXCHANGE CHROMATOGRAPHY

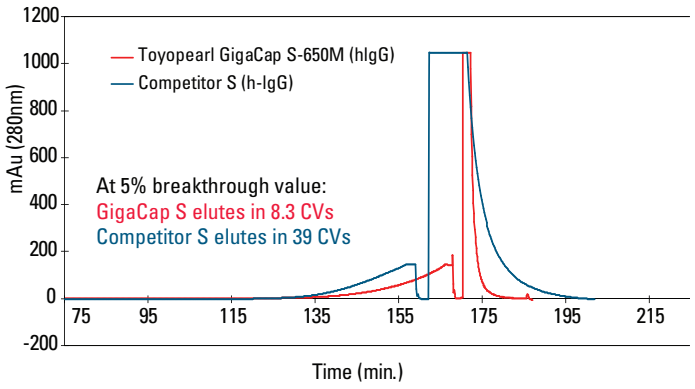
Figures 5, 6 and 7 show the breakthrough curves for the three TOYOPEARL GigaCap resins. They are compared where possible with the most current equivalent competitive resin. Each trace shows the dynamic binding capacity of the resin up to 10% breakthrough plus the elution profile for the target molecule. 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 separaion of process impurities.

FIGURE 5

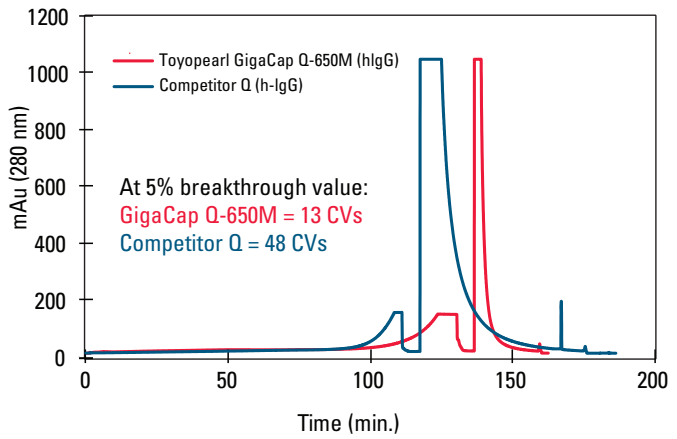
TOYOPEARL GigaCap S-650M VS. COMPETITOR S ELUTION POOL VOLUME COMPARISON



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 6

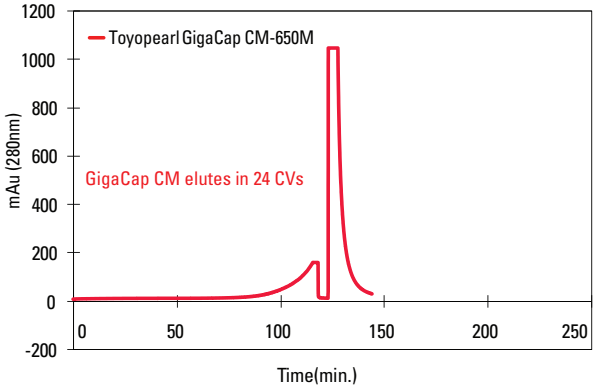
TOYOPEARL GigaCap Q-650M VS. COMPETITOR Q ELUTION POOL VOLUME COMPARISON



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 7

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

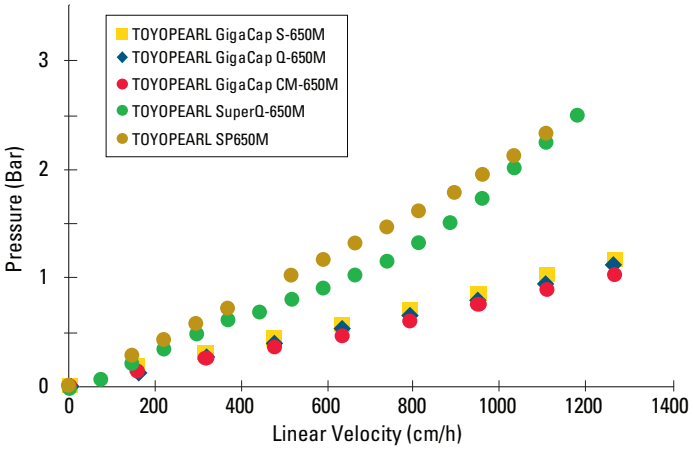
ION EXCHANGE CHROMATOGRAPHY

IMPACT OF PORE SIZE AND LIGAND ATTACHMENT ON DBC

Table III contains DBC data for five of our TOYOPEARL resins using three different sized proteins. 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 8

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 3 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 8) 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 our TOYOPEARL Instruction Manual for the 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 III

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

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 VI TOYOPEARL GigaCap series ion exchange resins have excellent alkaline stability. Figure 9 shows excellent CIP (cleaning in place) stability of the new TOYOPEARL GigaCap DEAE-650.

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 10, after 100 days of exposure to 1 mol/L NaOH, the DBC of TOYOPEARL Q-600C AR resin remains unchanged.

Figure 11 shows the preservation of selectivity after extensive exposure to caustic.

FIGURE 9

ALKALINE STABILITY OF TOYOPEARL GigaCap DEAE-650M

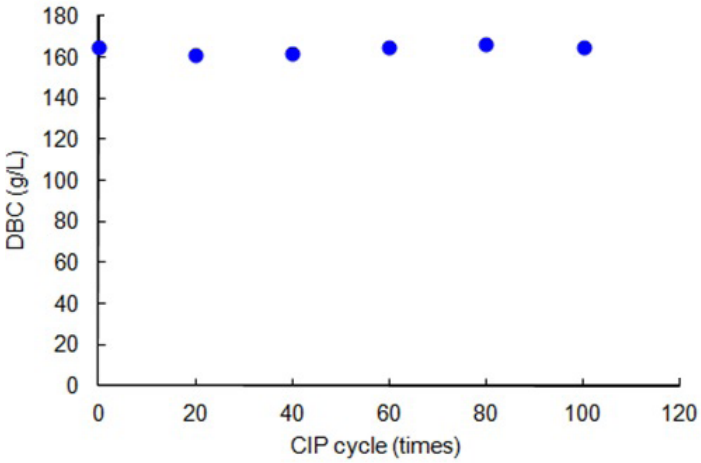


FIGURE 10

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

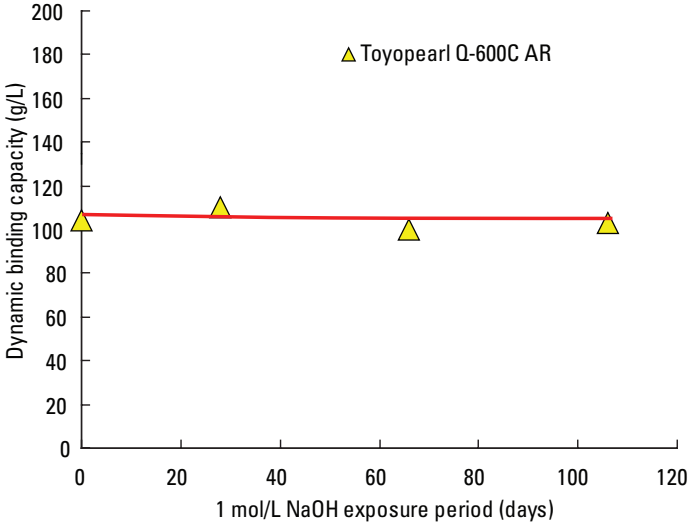
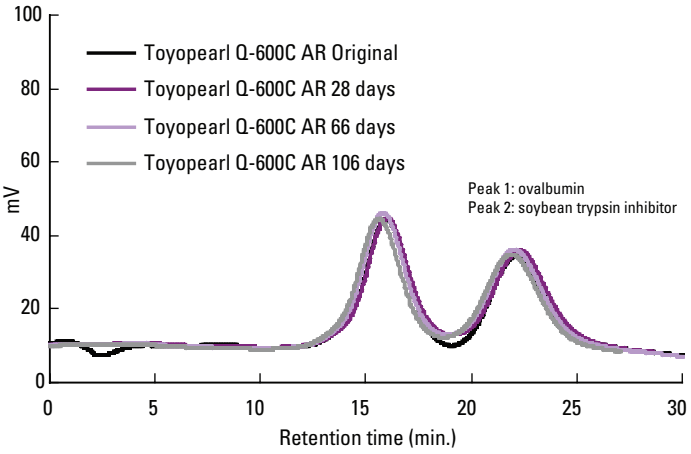


FIGURE 11

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

ION EXCHANGE CHROMATOGRAPHY

TABLE IV

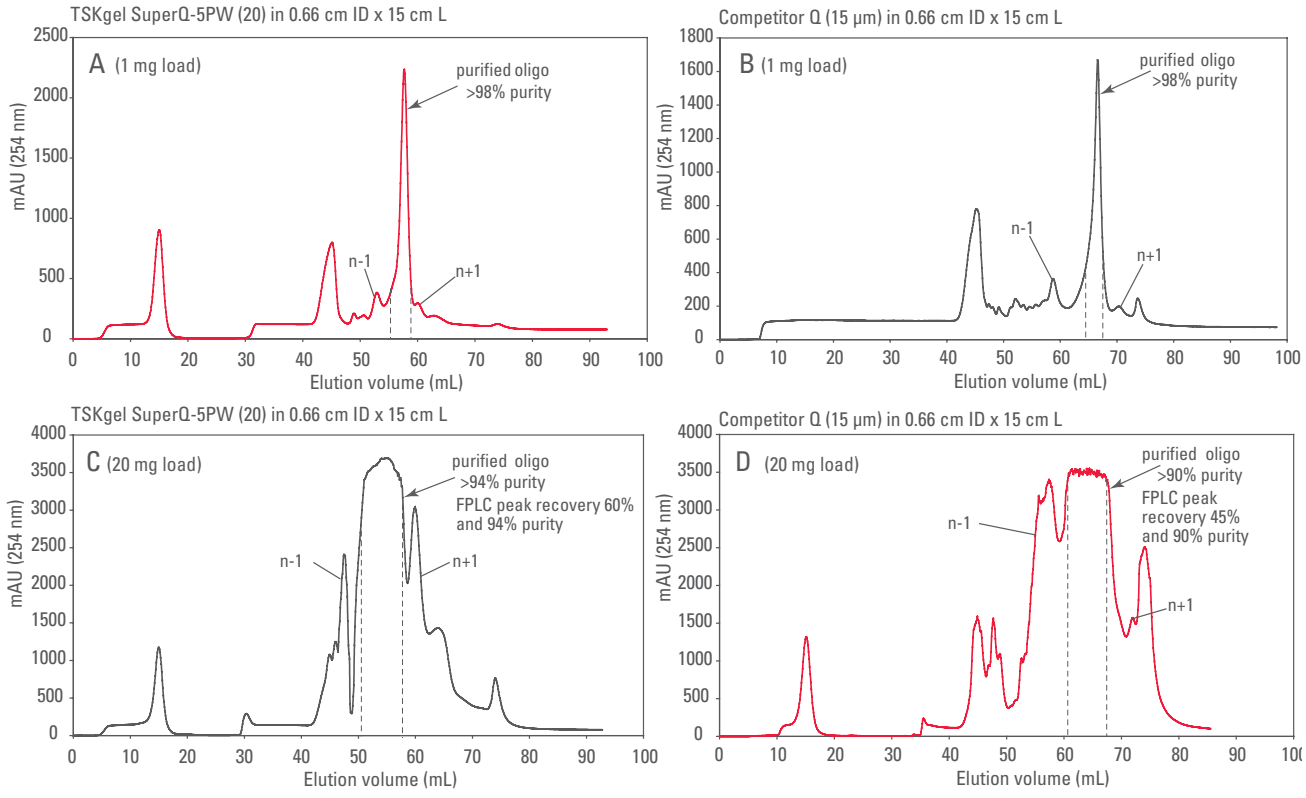
TOYOPEARL GigaCap RESIN BASE STABILITY

RESIN	STORAGE SOLUTION	TEST MOLECULE	CAPACITY CAPACITY	STARTING	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 12

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

ION EXCHANGE CHROMATOGRAPHY

OLIGONUCLEOTIDE PURIFICATIONS

Table V 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 type resin products 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 shown in Table IV) and its very high capacity can be used, although its selectivity is somewhat different than the other quaternary.

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 smaller particle, lower capacity resins. 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 12 shows a comparison of one smaller particle size, competitive product, which starts out having better resolution than TSKgel SuperQ-5PW (20) resin at 1 mg oligonucleotide/mL of resin. At 20 mg oligonucleotide/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.

TABLE V

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

ION EXCHANGE CHROMATOGRAPHY

PEPTIDE PURIFICATIONS

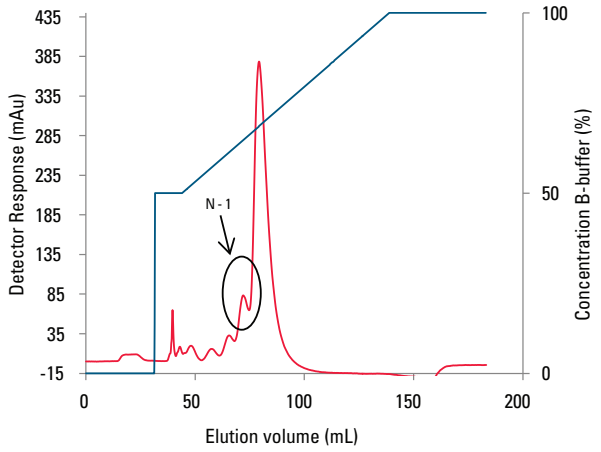
Cation exchange chromatography is commonly used for peptide purification. Table V shows the same particle size availability of TOYOPEARL and TSKgel PW strong cation exchange resins. Based on the needs for capacity and resolution, an appropriate S or SP resin is selected for a particular peptide application. 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 VI compares the 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 14.

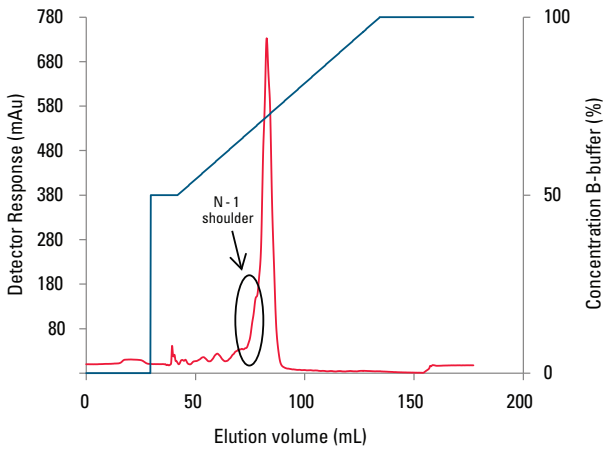
The new TOYOPEARL GigaCap Q-650S resin offers a low pressure alternative to oligonucleotide purification with TSKgel resins while preserving the selectivity, resolution and yields of those higher pressure processes.

FIGURE 13

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 VI

INSULIN DYNAMIC BINDING CAPACITIES

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

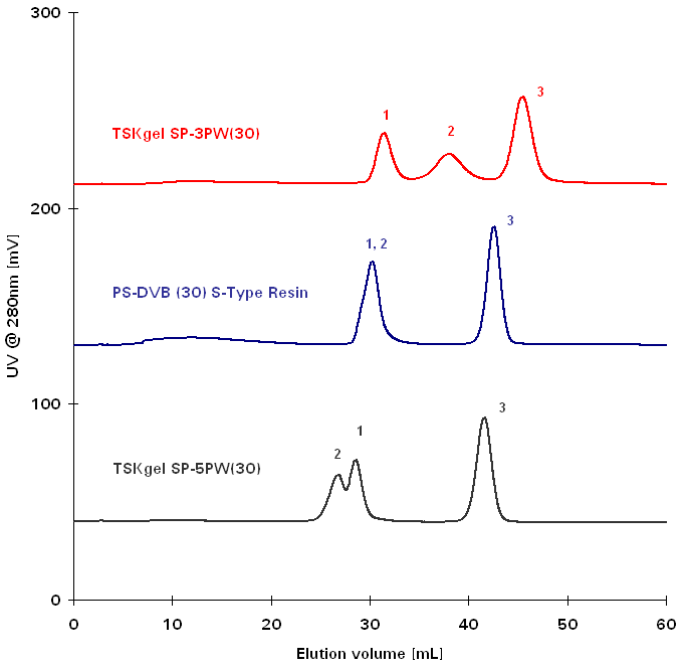
Figure 13 shows that the N-1 peak was slightly better resolved with the TSKgel SuperQ-5PW (20) than with the TOYOPEARL GigaCap Q-650S, perhaps due to the smaller particle size of the TSKgel resin. HPLC analysis of fractions taken across the peaks (data not shown) revealed that both resins were able to adequately resolve the full length oligonucleotide.

PEGylated PROTEINS

Ion exchange resins are frequently used for the purification of pegylated proteins. Figure 15 shows the breakthrough curves of five TOYOPEARL cation exchange resins for mono-pegylated lysozyme.

FIGURE 14

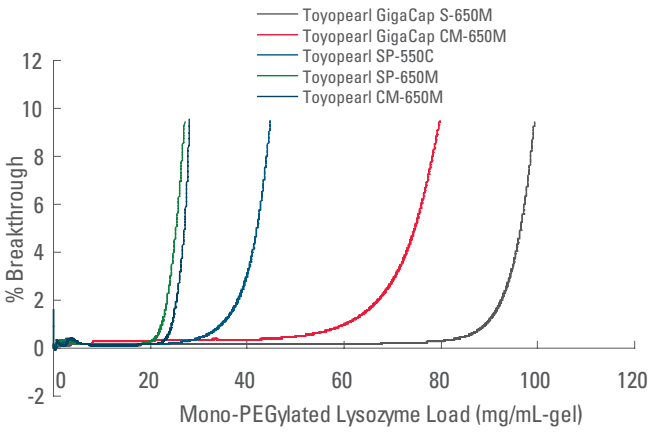
SELECTIVITY COMPARISON



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)

FIGURE 15

BREAKTHROUGH CURVES OF MONO-PEGYLATED LYSOZYME USING TOYOPEARL CATION EXCHANGE RESINS



Dynamic binding capacities were determined at 10% breakthrough Column size: 6 mm ID x 40 mm L; Sample: mono-PEGylated lysozyme; Loading buffer: 20 mmol/L phosphate buffer (pH= 7.0) Elution buffer: 20 mmol/L phosphate buffer (pH= 7.0) + 0.5 mol/L NaCl Linear velocity: 212 cm/h; Detection: UV @ 280 nm PEG MW= 5kDa

ION EXCHANGE CHROMATOGRAPHY

ToyoScreen PREPACKED COLUMNS FOR PROCESS DEVELOPMENT

ToyoScreen columns packed with the full range of our TOYOPEARL IEC products are available in 1 mL and 5 mL resin volumes. The ToyoScreen columns provide a convenient way to perform early resin screening for both target retention and recovery. Multiple columns can be connected in series for additional capacity or resolution. Please see the ordering information at the end of this section or contact us for more information on these products.

The most popular TOYOPEARL ion exchange resins are now also availabe as ToyoScreen RoboColumns. ToyoScreen RoboColumns are miniaturized chromatographic columns pre-packed with TOYOPEARL media. These columns are available in different volumes and can be operated with a robotic liquid handling system, such as the Freedom EVO® from TECAN. This approach allows automated high-throughput, small-scale biochromatographic separations of protein samples by running up to eight individual columns simultaneously.

LABPAK

For scientists wishing to develop a better physical understanding of the packing properties of TOYOPEARL and TSKgel ion exchange resins, we offer TOYOPEARL LabPaks containing small volumes of the bulk resins. Please see the ordering information at the end of this section or contact us for more information on these products.

SUMMARY

Since 2007, major additions have been made to the TOYOPEARL ion exchange resins offered by Tosoh Bioscience.The TOYOPEARL GigaGap family of resins and the new TOYOPEARL Q-600C AR media represent significant improvements in terms of dynamic binding capacity, elution kinetics, and alkaline stability. When these products are used, process developers have more options available to design and optimize their process to improve productivity and lower operating costs.

ION EXCHANGE CHROMATOGRAPHY

ORDERING INFORMATION

ToyoScreen PROCESS DEVELOPMENT COLUMNS FOR IEC

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

ToyoScreen RoboColumns FOR IEC

PART #	PRODUCT DESCRIPTION	PACKAGE
0045007	ToyScreen RoboColumn GigaCap DEAE-650M, 200 µL	0.2 mL x 8 columns
0045008	ToyScreen RoboColumn GigaCap DEAE-650M, 600 µL	0.6 mL x 8 columns
0045003	ToyScreen RoboColumn GigaCap Q-650M, 200 µL	0.2 mL x 8 columns
0045004	ToyScreen RoboColumn GigaCap Q-650M, 600 µL	0.6 mL x 8 columns
0045011	ToyScreen RoboColumn Q-600C AR, 200 µL	0.2 mL x 8 columns
0045012	ToyScreen RoboColumn Q-600C AR, 600 µL	0.6 mL x 8 columns
0045005	ToyScreen RoboColumn GigaCap CM-650M, 200 µL	0.2 mL x 8 columns
0045006	ToyScreen RoboColumn GigaCap CM-650M, 600 µL	0.6 mL x 8 columns
0045001	ToyScreen RoboColumn GigaCap S-650M, 200 µL	0.2 mL x 8 columns
0045002	ToyScreen RoboColumn GigaCap S-650M, 600 µL	0.6 mL x 8 columns

ION EXCHANGE CHROMATOGRAPHY

ORDERING INFORMATION

ToyoScreen COLUMN ACCESSORIES

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

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	15-25
0043280	IEXPAK PW (30) (SP-5PW, DEAE-5PW, SuperQ-5PW)	3 x 25 mL	20-40

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)	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

ION EXCHANGE CHROMATOGRAPHY

ORDERING INFORMATION

ANION EXCHANGE RESINS

TOYOPEARL BULK MEDIA

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	ION EXCHANGE CAPACITY (eq/L resin)	TYPICAL CAPACITY (g BSA/L resin)
0043271	TOYOPEARL QAE-550C	100	50-150	0.28-0.38	60-80
0014026		250			
0014704		1,000			
0014027		5,000			
0018365		50,000			
0021985	TOYOPEARL Q-600C AR	100	50-150	0.15-0.20	>120
0021986		250			
0021987		1,000			
0021988		5,000			
0021989		50,000			
0022881	TOYOPEARL GigaCap Q-650S	100	50-100	0.10-0.20	>170
0022882		250			
0022883		1,000			
0022884		5,000			
0022885		50,000			
0021854	TOYOPEARL GigaCap Q-650M	25	50-100	0.10-0.20	>162
0021855		250			
0021856		1,000			
0021857		5,000			
0021858		50,000			
0022865	TOYOPEARL GigaCap DEAE-650M	100	50-100	0.17-0.28	>156
0022866		250			
0022867		1,000			
0022868		5,000			
0022869		50,000			
0019823	TOYOPEARL SuperQ-650S	25	20-50	0.20-0.30	105-155
0017223		250			
0017224		1,000			
0017225		5,000			
0019679		50,000			
0043205	TOYOPEARL SuperQ-650M	100	40-90	0.20-0.30	105-155
0017227		250			
0017228		1,000			
0017229		5,000			
0021311		50,000			
0043275	TOYOPEARL SuperQ-650C	100	50-150	0.20-0.30	105-155
0017231		250			
0017232		1,000			
0017233		5,000			
0019804	TOYOPEARL DEAE-650S	25	20-50	0.08-0.12	25-35
0007472		250			
0014692		1,000			
0007973		5,000			
0021483		50,000			
0043201	TOYOPEARL DEAE-650M	100	40-90	0.08-0.12	25-35
0007473		250			
0014693		1,000			
0007974		5,000			
0018367		50,000			

ION EXCHANGE CHROMATOGRAPHY

ORDERING INFORMATION

ANION EXCHANGE RESINS

TOYOPEARL BULK MEDIA

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	ION EXCHANGE CAPACITY (eq/L resin)	TYPICAL CAPACITY (g BSA/L resin)
0007988	TOYOPEARL DEAE-650C	250	50-150	0.05-0.11	25-35
0014694		1,000			
0007989		5,000			

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	15-25	0.12-0.18	52-88
0018535		250			
0018546		1,000			
0018547		5,000			
0043283	TSKgel SuperQ-5PW (30)	25	20-40	0.12-0.18	52-88
0018536		250			
0018548		1,000			
0018549		5,000			
0043381	TSKgel DEAE-5PW (20)	25	15-25	0.05-0.11	25-45
0014710		250			
0014711		1,000			
0018436		5,000			
0043281	TSKgel DEAE-5PW (30)	25	20-40	0.05-0.11	20-40
0014712		250			
0014713		1,000			
0018370		5,000			

ION EXCHANGE CHROMATOGRAPHY

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)
0022875	TOYOPEARL GigaCap S-650S	25	50-100	0.14-0.18	>150 (γ-globulin)
0022876		250			
0022877		1,000			
0022878		5,000			
0022879		50,000			
0021833	TOYOPEARL GigaCap S-650M	100	50-100	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	50-100	0.17-0.28	>110 (γ-globulin)
0021947		250			
0021948		1,000			
0021949		5,000			
0021950		50,000			
0043272	TOYOPEARL SP-550C	100	50-150	0.14-0.18	80-120 (lysozyme)
0014028		250			
0014705		1,000			
0014029		5,000			
0018366		50,000			
0019822	TOYOPEARL SP-650S	25	20-50	0.13-0.17	40-60 (lysozyme)
0008437		250			
0014698		1,000			
0008438		5,000			
0021477		50,000			
0043202	TOYOPEARL SP-650M	100	40-90	0.13-0.17	40-60 (lysozyme)
0007997		250			
0014699		1,000			
0007998		5,000			
0018369		50,000			
0007994	TOYOPEARL SP-650C	250	50-150	0.12-0.18	35-55 (lysozyme)
0014700		1,000			
0007995		5,000			
0019803	TOYOPEARL CM-650S	25	20-50	0.08-0.12	30-50 (lysozyme)
0007474		250			
0014695		1,000			
0007971		5,000			
0043203	TOYOPEARL CM-650M	100	40-90	0.08-0.12	30-50 (lysozyme)
0007475		250			
0014696		1,000			
0007972		5,000			
0019839		50,000			
0007991	TOYOPEARL CM-650C	250	50-150	0.05-0.11	25-45 (lysozyme)
0014697		1,000			
0007992		5,000			
0019329		50,000			

ION EXCHANGE CHROMATOGRAPHY

ORDERING INFORMATION

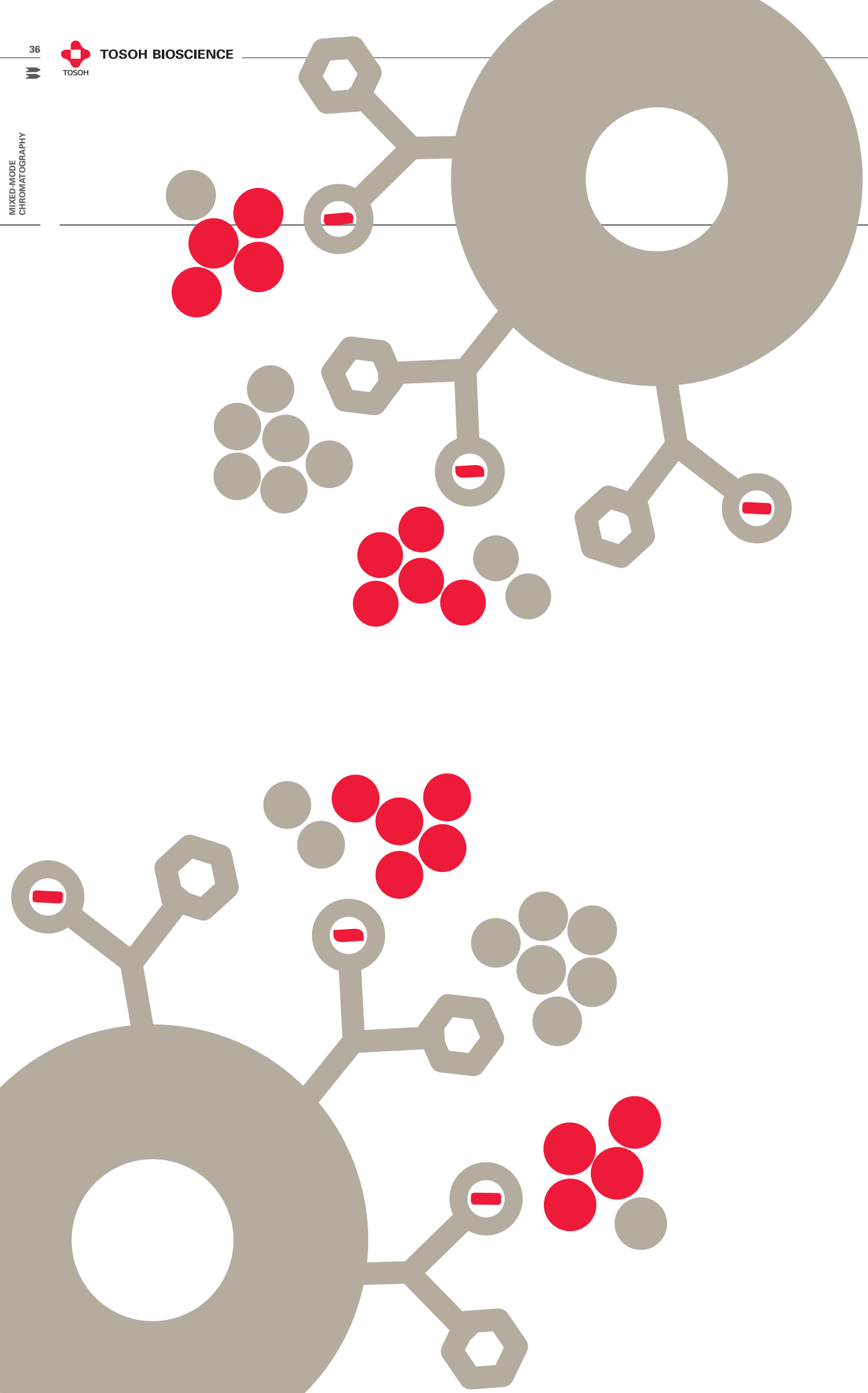
CATION EXCHANGE RESINS

TOYOPEARL BULK MEDIA

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	ION EXCHANGE CAPACITY (eq/L resin)	TYPICAL CAPACITY (g/L resin)
0021804	TOYOPEARL MegaCap II SP-550EC	100	100-300	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	20-40	0.07- 0.22	≥ 65
0021977		250			
0021978		1,000			
0021979		5,000			
0043382	TSKgel SP-5PW (20)	25	15-25	0.06-0.12	20-40
0014714		250			
0014715		1,000			
0018435		5,000			
0043282	TSKgel SP-5PW (30)	25	20-40	0.06-0.12	20-40
0014716		250			
0014717		1,000			
0018384		5,000			



MIXED-MODE CHROMATOGRAPHY

MIXED-MODE PRODUCTS

- TOYOPEARL MX-Trp-650M

TOSOH FACT

Mixed-mode or multimodal chromatography, refers to chromatographic methods that utilize more than one form of interactions between the stationary phase and analytes in order to achieve their separation. Before mixed-mode was considered as a chromatographic approach, secondary interactions were believed to be the main cause of peak tailing. However, it was discovered afterwards that secondary interactions can be applied for improving separation power. Tosoh Bioscience developed a high capacity mixed-mode resin mainly for purification of monoclonal antibodies and other proteins. The multimodal resin maintains dynamic binding capacity (DBC) at elevated feedstock or buffer conductivities.

MIXED-MODE CHROMATOGRAPHY

TOYOPEARL RESINS FOR MIXED-MODE CHROMATOGRAPHY

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

HOW DOES MIXED MODE CHROMATOGRAPHY WORK?

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 TOYOPEARL MX-Trp-650M greatly depends on pH (Figure 2). 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 1

TOYOPEARL MX-Trp-650M STRUCTURE

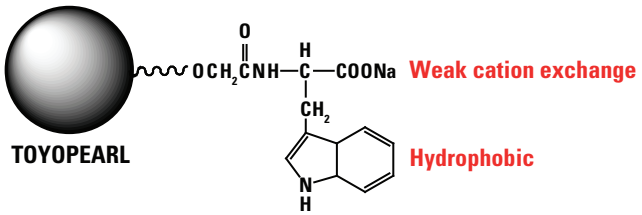
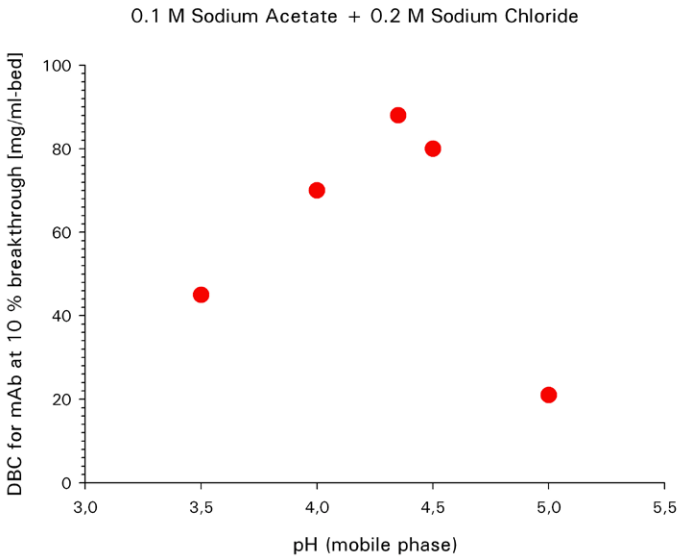


FIGURE 2

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.

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

HIGH BINDING CAPACITY AT HIGH CONDUCTIVITIES

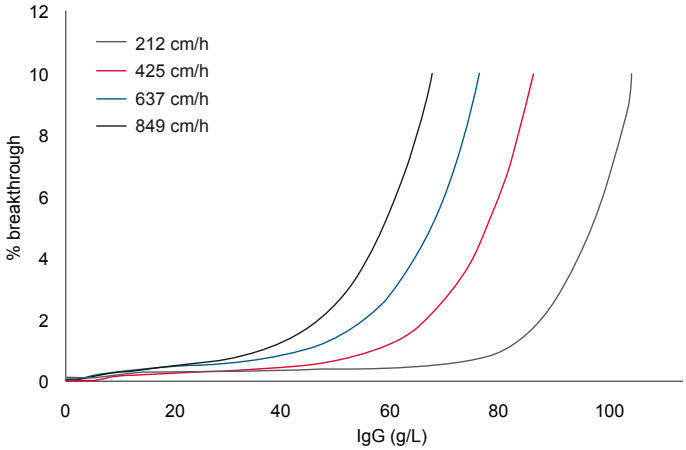
TOYOPEARL MX-Trp-650M exhibits dynamic binding capacities (DBC) for immunoglobulin G as high as 90-100 g/L at standard flow rates (Figure 3). At elevated flow rates/shorter residence times the binding capacity still remains high. Table 1 shows the DBC of the new resin at two feedstock conductivities: 12 mS/cm and 17 mS/cm. For comparison purposes, data for another agarose based multimodal cation exchanger (Brand M) is also shown. For both conductivity levels the new TOYOPEARL MX-Trp-650M resin shows much higher DBCs than the agarose based resin.

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 4).

FIGURE 3

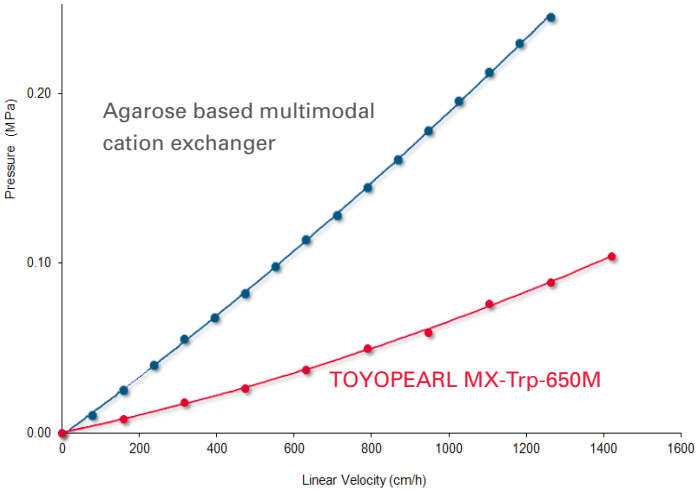
TOYOPEARL MX-Trp-650M DYNAMIC BINDING CAPACITY



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 4

TOYOPEARL MX-Trp-650M PRESSURE / FLOW CURVE



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

TABLE III

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);
Dynamic binding capacity (DBC) calculated at 10 % breakthrough.

MIXED-MODE CHROMATOGRAPHY

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 5).

PURIFICATION OF IMMUNOGLOBULINS

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

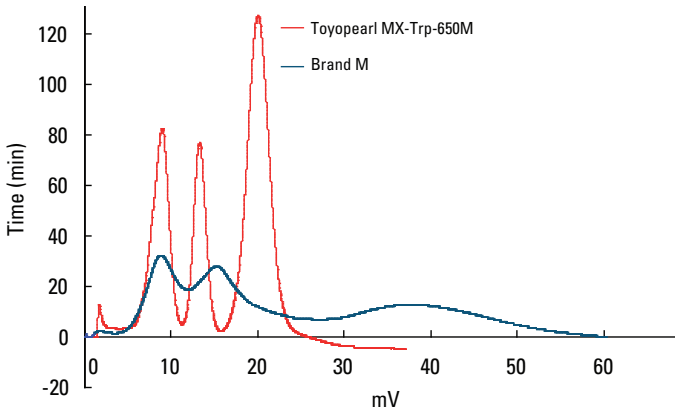
Antibodies captured by protein A affinity chromatography might be loaded at pH 4.5 and eluted with increased salt concentration and pH. Elution of various IgG monomers and their well separated aggregates was successful using an overall salt concentration of 0.5 mol/L and pH 5.5 (Figure 6). Monomer purities of up to 98.5 % can be achieved.

ToyoSCREEN PREPACKED COLUMNS FOR PROCESS DEVELOPMENT

ToyoScreen columns packed with TOYOPEARL MX-Trp-650M are available in 1 mL and 5 mL resin volumes. ToyoScreen columns provide a convenient way to perform early resin screening for both target retention and recovery. Multiple columns can be connected in series for additional capacity or resolution. ToyoScreen 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, small-scale biochromatographic separations of protein samples by running up to eight individual columns simultaneously. ToyoScreen RoboColumns packed with TOYOPEARL MX-Trp-650M are available with 200 µL and 600 µL resin volumes. Please see the ordering information at the end of this section or contact us for more information on these products.

FIGURE 5

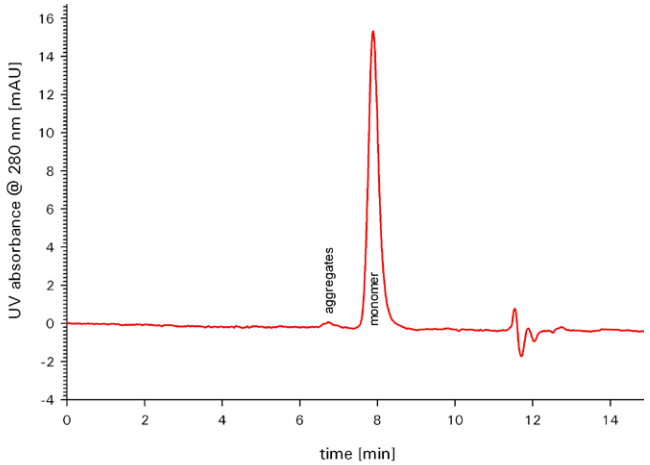
GOOD PEAK SHAPE AND HIGH RESOLUTION



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

FIGURE 6

PURITY CHECK FOR mAb FRACTION



Column: TSKgel G3000SW$_{XL}$ (7.8 mm ID X 30 cm);
Mobile phase: 0.1 mol/L phosphate buffer (pH 6.7) + 0.1 mol/L Na_2SO_4;
Flow rate: 1.0 mL/min; Detection: UV @ 280 nm
Sample: pooled monoclonal antibody fractions eluted from TOYOPEARL MX-Trp-650M @ 100 mmol/L acetate buffer pH 5.5 + 0.4 mol/L NaCl; Injection Vol.: 20 µL

MIXED-MODE CHROMATOGRAPHY

ORDERING INFORMATION

ToyoScreen PROCESS DEVELOPMENT COLUMNS FOR MIXED-MODE:

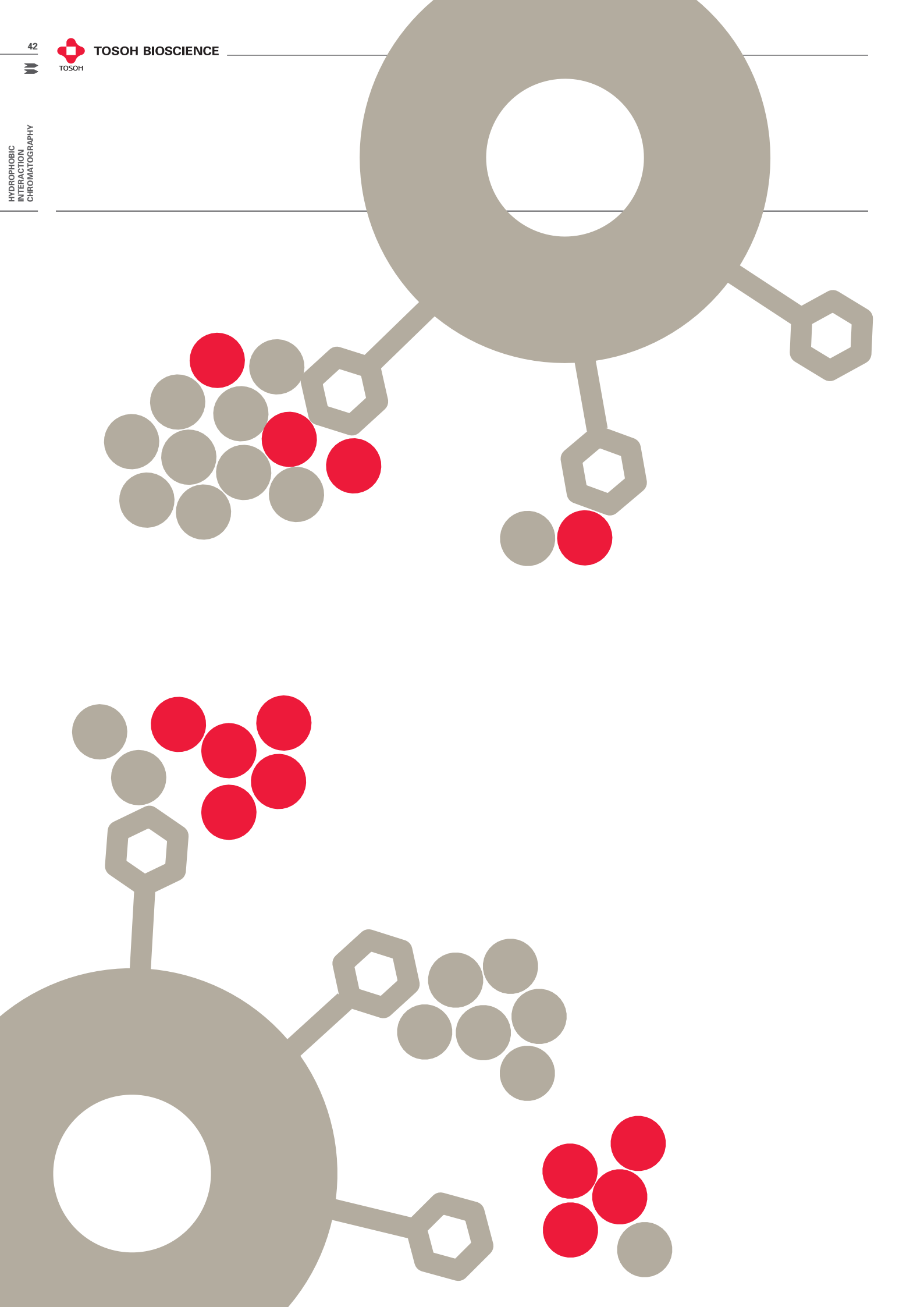
PART#	PRODUCT DESCRIPTION	PACKAGE
0022824	ToyoScreen MX-Trp-650M	1 mL x 6 (each)
0022825	ToyoScreen MX-Trp-650M	5 mL x 6 (each)
0045051	ToyoScreen RoboColumn MX-Trp-650M	200 µL x 8 (each)
0045052	ToyoScreen RoboColumn MX-Trp-650M	600 µL x 8 (each)

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	50-100	>75
0022818	TOYOPEARL MX-Trp-650M	100		
0022819	TOYOPEARL MX-Trp-650M	1,000		
0022820	TOYOPEARL MX-Trp-650M	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 FACT

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

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

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

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

TOYOPEARL RESINS FOR HYDROPHOBIC INTERACTION CHROMATOGRAPHY

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.

HOW DOES HIC WORK?

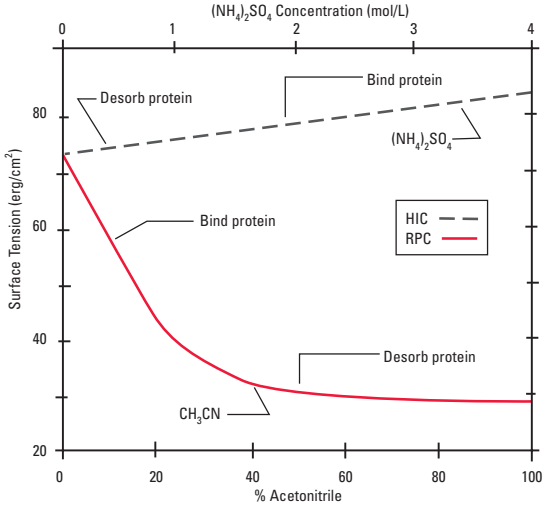
Proteins and other molecules with hydrophobic surfaces are attracted to the hydrophobic ligands of both reversed phase (RPC) and HIC resins. RPC resins have higher surface coverage and/or more hydrophobic ligand compared to HIC resins. Because of this, in a RPC separation the target binding readily occurs 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/cm^2)
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

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

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

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 (polypropyleneglycol), Phenyl, Butyl, and Hexyl (Figure 2).

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 and II show 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 irreversably 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.

FIGURE 2

HIC LIGAND CANDIDATES

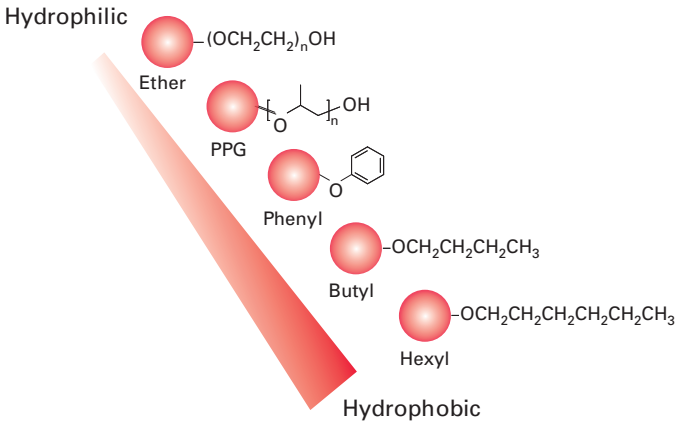


TABLE I

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.*

TABLE II

RECOVERY OF ENZYMATIC ACTIVITY OF PROTEINS

TOYOPEARL HIC resin	Protein	% Activity recovery
Phenyl-650	Phytochrome	79
Butyl-650	Halophilic protease	85
Butyl-650	Poly (3-hydroxybutyrate) depolymerase	88
Butyl-650	Aculeacin-A acylase	82
Butyl-650	Opine dehydrogenase	81

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

The retention and selectivity of protein standards on TOYOPEARL HIC resins using the ToyoScreen process development columns are shown in Figure 3.

INFLUENCE OF SALT TYPE

In addition to the hydrophobicity of the ligand, the selectivity in HIC is influenced by the eluent salt type. Figure 4 demonstrates the effect of salt type on the resolution factor of different protein pairs.

The Hofmeister lyotropic salt series shown in Figure 5 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. Figure 5 indicates that different salt systems may generate a variety of adsorption and desorption selectivities for each resin. This feature of HIC provides an additional parameter for the optimization of a process step.

FIGURE 5

HOFMEISTER LYOTROPIC SALT SERIES

For anions

SO_4^{2-} > HPO_4^{2-} > CH_3COO^- > halide > NO_3^- > ClO_4^- > SCN^-

For cations

$(CH_3)_4N^+$ > NH_4^+ > K^+ > Na^+ > Cs^+ > Li^+ > Mg^{2+} > Ca^{2+} > Ba^{2+}

Ammonium sulfate and sodium sulfate are the most commonly used salts in HIC. NaCl is often used as well.

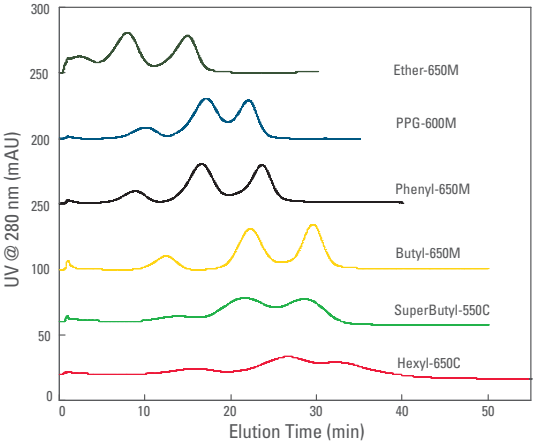
FIGURE 6

METHACRYLIC BASE BEADS AVAILABLE FOR HIC

Pore size (nm)	5	12,5	40-50	75	100	>100	>170
Product name							
TOYOPEARL HW-type	40	50	55	60	65	75	80
TSKgel PW-type	G1000	G2000	G4000	-	G5000	G6000	-

FIGURE 3

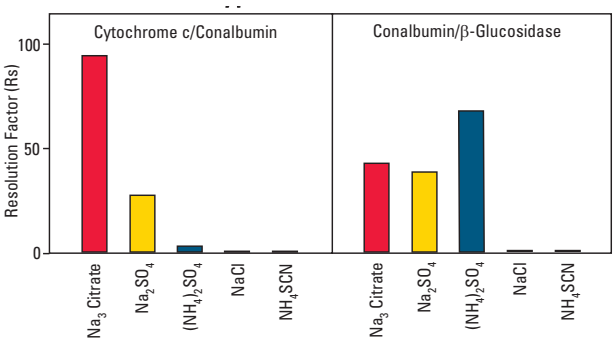
SCREENING OF TOYOPEARL HIC RESINS - STANDARD PROTEINS



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 4

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)

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

PARTICLE SIZE OPTIMIZATION

TOYOPEARL and TSKgel PW-type methacrylic base beads incorporate the same polymer chemistry (see Figure 6 or refer to SEC section for a more detailed discussion) and are available in a variety of particle sizes:

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

In many cases analytical columns are available packed with 10 micron particles having the same selectivity as the process media. Figure 7 shows the variety of ligands and particle sizes available for HIC process-scale applications. This figure also depicts where each particle size is used in a chromatographic manufacturing train (i.e. for capture, intermediate purification, or polishing).

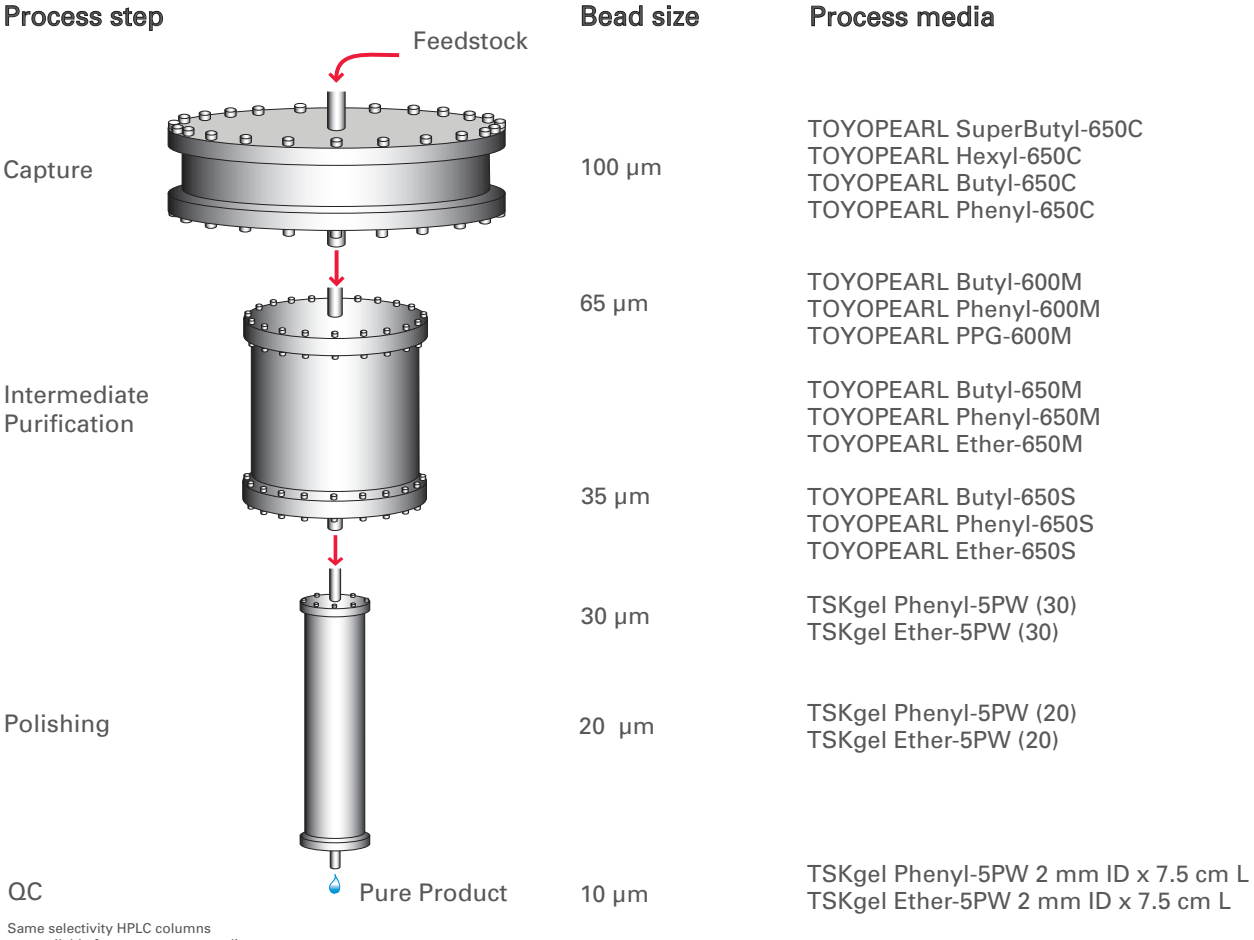
MECHANICAL STABILITY

The semi-rigid polymeric backbone of TOYOPEARL and TSKgel HIC resins permits high flow rates for maximum throughput and productivity. TOYOPEARL resins may be operated up to 3 bar and TSKgel PW-type resins may be operated up to 20 bar. The pressure-flow characteristics for each particle size grade of TOYOPEARL Phenyl-650 resins are shown in Figure 8. The beads are compatible with both fixed bed and dynamic axial compression columns.

CHEMICAL STABILITY

The polymeric structure of these products also makes them resistant to a wide range of pH conditions and ionic strengths. In addition, the hydroxylated surface of the base bead reduces non-specific binding of proteins.

FIGURE 7



HYDROPHOBIC INTERACTION CHROMATOGRAPHY

PORE SIZE OPTIMIZATION

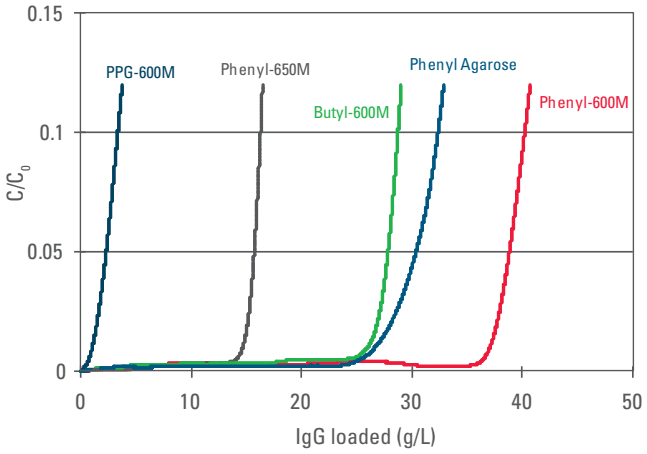
Most TOYOPEARL HIC products are derived from the versatile size exclusion resin, TOYOPEARL HW-65 (100 nm mean pore size), as the base bead for the majority of protein separations. But the pore size and accessible surface area of TOYOPEARL resins can be optimized for a given protein. More accessible surface 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.

HIGH DYNAMIC BINDING CAPACITY TOYOPEARL PROCESS RESINS

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

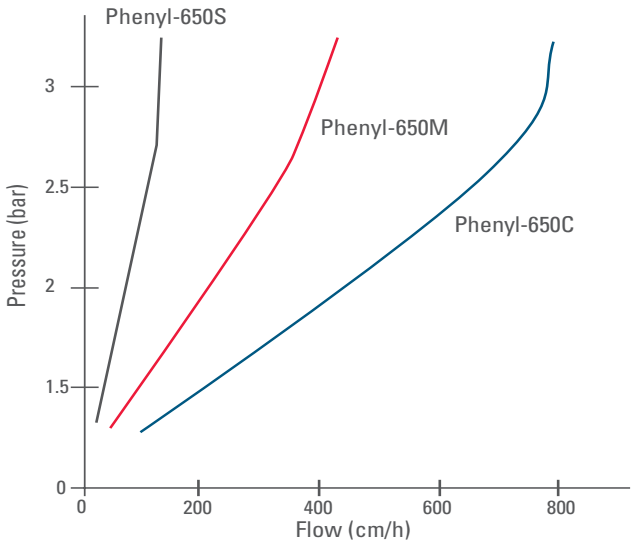
BREAKTHROUGH CURVES OF POLYCLONAL IgG ON VARIOUS HIC RESINS



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 8

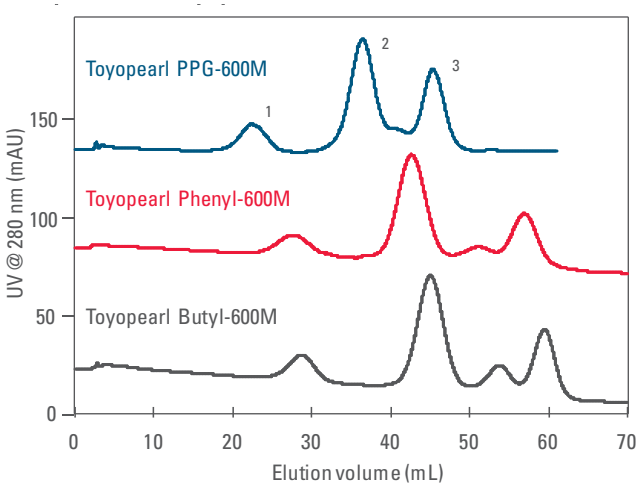
PRESSURE-FLOW CURVE FOR TOYOPEARL PHENYL-650 RESINS OF VARIOUS PARTICLE SIZE



Column: TOYOPEARL Phenyl-650C, M and S, 25 mm ID x 25 cm L
Mobile phase: 2 mol/L $(NH_4)_2SO_4$

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

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

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.

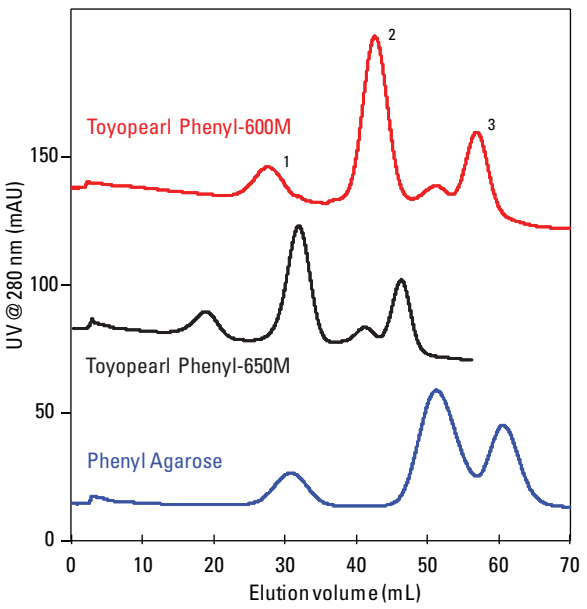
ToyoScreen PREPACKED COLUMNS FOR PROCESS DEVELOPMENT

ToyoScreen columns packed with the full range of our TOYOPEARL HIC products are available in 1 mL and 5 mL resin volumes. They provide a convenient way to screen different resins for both target retention and recovery. Multiple columns can be connected in series for additional separation. Please see the ordering information at the end of this section or contact us for more information on these products.

ToyoScreen 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, small-scale biochromatographic separations of protein samples by running up to eight individual columns simultaneously. ToyoScreen RoboColumns packed with the most popular TOYOPEARL HIC media are available with 200 µL and 600 µL resin volumes.

FIGURE 11

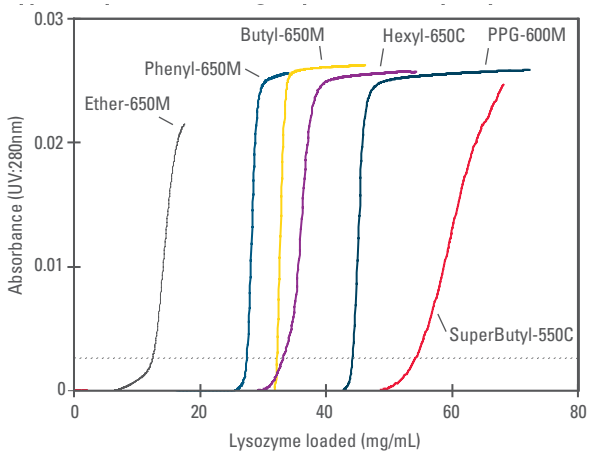
SELECTIVITY COMPARISON OF PHENYL-TYPE RESINS



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 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

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

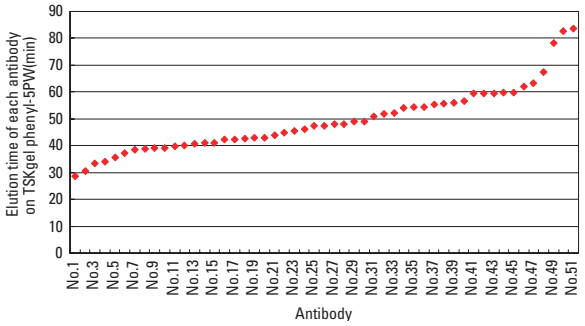
MONOCLONAL ANTIBODIES

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 (Figure 2, page 33) 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

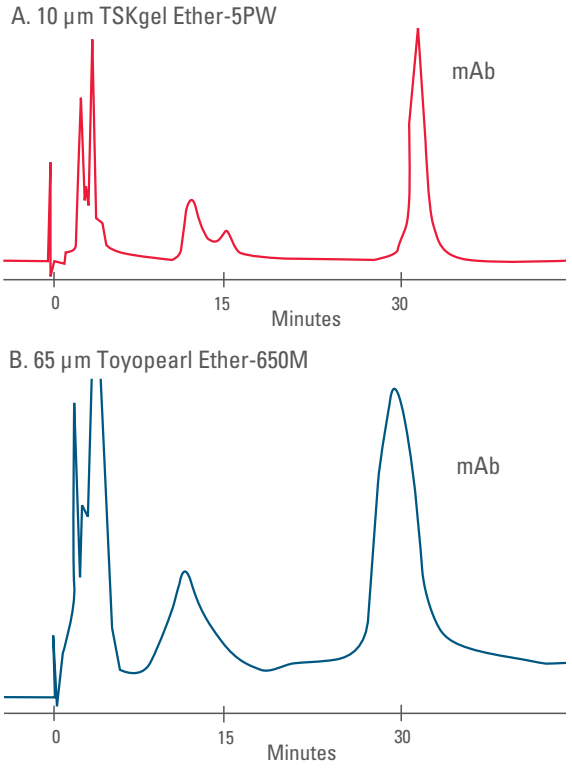
HYDROPHOBIC DIVERSITY OF MOUSE MONOCLONALS



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 antibodies

FIGURE 15

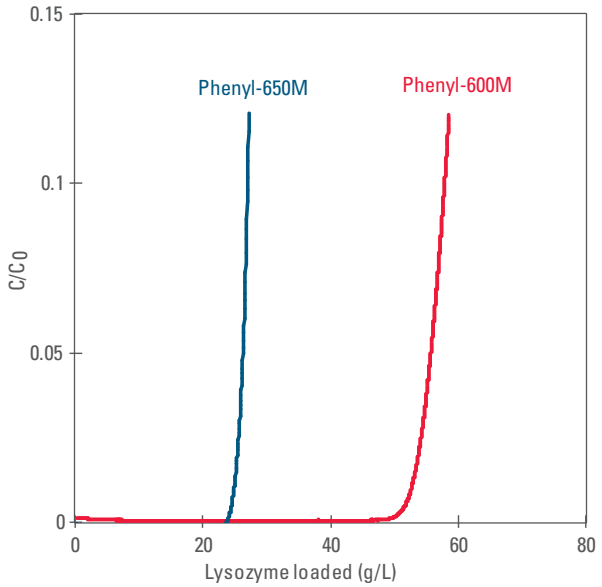
PURIFICATION OF mAbs FROM ASCITES FLUID



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

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 $(NH_4)_2SO_4$
Linear velocity: 300 cm/h; Detector: UV @ 280 nm

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

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.

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

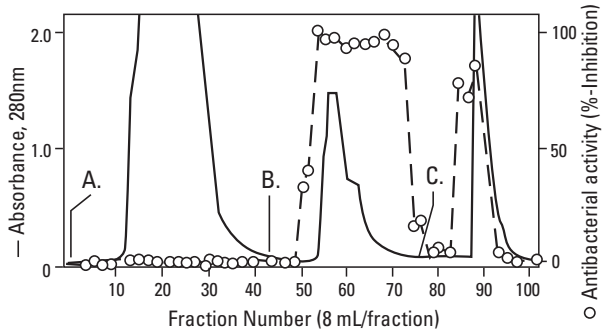
Because misfolded proteins will generally be more hydrophobic than the native protein, TOYOPEARL Butyl-650M resin is used frequently for the removal of misfolded proteins. In many cases flow through chromatography can be accomplished under eluent conditions binding the misfolds while allowing the target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

Go to www.separations.eu.tosohbioscience.com, 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.

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

ORDERING INFORMATION

ToyoScreen RoboColumns COLUMNS FOR HIC:

PART #	PRODUCT DESCRIPTION	PACKAGE
0045031	ToyoScreen RoboColumn Phenyl-600M, 200 µL	0.2 mL x 8 columns
0045032	ToyoScreen RoboColumn Phenyl-600M, 600 µL	0.6 mL x 8 columns
0045033	ToyoScreen RoboColumn Butyl-600M, 200 µL	0.2 mL x 8 columns
0045034	ToyoScreen RoboColumn Butyl-600M, 600 µL	0.6 mL x 8 columns
0045035	ToyoScreen RoboColumn PPG-600M, 200 µL	0.2 mL x 8 columns
0045036	ToyoScreen RoboColumn PPG-600M, 600 µL	0.6 mL x 8 columns
0045037	ToyoScreen RoboColumn Phenyl-650M, 200 µL	0.2 mL x 8 columns
0045038	ToyoScreen RoboColumn Phenyl-650M, 600 µL	0.6 mL x 8 columns

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

ORDERING INFORMATION

ToyoScreen PROCESS DEVELOPMENT COLUMNS FOR HIC

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

ToyoScreen COLUMN ACCESSORIES

PART #	PRODUCT DESCRIPTION
0021400	ToyoScreen Column Holder

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

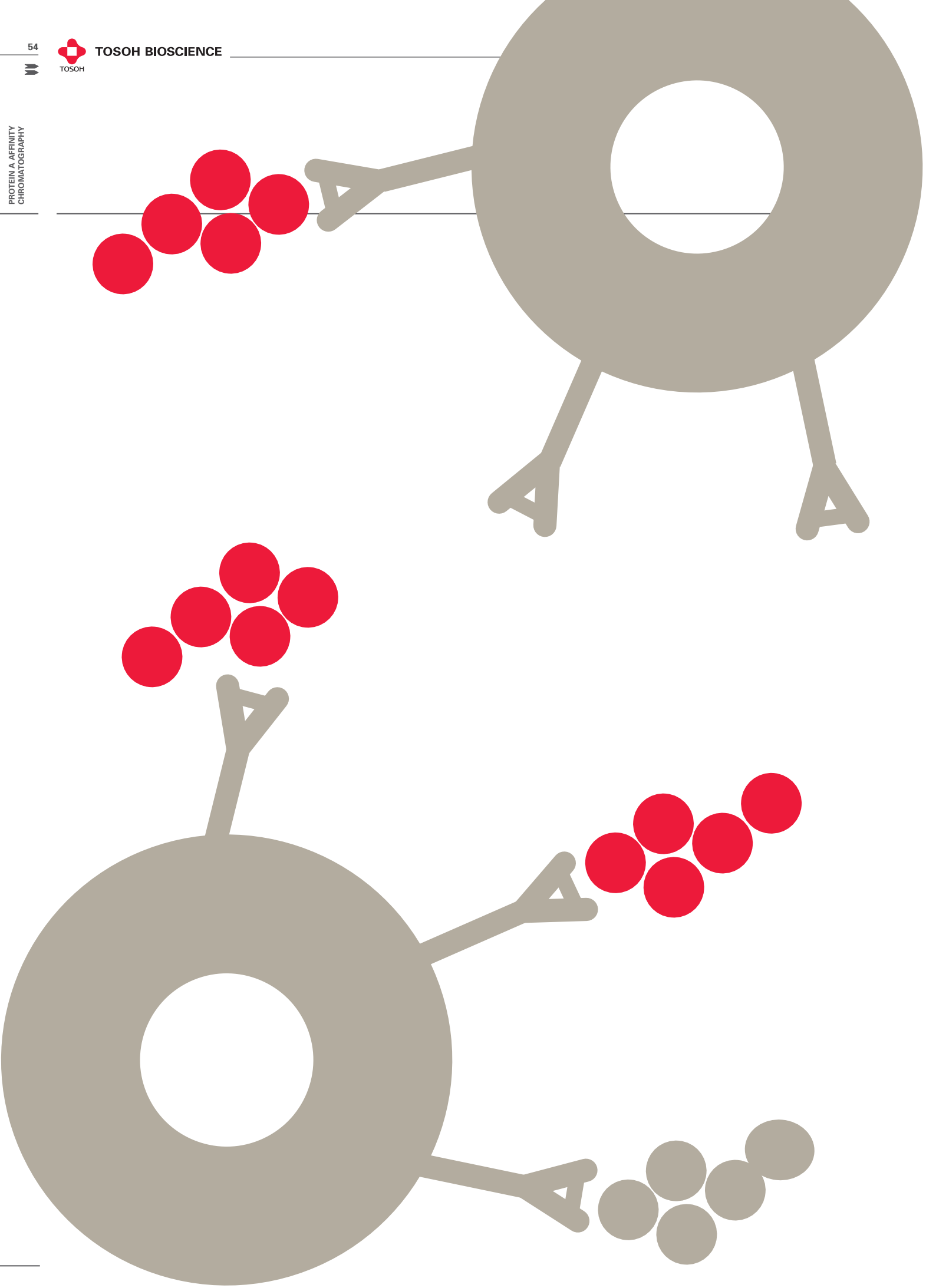
TOYOPEARL HIC RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	TYPICAL CAPACITY (g LYSOZYME/L RESIN)
0043151	TOYOPEARL Ether-650S	25	20-50	10-30
0016172		100		
0016174		1,000		
0016176		5,000		
0019805	TOYOPEARL Ether-650M	25	40-90	10-30
0016173		100		
0016175		1,000		
0016177		5,000		
0021301	TOYOPEARL PPG-600M	25	40-90	45-55
0021302		100		
0021303		1,000		
0021304		5,000		
0021305		50,000		
0021887	TOYOPEARL Phenyl-600M	25	40-90	45-65
0021888		100		
0021889		1,000		
0021890		5,000		
0021891		50,000		
0043152	TOYOPEARL Phenyl-650S	25	20-50	30-50
0014477		100		
0014784		1,000		
0014935		5,000		

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

ORDERING INFORMATION

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)	TYPICAL CAPACITY (g LYSOZYME/L RESIN)
0019818	TOYOPEARL Phenyl-650M	25	40-90	30-50
0014478		100		
0014783		1,000		
0014943		5,000		
0018364		50,000		
0043126	TOYOPEARL Phenyl-650C	25	50-150	30-50
0014479		100		
0014785		1,000		
0014944		5,000		
0043153	TOYOPEARL Butyl-650S	25	20-50	30-50
0007476		100		
0014701		1,000		
0007975		5,000		
0018826		50,000		
0019802	TOYOPEARL Butyl-650M	25	40-90	30-50
0007477		100		
0014702		1,000		
0007976		5,000		
0018355		50,000		
0043127	TOYOPEARL Butyl-650C	25	50-150	30-50
0007478		100		
0014703		1,000		
0007977		5,000		
0021448	TOYOPEARL Butyl-600M	25	40-90	40-60 (g/L γ-globulin)
0021449		100		
0021450		1,000		
0021451		5,000		
0021452		50,000		
0019955	TOYOPEARL SuperButyl-550C	25	50-150	52-70
0019956		100		
0019957		1,000		
0019958		5,000		
0019959		50,000		
0044465	TOYOPEARL Hexyl-650C	25	50-150	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		



AFC
PROTEIN A AFFINITY CHROMATOGRAPHY

PROTEIN A AFFINITY PRODUCTS

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

TOSOH FACT

TOYOPEARL AF-rProtein A HC-650F is an ultra-high capacity affinity resin designed for high throughput processing. It is the third generation of TOYOPEARL Protein A media. It is a rigid, alkaline resistant protein A affinity resin that offers the largest binding capacity for IgG of all base stable protein A media currently available on the market.

PROTEIN A AFFINITY CHROMATOGRAPHY

TOYOPEARL RESINS FOR PROTEIN A AFFINITY CHROMATOGRAPHY

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 or mixed-mode polishing steps in order to remove nucleic acids, aggregates and leached protein A.

The first protein A affinity resins were introduced in the 1970s based on native protein A ligands derived from the bacterium *Staphylococcus aureus*. 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.

Tosoh Bioscience offers two protein A affinity resins, both based on alkaline stable, recombinant ligands coupled to the proven TOYOPEARL polymethacrylate matrix. The new ultra-high capacity TOYOPEARL AF-rProtein A HC-650M excels all other commercially available protein A media with regard to its IgG binding capacity.

PROTEIN A CHROMATOGRAPHY – HOW DOES IT WORK

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 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.

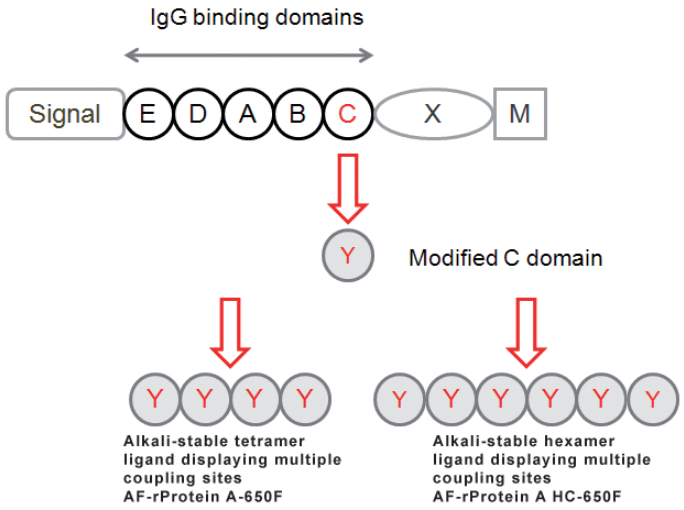
TOYOPEARL PROTEIN A RESINS

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 1).

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 also for a high resistance to alkaline solutions. Both resins are based on the TOYOPEARL HW-65F base bead with a particle size of 45 µm.

FIGURE 1

RECOMBINANT PROTEIN A DERIVED LIGANDS



FEATURES

- High IgG binding capacity
- Recombinant protein A ligand
- TOYOPEARL polymer matrix

BENEFITS

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

PROTEIN A AFFINITY CHROMATOGRAPHY

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

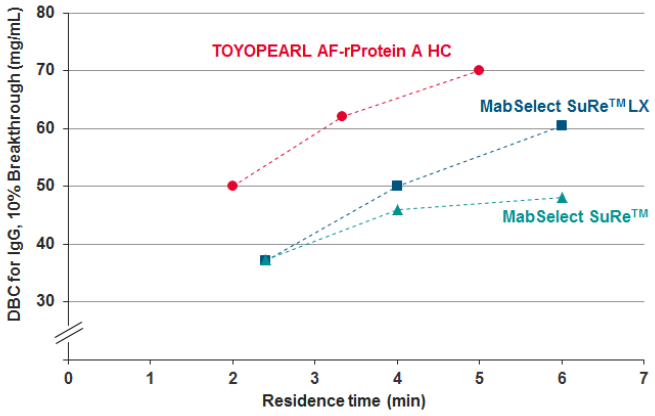
TOYOPEARL AF-rProtein A HC-650F is the newest affinity resin introduced by Tosoh Bioscience. It exhibits dynamic binding capacities of greater than 65 g/L at residence times of 5 minutes and greater than 50 g/L at 2 minutes residence time with feed stock concentrations from 1.0 g/L to 10.0 g/L (Figure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

Improved mass transfer characteristics allow it to maintain a larger percent of its capacity at lower residence times (Figure 3) relative to agarose based, caustic stable resins.

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 4). It maintains 80% of initial dynamic binding capacity after 40 CIP cycles with 0.5 mol/L NaOH (Figure 5).

FIGURE 3

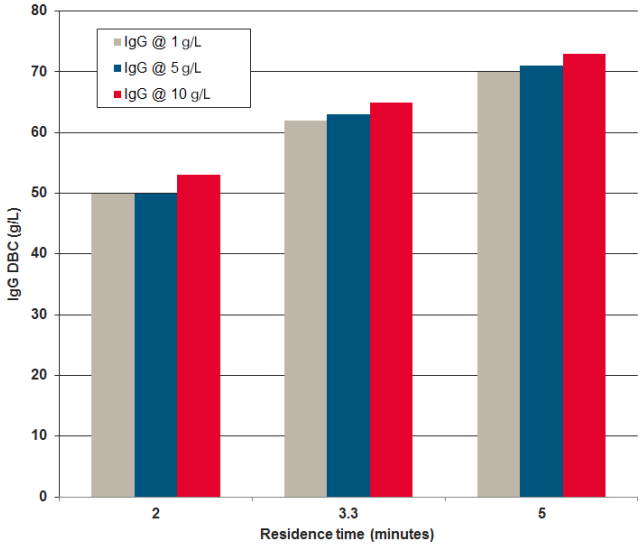
DBC OF HIGH CAPACITY PROTEIN A MEDIA



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 2

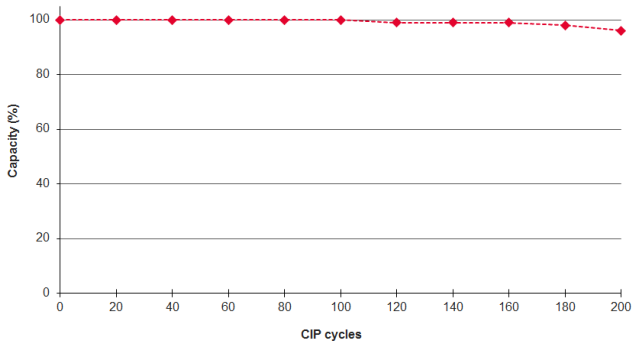
DBC AT VARIOUS LOADS AND RESIDENCE TIMES



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 4

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)

PROTEIN A AFFINITY CHROMATOGRAPHY

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 1).

Achievement of high linear velocities at relatively low pressure enables high throughput at production scale using equipment with moderate pressure limitations (Figure 6).

TABLE I

PROTEIN A LIGAND LEAKAGE

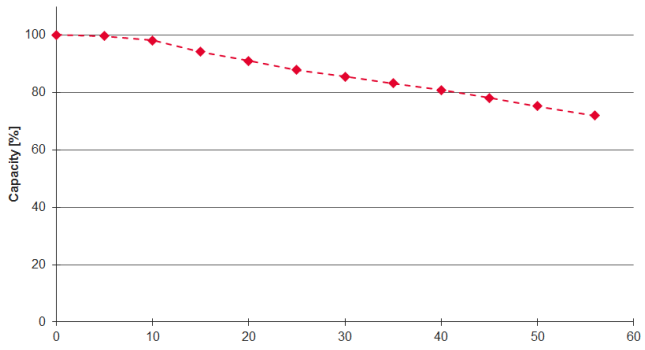
Amount of ligand leakage	Before CIP		After 200 CIP cycles	
	Elution Buffer		Elution Buffer	
	citrate (pH 3.0)	glycine-HCl (pH 3.0)	citrate (pH 3.0)	glycine-HCl (pH 3.0)
(ppm)	1.7	1.6	0.6	0.5

Amount of ligand leakage was determined with TOYOPEARL AF-rProtein A HC-650F ELISA

ppm=µg/g IgG

FIGURE 5

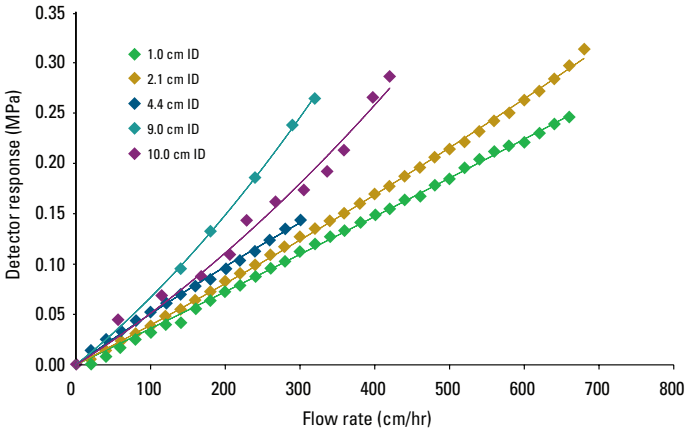
CIP STUDY WITH 0.5 M NaOH



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 6

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

PROTEIN A AFFINITY CHROMATOGRAPHY

TOYOPEARL AF-rPROTEIN A-650F

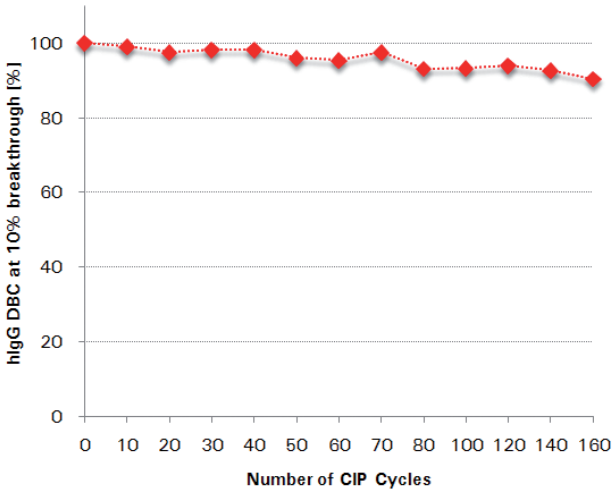
The standard TOYOPEARL AF-rProtein A-650F resin binds human and mouse immunoglobulin G, IgM, and Fab fragments. Typical static IgG binding capacity is > 45 mg/ml resin and typical dynamic IgG binding capacity at 10 % breakthrough is > 30 mg/mL resin at 2 minutes residence time (1 mg/mL protein load). Fast mass transfer kinetics support high binding capacities at high flow rates. IgG breakthrough curves (Figure 7) 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 8). 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 9 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 8

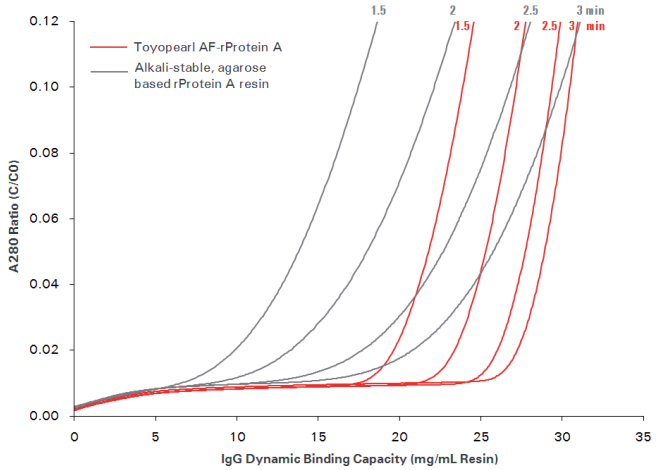
CLEANING-IN-PLACE STUDY WITH 0.1 M NaOH



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 binding buffer containing 0.1 mol/L NaOH, 16 min contact time
3 column volumes binding buffer pH 7.4

FIGURE 7

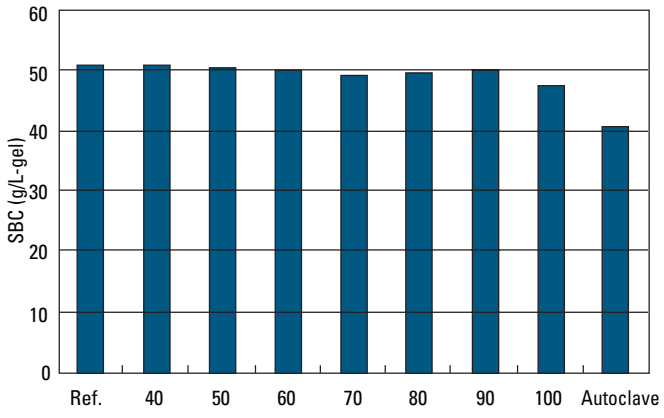
DYNAMIC BINDING CAPACITY



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; Sample conc.: 10 g/L; Residence time: 1.5, 2.0, 2.5, 3.0 min

FIGURE 9

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 is stable at 35 °C for least 3 years (data not shown)

PROTEIN A AFFINITY CHROMATOGRAPHY

PURIFICATION OF 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 pre-packed ToyoScreen columns or robotic high throughput screening devices with ToyoScreen RoboColumns.

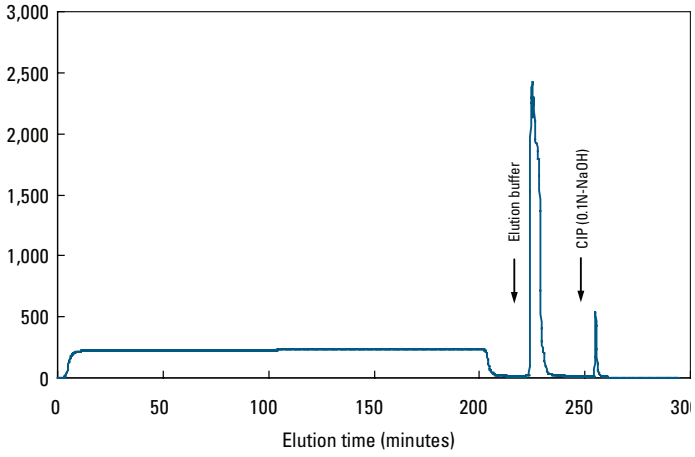
Suitable load/wash buffers are 20-100 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.2 - 7.5 or 100 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.2 - 7.5. Washing at reduced pH (e.g. pH 6) might further improve host cell protein reduction. Suitable elution buffers are 100 mmol/L citrate, 100 mmol/L acetate, 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 10) 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 11 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 even reached at 1 min. residence time.

FIGURE 10

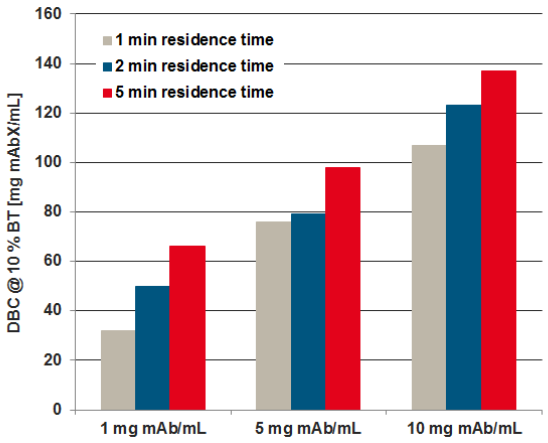
PURIFICATION OF MONOCLONAL ANTIBODY



Resin: TOYOPEARL Protein A; Column size: 5 mm ID × 5.0 cm L; Mobile phase: Buffer A: 20 mmol/L sodium phosphate containing 0.15 mol/L NaCl, pH 7.4, Buffer B: 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 IgG_1

FIGURE 11

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

ToyoScreen PREPACKED COLUMNS FOR PROCESS DEVELOPMENT

ToyoScreen columns packed with the TOYOPEARL AF-rProtein A resins are available in 1 mL and 5 mL resin volumes. ToyoScreen columns provide a convenient way to perform early resin screening for both target retention and recovery. Multiple columns can be connected in series for additional capacity. ToyoScreen RoboColumns are miniaturized chromatographic columns for operation with a robotic liquid handling system, such as the Freedom EVO® from TECAN. This approach allows automated highthroughput, small-scale biochromatographic separations of protein samples by running up to eight individual columns simultaneously. ToyoScreen RoboColumns packed with TOYOPEARL Protein A resins are available with 200 µL and 600 µL resin volumes.

PROTEIN A AFFINITY CHROMATOGRAPHY

ORDERING INFORMATION

ToyoScreen PROCESS DEVELOPMENT COLUMNS FOR AFC

PART #	PRODUCT DESCRIPTION	PACKAGE
0023430	ToyoScreen AF-rProtein A HC-650F	1 mL x 5 each
0023431	ToyoScreen AF-rProtein A HC-650F	5 mL x 1 each
0023432	ToyoScreen AF-rProtein A HC-650F	5 mL x 5 each
0022809	ToyoScreen AF-rProtein A-650F	1 mL x 5 each
0022810	ToyoScreen AF-rProtein A-650F	5 mL x 1 each
0022811	ToyoScreen AF-rProtein A-650F	5 mL x 5 each
0045061	ToyoScreen RoboColumn AF-rProtein A-650F	200 µL x 8 (each)
0045062	ToyoScreen RoboColumn AF-rProtein A-650F	600 µL x 8 (each)
0045063	ToyoScreen RoboColumn AF-rProtein A HC-650F	200 µL x 8 (each)
0045064	ToyoScreen RoboColumn AF-rProtein A HC-650F	600 µL x 8 (each)

ToyoScreen COLUMN ACCESSORIES

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

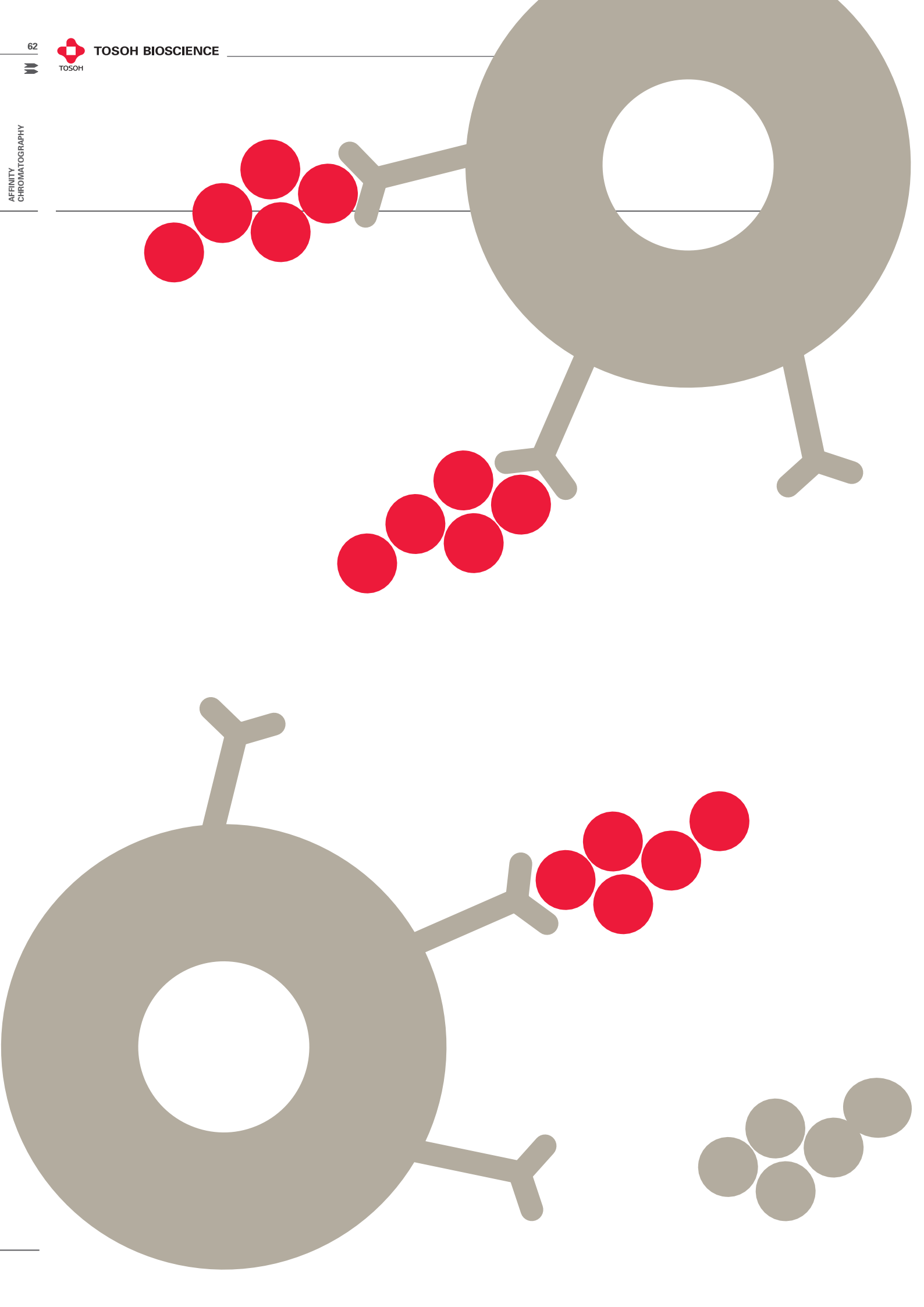
TOYOPEARL AFFINITY CHROMATOGRAPHY RESIN

GROUP SPECIFIC RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	TYPICAL CAPACITY
0023425	TOYOPEARL AF-rProtein A HC-650F NEW	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	

PROTEIN A IMMUNOASSAYS & STANDARDS

PART #	PRODUCT DESCRIPTION
0023433	Protein A-R40 ELISA Kit for TOYOPEARL AF-rProtein A HC-650F
0022815	Protein A-R28 ELISA Kit for TOYOPEARL AF-rProtein A-650F
0022836	Protein A-R28 STD 0.5 mL (10 mg/L) for TOYOPEARL AF-rProtein A-650F



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-Blue-650M
  - TOYOPEARL AF-Red-650M
  - TOYOPEARL AF-Heparin HC-650M

TOSOH FACT

Tosoh Bioscience GmbH offers workshops on chromatography in process development & production. These three day courses deliver a comprehensive background to bioprocess chromatographic purification as an integrated process technique. The workshops provide a balance of effective presentations and practical hands-on experience with process scale and methods development equipment under the guidance of qualified and knowledgeable Tosoh Bioscience technical experts. Every year more than 30 participants use this opportunity to broaden or refresh their chromatographic and downstream processing knowledge.

AFFINITY CHROMATOGRAPHY

TOYOPEARL AFFINITY RESINS FOR PROCESS SCALE CHROMATOGRAPHY

There are many custom designed affinity ligands available to the chromatographer. TOYOPEARL affinity chromatography resins are functionalized with chemically active groups or group-specific ligands. 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.

AFC – 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.

MECHANICAL & CHEMICAL STABILITY

TOYOPEARL resins remain dimensionally stable within wide extremes of pH and ionic strength. Moreover, the semi-rigid TOYOPEARL particles do not distort under flow rates that generate up to 3 bar pressure. 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 chromato-graphy using equipment with moderate pressure limitations. Sanitization or cleaning may be conducted with up to 0.5 mol/L NaOH or 0.5 mol/L HCl depending upon the ligand. In affinity chromatography, in particular, the choice of cleaning agent will be largely dependent upon the chemical stability of the ligand, rather than that of the base resin.

TABLE I

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

FEATURES

- Active, reactive and group specific resins
- Standard 100 nm pore size
- Porous, hydrophilic polymer matrix
- High mechanical stability

BENEFITS

- Broad range of applications
- High capacity for large biopolymers
- Suitable for laboratory and process scale purifications
- Constant bed volume over a wide range of buffer composition

AFFINITY CHROMATOGRAPHY

TOYOPEARL RESINS FOR AFFINITY LIGAND COUPLING

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 activated and reactive ligands are shown in Figure 1.

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

ACTIVATED TOYOPEARL AFFINITY RESINS

Toyopearl AF-Tresyl-650M

HW-65 — O-R-O-SO_2-CH_2-CF_3

Ligand Density: 80 µmol/g (dry)

Toyopearl AF-Epoxy-650M

HW-65 — O-R-O-CH_2-CH-CH_2 (O)

Ligand Density: 800 µmol/g (dry)

REACTIVE TOYOPEARL AFFINITY RESINS

Toyopearl AF-Formyl-650M

HW-65 — O-R-O-CH_2-CHO

Ligand Density: 60 meq/L

Toyopearl AF-Amino-650M

HW-65 — O-R-O-CH_2-CH(OH)-CH_2-NH_2

Ligand Density: 100 mmol/L

Toyopearl AF-Carboxy-650M

HW-65 — O-R-O-CH_2COOH

Ligand Density: 100 meq/L

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

ACTIVATED RESINS – READY FOR DIRECT LIGAND ATTACHMENT

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 2).

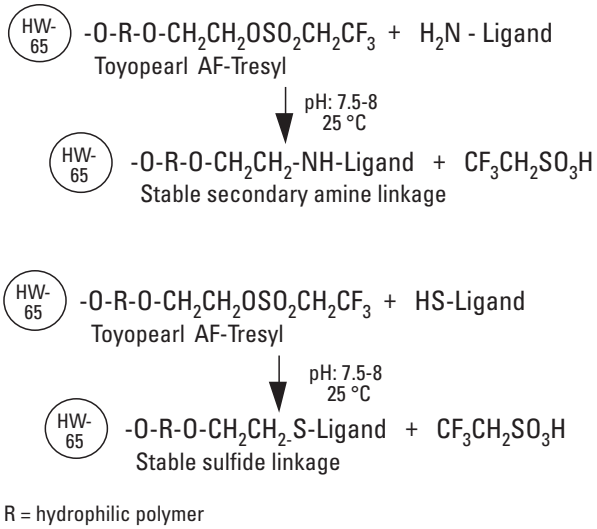
Under such conditions, even proteins of limited stability may be successfully coupled. Coupling leads to the formation of a highly stable secondary amine or thio-ether linkage. The optimized tresyl-density (ca. 20 µmol/mL hydrated resin) is sufficient to provide substantial protein binding while avoiding 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 3). 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 2

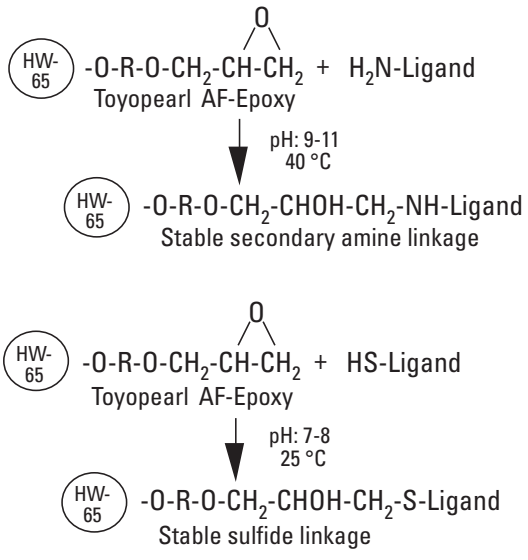
TOYOPEARL AF-TRESYL COUPLING PROCEDURE



R = hydrophilic polymer

FIGURE 3

TOYOPEARL AF-EPOXY COUPLING PROCEDURE



R = hydrophilic

AFFINITY CHROMATOGRAPHY

REACTIVE RESINS - REQUIRE ACTIVATION FOR LIGAND 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 4). 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.

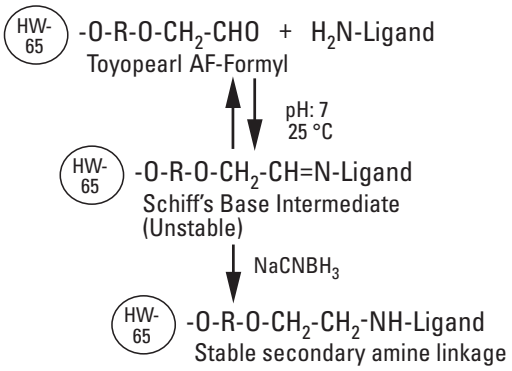
TOYOPEARL AF-Amino-650M resin may be used to couple ligands using their carboxyl groups (peptide bond formation) or aldehyde groups (reductive amination) as shown in Figure 5. Aldehyde groups may be present in a carbohydrate or glycoprotein ligand or may be introduced into the ligand by mild, periodate 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 6). Representative coupling densities are given in Table II.

FIGURE 4

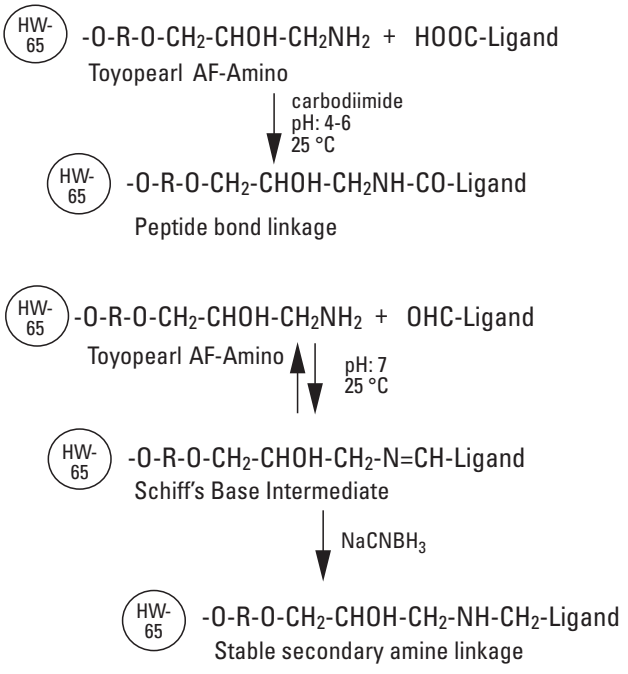
TOYOPEARL AF-FORMYL COUPLING PROCEDURE



R=hydrophilic polymer

FIGURE 5

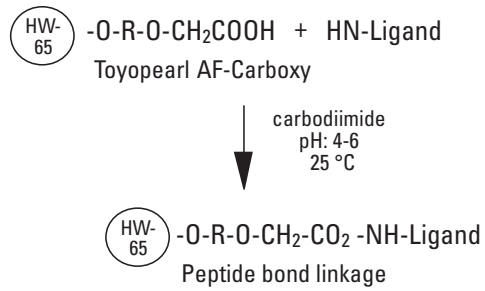
TOYOPEARL AF-AMINO COUPLING PROCEDURE



R = hydrophilic

FIGURE 6

TOYOPEARL AF-CARBOXY COUPLING PROCEDURE



R = hydrophilic polymer

AFFINITY CHROMATOGRAPHY

TOYOPEARL RESINS WITH GROUP SPECIFIC LIGANDS

The structures of TOYOPEARL group specific ligands are shown in Figure 7.

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-Blue HC-650M

Functionalized with Cibachron Blue F3G-A, TOYOPEARL AF-Blue HC-650M resin has excellent capacity for proteins, particularly albumin (Figure 8). In addition, this high capacity resin has improved caustic stability, reduced dye ligand leakage, and superior pressure-flow characteristics relative to more traditional agarose materials (Figure 9).

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.

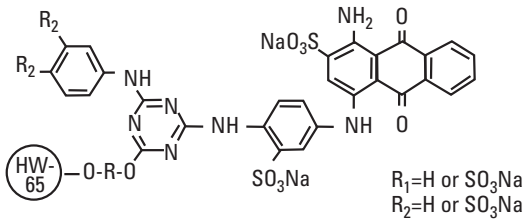
TOYOPEARL AF-Heparin HC-650M

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.

FIGURE 7

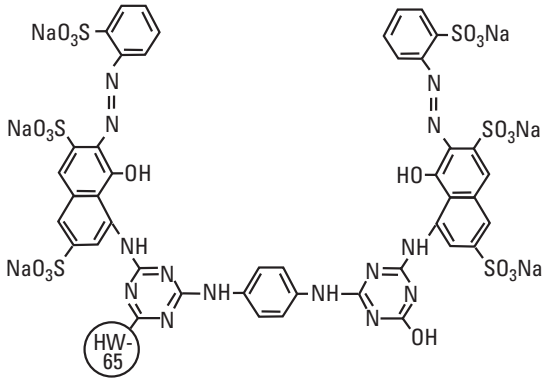
GROUP-SPECIFIC TOYOPEARL AFFINITY RESIN

Toyopearl AF-Blue HC

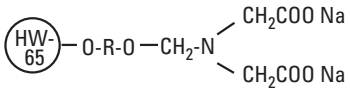


Ligand Density: 15 mmol/L

Toyopearl AF-Red

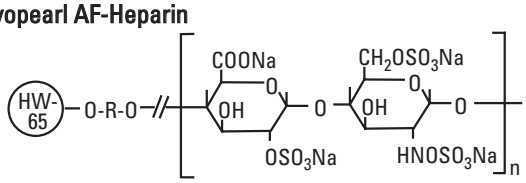


Toyopearl AF-Chelate



Ligand Density: 20 mmol/L

Toyopearl AF-Heparin



Approximate Ligand Density: 5 g/L

AFFINITY CHROMATOGRAPHY

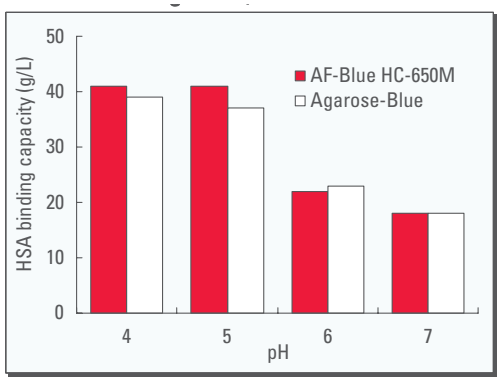
TABLE III

REPRESENTATIVE BINDING CAPACITIES FOR TOYOPEARL DYE-LIGAND AFFINITY MEDIA

Protein (mg/mL res±in)	AF-Blue HC-650M	AF-Red-650ML
Hexokinase	3	-
Bovine serum albumin	16	-
Human serum albumin	18 ±2.5	3.5 ±1
Lactate dehydrogenase	27	11

FIGURE 8

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

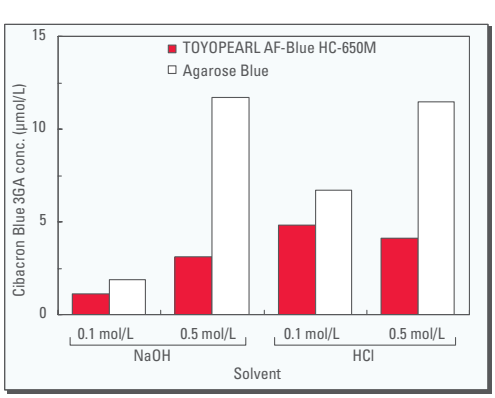


Conditions

- A 1.0 mL of adsorbent was washed with 10 mL of equilibration buffer (pH 4.0 and 5.0; 0.1 mol/L sodium acetate buffer, pH 6.0 and 7.0; 0.1 mol/L sodium phosphate buffer).
- A 5.0 mL of 1 % solution of human albumin desolved in each equilibration buffer was charged onto the column.
- After 10 min, unbound albumin was eluted and the column was washed with 10 mL of each equilibration buffer.
- Adsorbed human albumin was eluted with 0.1 mol/L sodium phosphate buffer at pH 7.0 containing 2.0 mol/L sodium chloride (desorption buffer) and 10 mL fractions were collected.
- Human albumin content was measured spectrophotometrically by using E 0.1 % at 280 nm = 0.55.

FIGURE 9

COMPARATIVE DYE LEAKAGE STUDY OF AF-BLUE HC-650M AND COMPETITOR BLUE @ 25 °C (AFTER 24 HOURS)



Conditions:

200 mg of each material was suspended in 4 mL of solvent and incubated at 25 °C and shaking for 24 h. The absorption at 620 nm of the supernatants were measured after appropriate adjustment to neutrality with known volume of acid or base. Dye concentrations were estimated assuming a molar extinction coefficient of 12,750 (L/M cm).

AFFINITY CHROMATOGRAPHY

ORDERING INFORMATION

ToyoScreen PROCESS DEVELOPMENT COLUMNS FOR AFC

PART #	PRODUCT DESCRIPTION	PACKAGE
0021384	ToyoScreen AF-Chelate-650M	1 mL x 6 each
0021385	ToyoScreen AF-Chelate-650M	5 mL x 6 each
0021386	ToyoScreen AF-Blue HC-650M	1 mL x 6 each
0021387	ToyoScreen AF-Blue HC-650M	5 mL x 6 each
0021388	ToyoScreen AF-Red-650ML	1 mL x 6 each
0021389	ToyoScreen AF-Red-650ML	5 mL x 6 each
0021390	ToyoScreen AF-Heparin HC-650M	1 mL x 6 each
0021391	ToyoScreen AF-Heparin HC-650M	5 mL x 6 each

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 RESIN

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

AFFINITY CHROMATOGRAPHY

ORDERING INFORMATION

TOYOPEARL AFFINITY CHROMATOGRAPHY RESIN

GROUP SPECIFIC RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	TYPICAL LIGAND DENSITY	TYPICAL CAPACITY
0019688	TOYOPEARL AF-Blue HC-650M	25	15 mmol/L	minimum 18 g/L
0019689		100		
0019690		1,000		
0019691		5,000		
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		

REACTIVE RESINS

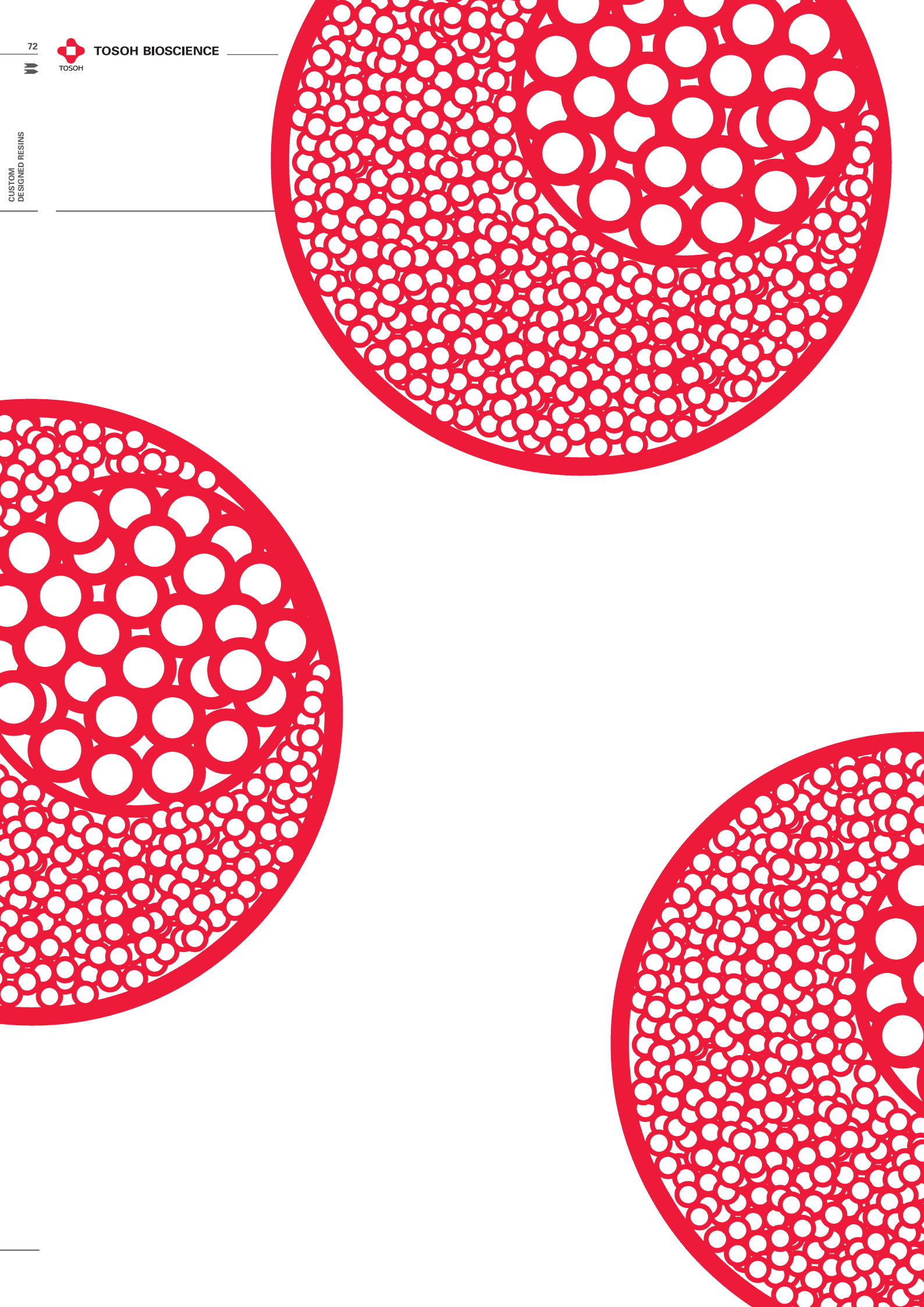
PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	TYPICAL LIGAND DENSITY	TYPICAL CAPACITY
0043411	TOYOPEARL AF-Amino-650M	10	70-130 meq/L	–
0008002		25		
0008039		100		
0018074		1,000		
0018316		5,000		
0043412	TOYOPEARL AF-Carboxy-650M	10	80-120 meq/L	–
0008006		25		
0008041		100		
0018827		1,000		
0018828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

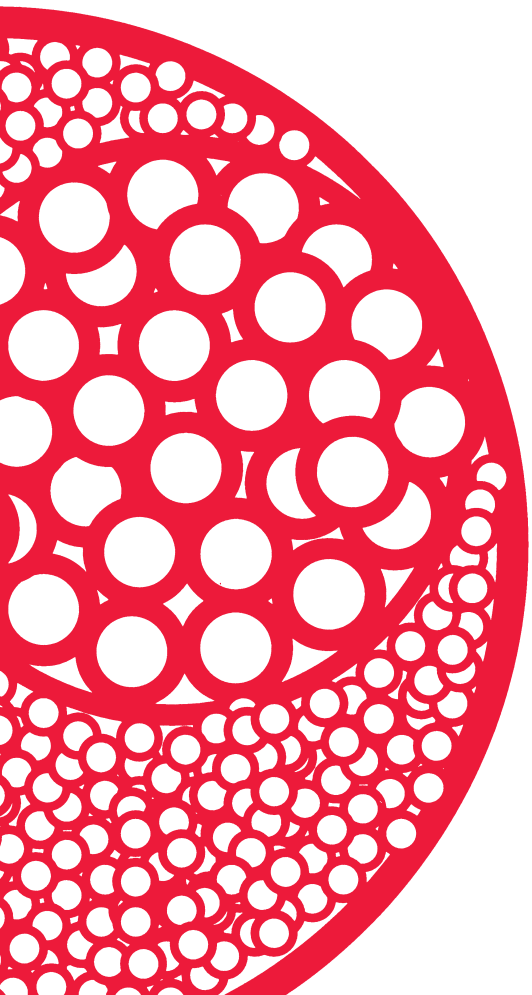
Conditions: All TOYOPEARL affinity resins are provided at a particle size of 65 µm. This particle size is ideal for both small and large scale separations.

*1 g yields approximately 3.5 mL of hydrated resin.



CUSTOM DESIGNED RESINS

CUSTOM DESIGNED RESINS



TOSOH FACT

Tosoh Bioscience provides solutions for today's biological purification needs. In fact, some of the first commercial HIC products were manufactured by Tosoh. We take pride in our ability to design new products based on existing chemistries to solve specific customer applications.

We encourage you to have a confidential discussion with us about your specific needs. Whether it is a surface modification of an existing product or the creation of a new one, we encourage you to call on us to meet your needs for a customized solution.

CUSTOM DESIGNED RESINS

OVERVIEW

Occasionally special purifcation needs require special adaptions to existing chromatographic resins for optimized operation of your process. This section explains in general terms Tosoh's custom resin development procedure.

TOSOH'S RESIN INNOVATION PROGRAM (TRIP)

Tosoh Corporation of Japan has introduced a new resin innovation program. Ideal candidates for this program are drug targets that have been selected for clinical trials which, if successful in the clinic, will be commercially manufactured. This program is not available for early stage laboratory research or the isolation of small amounts of new therapeutic proteins.

NEW PROJECT APPROVAL FOR TRIP

To receive a "Go" status for TRIP some evaluation is needed. During preliminary discussions, prior to the generation of scouting samples, an assessment is made of whether the project fits into Tosoh's scaffolding capabilities. If a fit is established, the projected liter volume for the new resin is reviewed. Decisions are based on the quantity projected for use after therapeutic approval and launch, but for high value added resins, projected commercial liter volumes of less than 100 liters have been approved.

NEW RESIN DEVELOPMENT TIME LINE

Tosoh Bioscience and Tosoh Corporation of Japan have the resources to develop custom resin products. The custom resin optimization timeline closely mirrors a customer's need to produce products for clinical trials. Only with detailed sample evaluation data from the customer, chemistry optimization and manufacturing lot release criteria can be established by Tosoh so a close cooperation between the customer and Tosoh is crucial.

TABLE 1

TRADITIONAL CHROMATOGRAPHIC LIGANDS

Anion Exchangers	Diethylaminoethyl (DEAE) Quaternary Anion Exchanger (QAE)
Cation Exchangers	Sulfopropyl (SP) Carboxymethyl (CM) Sulfonic Acid (S)
Hydrophobic Interaction	Hexyl Butyl Phenyl Polypropylene glycol (PPG) Ether

TABLE 2

TOSOH METHACRYLIC BASE BEADS USED FOR SEC

Pore size (nm)	5	12.5	40-50	75	100	>100	>170
Product name TOYOPEARL HW-Type	40	50	55	60	65	75	80
TSKgel PW-Type	G1000	G2000	G4000	G5000		G6000	

TABLE 3

AVAILABLE PARTICLE AND PORE SIZE COMBINATIONS

Pore Diameter (nm)	Particle Size (microns) 200	100	75	65	35	30	20	15
>170		☆						
>120	☆	☆						
100		☆		☆	☆	☆	☆	☆
75		☆	☆	☆				
40-50	☆	☆		☆	☆	☆	☆	☆
12.5				☆	☆			
5	☆	☆		☆	☆			

☆ commercial ☆ experimental not requested

7 x 8 = 56 possible combinations

CUSTOM DESIGNED RESINS

RESIN OPTIMIZATION (SEMI-CUSTOM COMMERCIAL RESINS)

In many cases the optimization of an already commercial resin can be accomplished by a more judicious selection of particle size, pore size, and currently available ligands (Table I). See the various combinations of these physical parameters in the Toyopearl SEC section of this catalog. When these products are combined with the comparable polymer chemistry TSKgel PW products (Table II) , there are 49 different combinations (Table III) to act as a scaffold for an improved resin product. All kinds of modes can be optimized.

CUSTOM RESINS

In more difficult situations custom resins can be developed for a customer. Ligand screening, selection, density, length of spacer arm, degree of crosslinking and degree of grafting all add to the fundamental scaffold of the semi-custom resins. The objective in designing a certain resin is to develop a bead that has maximum accessible surface area with an optimized ligand density using an appropriate spacer arm which fits the conformational attachment requirements of the target. These parameters can have a dramatic effect on the resin's chromatographic and economic performance.

GETTING STARTED

To initiate the TRIP program contact your local Business Development Manager. A period of due diligence will be started where the appropriate information can be exchanged in confidence between us. Detailed communications on these topics requires an in effect two way Confidential Information Disclosure Agreement between Tosoh Bioscience GmbH and the customer. If the project looks promising to both parties several laboratory scouting samples will be prepared and sent to the customer site for evaluation.

COMMERCIALIZATION

Once a "Go" decision is made, the performance specifications are determined from the optimized scouting samples, the custom resin enters into Tosoh's "ISO 9001" product commercialization process. Several small manufacturing batches are then made to set lot release criteria. Regulatory support studies are initiated at this time.

BEST OF ITS KIND

YOUR SPECIALIST IN SEPARATION

DISCOVER THE HIGHEST CAPACITY PROTEIN A RESIN ON THE MARKET

- SUPERIOR DYNAMIC BINDING CAPACITY FOR HUMAN IgG (≥ 70 g/L)
- CAPACITY INCREASES WITH FEEDSTOCK TITERS UP TO 10 g/L
- ALKALINE STABILITY THROUGH ENGINEERED RECOMBINANT LIGAND
- MINIMIZED LEACHING THROUGH MULTI-POINT ATTACHMENT

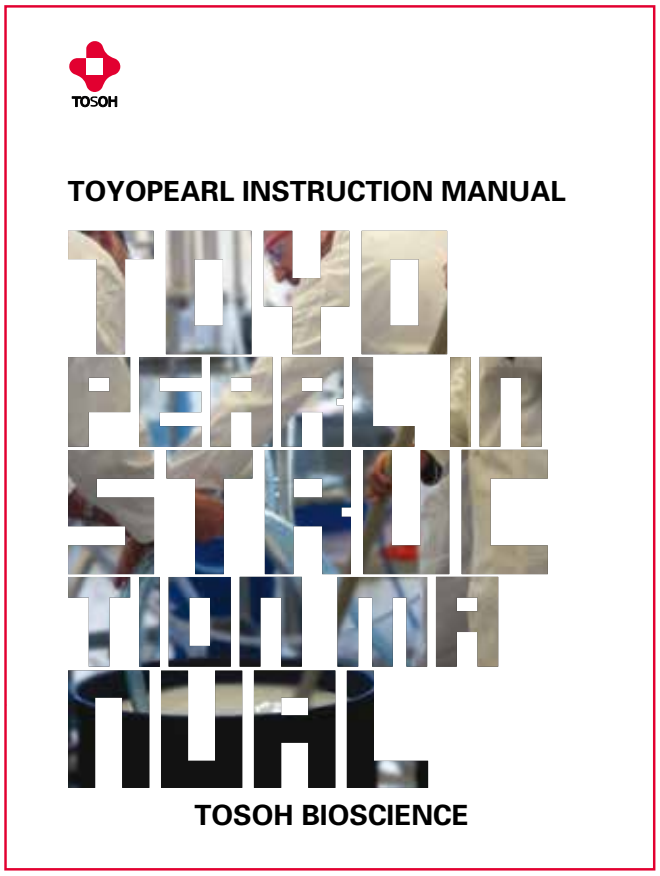
TOYOPEARL AF-rPROTEIN A HC-650F IS AN ULTRA-HIGH CAPACITY PROTEIN A AFFINITY RESIN DESIGNED FOR HIGH THROUGHPUT PROCESSING OF HIGH TITER FEEDSTOCK THAT CAN CONSIDERABLY IMPROVE PROCESS ECONOMICS. FIND OUT MORE ON WWW.TOSOHBIOSCIENCE.DE!

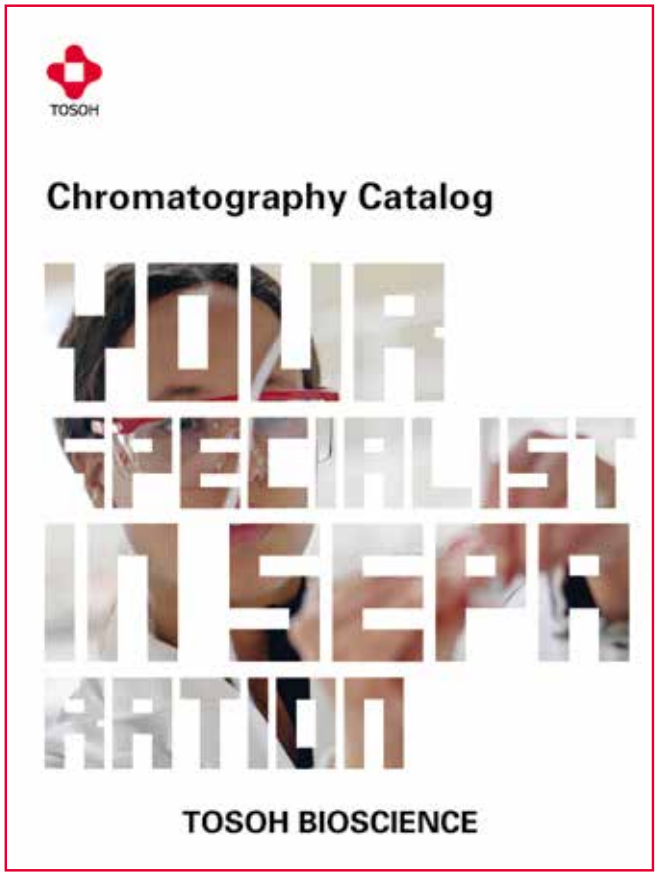
TOSOH BIOSCIENCE

FURTHER INFORMATION

For detailed Toyopearl packing instructions, request our **TOYOPEARL Instruction Manual**.

To get an overview about the whole range of TSKgel columns and small TOYOPEARL and TSKgel bulk media, please request our **Chromatography catalog**.





For a deeper insight into applications and all questions related to the practical use of TSKgel and TOYOPEARL, check out the website **www.tosohbioscience.de** and related catalogs or instruction manuals.

Our technical experts are happy to discuss your specific separation needs by phone: **+49 (0)711 13257-57** or **techsupport.tbg@tosoh.com**

TOSOH BIOSCIENCE

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