TOSOH

Chromatography Catalog

TOSOH BIOSCIENCE


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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, TSK-GEL 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 TSK-GEL 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	TOSOH CORPORATION ANNOUNCES THAT ALL TOSOH AFFILIATED SCIENTIFIC AND DIAGNOSTIC SYSTEM RELATED COMPANIES IN EUROPE, WILL BE UNIFIED UNDER THE NEW NAME TOSOH BIOSCIENCE.
2008	ECOSEC , THE 7TH GENERATION GPC SYSTEM IS INTRODUCED GLOBALLY

TOSOH BIOSCIENCE

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Introduction

Tosoh Bioscience is a major supplier of liquid chromatography products worldwide, particularly to the pharmaceutical and biotechnology industries. The company distributes and supports products manufactured by our parent company, Tosoh Corporation, which has offices and manufacturing facilities in Japan. Located in Stuttgart, Germany, Tosoh Bioscience GmbH provides sales and service to customers in Europe, the Middle East, South Asia and Africa.

This Chromatography Products Catalog describes analytical and semi-preparative TSK-GEL® prepacked columns for each of the major chromatography modes. It also gives a short overview of TOYOPEARL® and TSK-GEL bulk resins for laboratory scale purifications, as well as ToyoScreen process development columns.

TSK-GEL and Toyopearl products are used for the analysis, isolation and purification of proteins, peptides, DNA, oligonucleotides, antibiotics and other small molecular weight compounds. Our products are known for their ability to withstand high pressure, their robust chemical stability, and their superior performance for the recovery, concentration and purification of biomolecules.

What's new

TSK-GEL ODS-100V and ODS-100Z RPC Columns.

These new reversed phase columns incorporate unique bonding chemistry coupled with optimized endcapping, providing a universal solution for basic compounds, acidic compounds, polar compounds, and metabolites.

TSK-GEL ODS-140HTP RPC Columns.

These new reversed phase columns are designed for high throughput analysis. The particle size of 2.3 µm offers separation efficiencies at considerable lower back pressure when compared to sub 2 µm particles. Therefore the column is suitable for fast HPLC runs using a UPLC, pseudo UPLC or conventional HPLC system.

TSK-GEL AMIDE-80 3 µm HILIC Columns.

These new HILIC columns expand the portfolio of the well established Amide-80 columns. With a smaller particle size of 3 µm they are ideally suited for LC-MS analysis of polar compounds. They offer superior separation efficiency at shorter run times when compared to the 5µm material.

TSK-GEL PWXL-CP SEC Columns.

These columns were specifically developed for the analysis of water soluble cationic polymers. They eliminate adsorption of the polymer to the particle surface by incorporating a cationic functionality, thus allowing elution under low salt conditions.

TSK-GEL STAT IEC Columns.

TSK-GEL STAT nonporous ion exchange columns designed for high efficiency separation of biomolecules and low molecular weight compounds provide superior performance at reduced analysis time.

Toyopearl GigaCap IEC Resins.

The Toyopearl GigaCap series of ion exchange resins is designed for high throughput purification of biopharmaceuticals by packed bed chromatography. They exhibit high dynamic binding capacities of both large and small molecules at high linear flow rates.

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Toyopearl Phenyl-600M HIC resin.

Toyopearl Phenyl-600M is the third product in the Toyopearl '600M' series of HIC resins, optimized for monoclonal antibodies. It complements Toyopearl PPG-600M and Butyl-600M.

TSK-GEL columns

TSK-GEL columns are known worldwide for their dependability and suitability for a variety of chromatographic applications. Applications using TSK-GEL columns are continuously published in the scientific journals and are listed in the current U.S. Pharmacopoeia (see Appendix C). Prepacked columns contain TSK-GEL media designed for the analysis of proteins, peptides, biopolymers and low molecular weight compounds by size exclusion, ion exchange, hydrophobic interaction, reversed phase, affinity and normal phase chromatography. The packings in the columns are either silica-based or polymeric-based material, in particle sizes ranging from 2µm to 20µm.

Columns are available in analytical to preparative sizes, in stainless steel, PEEK®, or glass. To ensure specified column performance and to maximize the longevity of your Tosoh columns, please note the guidelines found in Appendix A for the use, cleaning, rehydration, and storage of your TSK-GEL columns.

ToyoScreen Process Development columns

ToyoScreen Process Development columns are available as an additional tool for convenient scale-up. These are easy-to-use, pre-packed columns containing Tosoh Bioscience's most popular Toyopearl resins. They provide a convenient, low-cost product for the evaluation of Toyopearl ligand chemistries. ToyoScreen Process Development columns are available in packages of six by 1mL and six by 5mL volumes for affinity, ion exchange and hydrophobic interaction chromatography.

Toyopearl and TSK-GEL LabPak Media

LabPak products are small package sizes of Toyopearl and TSK-GEL bulk media products. Typically they contain three or four different ligand types offered for a particular chromatography mode. They are useful for developmental scientists who wish to familiarize themselves with resin physical properties in differing buffer systems. The larger resin amounts in LabPak products allow the packing of wider bore and longer columns than what is available in the ToyoScreen products. This helps the developmental scientist or engineer to measure more accurately, under actual packing conditions, the resin's dynamic binding capacity, and selectivity.

TSK-GEL resins

The same media used in TSK-GEL columns are also available in bulk. They are offered in particle sizes of 20µm and 30µm, for ion exchange and hydrophobic interaction chromatography. TSK-GEL media are the most efficient packing materials available from Tosoh Bioscience for process chromatography. In tandem with their high efficiency, high mechanical stability and permeability, TSK-GEL resins are an excellent choice under medium to high pressure conditions.

Toyopearl resins

Toyopearl resins are hydrophilic, macroporous, bulk bioprocessing media suitable for large-scale chromatographic applications. Because of their polymeric backbone structure, the rigid Toyopearl packings assure excellent pressure/flow characteristics. The media are stable over the pH range of 2-12 for normal operating conditions and pH 2-14 for cleaning conditions. The particle sizes are: 20-50 µm superfine grade for the highest performance, 40-90 µm medium grade for economical purification, and 50-150 µm coarse grade for capture chromatography. Large pore sizes ensure greater capacity for high molecular weight molecules, while allowing faster separation and recycling times. Toyopearl media are available for size exclusion, ion exchange, hydrophobic interaction, and affinity chromatography.

For predictable results in scale-up, Toyopearl resins are based on the same chemistry as the prepacked TSK-GEL columns. This allows the seamless scale-up of methods developed on TSK-GEL columns to Toyopearl, without additional optimization at pilot scale. In addition, Toyopearl resins are also available in the ToyoScreen Process Development columns for convenient scouting and methods development.

Ordering Information

Tosoh Bioscience chromatography products are sold directly or can be purchased from distributors. An up-to-date list of distributors is available on our website (www.tosohbioscience.com). Orders may be placed by phone, fax or email. Tosoh Bioscience strives to ship all standard chromatography products within 24 hours of placing the order. Items that are not listed in the catalog may be provided as special (custom) products, which usually ship within four weeks. Contact your local Tosoh Bioscience office to discuss the availability of specialty products.
Contact us or a local chromatography products distributor for a copy of our terms and conditions of sale.

Tosoh Bioscience is fully committed to delivering quality products and service. TSK-GEL columns are accompanied by a chromatogram demonstrating the performance of a test mixture and by an OCS sheet that contains information about the Operating Conditions and Specifications for the column. Bulk TSK-GEL and Toyopearl media products are accompanied by a Certificate of Analysis. Despite our commitment to product quality, columns and resins occasionally perform differently than expected in a customer's application. Therefore, we ask you to inspect TSK-GEL and Toyopearl columns or media within 30 days of receipt by using the same conditions employed on the OCS sheet to ensure product performance. Let Tosoh Bioscience know within this 30-day period if the product does not meet the specifications on the OCS (Operating Conditions and Specifications) sheet and QC document, or as listed on the Certificate of Analysis. Subject to prior authorization, Tosoh Bioscience will accept the return of all products that do not perform according to specifications. If a product is authorized for return for reasons other than Tosoh Bioscience's error or because of a product defect, there will be a restocking charge of 10% of the list price or a minimum of 25 Euro.

For more information

Full descriptions and example applications of Tosoh Bioscience chromatography products are provided in this catalog. Our website provides complete product information as well as a literature library and chromatogram database (www.tosohbioscience.com). For pricing and availability, please contact our Customer Service department at +49(0) 711 13257 21. To receive a copy of our Process Chromatographic Media catalog or technical literature, please call +49(0)711 13257 0 or visit our website. A price list for Tosoh Bioscience Chromatography Products is published each December and may be requested by contacting the nearest Tosoh Bioscience office.

Safety Data and Warranty

Tosoh Bioscience provides Material Safety Data Sheets (MSDS) on all of its bulk resins. These sheets contain pertinent information that may be needed to protect employees and customers against any known health or safety hazards associated with our products. The end user is responsible for knowing all information and precautions disclosed in the MSDS and any other available materials provided by Tosoh Bioscience. The MSDS sets forth information concerning our products, describes precautions to be taken in the storage and handling of our products, and in the maintenance of the health and safety of persons exposed to our products, the public and the environment with respect to our products. The end user should convey such information and precautions to the persons who may be exposed to our products.

We also suggest contacting the supplier of other materials recommended for use with our products for appropriate health and safety precautions prior to their use.

Many of our bulk products are on file with the FDA in the form of Drug Master Files (DMF) or Chemistry Manufacturing and Controls (CMC) documents. Permission to reference these documents may be obtained upon written request. Direct inquiries can be send to our Technical Service, Tosoh Bioscience GmbH, Zettachring 6, 70567 Stuttgart, Germany. Tosoh Bioscience warrants that at the time of delivery each of our products will conform to the specifications there of contained in the Certificate of Analysis (COA) or the Operating Conditions and Specifications (OCS) sheet, as relevant, as will be provided together with such

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products; provided, however, that the foregoing warranty applies only if the products have been properly handled, stored and used by Buyer. **THIS WARRANTY IS GIVEN AND ACCEPTED IN LIEU OF ANY OTHER WARRANTIES AND REPRESENTATIONS, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR NONINFRINGEMENT OF INTELLECTUAL PROPERTY RIGHTS. IN NO EVENT SHALL TOSOH BIOSCIENCE BE LIABLE FOR SPECIAL, INDIRECT, INCIDENTAL OR CONSEQUENTIAL DAMAGES OR DAMAGES FOR LOST PROFIT OR LOSS OF USE AS A RESULT OF ANY CLAIM BY BUYER OR ANY ACT OR OMISSION OF TOSOH BIOSCIENCE.** Please refer to Tosoh Bioscience's terms and conditions of sale for additional information on our warranty.

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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 the end user of our products and the Technical Data are beyond the control of Tosoh Bioscience. The products should be tested against the Technical Data to determine if they will be suitable for the intended use and applications. 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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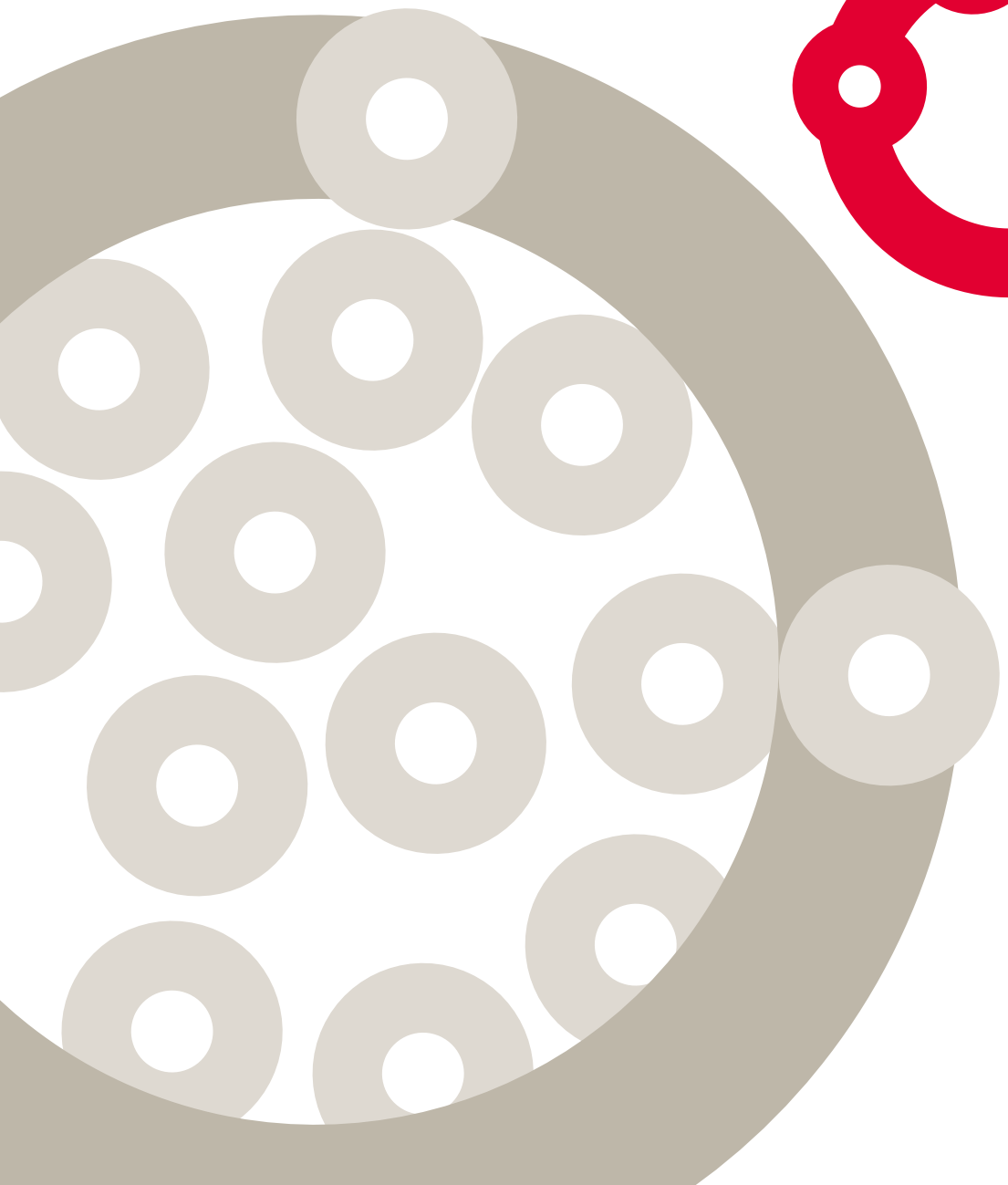
UPLC is a registered tradmark of Waters Corp.
Pyrex is a registered trademark of Corning Incorporated.
Teflon and Tefzel are registered trademarks of E.I. du Pont de Nemours and Company.



RPC REVERSED PHASE CHROMATOGRAPHY

RPC PRODUCTS

UNIVERSAL RP COLUMNS

TSKgel ODS-100V
TSKgel ODS-100Z

FAST RP COLUMNS

TSKgel ODS-140HTP

TRADITIONAL RP COLUMNS

TSKgel ODS-80TS
TSKgel ODS-80TM
TSKgel Octyl-80TM
TSKgel CN-80TS
TSKgel ODS-120A
TSKgel ODS-120T
TSKgel Super-ODS
TSKgel Super-Octyl
TSKgel Super-Phenyl
TSKgel OligoDNA RP
TSKgel TMS-250
TSKgel Octadecyl-NPR
TSKgel Octadecyl-2PW
TSKgel Octadecyl-4PW
TSKgel Phenyl-5PW RP

TOSOH FACT

Tosoh Bioscience, part of the Specialty Group Division of Tosoh Corporation, is a leading supplier of chromatographic columns, media and sophisticated clinical diagnostic systems.

TSK-GEL, Toyopearl and our other branded chromatography products have evolved over more than three decades from the measurement and analysis of polymers and organic compounds to development in the bioscience age with the analysis, separation and purification of proteins.

Experts and knowledgeable industry observers in areas from academia, government and scientific institutions praise the achievements of Tosoh Corporation in the fields of bioanalysis and purification.

UNIVERSAL REVERSED PHASE COLUMNS TSK-GEL ODS-100V AND TSK-GEL ODS-100Z

HIGHLIGHTS

- Ultra-pure silica minimizes sample adsorption
- High surface area (450m²/g) silica
- Spherical 3 and 5 µm particles with 100Å pores
- Very high column efficiency
- Moderate column back pressure
- Two levels of hydrophobicity:
  - 15% carbon (100V)
  - 20% carbon (100Z)
- Monomeric bonding chemistry
- Low residual silanol content

TSKgel ODS-100V & TSKgel ODS-100Z columns incorporate the best-in-class surface properties to limit secondary interactions of basic, acidic and chelating compounds. The ultra high purity Type B base silica contains negligible amounts of metal ion impurities.

TSKgel ODS-100V provides strong retention for polar compounds due to its lower C18 ligand density (15% carbon content). Proprietary monomeric bonded phase chemistry provides complete wetting and retention stability in 100% aqueous mobile phases.

The TSKgel ODS-100V line was expanded to include 3 µm packed columns. These columns are well suited for high throughput LC/MS applications, providing fast and efficient separations.

TSKgel ODS-100Z contains a high density (20% carbon content) monomeric C18 bonded phase for maximum retention and selectivity of small molecular weight compounds. Exhaustive endcapping prevents secondary interaction with residual silanol groups.

TABLE I

	TSKgel ODS-100V	TSKgel ODS-100Z
Carbon content	15%	20%
Particle size (µm)	3 and 5	5
Endcapped	Yes (1)	Yes (2)
Pore size (Å)	100	100
Preferred sample type	Polar, basic, acidic	Hydrophobic
Bonded phase structure	Monolayer	Monolayer
Specific surface area (m²/g)	450	450
*Asymmetry factor (10%)	0,90 - 1,15	0,90 - 1,15
*Theoretical plates	>14.000	>14.000

* Specifications for 4.6 mm ID x 15 cm L columns packed with 5 µm particles. Conditions: 70% methanol, 30% water; Flow Rate: 1 mL/min; Temp.: 40°C, N and AF are based on naphthalene peak. Typical pressure: 6 MPa

(1) Prepared by an incomplete first reaction with a difunctional octadecylsilane reagent, which is followed by endcapping with a mixture of two difunctional dialkylsilane reagents.

(2) Prepared by bonding the surface with a difunctional octadecylsilane reagent, followed by repeated endcapping with monofunctional trimethylsilane reagent.

RPC

APPLICATION OF TSK-GEL ODS-100V AND TSK-GEL ODS-100Z

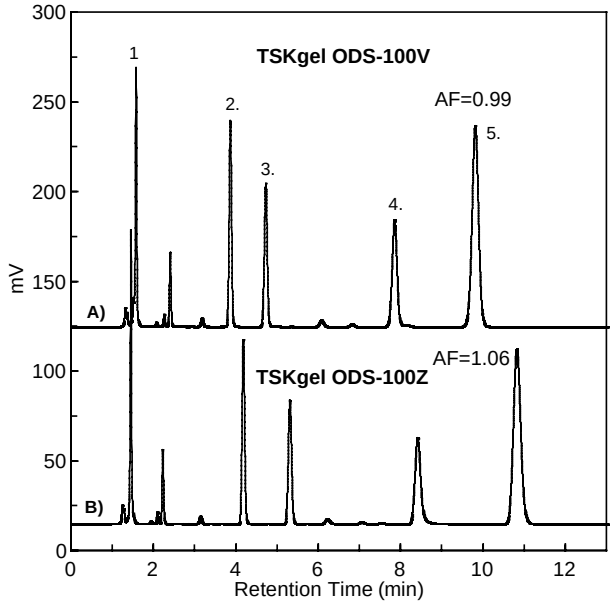
SRM 870

Standard Reference Material SRM 870 was developed by NIST (National Institute of Standards and Technology) as a means to classify the many commercially available reversed phase columns into closely-related groups. Amitriptyline, a tertiary amine, and quinizarin, a strong chelating compound, are included in the SRM 870 mixture, together with more traditional compounds. As shown in FIGURE 1, symmetrical peaks are obtained on TSKgel ODS-100V and TSKgel ODS-100Z for the compounds in this test mixture, clearly demonstrating the superior performance of these columns for the analysis of basic and chelating compounds.

Vitamins

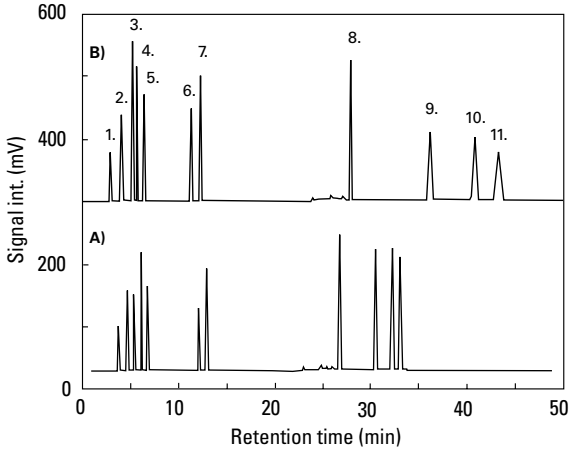
Simple and fast analysis of water- and lipid-soluble vitamins is possible on the TSKgel ODS-100V and TSKgel ODS-100Z columns, as shown in FIGURE 2. Clearly the TSKgel ODS-100Z column provides better overall resolution for the polar compounds in the mixture, while much shorter analysis time was obtained on TSKgel ODS-100V for the late eluting non-polar compounds.

FIGURE 1



Columns: (A) TSKgel ODS-100V 3µm (4.6mmID x 15cm)
(B) TSKgel ODS-100Z 3µm (4.6mmID x 15cm)
Eluent: 20mmol/L Phosphate buffer (pH 7.0) /MeOH (20/80)
Flow rate : 1.0mL/min
Detection: UV @ 254nm
Temp: 40°C
Inj. volume: 10µL
Sample: 1. Uracil, 2. Toluene, 3. Ethyl benzene, 4. Quinizarin, 5. Amitriptyline

FIGURE 2



Columns: A) TSKgel ODS-100V (4.6mm ID x 15cm)
B) TSKgel ODS-100Z (4.6mm ID x 15cm)
Eluent: A) 0.1% TFA in H_2O
B) 0.1% TFA in ACN
Gradient: 0 min (B: 0%) -- 20 min (B: 40%) -- 22min (B: 100%) -- 50min (B: 100%)
Flow rate: 1.0mL/min.
Temp.: 40˚C
Detection: UV @ 280nm
Inj. volume: 5µL
Samples: 1. L-Ascorbic acid, 2. Nicotinic acid, 3. Thiamine, 4. Pyridoxal, 5. Pyridoxine, 6. Caffeine, 7. Riboflavin, 8. Retinol, 9. δ-Tocopherol, 10. α-Tocopherol, 11. α-Tocopherol acetate

APPLICATION OF TSK-GEL ODS-100V AND TSK-GEL ODS-100Z

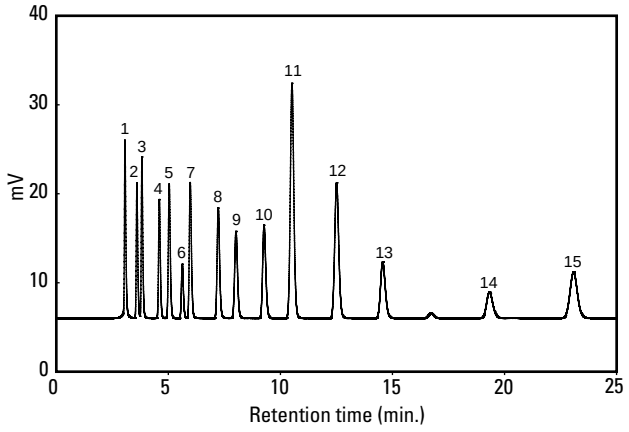
Organic Acids

Organic acids play an important role in many metabolic processes, fermentation and food products. FIGURE 3 shows a baseline separation of 15 organic acids in less than 25 minutes using a simple 0.1% phosphoric acid mobile phase.

Polymer Additives

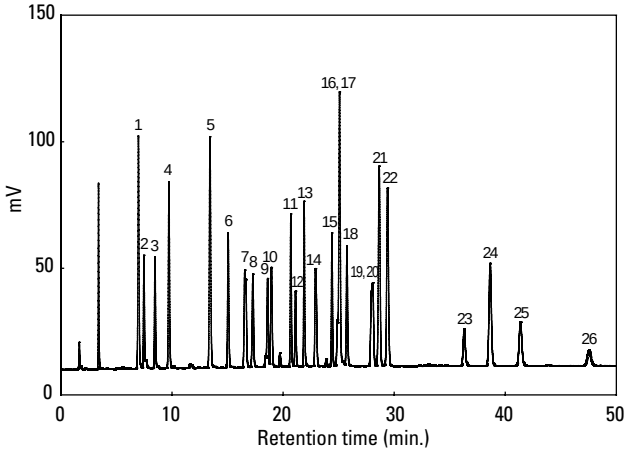
A baseline separation of 26 well known polymer additives is shown in FIGURE 4. Note that while a simple linear acetonitrile gradient was used, the column temperature was increased to 50°C to achieve the required baseline separation on a TSKgel ODS-100V column.

FIGURE 3



Column: TSKgel ODS-100V (4.6mm ID × 25cm)
Mobile phase: 0.1% H_3PO_4, pH 2.3
Flow rate: 1.0mL/min
Temp: 40°C
Inj. Volume: 10µL
Samples:
1. Oxalic acid (0.1mg/mL)
2. L-Tartaric acid (0.5mg/mL)
3. Formic acid (1.0mg/mL)
4. L-Malic acid (1.0mg/mL)
5. L-Ascorbic acid (0.1mg/mL)
6. Lactic acid (1.0mg/mL)
7. Acetic acid (1.0mg/mL)
8. Maleic acid (0.01mg/mL)
9. Citric acid (1.0mg/mL)
10. Succinic acid (1.0mg/mL)
11. Fumaric acid (0.025mg/mL)
12. Acrylic acid (0.1mg/mL)
13. Propionic acid (2.0mg/mL)
14. Glutaric acid (1.0mg/mL)
15. Itaconic acid (0.025mg/mL)

FIGURE 4



Column: TSKgel ODS-100V (4.6mm ID × 15cm)
Mobile phases: A) H_2O
B) ACN
Gradient: 0 min (B: 60%) -- 20 min (B: 100%)
Flow rate: 1.0mL/min
Temp: 50°C
Detection: UV (225nm)
Inj. Volume: 10µL
Concentration: 10mg/L each
Samples:
1. Cyasorb UV-24, 2. BHA, 3. Ionox 100,
4. Seesorb 101, 5. Tinuvin P, 6. Yoshinox SR,
7. Seesorb 202, 8. BHT, 9. Noclizer M-17,
10. Yoshinox 2246R, 11. Topanol CA,
12. Yoshinox 425, 13. Cyanox 1790,
14. Cyasorb UV-531, 15. Ionox 220,
16. Nonflex CBP, 17. Tinuvin 326,
18. Tinuvin 120, 19. Irganox 3114,
20. Uvtex OB, 21. Tinuvin 327, 22. Tinuvin 328,
23. Irganox 1010, 24. Irganox 1330,
25. Irganox 1076, 26. Irgafos 168

RPC

APPLICATION OF TSK-GEL ODS-100V AND TSK-GEL ODS-100Z

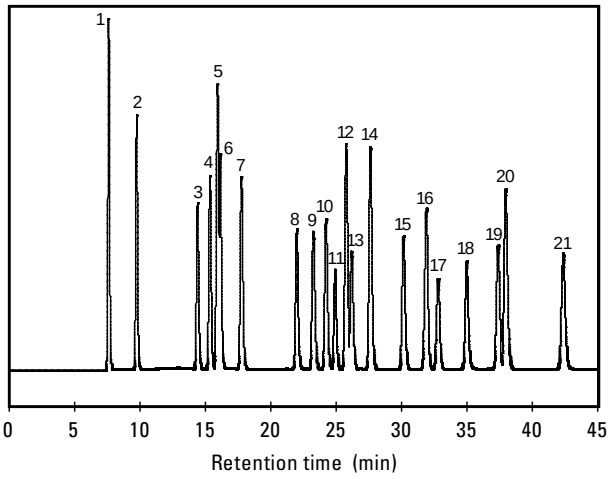
Nucleotides

The analysis of mono-, di-, and tri-phosphorylated nucleotides on a TSKgel ODS-100V column is shown below (FIGURE 5). The separation is accomplished by adding a short chain ion pairing agent, *t*-butylamine, and adjusting the mobile phase pH to 6.8.

Visit our website:

www.tosohbioscience.com for additional applications, product specifications and literature.
Or, contact our Technical Service specialists to discuss your specific application (+49 (0)711 13257 0) or Techsupport.Sep@tosoh.com.

FIGURE 5



Column:	TSKgel ODS-100V (4.6mm ID × 25cm)
Mobile phases:	A) 20 mmol/L *t*-butylamine + H_3PO_4 (pH 6.8)
	B) A/MeOH (90/10)
Gradient:	0 min (B: 0%) -- 35 min (B: 100%)
Flow rate:	1.0mL/min
Temp:	25°C
Detection:	UV (260nm)
Inj. Volume:	2µL
Concentration:	0.3g/L each
Samples:	1. CMP, 2. UMP, 3. CDP, 4. dUMP, 5. GMP, 6. IMP, 7. UDP, 8. CTP, 9. TMP, 10. GDP, 11. IDP, 12. AMP, 13. UTP, 14. dGMP, 15. TDP, 16. GTP, 17. ITP, 18. ADP, 19. TTP, 20. dAMP, 21. ATP

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Stainless steel columns								
21838	ODS-100V, 100 Å	1.0	3.5	3	≥ 2,900	0.02 - 0.05	0.22	150
21839	ODS-100V, 100 Å	1.0	5.0	3	≥ 4,500	0.02 - 0.05	0.22	150
21813	ODS-100V, 100 Å	2.0	3.5	3	≥ 4,000	0.15 - 0.18	0.22	150
21812	ODS-100V, 100 Å	2.0	5.0	3	≥ 5,700	0.15 - 0.18	0.22	150
21811	ODS-100V, 100 Å	2.0	7.5	3	≥ 8,600	0.15 - 0.18	0.22	210
21938	ODS-100V, 100 Å	2.0	10.0	3	≥ 11,500	0.15 - 0.18	0.22	240
21810	ODS-100V, 100 Å	2.0	15.0	3	≥ 17,500	0.15 - 0.18	0.22	250
21842	ODS-100V, 100 Å	3.0	5.0	3	≥ 6,000			150
21843	ODS-100V, 100 Å	3.0	7.5	3	≥ 9,000			210
21939	ODS-100V, 100 Å	3.0	10.0	3	≥ 12,000			240
21844	ODS-100V, 100 Å	3.0	15.0	3	≥ 18,000			240
21831	ODS-100V, 100 Å	4.6	5.0	3	≥ 6,500	0.7 - 1.0	1.2	150
21830	ODS-100V, 100 Å	4.6	7.5	3	≥ 9,750	0.7 - 1.0	1.2	200
21940	ODS-100V, 100 Å	4.6	10.0	3	≥ 13,000	0.7 - 1.0	1.2	240
21829	ODS-100V, 100 Å	4.6	15.0	3	≥ 19,500	0.7 - 1.0	1.2	240
21457	ODS-100V, 100 Å	2.0	5.0	5	≥ 3,300	0.15 - 0.18	0.22	180
21458	ODS-100V, 100 Å	2.0	15.0	5	≥ 11,000	0.15 - 0.18	0.22	180
21455	ODS-100V, 100 Å	4.6	15.0	5	≥ 14,000	0.7 - 1.0	1.2	180
21456	ODS-100V, 100 Å	4.6	25.0	5	≥ 23,000	0.7 - 1.0	1.2	210
21460	ODS-100Z, 100 Å	2.0	5.0	5	≥ 3,300	0.15 - 0.18	0.22	180
21459	ODS-100Z, 100 Å	2.0	15.0	5	≥ 11,000	0.15 - 0.18	0.22	180
21461	ODS-100Z, 100 Å	4.6	15.0	5	≥ 14,000	0.7 - 1.0	1.2	180
21462	ODS-100Z, 100 Å	4.6	25.0	5	≥ 23,000	0.7 - 1.0	1.2	180

Guard column products					
21814	ODS-100V Guard Cartridge, pk3	2.0	1.0	3	For all ODS-100V 2 mm ID columns
21453	ODS-100V Guard Cartridge, pk3	3.2	1.5	5	For all ODS-100V 4.6 mm ID columns
21841	ODS-100Z Guard Cartridge, pk3	2.0	1.0	5	For all ODS-100Z 2 mm ID columns
21454	ODS-100Z Guard Cartridge, pk3	3.2	1.5	5	For all ODS-100Z 4.6 mm ID columns
19308	Cartridge Holder	2.0	1.0		For 2 mm ID cartridges
19018	Cartridge Holder	3.2	1.5		For 4.6 mm ID cartridges

NOTE: Tosoh Bioscience offers guard columns and guard cartridges to protect your analytical column. Guard cartridges are usually delivered in packages of three and require the appropriate cartridge holder.
In general cartridges for 4.6 mm ID columns are produced in 3.2 mm ID and 1.5 cm length. They require the cartridge holder 19018. Guard cartridges for 2 mm ID columns are 2 mm ID x 1 cm L and require holder 19308.

RPC

FAST REVERSED PHASE COLUMNS TSK-GEL ODS-140HTP

HIGHLIGHTS

- Moderate pressure at high flow rates
- High resolution and high efficiency
- High throughput applications
- Compatible with HPLC and UPLC systems
- Moderate carbon content
- Polylayer bonding chemistry

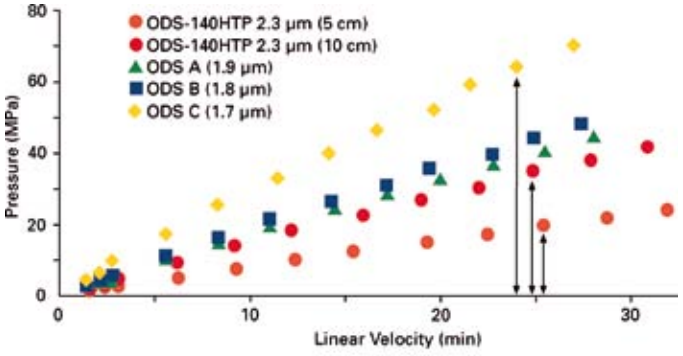
TSK-GEL ODS-140HTP columns were developed for use in high throughput applications, including drug discovery, pharmacokinetics and peptide digest separations. They are available in 2.1 mm ID columns with 5 cm and 10 cm lengths.

TSKgel ODS-140HTP columns are packed with 2.3 µm particles, providing high resolution and short analysis times at moderate pressure. The lower pressure drop reduces the burden on the hardware, allowing TSKgel ODS-140 HTP columns to be used with either UPLC (up to 9000 psi) or conventional HPLC systems. The backpressure of a TSKgel ODS-140 HTP column is less than half of the pressure of a sub-2 µm column of the same dimensions (FIGURE 6).

APPLICATIONS

Excellent resolution at high speed can be achieved on a TSK-gel ODS-140HTP column wih the separation of a ß-lactoglobulin trytic digest (see FIGURE 7). Peak capacity improved when using a longer gradient time.

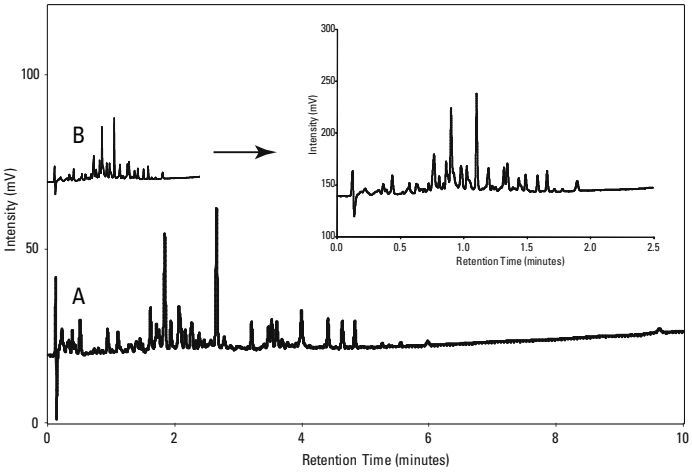
FIGURE 6



Conditions

Column:	TSKgel ODS-140HTP 2.3 µm (2.0 mm ID x 5.0 cm, 10 cm L) Sub-2 µm ODS columns (2,1 mm ID x 5.0 cm L)
Eluent:	H_2O/CH_3CN = 50/50

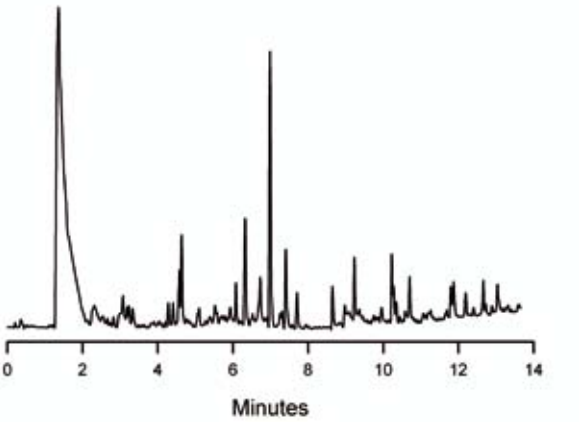
FIGURE 7



Column:	TSKgel ODS-140HTP, 2.3 µm, (2.1mm ID x 5 cm)
Eluent:	A: H_2O/ACN (95/5) + 0.1% TFA B: H_2O/ACN (50/50) + 0.1% TFA
Flow rate:	1.0 mL/min
Detection:	UV@220nm
Temperature:	40ºC
Injection volume:	10 µL
Gradient:	0-100%B (Linear gradient)
Gradient time:	A: 10 min, B: 2.5 min
Sample:	β-lactoglobulin tryptic digest

In Vietnamese and Chinese traditional medicine, hot aqueous extract of Crinum latifolium is used because of its antitumor activity. Crinum latifolium is thought to possess antiviral and immunostimulative properties and shows immunomodulatory properties in human peripheral blood mononuclear cells. The analysis of products derived from plant extracts is a challenging chromatographic task. Due to the high number ob components the column needs to provide a high peak capacity, as shown in FIGURE 8.

FIGURE 8



Column:	TSKgel ODS-140HTP 2.3 µm, 2.1 mm ID x 10 cm L
Sample:	Crinum latifolium L extract, 2 µl
Eluent:	A: water B: acetonitrile
Gradient:	0 min (5%B) 1 2 min (5%B) 4 min (30%B) → 15 min (68%B) 15 1 min (100%B) 20 min (100%B)
Flow rate:	0.4 ml/min
Temp.:	40°C
Detection:	UV @ 220 nm
Sampling rate:	80 Hz

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Pore Size (Å)	Matrix
Stainless steel columns						
21927	TSKgel ODS-140HTP	2.1	5.0	2.3	140	Silica
21928	TSKgel ODS-140HTP	2.1	10.0	2.3	140	Silica

RPC

TRADITIONAL RP COLUMNS
TSK-GEL ODS-80TS TSK-GEL ODS-80TM TSK-GEL OCTYL-80TS TSK-GEL CN-80TS

HIGHLIGHTS

- ODS-80 is prepared from spherical silica with 80 Å pores
- Silica surface is metal free, minimizing solute interactions with residual silanol groups
- Monomeric-bonded phase chemistry for optimal lot-to-lot reproducibility
- Very high column efficiency
- High (80TM) or complete (80TS) endcapping shields the silica surface from participating in solute retention through ionic interaction
- Particles contain 80 Å pores for fast mass transfer of solutes in the 100 to 6,000 Da MW range
- Available in particle sizes of 5 µm, 10 µm, and 20 µm
- Large surface area and high sample capacity
- Hardware: stainless steel columns for analytical, semi-preparative, and preparative separations

2 mm ID Columns

TSKgel ODS-80TS columns are available with a 2 mm ID. Compared with conventional 4.6 mm ID columns, these columns offer the benefits of improved sensitivity and reduced solvent consumption. 2 mm ID columns are operated at lower flow rates, making them more suitable for LC/MS applications.

APPLICATIONS

TSK-GEL ODS-80TM

- Hydrophobic and hydrophilic peptides, synthetic peptides, purity check, peptide mapping
- General purpose column for low MW pharmaceuticals, basic compounds, nucleosides, nucleotides, purines and pyrimidines

TSK-GEL ODS-80TS

- Complete endcapping makes the TSKgel ODS-80TS a good choice for strongly basic compounds and for applications that require operation at pH 7.5

TSK-GEL Octyl-80TS

- Faster kinetics than ODS, but lower hydrophobic selectivity
- Lower hydrophobic selectivity of Octyl versus ODS

TSK-GEL CN-80TS

- Alternative to ODS and Octyl columns for analysis of polar compounds
- Solvent strength should be reduced to obtain similar retention to Octyl and ODS columns when separating non-polar compounds

TSK-GEL ODS-120A TSK-GEL ODS-120T

HIGHLIGHTS

- TSKgel ODS-120 contains polymeric-bonded octadecyl groups on 120Å pore size silica
- TSKgel ODS-120A is not endcapped; TSKgel ODS-120T is endcapped with trimethylsilyl groups
- TSKgel 120T columns are available in 2 mm ID format
- Available in 5 µm and 10 µm particle sizes in analytical and semi-preparative columns respectively. Larger particle sizes are available in preparative columns
- Hardware: stainless steel columns for analytical, semi-preparative, and preparative separations

APPLICATIONS

TSK-GEL ODS-120A

- Polymeric bonded ODS exhibits improved peak shape for the separation of complex geometric isomers, such as polynuclear aromatic hydrocarbons (PAH)
- TSKgel ODS-120A and 120T provide a similar separation at low pH for a mixture of catecholamines, while at pH 6 the basic solutes interact with negatively charged silanol groups on 120A, but not on 120T

TSK-GEL ODS-120T

- Endcapped ODS-120T is an alternative to ODS-80TM for peptide and protein separations

TSK-GEL SUPER-ODS TSK-GEL SUPER-OCTYL TSK-GEL SUPER PHENYL

HIGHLIGHTS

- The silica particles used in Super series columns are monodisperse spherical 2 µm beads with 110 Å pores
- TSKgel Super-ODS, Super-Octyl and Super-Phenyl packings are bonded with, respectively, C18, C8 and phenyl functional groups. The bonded phases have a polymeric structure. An exhaustive endcapping reaction minimizes the presence of residual silanol groups
- 2 µm particles provide superior resolution and speed, as well as improved sensitivity
- Pressure drop is not excessive due to the monodisperse particle size distribution
- Stainless steel columns are available with 4.6 mm and 2 mm ID formats

APPLICATIONS

Super-ODS, Super-Octyl, Super-Phenyl

- Recommended for small molecular weight compounds (<10,000Da) such as peptides, amino acids, tryptic digests, nucleotides, pharmaceutical molecules, and food and beverage samples.

Optimizing Results with Super Series Columns

Super series columns can be used on a regular HPLC system if the dead volume is minimized, although optimal results are obtained with an HPLC system designed for 2 mm or smaller ID columns.

The following recommendations are for 4.6 mm ID columns. Use proportionately lower values for 2 mm ID columns.

1. A guard filter is highly recommended to reduce particulate contamination from the sample or system components.
2. Keep sample volume less than 10 µL.
3. To ensure minimal extra-column volume, keep tubing as short as possible (extra-column volume less than 5 µL between column and detector).
4. Conventional 0.1 mm ID connecting tubing may be used (0.005).
5. The smallest detector time constant should be selected (if possible, less than 50 ms).
6. The detector flow cell should be 2 µL or less for best results. A standard HPLC flow cell (10 µL) can be used as an alternative, however, it is recommended that the heating coil is removed.

TSK-GEL OLIGODNA RP TSK-GEL TMS-250

HIGHLIGHTS

- TSKgel OligoDNA RP and TSKgel TMS-250 both incorporate 5 µm spherical porous silica with 250 Å pores to allow unhindered access by large oligonucleotides and proteins respectively
- TSKgel OligoDNA RP contains a monomeric C18 bonded phase that is not endcapped
- TSKgel TMS-250 is exhaustively and repeatedly reacted with trimethyl silyl groups. Standard nomenclature designates the bonded phase as C1
- TSKgel OligoDNA RP is available in 4.6 mm ID and 7.8 mm ID (both 15 cm length), while TSKgel TMS-250 is only available in 4.6 mm ID x 7.5 cm L

APPLICATIONS

TSK-GEL OLIGODNA RP

- Ideal for the purification and analysis of oligonucleotides (up to 500-mer), RNAs, and DNA fragments
- Possesses high-resolving power for octamers of similar sequence

TSK-GEL TMS-250

- Recommended for the analysis of proteins
- The “wide-pore” TMS-250 packing can accommodate large proteins, such as aldolase (158,000 Da).

RPC

TSK-GEL OCTADECYL-NPR TSK-GEL OCTADECYL-2PW TSK-GEL OCTADECYL-4PW TSK-GEL PHENYL-5PW RP

HIGHLIGHTS

- Polymer-based RPC columns are chemically stable at pH 2-12, allowing operation at basic pH where silica-based columns have limited chemical stability.
- Polymer-based TSK-GEL RPC columns can be cleaned and impurities removed by using either strong acid or base.
- Polymer-based TSK-GEL RPC columns are available packed with nonporous resins (NPR) or with porous resins of various pore sizes. The proper column to use is selected based on sample MW or application.
- 2.5 µm particle size TSKgel Octadecyl-NPR resin features fast kinetics resulting in high column efficiency and quantitative protein recovery at sub-microgram loads.
- TSKgel Octadecyl-2PW is based on 5 µm particle size G2000PW resin with 125 Å pores.
- TSKgel Octadecyl-4PW is based on 7 µm particle size G4000PW resin, which contains 500 Å pores.
- TSKgel Phenyl-5PW RP is based on 10 µm particle size G5000PW resin, which has an average pore size of 1000 Å. In comparison with the Phenyl-5PW packing material used in HIC, the greater level of hydrophobicity in TSKgel Phenyl-5PW RP makes this material more suitable for use in RPC.

APPLICATIONS

TSK-GEL OCTADECYL-NPR

- High efficiency purification of proteins and peptides at sub-microgram loads
- Nonporous particles are stable to higher pressures than porous particles
- Improved recovery at low sample concentration over traditional porous resins

TSK-GEL OCTADECYL-2PW

- For analyzing small MW pharmaceutical compounds at basic pH
- Faster analysis than competitive polymeric reversed phase packings

TSK-GEL OCTADECYL-4PW

- Recommended for peptides and small proteins

TSK-GEL PHENYL-5PW RP

- Ideal for the separation of proteins, including high MW
- Able to handle high loads (high capacity)

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ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Stainless steel columns								
18150	ODS-80Ts, 80 Å	2.0	15.0	5	≥≥ 11,000	0.15 - 0.18	0.22	200
18151	ODS-80Ts, 80 Å	2.0	25.0	5	≥ 18,000	0.15 - 0.18	0.22	300
17200	ODS-80Ts, 80 Å	4.6	7.5	5	≥ 4,500	0.8 - 1.0	1.2	100
17201	ODS-80Ts, 80 Å	4.6	15.0	5	≥ 11,000	0.8 - 1.0	1.2	200
17202	ODS-80Ts, 80 Å	4.6	25.0	5	≥ 18,000	0.8 - 1.0	1.2	300
17380	ODS-80Ts, 80 Å	21.5	30.0	10	≥ 6,000	4.0 - 6.0	12.0	60
16651	ODS-80TM, 80 Å	4.6	7.5	5	≥ 4,500	0.8 - 1.0	1.2	100
08148	ODS-80TM, 80 Å	4.6	15.0	5	≥ 11,000	0.8 - 1.0	1.2	200
08149	ODS-80TM, 80 Å	4.6	25.0	5	≥ 18,000	0.8 - 1.0	1.2	300
14001	ODS-80TM, 80 Å	7.8	30.0	10	≥ 6,000	1.0 - 2.0	3.0	80
14002	ODS-80TM, 80 Å	21.5	30.0	10	≥ 6,000	4.0 - 6.0	12.0	60
17344	Octyl-80Ts, 80 Å	4.6	15.0	5	≥ 11,000	0.8 - 1.0	1.2	200
17345	Octyl-80Ts, 80 Å	4.6	25.0	5	≥ 18,000	0.8 - 1.0	1.2	300
17348	CN-80Ts, 80 Å	4.6	15.0	5	≥ 11,000	0.8 - 1.0	1.2	200
17349	CN-80Ts, 80 Å	4.6	25.0	5	≥ 18,000	0.8 - 1.0	1.2	300
Guard column products								
19325	ODS-80Ts Guard cartridge, pk 3	2.0	1.0	5	For all 2 mm ID ODS-80Ts columns			
19011	ODS-80Ts Guard cartridge, pk 3 -NEW-	3.2	1.5	5	For all 4.6 mm ID ODS-80Ts columns, replaces P/N 17242			
19012	Octyl-80Ts Guard cartridge, pk 3 -NEW-	3.2	1.5	5	For all 4.6 mm ID Octyl-80Ts columns, replaces P/N 17378			
17385	ODS-80Ts Guard column	21.5	7.5	10	For P/N 17380			
14098	ODS-80TM Guard column	21.5	7.5	10	For P/N 14002			
19004	ODS-80TM Guard cartridge, pk 3 -NEW-	3.2	1.5	5	For 4.6 mm ID ODS-80TM columns, replaces P/N 17242			
19013	CN-80Ts Guard cartridge, pk 3 -NEW-	3.2	1.5	5	For 4.6 mm ID CN-80Ts columns, replaces P/N 17379			
19308	Cartridge holder				For 2.0 mm ID Guard cartridges			
19018	Cartridge holder				For 3.2 mm ID Guard cartridges, replaces P/N 14100			
Stainless steel columns								
07636	ODS-120A, 120 Å	4.6	15.0	5	≥ 7,000	0.8 - 1.0	1.2	150
07124	ODS-120A, 120 Å	4.6	25.0	5	≥ 10,000	0.8 - 1.0	1.2	200
07129	ODS-120A, 120 Å	7.8	30.0	10	≥ 6,000	1.0 - 2.0	3.0	75
06172	ODS-120A, 120 Å	21.5	30.0	10	≥ 6,000	4.0 - 6.0	12.0	60
18152	ODS-120T, 120 Å	2.0	15.0	5	≥ 6,500	0.15 - 0.18	0.22	150
18153	ODS-120T, 120 Å	2.0	25.0	5	≥ 10,000	0.15 - 0.18	0.22	200
07637	ODS-120T, 120 Å	4.6	15.0	5	≥ 7,000	0.8 - 1.0	1.2	150
07125	ODS-120T, 120 Å	4.6	25.0	5	≥ 10,000	0.8 - 1.0	1.2	200
07130	ODS-120T, 120 Å	7.8	30.0	10	≥ 6,000	1.0 - 2.0	3.0	75
07134	ODS-120T, 120 Å	21.5	30.0	10	≥ 6,000	3.0 - 6.0	12.0	60
07433	ODS-120T, 120 Å	55.0	30.0	20	≥ 4,500	45.0 - 80.0	100.0	20
07434	ODS-120T, 120 Å	55.0	60.0	20	≥ 9,000	40.0 - 60.0	75.0	35
Guard column products								
19325	ODS-120T Guard cartridge, pk 3	2.0	1.0	5	For all 2 mm ID ODS-120T columns			
07642	ODS-120T/A Guard column	21.5	7.5	10	For P/Ns 06172 and 07134			
19006	ODS-120T Guard cartridge, pk 3 -NEW-	3.2	1.5	5	For 4.6 mm ID ODS-120T columns, replaces P/N 14125			
19005	ODS-120A Guard cartridge, pk 3 -NEW-	3.2	1.5	5	For 4.6 mm ID ODS-120A columns, replaces P/N 14125			
19018	Guard cartridge holder	3.2	1.5		For 3.2 mm ID Guard cartridges, replaces P/N 14100			
19308	Guard cartridge holder	2.0	1.5		For all 2 mm ID Guard cartridges			

RPC

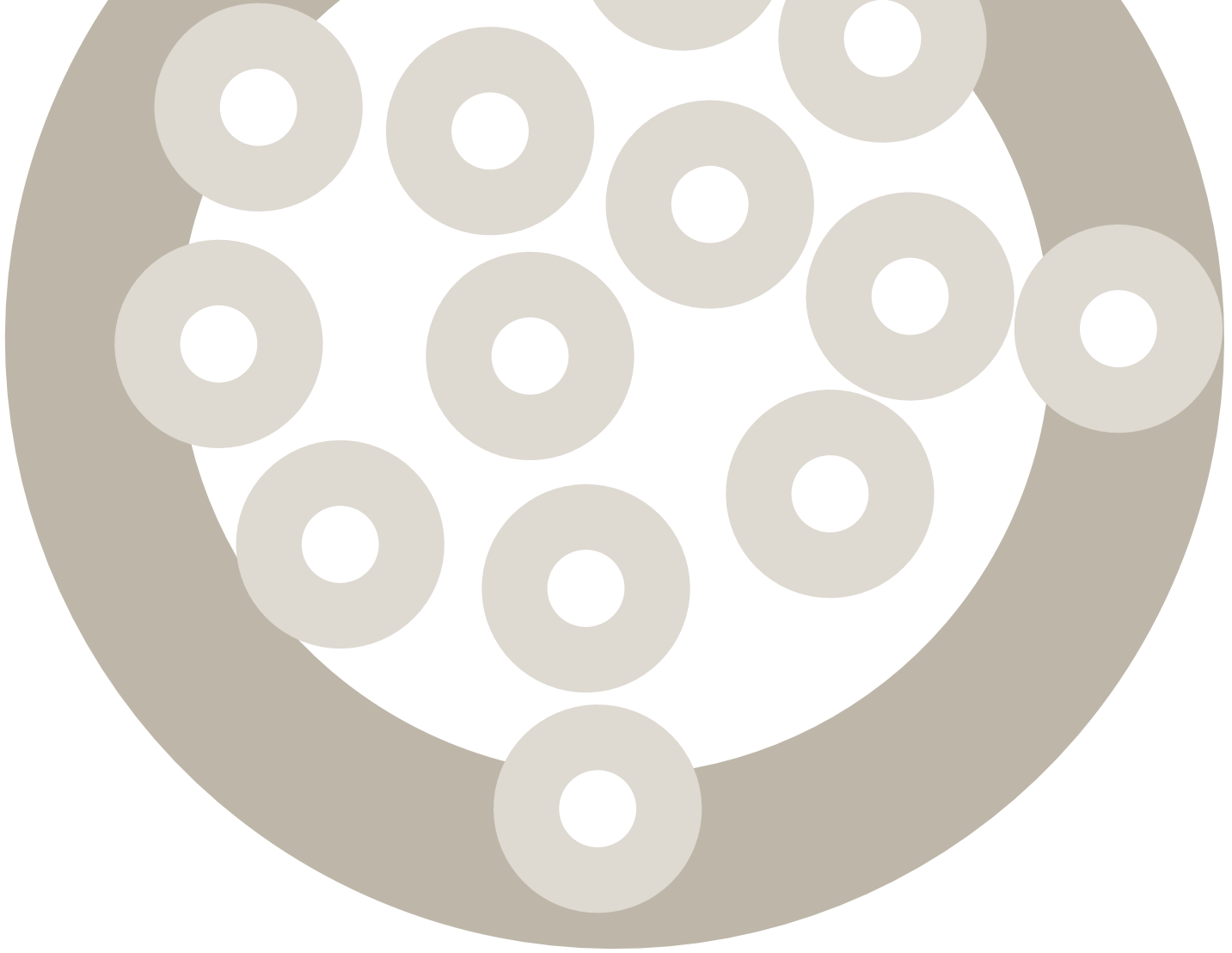
Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Stainless steel columns								
20015	Super-ODS, 110 Å	1.0	5.0	2	≥ 15,000	0.03 - 0.05	0.06	150
20016	Super-ODS, 110 Å	1.0	10.0	2		0.03 - 0.05	0.06	150
19541	Super-ODS, 110 Å	2.0	5.0	2	≥ 6,000	0.15 - 0.2	0.25	250
19542	Super-ODS, 110 Å	2.0	10.0	2	≥ 12,000	0.15 - 0.2	0.25	250
18154	Super-ODS, 110 Å	4.6	5.0	2	≥ 8,000	1.0 - 2.5	4.0	300
18197	Super-ODS, 110 Å	4.6	10.0	2	≥ 16,000	1.0 - 2.5	4.0	300
20013	Super-Octyl, 110 Å	2.0	5.0	2	≥ 15,000	0.15 - 0.20	0.25	150
20014	Super-Octyl, 110 Å	2.0	10.0	2	≥ 5,000	0.15 - 0.20	0.25	300
18275	Super-Octyl, 110 Å	4.6	5.0	2	≥ 8,000	1.0 - 2.5	4.0	300
18276	Super-Octyl, 110 Å	4.6	10.0	2	≥ 16,000	1.0 - 2.5	4.0	300
20017	Super-Phenyl, 110 Å	2.0	5.0	2	≥ 3,000	0.15 - 0.20	0.25	80
20018	Super-Phenyl, 110 Å	2.0	10.0	2	≥ 6,000	0.15 - 0.20	0.25	150
18277	Super-Phenyl, 110 Å	4.6	5.0	2	≥ 8,000	1.0 - 2.5	4.0	300
18278	Super-Phenyl, 110 Å	4.6	10.0	2	≥ 16,000	1.0 - 2.5	4.0	300
Guard column products								
19672	Guard cartridge, pk 3	2.0	1.0	2	For 2 mm ID Super-ODS columns			
19308	Cartridge holder				For P/N 19672			
18207	Guard cartridge, pk 3	4.0	4.0	2	For 4.6 mm ID columns (Super-ODS, -Octyl, -Phenyl)			
18206	Cartridge holder				For P/N 18207			
Stainless steel columns								
13352	OligoDNA RP, 250 Å	4.6	15.0	5	≥ 7,000	0.6 - 1.0	1.2	120
13353	OligoDNA RP, 250 Å	7.8	15.0	5	≥ 7,000	2.0 - 3.0	3.2	120
07190	TMS-250, 250 Å	4.6	7.5	10	≥ 1,500	0.5 - 0.8	1.0	20
Glass columns								
14006	Phenyl-5PW RP Glass, 1000 Å	5.0	5.0	10	≥ 400	0.5 - 1.0	1.2	20
14007	Phenyl-5PW RP Glass, 1000 Å	8.0	7.5	10	≥ 700	1.0 - 2.0	2.5	20
Stainless steel columns								
14005	Octadecyl-NPR nonporous	4.6	3.5	2.5	≥ 1,000	1.0 - 1.5	1.6	200
18754	Octadecyl-2PW, (100 - 200 Å)	2.0	15.0	5	≥ 5,000	0.07 - 0.11	0.14	70
17500	Octadecyl-2PW, (100 - 200 Å)	4.6	15.0	5	≥ 6,000	0.4 - 0.6	1.2	100
17501	Octadecyl-2PW, (100 - 200 Å)	6.0	15.0	5	≥ 6,000	0.5 - 1.0	1.5	100
18755	Octadecyl-4PW, 500 Å	2.0	15.0	7	≥ 2,000	0.08 - 0.17	0.22	100
13351	Octadecyl-4PW, 500 Å	4.6	15.0	7	≥ 2,000	0.5 - 1.0	1.2	120
16257	Octadecyl-4PW, 500 Å	21.5	15.0	13	≥ 2,000	3.0 - 6.0	8.0	35
16258	Octadecyl-4PW, 500 Å	55.0	20.0	20	≥ 1,700	30.0 - 50.0	60.0	5
18756	Phenyl-5PW RP, 1000 Å	2.0	7.5	10	≥ 400	0.05 - 0.1	0.12	10
08043	Phenyl-5PW RP, 1000 Å	4.6	7.5	10	≥ 500	0.5 - 1.0	1.2	30
16260	Phenyl-5PW RP, 1000 Å	21.5	15.0	13	≥ 1,000	6.0 - 8.0	8.0	30
16261	Phenyl-5PW RP, 1000 Å	55.0	20.0	20	≥ 700	–	–	–

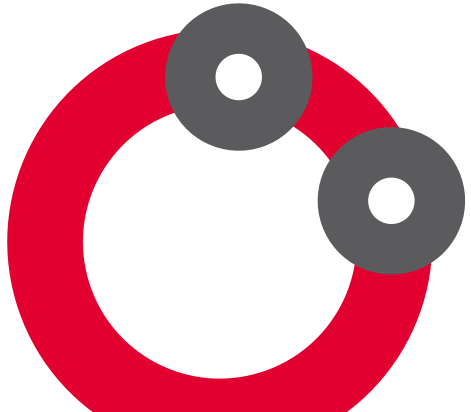
Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	
Guard column products					
14022	Phenyl-5PW RP Guardgel Kit, Glass			20	For P/Ns 14006 and 14007
42159	Phenyl-5PW RP Cartridge, pk 3	2.0	1.0	10	For P/N 18756
19007	Phenyl-5PW RP Cartridge, pk 3 -NEW-	3.2	1.5	10	For P/N 08043, Replaces 14126
16262	Phenyl-5PW RP Guard column	45.0	5.0	20	For P/N 16261
42161	Octadecyl-2PW Cartridge, pk 3	2.0	1.0	5	For P/N 18754
17502	Octadecyl-2PW Guard column	4.6	1.0	5	For P/N 17500
17503	Octadecyl-2PW Guard column	6.0	1.0	5	For P/N 17501
42160	Octadecyl-4PW Cartridge, pk 3	2.0	1.0	7	For P/N 18755
19008	Octadecyl-4PW Cartridge, pk 3 -NEW-	3.2	1.5	7	For P/N 13351, Replaces P/N14127
16749	Octadecyl-4PW Prep Guardgel Kit			13	For P/N 16257
16259	Octadecyl-4PW Guard column	45.0	5.0	20	For P/N 16258
19308	Guard cartridge holder	2.0	1.0		For all 2 mm ID Guard cartridges
19018	Guard cartridge holder	3.2	1.5		For 4.6 mm ID Octadecyl 4-PW and Phenyl-5PW RP Guard cartridges, Replaces P/N 14100

NOTE: Tosoh Bioscience offers guard columns and guard cartridges to protect your analytical column. Guard cartridges are usually delivered in packages of three and require the appropriate cartridge holder.

In general cartridges for 4.6 mm ID columns are produced in 3.2 mm ID and 1.5 cm length. They require the cartridge holder 19018. Guard cartridges for 2 mm ID columns are 2 mm ID x 1 cm L and require holder 19308.

RPC





HILIC
HYDROPHILIC INTERACTION CHROMATOGRAPHY

HILIC PRODUCTS

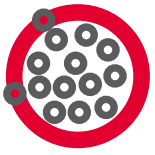
- TSKgel Amide-80

NEW

3 µm Particle Size

TOSOH FACT

The first columns used in chromatography were glass, both for liquid-solid chromatography by Tswett in his separation of plant pigments and by James and Martin in their first gas chromatograph. However, as the technique developed and particle size was reduced, the length of the columns in liquid chromatography was decreased. This resulted in the columns having to be operated at higher pressures. To accommodate these higher pressures, stainless steel columns were introduced. Tosoh introduced its first HPLC (GPC) columns in 1971, which were composed of stainless steel. Recently, columns packed in PEEK, a biocompatable fluorocarbon polymer, became available. PEEK can withstand the pressures commonly encountered in HPLC.



INTRODUCTION TO TSK-GEL HILIC COLUMNS

HIGHLIGHTS

- Stable bonding chemistry
- Unique polar phase
- Handles a wide spectrum of sample polarities
- Stable in 100% organic
- Separates many different types of polar molecules
- New 3 µm particle size for LC/MS analysis

Hydrophilic interaction chromatography (HILIC) is used primarily for the separation of polar and hydrophilic compounds. HILIC has similarities with traditional normal phase chromatography, but the mobile phases for HILIC are similar to those known from reversed phase chromatography (RPC). They include polar organic solvents like methanol or acetonitrile and water. Compared to RPC the elution order in HILIC mode is inversed for most substances.

HILIC is often used to separate hydrophilic compounds such as peptides, carbohydrates and small polar drug candidates or metabolites. Hydrophilic compounds are retained on the polar-bonded phase column while nonpolar sample impurities elute unretained in the void volume. In addition it is ideally suited for sensitive LC-MS analysis of water soluble polar compounds because the high organic content in the mobile phase provides rapid evaporation of solvent during electrospray ionization.

The TSK-GEL AMIDE-80 column offers an excellent alternative to amino-bonded stationary phases and consists of 3, 5 or 10 µm silica particles in a stainless steel format. Spherical silica particles are covalently bonded with carbamoyl groups. Based on hydrogen bonds the aqueous content of the mobile phase creates a water-rich layer on the particle surface. This allows for partitioning of polar compounds between the more organic mobile phase and the aqueous layer. The number of polar groups, as well as the conformation and solubility of the sample in the mobile phase determines the elution order. Typical mobile phases consist of acetonitrile buffer mixtures. Samples are eluted from the column by increasing the percentage of the aqueous component.

For years TSKgel Amide-80 columns have been the standard for the analysis of glycans. TSKgel Amide-80 columns packed with 3 µm particles are the newest addition to the TSKgel Amide-80 series. The 3 µm HILIC columns reduce analysis time and improve peak capacity and sensitivity for HPLC and LC-MS analysis.

Column Operation and Specifications

TSKgel Amide-80 columns can be operated over a broad range of mobile phase conditions for use with many sample polarities. Factors to consider when employing this column include:

Sample Loading Capacity: this is dependent upon the polarity of the mobile phase. Loading capacity increases with decreasing mobile phase polarity. For example, the highest loading capacity for mannitol (200 µg) occurs with a mobile phase of 75:25 acetonitrile/water. However, <100 µg of mannitol can be loaded in a mobile phase of 65:35 acetonitrile/water. The maximum sample volume for a 4.6 mm ID x 25 cm L Amide-80 analytical column is 50 µL.

Pressure Limitations: Column pressure drop varies with mobile phase viscosity. For mobile phases containing high water concentrations, the back-pressure should be < 120 kg/cm^2 for 1 mm ID columns, < 150 kg/cm^2 for 2 mm ID columns, <150 kg/cm^2 for 4.6 mm ID columns, < 70 kg/cm^2 (for 7.8 mm ID columns, and < 30 kg/cm^2 for 21.5 mm ID columns.

Temperature Range: the TSKgel Amide-80 column can be operated over a temperature range of 4-80°C (4-40°C for Amide-80 3µm). In general, retention times for carbohydrates decrease with increasing temperature, thereby shortening analysis time. Below certain temperatures some carbohydrates may elute as split peaks. In this case, column heating or addition of triethylamine to the mobile phase is required.

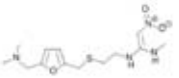
Choice of Mobile Phase: the pH range of the TSKgel Amide-80 column is 2.5-7.5 with a maximum salt concentration of 100 mmol/L. The column is stable in 100% organic; however, a combination of aqueous and organic solvents is necessary in order to create the water-rich surface layer. Elution volume can be controlled by the mobile phase polarity. As the mobile phase polarity decreases (higher organic content) the sample is retained longer on the column. For example, oligosaccharides require 40-50% water in the mobile phase in order to elute from the Amide-80 column.

HILIC

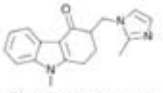
Small polar molecule drugs:

TSKgel Amide-80 is a valuable tool for the analysis of small, polar molecule drugs that cannot be retained very well by reversed phase LC columns.

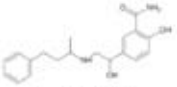
FIGURE 1 compares the separation of polar, drug standards with detection by electrospray ionization mass spectroscopy (ESI-MS) in HILIC mode compared to reversed phase mode. Due to the high organic content of the eluent HILIC analysis provides increased detection sensitivity.



Ranitidine
315/176



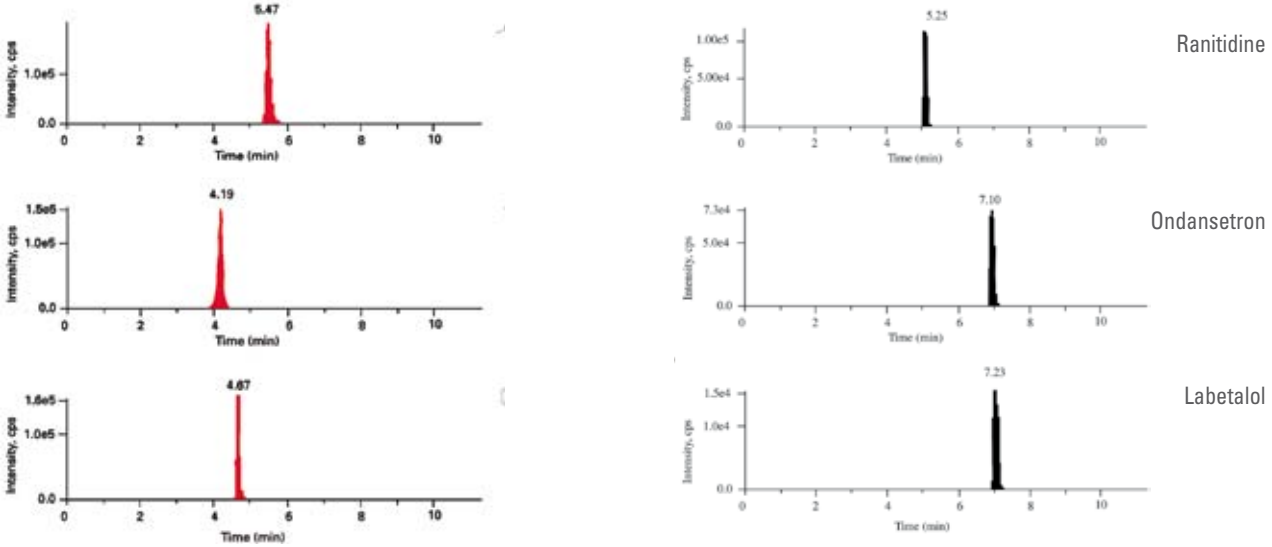
Ondansetron
294/212



Labetalol
329/162

FIGURE 1

ESI-LC-MS analysis of basic drugs on HILIC and Reversed Phase columns with small particles



Column:	TSKgel Amide-80 3 µm (2.0 mm ID x 15 cm L)	Column:	TSKgel ODS-100V 3 µm (2.0 mm ID x 15 cm L)
Eluent :	A: 10 mM Ammoniumformiate (pH 3.75)	Eluent :	A: 10 mM Ammoniumformiate (pH 3.75)
	B: ACN		B: ACN
Gradient :	0 min (B 90%) -> 10 min (B 40%) ->13 min (B 40%)	Gradient :	0 min (B 0%) -> 10 min (B 80%) ->13 min (B 80%)
Flow rate :	0.2 mL/min	Flow rate :	0.2 mL/min
Inj. volume :	5 µL (50 µg/L)	Inj. volume :	5 µL (50 µg/L)
Detection :	QTrap® LC-MS/MS (Applied Biosystems), ESI+	Detection :	QTrap® LC-MS/MS (Applied Biosystems), ESI+

Polyalcohols

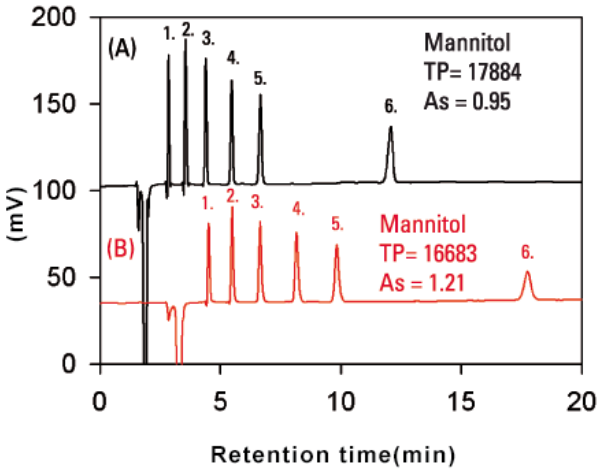
Polyalcohols are typically separated with a mobile phase of organic solvent and water as shown in FIGURE 2 for a 3 µm TSKgel Amide-80 column compared to a 5 µm column.

Oligosaccharides

The TSKgel Amide-80 can separate oligosaccharides very rapidly and efficiently. FIGURE 3 shows a separation of a ß-cyclodextrin hydrolysate in less than 10 minutes. The labels indicate the number of base sugars such as glucose in each oligomer.

FIGURE 2

Separation of polyalcohols on TSGgel Amide-80 3 µm and 5 µm

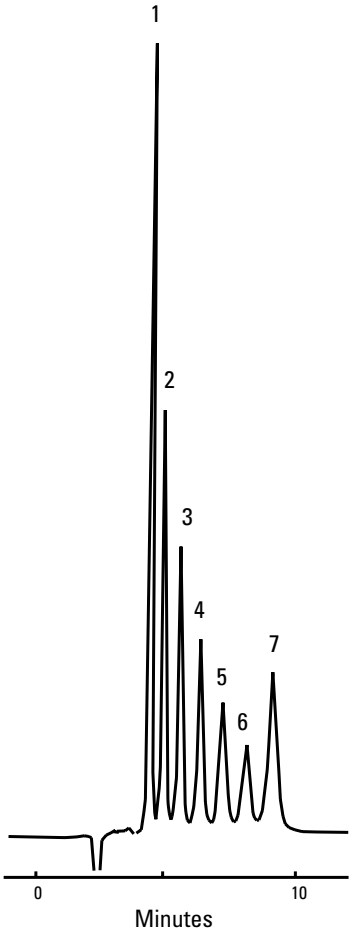


Conditions

Column:	A) TSKgel Amide-80 3 µm (4.6 mm ID x 15 cm L)
	B) TSKgel Amide-80 5 µm (4.6 mm ID x 25 cm L)
Eluent:	H_2O/CH_3CN = 25/75
Flow rate:	1.0 mL/min
Detection:	Refractive index
Temp.:	25 °C
Inj. volume :	10 µL
Samples:	1. Ethyleneglycol 2. Glycerin
	3. Erythritol 4. Xylitol
	5. Mannitol 6. Inositol

FIGURE 3

Separation of β-cyclodextrin hydrolysate on TSKgel Amide-80 column



Column:	TSKgel Amide-80, 4.6mm ID x 25cm
Sample:	2µL, β-cyclodextrin hydrolysate, 1-7 degrees of polymerization (4.6mg/mL)
Elution:	ACN/water (55/45)
Flow Rate:	1.0mL/min
Detection:	refractive index detector
Temperature:	25°C

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Or, contact our Technical Service specialists to discuss your specific application:

techsupport.sep@tosoh.com

+49 (0)711 13257 0.

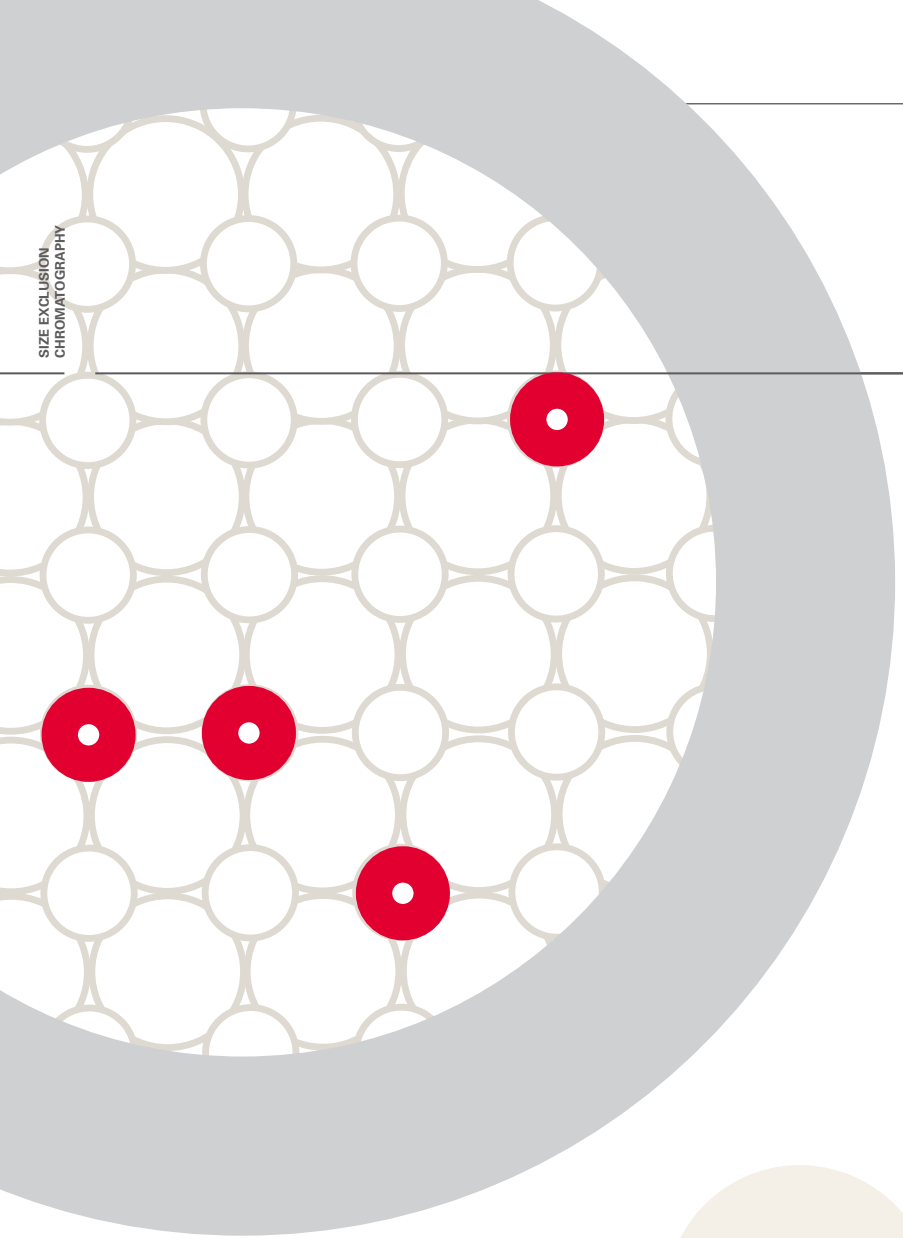
HILIC

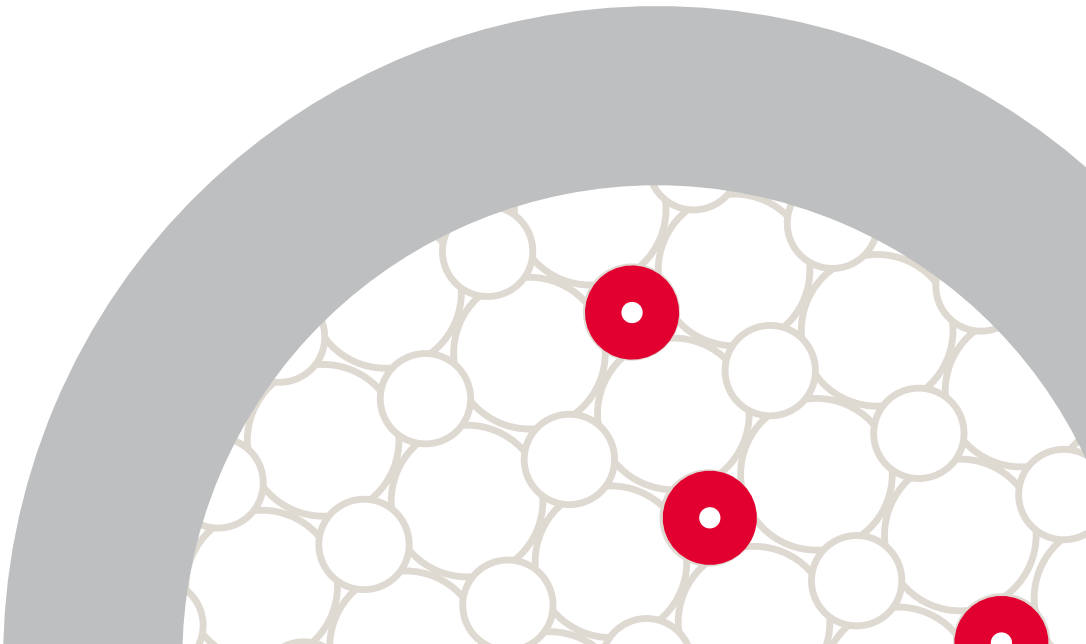
ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Stainless steel columns								
21864	Amide-80 -NEW-	2.0	5.0	3	≥ 3,500			200
21865	Amide-80 -NEW-	2.0	15.0	3	≥ 13,000			200
21866	Amide-80 -NEW-	4.6	5.0	3	≥ 6,000			200
21867	Amide-80 -NEW-	4.6	15.0	3	≥ 18,500			200
20009	Amide-80	1.0	5.0	5	≥ 300	0.03 - 0.05	0.06	30
20010	Amide-80	1.0	10.0	5	≥ 600	0.03 - 0.05	0.06	60
21486	Amide-80 -NEW-	1.0	15.0	5	≥ 4,000	0.03 - 0.05	0.06	90
21487	Amide-80 -NEW-	1.0	25.0	5	≥ 6,000	0.03 - 0.05	0.06	120
19694	Amide-80	2.0	5.0	5	≥ 1,000	0.15 - 0.20	0.25	40
19695	Amide-80	2.0	10.0	5	≥ 2,000	0.15 - 0.20	0.25	80
19696	Amide-80	2.0	15.0	5	≥ 4,000	0.15 - 0.20	0.25	100
19697	Amide-80	2.0	25.0	5	≥ 6,000	0.15 - 0.20	0.25	150
19532	Amide-80	4.6	5.0	5	≥ 2,500	0.8 - 1.0	1.2	50
19533	Amide-80	4.6	10.0	5	≥ 4,000	0.8 - 1.0	1.2	50
13071	Amide-80	4.6	25.0	5	≥ 8,000	0.8 - 1.0	1.2	150
14459	Amide-80	7.8	30.0	10	≥ 5,000	1.0 - 2.0	3.0	70
14460	Amide-80	21.5	30.0	10	≥ 8,000	4.0 - 6.0	8.0	30

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	
Guard column products					
21862	Amide-80 Guard cartridge, pk 3 -NEW-	2.0	1.0	3	For 2.0 mm ID columns
21863	Amide-80 Guard cartridge, pk 3 -NEW-	3.2	1.5	3	For 4.6 mm ID columns
21941	Amide-80 Guard cartridge, pk 3	2.0	1.0	5	For all 2 mm ID columns
19021	Amide-80 Guard column	4.6	1.0	5	For all 4.6 mm ID columns
19010	Amide-80 Guard cartridge, pk 3 -NEW-	3.2	1.5	5	For all 4.6 mm ID columns
14461	Amide-80 Guard column	21.5	7.5	10	For 21.5 mm ID column
19308	Amide-80 Guard cartridge holder				For 2 mm ID x 1 cm L guard cartridges
19018	Amide-80 Guard cartridge holder				For 3.2 mm ID x 1.5 cm L guard cartridges

NOTE: Tosoh Bioscience offers guard columns and guard cartridges to protect your analytical column. Guard cartridges are usually delivered in packages of three and require the appropriate cartridge holder.
In general cartridges for 4.6 mm ID columns are produced in 3.2 mm ID and 1.5 cm length. They require the cartridge holder 19018. Guard cartridges for 2 mm ID columns are 2 mm ID x 1 cm L and require holder 19308.





SEC
SIZE EXCLUSION CHROMATOGRAPHY

SEC PRODUCTS

TSK-GEL SW-type

TSKgel SW
TSKgel SW_{XL}
TSKgel SuperSW

TSK-GEL PW-type

TSKgel PW
TSKgel PW_{XL}
TSKgel PW_{XL}-CP

TSK-GEL Alpha-type

TSKgel Alpha
TSKgel SuperAW

TSK-GEL H-type

TSKgel H_{XL}
TSKgel H_{HR}
TSKgel $Super_{H}$
TSKgel $Super_{HZ}$

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 had particle sizes from 10 to 13 µm and were quickly adopted and referred to as the standard for analytical SEC on FPLC and HPLC systems.

As new packing materials were discovered and new bonding chemistries developed, the SEC product line has grown into four major classes of SEC columns. The following pages will help you choose the best column for your application.

INTRODUCTION TO TSK-GEL SIZE EXCLUSION COLUMNS

Gel Filtration Chromatography (GFC)

GFC is popular among biochemists for the isolation of proteins, for the removal of aggregates, to desalt a protein sample, to separate nucleic acid fractions, or to characterize water-soluble polymers used in food products, paints, pharmaceutical preparations, etc. Available TSK-GEL products are classified by application area and particle composition.

Each of the types below is described in detail in this chapter.

Application Area: Proteins and other biopolymers

Base material: silica

- SW
- SW_{XL}
- SuperSW

These columns are ideal for proteins and nucleic acids using an aqueous buffer as mobile phase.

Application Area: Water-soluble polymers

Base material: polymethacrylate

- PW
- PW_{XL}
- PW_{XL}-CP

These columns are ideal for industrial polymers, oligosaccharides, nucleic acids and small viruses using aqueous buffer or salt solutions as mobile phase. The PW_{XL}-CP columns are developed to facilitate SEC separation of cationic polymer under low salt conditions.

Application Area: Water- and organic-soluble polymers

Base material: polyvinyl

- Alpha
- SuperAW

These columns are ideal for industrial polymers soluble in water, buffers and many organic solvents.

Gel Permeation Chromatography (GPC)

GPC plays an important role in the characterization of organic-soluble polymers in the chemical and petrochemical industries. TSK-GEL GPC columns contain particles prepared from polystyrene crosslinked with divinylbenzene. Available products are grouped according to their relative lack of adsorptive properties and the speed of analysis.

Each of the types below is described in detail in this chapter.

Application Area: Organic-soluble polymers

Base material: polystyrene

Ultra-low adsorption columns with limited solvent range

- SuperHZ (high throughput)
- H_{XL} (conventional)

Low adsorption columns with expanded solvent range

- SuperH (high throughput)
- H_{HR} (conventional)

FEATURES

- Rigid hydrophilic and hydrophobic packings
- Four series of SEC columns with diferent ranges of solvent compatibility
- Easy scale up

BENEFITS

- Minimal swelling and excellent physical strength
- Low adsorption resulting in high mass recovery
- Suitable for both types of size exclusion, aqueous (GFC) and non-aqueous (GPC)
- Analytical and preparative pre-packed SEC column

SEC

SUMMARY OF TSK-GEL SIZE EXCLUSION COLUMN LINES

Characteristics of TSK-GEL Size Exclusion Column Lines

Column Line	TSK-GEL SW	TSK-GEL PW	TSK-GEL Alpha TSK-GEL SuperAW	TSK-GEL H
Particle Composition	Silica	Methacrylate	Polyvinyl	PS-DVB
No. of Available Pore Sizes	3	6	6	6
pH Stability	2.5 - 7.5	2.0 - 12.0	2.0 - 12.0	1.0 - 14.0
Solvent Compatibility	100% polar	50% polar	100% polar and nonpolar	100% nonpolar, limited polar
Max. Temperature	30°C	80°C*	80°C	60-80°C (H_{XL} and SuperHZ) 140°C (H_{HR} and SuperH)
Pressure (kg/cm^2)**	10-120	4-40	20-60	15-60
Application Focus	proteins	water-soluble polymers	intermediate polar polymers	organic-soluble polymers

* *Except for the TSKgel G-DNA-PW, which can be operated up to 50°C and the 55 mm ID TSK-GEL PW-type columns, which can be operated up to 60°C. When operating below 10°C, reduce the flow rate to ensure that the maximum pressure is not exceeded.*

** *Depends on column dimensions and particle size.*

Note: The operating conditions and specifications for each column are listed on the Operating Conditions and Specifications sheet (OCS) shipped with the column and in the Ordering Information section at the end of each section.

COLUMN SELECTION GUIDE FOR TSK-GEL GEL FILTRATION COLUMNS

Sample			*Column selection*	*Selection criteria*	
			First choice	*Alternative*	
Carbohydrates	polysaccharides		TSKgel GMPW$_{XL}$	G5000PW$_{XL}$ and G3000PW$_{XL}$	large pore size, linear calibration curve, small particles, high resolving power
	oligosaccharides		TSKgel G-Oligo-PW	G2500PW$_{XL}$	small particles, high resolving power
Nucleic acids	DNA fragments	large	TSKgel G-DNA-PW or TSKgel G5000PW$_{XL}$		large pore size, small particles, high resolving power
		medium and small	TSKgel G4000SW$_{XL}$, TSKgel BioAssist G4SW$_{XL}$, TSKgel SuperSW3000, or G3000SW$_{XL}$, TSKgel BioAssist G3SW$_{XL}$		suitable pore sizes
	RNA		TSKgel G4000SW$_{XL}$ TSKgel BioAssist G4SW$_{XL}$ TSKgel SuperSW3000, or G3000SW$_{XL}$, TSKgel BioAssist G3SW$_{XL}$		suitable pore sizes
	oligonucleotides		TSKgel G2500PW$_{XL}$		small pore size, ionic interaction
Proteins	normal size small-medium proteins		TSKgel SuperSW3000, G3000SW$_{XL}$, SKgel BioAssist G3SW$_{XL}$ TSKgel G4000SW$_{XL}$, TSKgel BioAssist G4SW$_{XL}$ TSKgel SuperSW2000, or G2000SW$_{XL}$, TSKgel BioAssist G2SW$_{XL}$	G3000PW$_{XL}$ or G4000PW$_{XL}$	small particles small to medium range pore sizes
	large proteins	low density lipoprotein	TSKgel G6000PW$_{XL}$ or TSKgel G5000PW$_{XL}$		large pore sizes
		gelatin	TSKgel GMPW$_{XL}$	G5000PW$_{XL}$ and G3000PW$_{XL}$	large pore size, linear calibration curve
Peptides	large		TSKgel SuperSW3000, G3000SW$_{XL}$, TSKgel BioAssist G3SW$_{XL}$ or G2000SW$_{XL}$, TSKgel BioAssist G2SW$_{XL}$	SuperSW2000 or G3000PW$_{XL}$	small to medium range pore size, versatile
	small		TSKgel G2500PW$_{XL}$	SuperSW2000 or G2000SW$_{XL}$	linear calibration curve, high resolving power
Viruses			TSKgel G6000PW$_{XL}$ or TSKgel G5000PW$_{XL}$		large pore size, high resolving power
Synthetic polymers			TSKgel GMPW$_{XL}$ or TSKgel Alpha-M	G5000PW$_{XL}$ and G3000PW$_{XL}$ or Alpha-5000 and Alpha-3000	large pore size, low adsorption, linear calibration curve
	cationic		TSKgel G3000PW$_{XL}$-CP, TSKgel G5000PW$_{XL}$-CP TSKgel G6000PW$_{XL}$-CP		medium to large pore size, low adsorption, linear calibration curve
Synthetic oligomers	nonionic		TSKgel G-Oligo-PW, TSKgel G2500PW$_{XL}$ or TSKgel Alpha-2500	G2500PW or SuperAW2500	small pore size, high resolving power
	anionic		TSKgel G2500PW$_{XL}$ or TSKgel Alpha-2500	G2500PW or SuperAW2500	small pore size, ionic interaction

SEC

TSK-GEL SW, SW_{XL} AND SUPERSW GEL FILTRATION COLUMNS

HIGHLIGHTS

- TSK-GEL SW-type columns (SW, SW_{XL} and SuperSW column lines) are all based on spherical silica particles with very high internal pore volumes.
- Silica particles in SW-type columns are chemically bonded with a stationary phase containing polar diol groups.
- SW-type columns feature low residual adsorption and very high pore volumes, which are essential characteristics of high performance gel filtration columns.
- SW and SW_{xl} columns lines are available in three pore size ranges with nominal pore sizes of 125 Å, 250 Å and 450 Å. SuperSW and QC-PAK column lines are available in 125 Å and 250 Å pores.
- SW columns are packed with 10 micron (G2000SW and G3000SW) or 13 micron (G4000SW) particles. SW_{XL} columns contain 5 micron ($G2000SW_{XL}$ and $G3000SW_{XL}$) or 8 micron ($G4000SW_{XL}$) particles. SuperSW columns contain 4 micron particles (SuperSW2000 and SuperSW3000)
- All SW-type columns are available in stainless steel hardware. SW and QC-PAK columns are also available in glass, while SW_{XL} columns are also available in PEEK hardware.

Recommendations for TSK-GEL SW series selection

Samples of unknown molecular weight

TSKgel $G3000SW_{XL}$ is the ideal scouting column. If the protein of interest elutes near the exclusion volume, then $G4000SW_{XL}$ is the logical next step. Conversely, if the protein of interest elutes near the end of the chromatogram, try the $G2000SW_{XL}$.

Proteins (general)

Choose one of the TSK-GEL SW_{XL} columns using the calibration curves on page 34 to select the appropriate pore size based on knowledge or estimate of protein size.

Monoclonal antibodies

TSKgel $G3000SW_{XL}$ is commonly used for quality control. TSKgel SuperSW3000 is utilized when sample is limited or at very low concentration.

Peptides

TSKgel $G2000SW_{XL}$ is the first selection for the analysis of peptides. TSKgel SuperSW2000 is utilized when sample is limited or at very low concentration.

Other

The use of TSK-GEL SuperSW columns requires optimization of the HPLC system with respect to extra-column band broadening. Capillary tubing ID, injection volume, detector cell volume, and detector time constant all need to be reduced to fully benefit from the high column efficiency and small peak volumes of the SuperSW columns. Use SW columns when not sample limited or when larger amounts of sample need to be isolated.

Properties and separation ranges for TSK-GEL SW-type packings

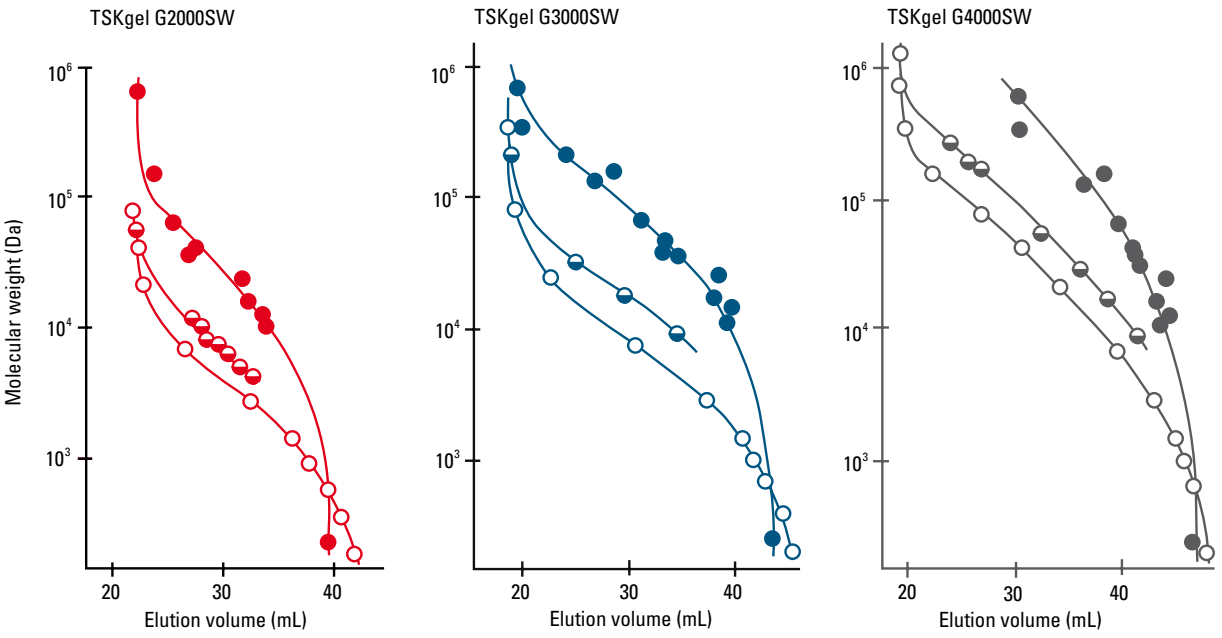
TSK-GEL packing	Particle Size (µm)	Pore Size (Å)	Molecular weight of sample (Da)		
			Globular proteins	Dextrans	Polyethylene glycols and oxides
SuperSW2000	4	125	$5 \times 10^3 – 1.5 \times 10^5$	$1 \times 10^3 – 3 \times 10^4$	$5 \times 10^2 – 15 \times 10^3$
$G2000SW_{XL}$/BioAssist $G2SW_{XL}$	5	125	$5 \times 10^3 – 1.5 \times 10^5$	$1 \times 10^3 – 3 \times 10^4$	$5 \times 10^2 – 15 \times 10^3$
QC-PAK TSK 200	5	125	$5 \times 10^3 – 1.5 \times 10^5$	$1 \times 10^3 – 3 \times 10^4$	$5 \times 10^2 – 15 \times 10^3$
G2000SW	10, 13, 20	125	$5 \times 10^3 – 1 \times 10^5$	$1 \times 10^3 – 3 \times 10^4$	$5 \times 10^2 – 15 \times 10^3$
SuperSW3000	4	250	$1 \times 10^4 – 5 \times 10^5$	$2 \times 10^3 – 7 \times 10^4$	$1 \times 10^3 – 3.5 \times 10^4$
$G3000SW_{XL}$/BioAssist $G3SW_{XL}$	5	250	$1 \times 10^4 – 5 \times 10^5$	$2 \times 10^3 – 7 \times 10^4$	$1 \times 10^3 – 3.5 \times 10^4$
QC-PAK TSK 300	5	250	$1 \times 10^4 – 5 \times 10^5$	$2 \times 10^3 – 7 \times 10^4$	$1 \times 10^3 – 3.5 \times 10^4$
G3000SW	10, 13, 20	250	$1 \times 10^4 – 5 \times 10^5$	$2 \times 10^3 – 7 \times 10^4$	$1 \times 10^3 – 3.5 \times 10^4$
$G4000SW_{XL}$/BioAssist $G4SW_{XL}$	8	450	$2 \times 10^4 – 7 \times 10^6$	$4 \times 10^3 – 5 \times 10^5$	$2 \times 10^3 – 2.5 \times 10^5$
G4000SW	13, 17	450	$2 \times 10^4 – 7 \times 10^6$	$4 \times 10^3 – 5 \times 10^5$	$2 \times 10^3 – 2.5 \times 10^5$

Data generated using the following conditions:
Columns: Two 4 µm, 4.6 mm ID x 30cm L TSK-GEL SuperSW columns in series; two 5 µm, 7.8 mm ID x 30 cm L TSK-GEL SW_{XL} columns in series; two 10 µm, 7.5 mm ID x 60 cm L TSK-GEL SW columns in series
Elution: Globular proteins: 0.3 mol/L NaCl in 0.1 mol/L (0.05 mol/L for SW_{XL} columns) phosphate buffer, pH 7.0
Dextrans and polyethylene glycols and oxides (PEOs): distilled water

CALIBRATION CURVES FOR TSK-GEL SW-TYPE GEL FILTRATION COLUMNS

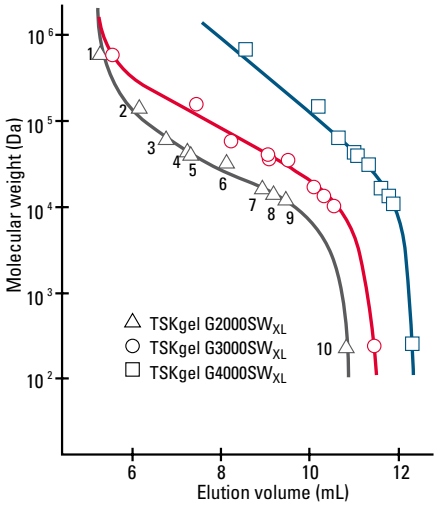
The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

Polyethylene oxide, dextran and protein calibration curves for TSK-GEL SW columns



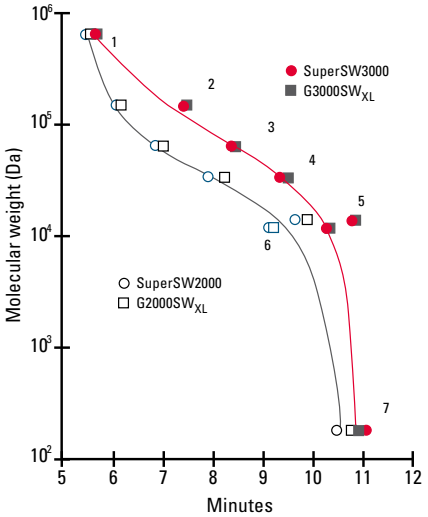
Column: TSK-GEL SW, two 7.5 mm ID x 60 cm L columns in series
Sample: ● proteins, ○ polyethylene oxides, ◒ dextrans
Elution: dextrans and polyethylene oxides: distilled water; proteins: 0.3 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.0
Flow Rate: 1.0 mL/min
Detection: UV @ 220 nm and RI

Protein calibration curves for TSK-GEL SW_{XL} columns



Column: TSK-GEL SW_{XL} columns, 5 or 8 μm ,7.8 mm ID x 30 cm L
Sample: 1. thyroglobulin (660,000 Da); 2. IgG (160,000 Da);
3. BSA (67,000 Da); 4. ovalbumin (43,000 Da);
5. peroxidase (40,200 Da); 6. β-lactoglobulin (18,400 Da);
7. myoglobin (16,900 D a); 8. ribonuclease A (12,600 Da);
9. cytochrome C (12,400 Da); 10. glycine tetramer (246 Da)
Elution: 0.3 mol/L NaCl in 0.1 mol/L sodium phosphate buffer, pH 7.0
Detection: UV @ 220 nm

Calibration curves for TSK-GEL SuperSW and SW_{XL}



Sample: proteins: 1. thyroglobulin (660,000 Da);
2. γ-globulin (150,000 Da); 3. BSA (67,000 Da);
4. β-lactoglobulin (18,400 Da); 5. lysozyme (14,500 Da);
6. cytochrome C (12,400 Da); 7. triglycine (189 Da)
Elution: 0.15 mol/L phosphate buffer (pH 6.8)
Flow Rate: 0.35 mL/min for SuperSW; 1.0 mL/min for SW_{XL}
Temperature: 25°C
Detection: UV @ 280 nm (220 nm for triglycine)

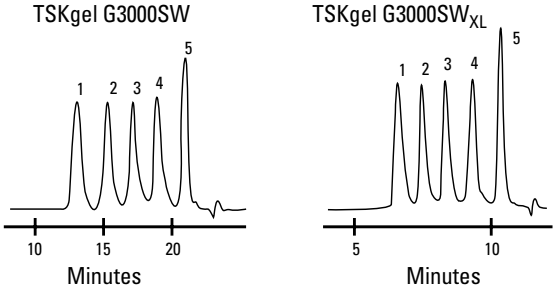
SEC

COMPARING TSK-GEL SW, SW_{XL} AND SUPERSW GEL FILTRATION COLUMNS

FIGURE 1 shows the increased resolution and faster separation time with a protein standard mixture on the TSK-GEL SW_{XL} compared to TSK-GEL SW. This is due to the smaller particle size (5 vs. 10 µm) of the TSK-GEL SW_{XL} packing material.
FIGURE 2 & FIGURE 3 show the increased resolution and sensitivity of the TSK-GEL SuperSW columns compared to TSK-GEL SW_{XL} columns. This is due to the smaller particle size (4 vs. 5 µm) coupled with a narrow column (4.6 mm ID).

FIGURE 1

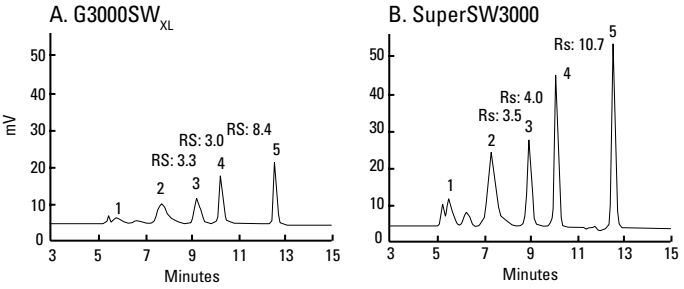
Higher resolution with 5µm TSK-GEL SW_{XL} compared with 10µm TSK-GEL SW columns



Column: Left: TSK-GEL SW, two 10µm, 7.5mm ID x 30cm columns in series
Right: TSK-GEL SW_{XL}, one 5µm, 7.8mm ID x 30cm column
Sample: 1. glutamate dehydrogenase (280 kDa)
2. lactate dehydrogenase (132 kDa)
3. enolase (67 kDa)
4. adenylate kinase (21 kDa)
5. cytochrome C (12,4 kDa)
Elution: 0.3 mol/L NaCl in 0.05 mol/L phosphate buffer pH 6.9
Flow Rate: 1.0mL/min
Detection: UV @ 220nm

FIGURE 2

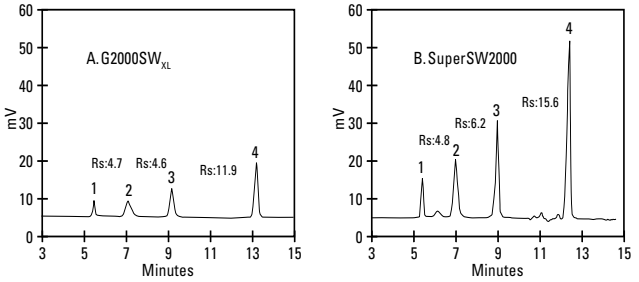
Comparison of TSKgel SuperSW3000 and TSKgel $G3000SW_{XL}$ for the separation of proteins



Column: A.TSKgel $G3000SW_{XL}$, 7.8mm ID x 30cm;
B. TSKgel SuperSW3000, 4.6mm ID x 30cm
Sample: 5µL of a mixture of 1. thyroglobulin, 0.5mg/mL (660,000Da);
2. γ-globulin, 1.0mg/mL; (150,000 Da);
3. ovalbumin, 1.0mg/mL (43,000Da);
4. ribonuclease A, 1.5mg/mL (12,600Da);
5. *p*-aminobenzoic acid, 0.01mg/mL (137Da)
Elution: 0.1mol/L $NaSO_4$ in 0.1mol/L in phosphate buffer with 0.05% NaN_3, pH 6.7
Flow Rate: 1.0mL/min for $G3000SW_{XL}$; 0.35mL/min for SuperSW3000
Temp: 25°C
Detection: UV @ 220nm

FIGURE 3

Comparison of TSKgel SuperSW2000 and TSKgel $G2000SW_{XL}$ for the separation of proteins



Column: A. TSKgel $G2000SW_{XL}$, 7.8mm ID x 30cm;
B. TSKgel SuperSW2000, 4.6mm ID x 30cm
Sample: 1. thyroglobulin (0.2mg/mL); 2. albumin (1.0mg/mL);
3. ribonuclease A (1.0mg/mL); 4. *p*-aminobenzoic acid (0.01mg/mL)
Inj. Volume: 5µL
Elution: 0.1mol/L phosphate buffer + 0.1mol/L Na_2SO_4 + 0.05% NaN_3 (pH 6.7)
Flow Rate: 0.35mL/min for SuperSW2000; 1.0mL/min for $G2000SW_{XL}$
Temp: 25°C
Detection: UV @ 280nm

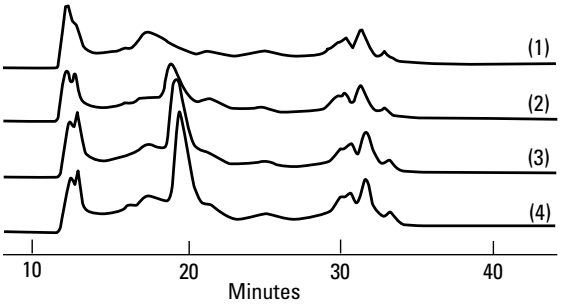
APPLICATIONS OF TSK-GEL SW-TYPE GEL FILTRATION COLUMNS

Proteins

The effect of different concentrations of surfactant on the separation of membrane proteins is seen in FIGURE 4. As the concentration of octaethyleneglycol dodecylether increases to 0.05%, the main peak becomes sharper and recovery increases. The TSKgel SuperSW3000 provides an excellent high resolution separation of IgG_1 from mouse ascites fluid as can be seen in FIGURE 5.

FIGURE 4

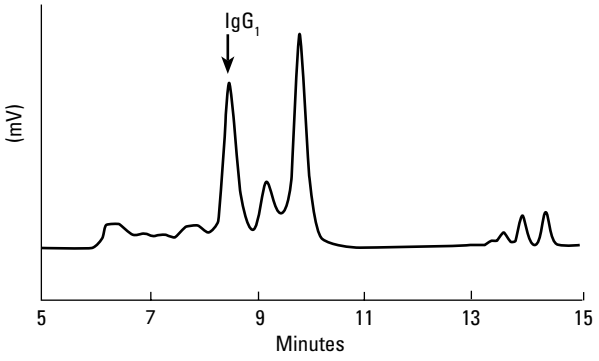
Separation of membrane protein by SEC with different surfactant concentration in the eluent



Column:	TSKgel G3000SW, 7.5mm ID x 60cm
Sample:	Membrane protein from a crude extract from rat liver microsome
Elution:	(0.2mol/L sodium chloride + 20% glycerol + octaethyleneglycol dodecylether) in 50mmol/L phosphate buffer, pH7.0. Note: concentration of surfactant: (1) 0.005%, (2) 0.01%, (3) 0.025%, (4) 0.05%
Flow Rate:	1.0mL/min
Detection:	UV @ 280nm

FIGURE 5

The separation of monoclonal antibody (IgG_1) from mouse ascites fluid



Column:	TSKgel SuperSW3000, 4.6mm ID x 30cm
Sample:	5µm of IgG_1 from mouse ascites
Elution:	0.2mol/L phosphate buffer, pH 6.7
Flow Rate:	0.35mL/min
Detection:	UV @ 280nm micro flow cell

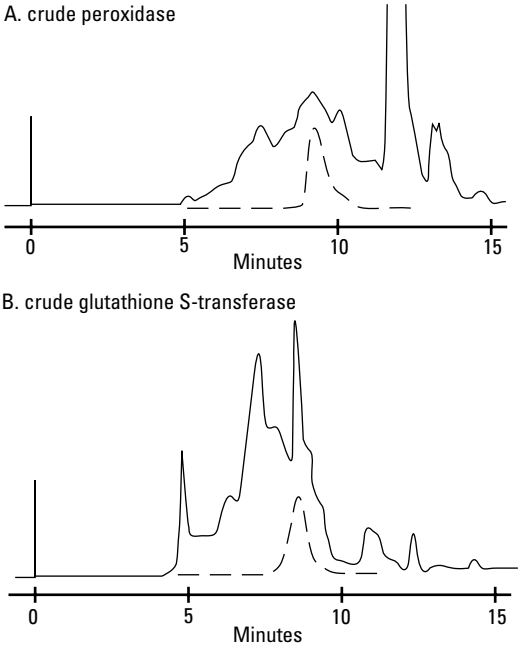
Enzymes

Mobile phase conditions in GFC are optimized to ensure little or no interaction of the sample with the packing material. This gentle technique allows for high recovery of enzymatic activity. For example, crude samples of peroxidase and glutathione S-transferase were separated in only 15 minutes on a TSKgel $G3000SW_{XL}$ column and activity recovery was 98% and 89%, respectively.

The elution profiles of the separations in FIGURE 6 show that all of the activity eluted in a narrow band of about 1.5 mL.

FIGURE 6

Separation of crude protein samples on TSKgel $G3000SW_{XL}$



Column:	TSKgel $G3000SW_{XL}$, 5µm, 7.8mm ID x 30cm
Sample:	A. crude peroxidase from Japanese radish, 0.15mg in 0.1mL B. crude glutathione S-transferase from guinea pig liver extract, 0.7mg in 0.1mL
Elution:	0.3mol/L NaCl in 0.05mol/L phosphate buffer, pH 7
Flow Rate:	1.0mL/min
Detection:	UV @ 220nm (solid line) and enzyme assay tests (dashed line)
Recovery:	enzymatic activity recovered was 98% in A and 89% in B

SEC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Glass columns								
16214	QC-PAK GFC 200GL	8.0	15	5	≥ 10,000	0.5 - 1.0	1.2	40
16216	QC-PAK GFC 300GL	8.0	15	5	≥ 10,000	0.5 - 1.0	1.2	40
08799	G2000SW, Glass	8.0	30	10	≥ 10,000	0.4 - 0.8	0.8	20
08800	G3000SW, Glass	8.0	30	10	≥ 10,000	0.4 - 0.8	0.8	20
08801	G4000SW, Glass	8.0	30	13	≥ 8,000	0.4 - 0.8	0.8	20
14464	G3000SW, Glass	20.0	30	13	≥ 6,000	3.0 -6.0	8.0	8
Stainless steel columns								
18674	SuperSW2000	4.6	30	4	≥ 30,000	0.1 -0.35	0.4	120
21845	SuperSW3000 -NEW-	1.0	30	4	≥ 18,000	0.016	0.02	120
21485	SuperSW3000 -NEW-	2.0	30	4	≥ 25,000	0.065	0.075	120
18675	SuperSW3000	4.6	30	4	≥ 30,000	0.1 - 0.35	0.4	120
08540	G2000SW$_{XL}$	7.8	30	5	≥ 20,000	0.5 -1.0	1.2	70
08541	G3000SW$_{XL}$	7.8	30	5	≥ 20,000	0.5 - 1.0	1.2	70
08542	G4000SW$_{XL}$	7.8	30	8	≥ 16,000	0.5 - 1.0	1.2	35
16215	QC-PAK GFC 200	7.8	15	5	≥ 10,000	0.5 -1.0	1.2	40
16049	QC-PAK GFC 300	7.8	15	5	≥ 10,000	0.5 -1.0	1.2	40
05788	G2000SW	7.5	30	10	≥ 10,000	0.5 -1.0	1.2	20
05789	G3000SW	7.5	30	10	≥ 10,000	0.5 -1.0	1.2	25
05790	G4000SW	7.5	30	13	≥ 8,000	0.5 -1.0	1.2	15
05102	G2000SW	7.5	60	10	≥ 20,000	0.5 -1.0	1.2	40
05103	G3000SW	7.5	60	10	≥ 20,000	0.5 -1.0	1.2	50
05104	G4000SW	7.5	60	13	≥ 16,000	0.5 -1.0	1.2	30
06727	G2000SW	21.5	30	10	≥ 10,000	3.0 -6.0	8.0	10
06728	G3000SW	21.5	30	10	≥ 10,000	3.0 -6.0	8.0	15
06729	G4000SW	21.5	30	13	≥ 8,000	3.0 - 6.0	8.0	10
05146	G2000SW	21.5	60	13	≥ 20,000	3.0 -6.0	8.0	20
05147	G3000SW	21.5	60	13	≥ 20,000	3.0 -6.0	8.0	30
05148	G4000SW	21.5	60	17	≥ 16,000	3.0 -6.0	8.0	20
07428	G2000SW	55.0	30	20	≥ 400	25.0 - 40.0	50.0	10
07481	G3000SW	55.0	30	20	≥ 4,000	15.0 -25.0	50.0	10
07429	G2000SW	55.0	60	20	≥ 750	20.0 -30.0	35.0	15
07482	G3000SW	55.0	60	20	≥ 9,000	15.0 - 25.0	50.0	15
PEEK Columns								
20027	BioAssist G2SW$_{XL}$	7.8	30	5	≥ 20,000	0.5 - 1.0	1.2	70
20026	BioAssist G3SW$_{XL}$	7.8	30	5	≥ 20,000	0.5 - 1.0	1.2	70
20025	BioAssist G4SW$_{XL}$	7.8	30	8	≥ 16,000	0.5 - 1.0	1.2	35

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	
Guard column products					
08805	SW Guard column, Glass	8.0	4.0		For all 8 mm ID SW glass columns
14465	SW Guard column, Glass	20.0	4.0		For P/N 14464 SW glass columns
18762	SuperSW Guard column	4.6	3.5		For 4.6 mm ID SuperSW columns (contains SuperSW3000 packing)
08543	SW_{XL} Guard column	6.0	4.0		For all SW_{XL} columns and P/Ns 16215 and 16049 (contains 3000SW_{XL} packing)
18008	SW_{XL} Guard column, PEEK	6.0	4.0		For all BioAssist SW_{XL}, PEEK columns
05371	SW Guard column	7.5	7.5		For all 7.5 mm ID SW columns (contains 3000SW packing)
05758	SW Guard column	21.5	7.5		For all 21.5 mm ID SW columns
07427	SW Guard column	45.0	5.0		For 55 mm ID SW columns
Bulk packing					
08544	SW_{XL} Top-Off, 1g wet gel			5	For SW_{XL} and QC-PAK columns
06819	SW Top-Off, 1g wet gel			10	For all 7.5 mm ID SW columns

SEC

TSK-GEL PW and TSK-GEL PW$_{XL}$ columns

Gel Filtration Chromatography of water soluble polymers

HIGHLIGHTS

- Hydrophilic, rigid, spherical, porous methacrylate beads
- Excellent chemical and mechanical stability
- pH range of 2 to 12, with up to 50% organic solvent
- Temperatures up to 80°C (50°C for TSKgel G-DNA-PW)
- Wide separation range up to 8 x 10^6 Da for linear polymers
- PEEK column hardware available for G6000PW packings for ultra-low sample adsorption during virus analysis
- Available in analytical, semi-preparative and preparative stainless steel columns TSK-GEL
- New PW$_{XL}$-CP columns designed for low salt SEC separations of cationic polymers.

Polymeric TSK-GEL PW and TSK-GEL PW$_{XL}$ columns are designed for GFC of water soluble organic polymers, polysaccharides, oligosaccharides, DNA and RNA. For the analysis of proteins and peptides SW type columns are recommended.

A number of specialty columns include columns for samples with a broad molecular weight range, oligosaccharides, DNA, and RNA. For analytical purposes the TSK-GEL PW$_{XL}$ columns are preferred. For preparative work, or for other cases in which large amounts of sample must be used, the 60 cm TSK-GEL PW columns are recommended because of their increased loading capacity. TSK-GEL PW$_{XL}$-CP columns are especially suited for the separation of cationic polymers.

PROPERTIES AND SEPARATION RANGES FOR TEK-GEL PW-TYPE PACKINGS

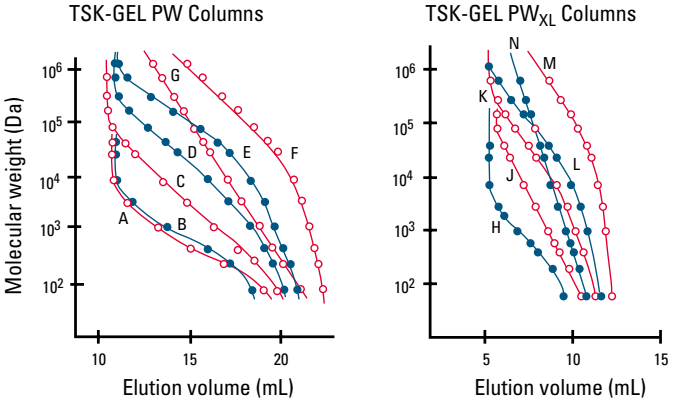
			Molecular weight of sample (Da)		
TSK-GEL packing	**Particle Size* (µm)**	**Pore Size (Å)**	**Polyethylene glycols and oxides**	**Dextrans****	**Globular proteins****
G2000PW	10	125	< 2 x 10^3		< 5 x 10^3
G2500PW$_{XL}$	7	< 200		< 3 x 10^3	< 8 x 10^3
G2500PW	12, 17, 20	< 200	< 3 x 10^3		
G3000PW$_{XL}$	7	200	< 5 x 10^4	< 6 x 10^4	5 x 10^2 - 8 x 10^5
G3000PW	10, 17, 20	200	< 5 x 10^4		
G3000PW$_{XL}$-CP	7	< 200	< 5 x 10^4		
G4000PW$_{XL}$	10	500	< 3 x 10^5	1 x 10^3 - 7 x 10^5	1 x 10^4 - 1.5 x 10^6
G4000PW	17, 20	500	< 3 x 10^5		
G5000PW$_{XL}$	10	1000	< 1 x 10^6	5 x 10^4 - 2.5 x 10^6	< 1 x 10
G5000PW	17, 20	1000	< 1 x 10^6		
G5000PW$_{XL}$-CP	10	< 1000	< 5 x 10^4		
G6000PW$_{XL}$	13	> 1000	< 8 x 10^6	5 x 10^5 - 5 x 10^7	< 2 x10^8
G6000PW / BioAssist G6PW	17	> 1000	< 8 x 10^6		
G6000PW$_{XL}$-CP	13	> 1000	< 8 x 10^6		
GMPW$_{XL}$	13	< 100 - 1000	5 x 10^2 - 8 x 10^6	< 5 x 10^7	< 2 x 10^8
GMPW	17	< 100 - 1000	5 x 10^2 - 8 x 10^6		
G-Oligo-PW	7	125	< 3 x 10^3		< 3 x 10^3
G-DNA-PW	10	> 1000	< 8 x 10^6	<5 x 10^7	< 2 x 10^8

Column: *TSK-GEL PW columns, 7.5 mm ID x 60 cm L; TSKgel PW$_{XL}$, TSKgel PW$_{XL}$-CP, G-Oligo-PW & G-DNA-PW, 7.8 mm ID x 30 cm L*
Elution: *Polyethylene glycols and oxides: distilled water; dextrans and proteins: 0.2 mol/L phosphate buffer, pH 6.8*
Flow Rate: *1.0 ml/min*
Note: **Larger particle sizes of each group are for 21.5 mm ID x 60 cm L semi-preparative and 55 mm or 108 mm ID x 60 cm L preparative columns.* ***Maximum separation range determined from estimated exclusion limits.*

CALIBRATION CURVES FOR TSK-GEL PW-TYP COLUMNS

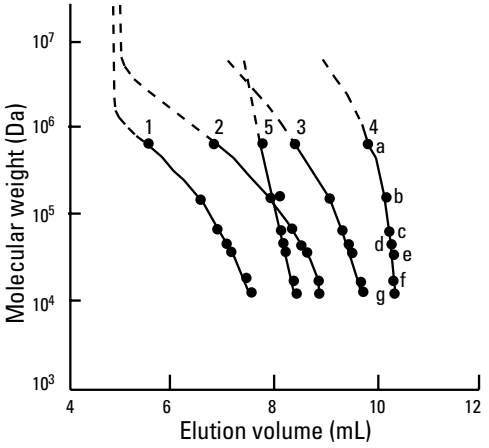
The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

Polyethylene glycol and oxide calibration curves on TSK-GEL PW and TSK-GEL PW_{XL} columns



Column: TSK-GEL PW columns: A. G2000PW, B. G2500PW, C. G3000PW D. G4000PW, E. G5000PW, F. G6000PW, G. GMPW, all 7.5mm ID x 60 cm
TSK-GEL PW_{XL} columns: H. $G2500PW_{XL}$, J. $G3000PW_{XL}$, K. $G4000PW_{XL}$, L. $G5000PW_{XL}$, M. $G6000PW_{XL}$, N. $GMPW_{XL}$, all 7.8 mm ID x 30 cm
Elution: distilled water
Flow Rate: 1.0 m L/min
Detection: RI

Protein calibration curves on TSK-GEL PW_{XL} columns

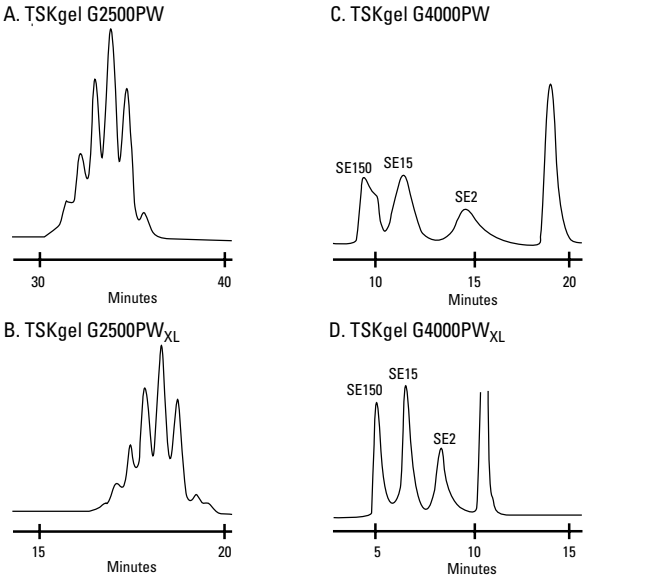


Column: 1. $G3000PW_{XL}$, 2. $G4000PW_{XL}$, 3. $G5000PW_{XL}$, 4. $G6000PW_{XL}$, 5. $GMPW_{XL}$
Sample: a. thyroglobulin (660,000 Da), b. γ-globulin (150,000 Da), c. albumin (67,000 Da), d. ovalbumin (43,000 Da), e. β-lactoglobulin (36,000 Da), f. myoglobin (16,900 Da), g. cytochrome C (12,400 Da)
Elution: 0.2 mol/L phosphate buffer (pH 6.8)
Flow Rate: 1.0 mL/min
Detection: UV @ 280 nm

COMPARISON BETWEEN TSK-GEL PW AND TSK-GEL PWXL

The smaller particle sizes of the TSK-GEL PW_{XL} columns provide almost 1.5 times the resolution of their TSK-GEL PW counterparts. With shorter TSK-GEL PW_{XL} columns, similar or higher resolution separations are possible in less than half the time.

Faster analysis and higher resolution with TSK-GEL PW_{XL} columns



Column: A. TSKgel G2500PW, two 10µm, 7.5mm ID x 60cm columns in series
B. TSKgel $G2500PW_{XL}$, two 6µm, 7.5mm ID x 60cm columns in series
C. TSKgel G4000PW, 17µm, 7.5mm ID x 60cm
D. TSKgel $G4000PW_{XL}$, 10µm, 7.8mm ID x 30cm
Sample: A. & B.: polyethylene glycol 200
C. & D.: polyethylene oxide standards: SE-150, SE-15 and SE-2 in 100µL
Elution: A. & B.: distilled water; C. & D.: 0.1mol/L NaCl
Flow Rate: 1.0mL/min
Temp.: A. & B.: 25°C; C. & D.: 50°C
Detection: RI

SEC

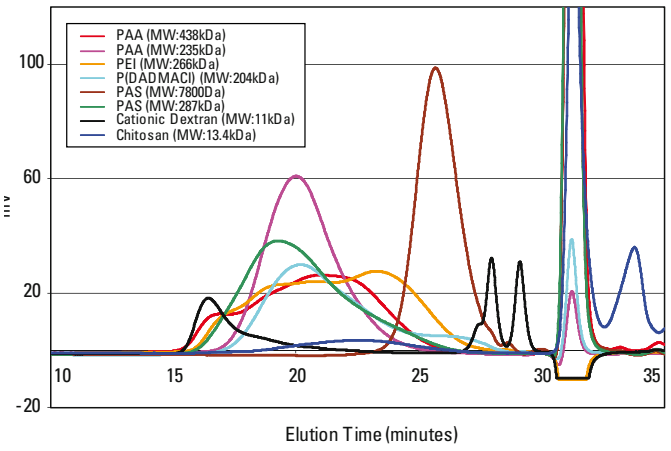
COLUMNS FOR SPECIFIC APPLICATIONS

TSK-GEL PWXL-CP

The new TSK-GEL PW_{XL}-CP columns are designed to facilitate the separation of cationic polymers by SEC at low salt conditions. They are based on the well known PW-type of polymeric resins for aqueous SEC. Cationic surface modification enables low salt elution of cationic polymers with high recoveries. The columns show high theoretical plate numbers, linear calibration curves and high durability. They are produced with three pore sizes for diffrent ranges (G3000-, G5000- and G6000PW_{XL}-CP). FIGURE 7 shows the analysis of various cationic polymers on a series of TSKgel PW_{XL}-CP columns.

FIGURE 7

Separation of cationic polymers



Columns:	TSKgel G3000PW_{XL}-CP, 7µm (7.8mm ID x 30cm), TSKgel G5000PW_{XL}-CP, 10µm (7.8mm ID x 30cm), TSKgel G6000PW_{XL}-CP, 13µm (7.8mm ID x 30cm)
Eluent:	0.1mol/L $NaNO_3$
Flow Rate:	1mL/min
Detection:	RI
Temperature:	25°C
Sample Load:	3g/L, 100µL

TSK-GEL G-Oligo-PW

The specialty column TSKgel G-Oligo-PW is designed for high resolution separations of nonionic and cationic oligomers. Because of the presence of residual cationic groups, this column is not recommended for separating anionic materials. The polyethylene glycol and polythylene oxide calibration curves for TSKgel G-Oligo-PW (not shown) are identical to the calibration curve for TSKgel G2500PW_{XL} (shown on the previous page).

TSK-GEL G-DNA-PW

The TSKgel G-DNA-PW column is dedicated to the separation of large polynucleotides, such as DNA and RNA fragments of 500 to 5,000 base pairs. The exclusion limits for double-stranded DNA fragments are lower than those for rRNAs, indicating that double-stranded DNA fragments have a larger molecular size in solution than rRNAs of the same molecular weight. The packing of the TSKgel G-DNA-PW column has very large pores (>1000 Å) and a small particle size (10 µm).

For the separation of large DNA fragments greater than 1,000 base pairs, a four-column system is typically required. Baseline resolution of DNA fragments up to 7,000 base pairs can be achieved, provided there is a two-fold difference in the chain length of the fragments.

TSK-GEL GMPW and TSK-GEL GMPWXL

When the molecular weight range of the sample is broad or unknown, Tosoh Bioscience offers two mixed-bed columns, TSKgel GMPW and TSKgel GMPW$_{XL}$, for analysis. The TSKgel GMPW column and its high resolution counterpart, TSKgel GMPW$_{XL}$, are packed with the G2500, G3000 and G6000 PW or corresponding PW_{XL} resins. They offer a broad molecular weight separation range. As shown on the previous page, the calibration curve for polyethylene glycols and oxides on these mixed-bed columns is fairly shallow and is linear over the range of 100-1,000,000 Da.

The introduction of mixed-bed columns has made the problems of analyzing polydisperse samples much easier. Previously, many two-column systems such as TSKgel G3000PW and TSKgel G6000PW, were required to achieve good resolution with wide MW-range samples. The substitution of a TSK-GEL GMPW series column can save both time and money compared with multi-column systems.

OPTIMIZING GEL FILTRATION WITH TSK-GEL PW AND TSK-GEL PW$_{XL}$ COLUMNS

Selecting Mobile Phase Buffers

SEC separation is based on the difference of apparent molecular size with no additional interaction between the column matrix and the sample molecules. In practice, however, a small number of weakly charged groups on the surface of PW-type packings can cause changes in elution order from that of an ideal system. The eluent composition can vary greatly with TSK-GEL PW columns to be compatible with a wide range of neutral, polar, anionic, and cationic samples. The table below lists appropriate eluents for GFC of major polymer types.

For some nonionic, nonpolar polymers, such as polyethylene glycols, ideal size exclusion behavior can be obtained by using distilled water. More polar ionic polymers may exhibit abnormal peak shapes or minor peaks near the void volume when eluted with distilled water, due to ionic interactions between the sample and residual charged groups on the resin surface. To eliminate ionic interactions, a neutral salt such as sodium nitrate or sodium sulfate should be added. Generally, a salt concentration of 0.1 to 0.5 mol/L is sufficient to overcome undesirable ionic interactions.

Hydrophobic Samples

TSK-GEL PW-type resins are more hydrophobic than polysaccharide gels such as cross-linked dextran. Depending on the sample, this can lead to hydrophobic interaction as a secondary retention mechanism. The extent of hydrophobic interaction increases as the salt concentration of the eluent increases, but it can be reduced by the addition of an organic modifier such as acetonitrile. Water-soluble organic solvents are frequently used as modifiers to suppress hydrophobic interactions between the sample and the resin surface.

Modifiers are also used for optimizing the elution of both charged and neutral hydrophobic polymers. Typical examples for a variety of sample types are given in the table below. All TSK-GEL PW-type column packings are compatible with 20 % aqueous solutions of methanol, ethanol, propanol, acetonitrile, dimethyl formamide, dimethyl sulfoxide, formic acid, and acetic acid. In addition, these columns can be operated in 50 % aqueous acetone.

RECOMMENDED ELUENTS FOR GFC OF WATER-SOLUBLE POLYMER ON TSK-GEL PW-TYPE COLUMNS

Type of polymer	Typical sample	Suitable eluent
Nonionic hydrophilic	polyethylene glycol soluble starch, methyl cellulose, pullulan dextran, hydroxyethyl cellulose, polyvinyl alcohol, polyacrylamide	distilled water 0.01N NaOH 20% DMSO Buffer or salt solution (e.g., 0.1–0.5 mol/L $NaNO_3$)
Nonionic hydrophobic	polyvinylpyrrolidone	Buffer or salt solution with organic solvent (e.g., 20% ACN in 0.1mol/L $NaNO_3$)
Anionic hydrophilic	sodium chondroitin sulfate, sodium alginate, carboxymethyl cellulose, sodium polyacrylate, sodium hyaluronate	Buffer or salt solution (e.g., 0.1 mol/L $NaNO_3$)
Anionic hydrophobic	sulfonated lignin sodium salt, sodium polystyrenesulfonate	Buffer or salt solution with organic solvent (e.g., 20% ACN in 0.1 mol/L $NaNO_3$)
Cationic hydrophilic	glycol chitosan, DEAE-dextran, poly(ethyleneimine), poly(trimethylaminoethyl methacrylate) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L Na_2SO_4, or 0.8 mol/L $NaNO_3$ (0.1 mol/L $NaNO_3$ for PW$_{XL}$-CP type)
Cationic hydrophobic	poly(4-vinylbenzyltrimethylammonium chloride), poly(N-methyl-2-vinylpyridinium) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L Na_2SO_4
Amphoteric hydrophilic	peptides, proteins, poly-and oligosaccharides, DNA, RNA	Buffer or salt solution (e.g., 0.1 mol/L $NaNO_3$)
Amphoteric hydrophobic	blue dextran, collagen, gelatin, hydrophobic proteins hydrophobic peptides	Buffer or salt solution with organic solvent (e.g., 20% ACN in 0.1 mol/L $NaNO_3$ or 35–45% ACN in 0.1% TFA)

SEC

APPLICATIONS OF TSK-GEL PW-TYPE GEL FILTRATION COLUMNS

Nucleic acids

Desalting of nucleosides can be accomplished using TSKgel G2500PW$_{XL}$, as depicted in FIGURE 8. Clearly, adenosine elutes after the void volume in the unbuffered water mobile phase.

Polysaccharides

TSK-GEL PW columns are recommended for polysaccharide analysis due to their ability to separate a wide molecular weight distribution. Nonionic polysaccharides are the least complicated molecules to analyze by SEC because they seldom exhibit secondary interactions with the solid support. TSKgel G5000PW and TSKgel G3000PW in series are effective for the characterization of clinical dextran.

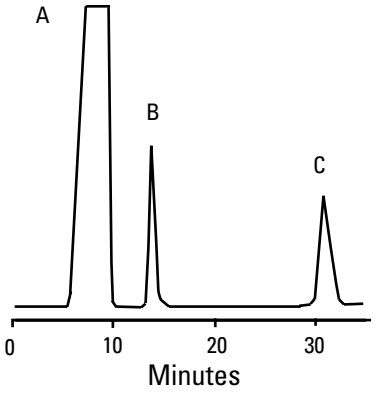
Cationic samples can be adsorbed on the resin by electrostatic interaction. If the polymer is strongly cationic, a fairly high salt concentration is required to prevent ionic interactions with conventional SEC packings. A mobile phase of 0.5 mol/L acetic acid with 0.3 mol/L Na_2SO_4 can also be used.

The new TSKgel PW$_{XL}$-CP series enables elution of water soluble, cationic polymers under low salt conditions (e.g. 0.1 mol/L $NaNO_3$).

An effective separation of the anionic hydrophilic glucosaminoglycan, hydraluronic acid, is shown in FIGURE 9 on a TSKgel G6000PW and TSKgel G4000PW column in series with a 0.2 mol/L sodium chloride mobile phase.

FIGURE 8

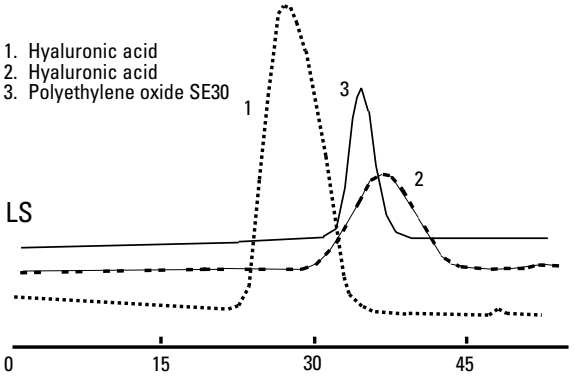
Desalting of nucleosides



Column: TSKgel G2500PW$_{XL}$, 7.8mm ID x 30cm
Sample: A. 0.5mol/L NaCl; B. uridine; C. adenosine
Eluent: distilled water
Flow Rate: 1.0mL/min
Detection: UV @ 260nm

FIGURE 9

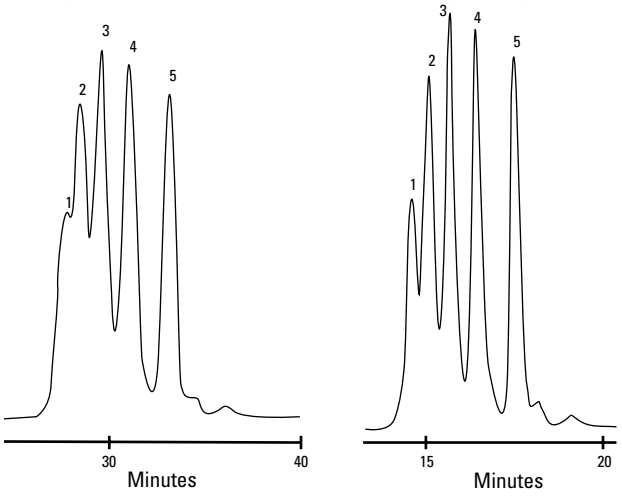
Separation of hyaluronic acid



Column: TSKgel G6000PW + G4000PW, two 7.5mm ID x 60cm columns in series
Sample: hyaluronic acid
Elution: 0.2mol/L NaCl
Flow Rate: 0.9mL/min
Temp: 40°C

FIGURE 10

Faster analysis and higher resolution of chito-oligosaccharides on a TSKgel G-Oligo-PW column



Column: A. TSKgel G2000PW, two 10µm, 7.5mm ID x 60cm columns in series
B. TSKgel G-Oligo-PW, two 6µm, 7.8mm ID x 30cm columns in series
Sample: 1. chitohexaose, 2. chitopentaose, 3. chitotetraose, 4. chitotriose, 5. chitobiose.
Elution: distilled water
Flow Rate: 1.0mL/min
Detection: RI

Oligomers

The TSKgel G-Oligo-PW column is designed for high resolution separations of nonionic and cationic oligomers. FIGURE 10 demonstrates excellent resolution of chito-oligosaccharides obtained by using the smaller, 6 µm particle size packing in TSKgel G-Oligo-PW columns as compared with the resolution obtained with a TSKgel G2000PW column. The pore sizes in both TSKgel G-Oligo-PW and TSKgel G2000PW columns are about 125 Å and both resins bear approximately 0.2 µeq/mL of cationic groups. Because of the presence of cationic groups, neither column is recommended for separating anionic materials. However, for nonionic oligomers, TSKgel G-Oligo-PW columns provide higher resolution than TSKgel G2500PW$_{XL}$ columns.

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Stainless steel columns								
08031	G-Oligo-PW	7.8	30	7	≥ 14,000	0.5 - 0.8	1.0	40
08032	G-DNA-PW	7.8	30	10	≥ 10,000	0.2 - 0.5	0.6	20
08020	G2500PW$_{XL}$	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	40
08021	G3000PW$_{XL}$	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	40
08022	G4000PW$_{XL}$	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	20
08023	G5000PW$_{XL}$	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	20
08024	G6000PW$_{XL}$	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	20
08025	GMPW$_{XL}$	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	20
21873	G3000PW$_{XL}$-CP	7.8	30	7	≥ 16,000		1.0	55
21874	G5000PW$_{XL}$-CP	7.8	30	10	≥ 10,000		1.0	25
21875	G6000PW$_{XL}$-CP	7.8	30	13	≥ 7,000		1.0	20
05761	G2000PW	7.5	30	12	≥ 5,000	0.5 - 1.0	1.2	20
08028	G2500PW	7.5	30	12	≥ 5,000	0.5 - 1.0	1.2	20
05762	G3000PW	7.5	30	12	≥ 5,000	0.5 - 1.0	1.2	20

SEC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
05763	G4000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.2	10
05764	G5000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.2	10
05765	G6000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.2	10
08026	GMPW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.2	10
05105	G2000PW	7.5	60	12	≥ 10,000	0.5 - 1.0	1.2	40
08029	G2500PW	7.5	60	12	≥ 10,000	0.5 - 1.0	1.2	40
05106	G3000PW	7.5	60	12	≥ 10,000	0.5 - 1.0	1.2	40
05107	G4000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	1.2	20
05108	G5000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	1.2	20
05109	G6000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	1.2	20
08027	GMPW	7.5	60	17	≥ 6,000	0.5 - 1.0	1.2	20
08030	G2500PW	21.5	60	17	≥ 10,000	1.6 - 6.0	8.0	20
05151	G3000PW	21.5	60	17	≥ 10,000	1.6 - 6.0	8.0	20
05152	G4000PW	21.5	60	20	≥ 6,000	1.6 - 6.0	8.0	10
05153	G5000PW	21.5	60	20	≥ 6,000	1.6 - 6.0	8.0	10
07926	G3000PW	55.0	60	20	ND	15.0 - 25.0	30.0	15
07927	G5000PW	55.0	60	20	≥ 5,000	15.0 - 25.0	30.0	15
PEEK columns								
20024	BioAssist G6PW	7.8	30	17	≥ 3,000	0.5 - 1.0	1.2	10
Guard columns								
08034	Oligo Guard column	6.0	4.0	13	For 7.8 mm ID G-Oligo-PW columns			
08033	PW_{XL} Guard column	6.0	4.0	12	For 7.8 mm ID PW_{XL} and G-DNA-PW columns (contains TSKgel G3000PW packing)			
21876	PW_{XL}-CP Guard column	6.0	4.0	13	For 7.8 mm ID PW_{XL} -CP columns			
06763	PW Guard column	7.5	7.5	13	For 7.5 mm ID G1000PW and G2000PW columns (contains TSKgel G2000PW packing)			
06762	PW Guard column	7.5	7.5	13	For 7.5 mm ID G2500PW through GMPW columns			
06758	PW Guard column	21.5	7.5	17	For 21.5 mm ID G2500PW through G5000PW columns			
07924	PW Guard column	45.0	5.0	20	For 55 mm ID G3000PW through G5000PW columns			
Bulk packing								
08035	PW_{XL} Top-Off, 1 g wet resin			10	For all PW_{XL} and G-DNA-PW columns			

TSK-GEL ALPHA AND SUPERAW GEL FILTRATION COLUMNS

Gel Filtration and Gel Permeation Chromatography of water-soluble and polar organic-soluble polymers

HIGHLIGHTS

- A unique hydrophilic, polyvinyl resin is available in conventional column dimensions (Alpha) and high throughput column format (SuperAW).
- Exhibits strong mechanical stability and minimal swelling characteristics
- A wide range of solvent compatibility, from 100% water to 100% non-polar organic solvents
- The reduced particle size and shorter column length of TSK-GEL SuperAW columns provide equivalent resolution in one half the time for high throughput applications.
- Unlike polystyrene-divinylbenzene (PS-DVB) resins that may adsorb polymers due to hydrophobic interaction, both the TSK-GEL Alpha and SuperAW columns allow for the separation of polymers soluble in methanol.
- Provide accurate molecular weight determination of samples in dimethyl formamide and exhibit normal retention of polystyrene polymers
- System peaks from salts in the eluent elute away from the oligomer of interest, providing accurate MW determinations.

Column Selection

The TSK-GEL Alpha Series consists of six columns with three particle sizes: 7, 10, and 13 µm. These columns span a wide MW separation range from 100 to more than 1 x 10^6 Da when using polyethylene oxide (PEO) as a MW standard. Exclusion limits for the TSK-GEL Alpha columns for polyethylene oxide (PEO), polyethylene glycols (PEG) and polystyrenes (PS) are shown in the table below. Calibration curves for the TSK-GEL Alpha Series columns are shown on the next page for polyethylene oxide, polyethylene glycol and polystyrene standards.

The TSK-GEL SuperAW series contains a similar chemistry as the TSK-GEL Alpha series but offers the benefit of smaller particle sizes (4 µm to 9 µm) and smaller column dimensions. Reductions in analysis time and mobile phase consumption make SuperAW columns ideal for high throughput applications. TSK-GEL Alpha and SuperAW columns are offered in 5 discrete exclusion ranges and 1 mixed bed. Both column types can accommodate polymer standards up to several million Dalton molecular weight (see calibration curves on the next page).

Exclusion limits for TSK-GEL Alpha Series and SuperAW Series columns

Column	Particle Size (µm)	Exclusion limit (Da) for various standards and eluents		
		PEO[a]/H_2O	PS[b]/10mmol/L LiBr in DMF	PEG[c]/10mmol/L LiBr in MeOH
Alpha-2500	7	5 x 10^3	1 x 10^4	1 x 10^4
Alpha-3000	7	9 x 10^4	1 x 10^5	6 x 10^4
Alpha-4000	10	4 x 10^5	1 x 10^6	3 x 10^6
Alpha-5000	10	1 x 10^6	7 x 10^6	N.D.
Alpha-6000	13	> 1 x 10^7	> 1 x 10^7	N.D.
Alpha-M	13	> 1 x 10^7	> 1 x 10^7	N.D.
SuperAW2500	4	5 x 10^3	8 x 10^3	1 x 10^4
SuperAW3000	4	9 x 10^4	8 x 104	1 x 10^5
SuperAW4000	6	1 x 10^6	6 x 10^5	6 x 10^5
SuperAW5000	7	1 x 10^{6*}	N.D.	N.D.
SuperAW6000	9	1 x 10^{7*}	N.D.	N.D.
SuperAWM-H	9	1 x 10^{7*}	N.D.	N.D.

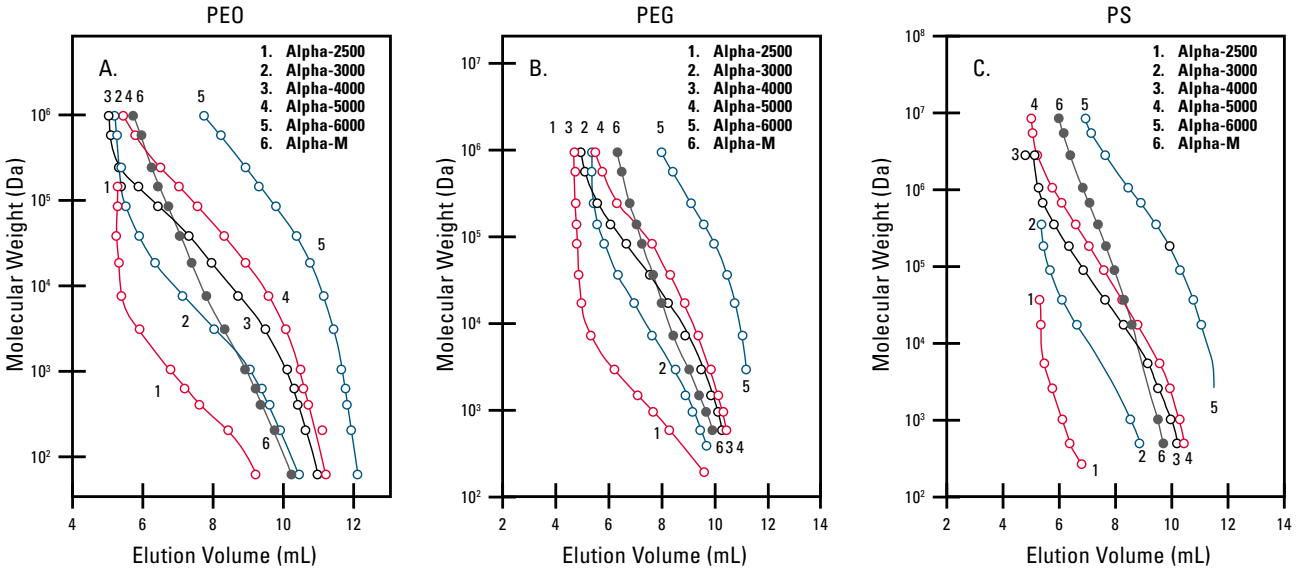
N.D. = not determined a Polyethylene oxide b Polystyrene divinyl benzene c Polyethylene glycol
* Exclusion limit for SuperAW5000, SuperAW6000, and SuperAWM-H are estimated, respectively

SEC

CALIBRATION CURVES FOR TSK-GEL ALPHA AND SUPERAW GEL FILTRATION COLUMNS

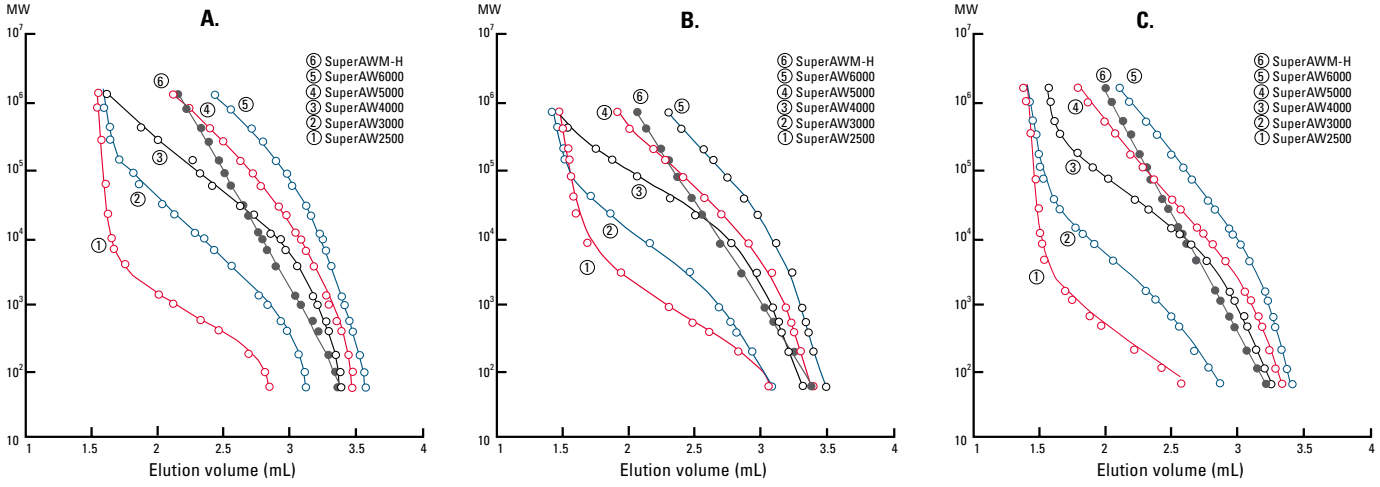
The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

Polyethylene oxide (PEO), polyethylene glycol (PEG) and polystyrene (PS) calibration curves for TSK-GEL Alpha columns



Column: TSK-GEL Alpha Series, 7.8 mm ID x 30 cm L
Eluent: A. H_2O; B. 10 mmol/L LiBr in Methanol; C. 10 mmol/L LiBr in DMF
Flow Rate: 1.0 mL/min
Temperature: A. 25°C; B. 25°C; C. 40°C
Detection: RI

Calibration curves for TSK-GEL SuperAW Series in different solvents with different polarity



Column: TSK-GEL SuperAW Series (6.0 mm ID x 15 cm L)
Eluent: A. Water; B. MeOH containing 10 mmol/L LiBr; C. DMF containing 10 mmol/L LiBr
Flow rate: 0.6 mL/min
Temperature: 25°C
Detection: Refractive index detector
Samples: Standard polyethylene oxide, polyethylene glycol, ethylene glycol

APPLICATIONS OF TSK-GEL ALPHA AND SUPERAW GEL FILTRATION COLUMNS

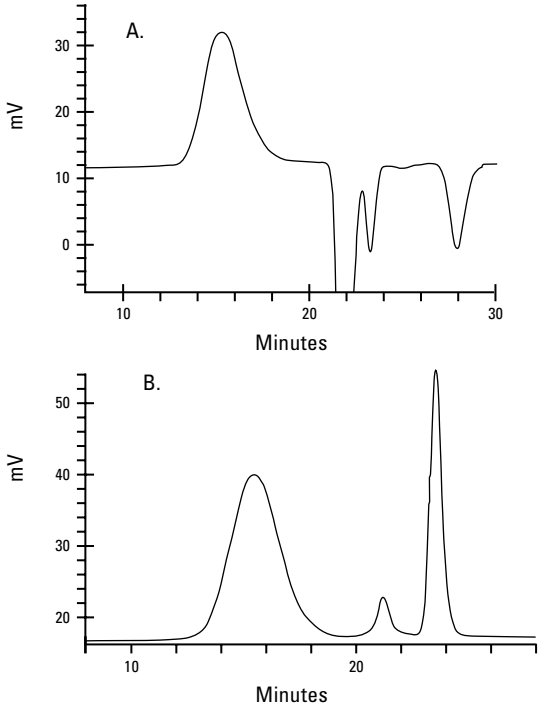
The versatility of using TSK-GEL Alpha columns with various polar solvents is illustrated in FIGURE 11 for the analysis of cellulose derivatives. A TSKgel Alpha-M column was used to separate ethylcellulose with the polar solvent DMF and ethylhydroxyethyl cellulose with methanol.

The separation of polyvinylalcohol with different degrees of saponification is shown in FIGURE 12. This separation was performed with a TSKgel Alpha-5000 and a TSKgel Alpha-3000 column in series using a hexafluoroisopropanol mobile phase.

FIGURE 13 shows that the column efficiency of TSK-GEL SuperAW series columns is maintained in a wide variety of polar organic solvents.

FIGURE 11

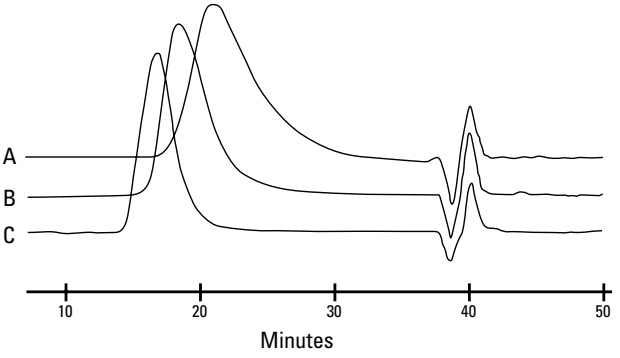
TSKgel Alpha-M separation of cellulose derivatives



Column:	TSKgel Alpha-M, 7.8mm ID x 30cm
Sample:	A. 50µL ethylcellulose, 0.1%; B. 50µL ethylhydroxyethylcellulose, 0.1%
Elution:	A. 10mmol/L LiBr in DMF; B. 10mmol/L LiBr in methanol
Flow Rate:	0.5mL/min
Temperature:	40°C
Detection:	RI

FIGURE 12

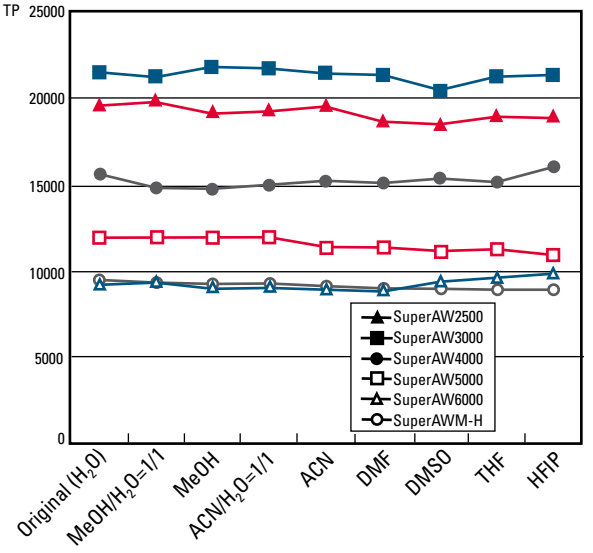
Polyvinylalcohol characterization using TSK-GEL Alpha-5000 and Alpha-3000 columns in series



Column:	TSKgel Alpha-5000 and Alpha-3000, 7.8mm ID x 30cm in series
Sample:	degree of saponification of polyvinyl alcohol: A. 75%; B. 88%; C. 100%
Eluent:	hexafluoroisopropanol (HFIP)
Flow Rate:	0.5mL/min
Temperature:	40°C
Detection:	RI

FIGURE 13

Solvent Compatibility of TSK-GEL SuperAW Series



Column:	TSK-GEL SuperAW Series (6.0 mm ID x 15 cm L)
Eluent:	Water
Flow rate:	0.6 mL/min
Temperature:	25°C
Detection:	Refractive index detector
Sample:	Ethylene glycol
Inj. volume:	5 µL (2.5 g/L)

SEC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Stainless steel columns								
18339	Alpha-2500	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	40
18340	Alpha-3000	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	40
18341	Alpha-4000	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	30
18342	Alpha-5000	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	30
18343	Alpha-6000	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	20
18344	Alpha-M (mixed bed)	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	20
Guard columns								
18345	Alpha Guard column	6	4	13	For all Alpha columns			
VMpak columns*								
20011	VMpak-25	2.0	5	7	≥ 1,000	0.1 - 0.2	0.25	20
20012	VMpak-25	2.0	15	7	≥ 3,000	0.1 - 0.2	0.25	60
Stainless steel columns								
19315	SuperAW2500	6.0	15	4	≥ 16,000	0.3 - 0.6	0.6	60
19316	SuperAW3000	6.0	15	4	≥ 16,000	0.3 - 0.6	0.6	60
19317	SuperAW4000	6.0	15	6	≥ 10,000	0.3 - 0.6	0.6	40
19318	SuperAW5000	6.0	15	7	>10,000	0.3 - 0.6	0.6	30
19319	SuperAW6000	6.0	15	9	>7,000	0.3 - 0.6	0.6	20
19320	SuperAWM-H	6.0	15	9	>7,000	0.3 - 0.6	0.6	20
Guard columns								
19321	SuperAW-L Guard Column	4.6	3.5	7	For SuperAW2500-4000 columns.			
19322	SuperAW-H Guard Column	4.6	3.5	23	For SuperAW5000-AWM-H columns			

*TSK-GEL VMpak-25 series contains a similar packing as TSKgel Alpha-2500. It can be used for multimodal LC/LC-MS separations.

TSK-GEL H_{XL}, H_{HR}, SUPERH AND SUPERHZ GEL PERMEATION COLUMNS

Polymer-based columns for Gel Permeation Chromatography of organic-soluble polymers

HIGHLIGHTS

- Porous, highly cross-linked, spherical polystyrene divinylbenzene (PS-DVB) resin.
- Four different TSK-GEL H-type columns are available. Each of these are packed with different particle sizes (see table below).
- H-type packings are available in eight pore sizes.
- Expanded molecular weight ranges with exclusion limits from 1,000 Da to an estimated 4 x 10^8 Da
- Minimal shrinking and swelling of the column bed
- Chemically and thermally stable
- Use 4.6 mm ID SuperH and SuperHZ columns for reduced solvent consumption in high throughput analysis.
- Novel multi-pore distribution in the TSKgel MultiporeH_{XL}-M column provides linear calibration curves over a wider MW range.
- Mixed bed columns with optimized particle and pore sizes to prevent polymer sheering.
- Semi-micro SuperHZ columns now available as multipore columns with linear calibration curves.

TSK-GEL H-type packings are available in eight pore sizes and span four different column chemistries. For polymer samples with a broad molecular range, packing of several pore sizes are provided in the mixed bed columns: TSK-GEL SuperHZM series, TSK-GEL SuperHM series, TSKgel GMH_{XL}, TSKgel GMH_{HR}, and selected high temperature versions provide linear calibration curves up to several million Daltons (see page 51).

Column Selection

The Super prefix refers to the efficiency of the column. The Super series columns contain ultra efficient particles as small as 3 µm, housed in 15 cm length columns. The smaller particle allows for equivalent resolution to conventional H_{XL} columns, with 50% less run time due to the shorter column length. The Super series columns are an excellent choice for high throughput polymer analysis.

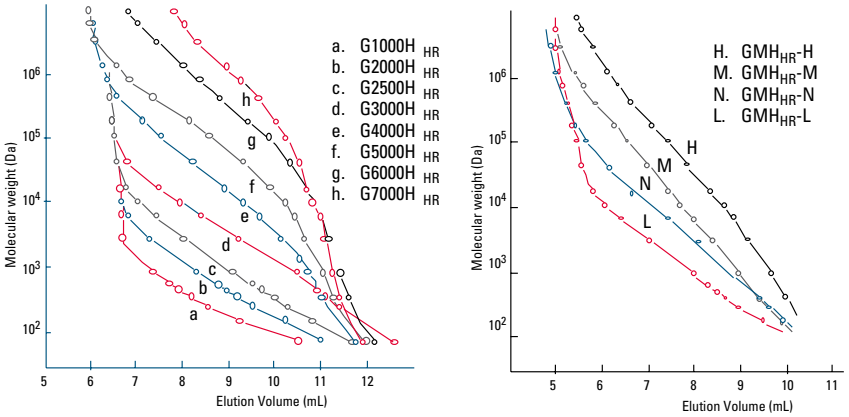
Series Type	SuperHZ	H_{XL}	SuperH	H_{HR}
Application focus	High-throughput polymer analysis with ultra low polymer adsorption. Limited solvent compatibility range.	Conventional polymer analysis with ultra low polymer adsorption. Limited solvent compatibility range.	High-throughput polymer analysis with expanded solvent compatibility.	Conventional polymer analysis with expanded solvent compatibility range.
Particle size	3, 5 and 10 µm, depending on pore size	5, 6 and 9 µm, depending on pore size	3 and 5 µm, depending on pore size	5 µm
Theoretical plates[1]	16,000/15 cm column	16,000/30 cm column	16,000/15 cm column	16,000/30 cm column
Maximum temperature	G1000 - G4000 60°C G5000 - mixed 80°C	G1000 - G4000 60°C G5000 - mixed 80°C	140°C	140°C
Standard shipping solvent	THF	THF[2]	THF[2]	THF[2]
THF can be switched to	benzene, chloroform, toluene, xylene, dichloromethane[3] and dicholoroethane[3]		see our website for detailed information	
Other shipping solvents available?	yes[4]		no	
Number of solvent substitutions	One time only		Several[5]	
Solvent exchange instructions	Linear gradient with a 2%/min rate of change at a flow rate <0.25 mL/min.	Linear gradient with a 2%/min rate of change at a flow rate <0.5 mL/min.	Linear gradient with a 2%/min rate of change according to flow rates listed on our website.	

1) Theoretical plates listed are based on smallest particle size listed
2) High-temperature columns (HT) are shipped with ODCB (Orthochlorodivinylbenzene) as standard shipping solvent.
3) Switching from THF to dichloromethane and dichloroethane is not recommended for G1000 pore size columns.
4) See our website for available shipping solvents
5) After switching to a very polar solvent such as acetone, switching back to a nonpolar solvent is not recommended.

SEC

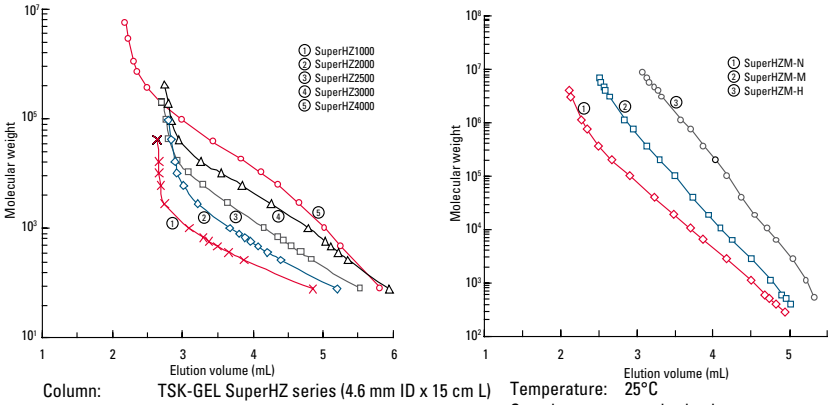
CALIBRATION CURVES FOR TSK-GEL H-TYPE GELPERMEATION COLUMNS

Calibration curves for TSK-GEL H_{HR} columns with polystyrene standards



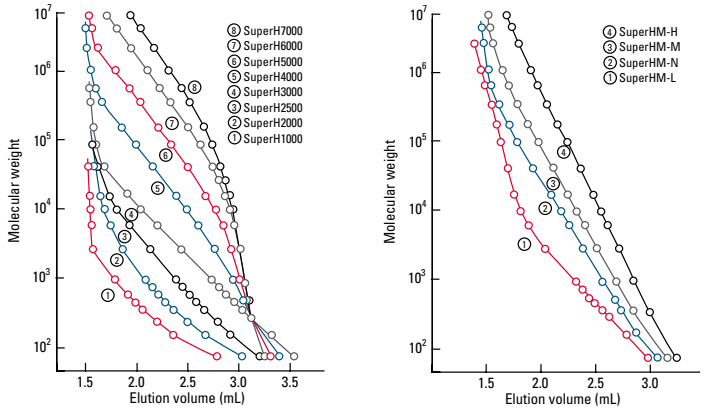
Column: TSKgel H_{HR} series (7.8 mm ID x 30 cm L)
Sample: polystyrene standards
Elution: THF
Flow Rate: 1.0 mL/min
Temperature: 25°C
Detection: UV @ 254 nm

Calibration curves for TSK-GEL SuperHZ columns with polystyrene standards



Column: TSK-GEL SuperHZ series (4.6 mm ID x 15 cm L)
Eluent: THF
Flow rate: 0.35 mL/min
Temperature: 25°C
Sample: standard polystyrene
Inj. volume: 2 µL

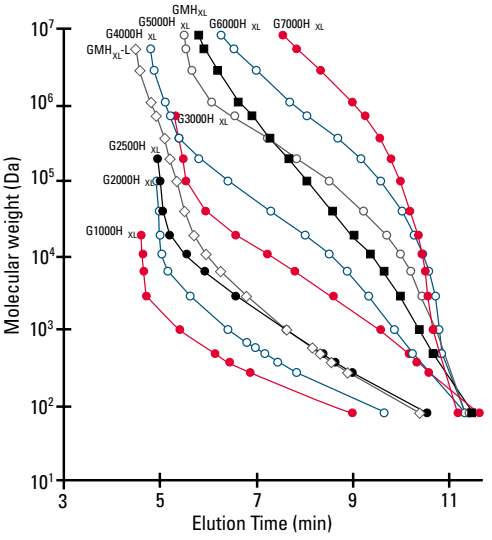
Calibration curves for TSK-GEL SuperH columns with polystyrene standards



Column: TSK-GEL SuperH series (6.0 mm ID x 15 cm L)
Eluent: THF
Flow rate: 0.6 mL/min
Temperature: 25°C
Detection: UV@254 nm
Sample: polystyrene standards

The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

Calibration curves for TSK-GEL H_{XL} columns with polystyrene standards



Column size: 7.8 mm ID x 30 cm L
Sample: polystyrene standards
Eluent: THF
Flow Rate: 1.0 mL/min
Temperature: 25°C
Detection: UV @ 254 nm

MULTI-PORE SIZE DISTRIBUTION IN A POLYSTERENE PACKING MATERIAL

Novel approach to GPC of samples with a wide range of molecular weights

The TSKgel MultiporeH$_{XL}$-M column offers a unique packing material and a novel strategy for the precise analysis of polymers by Gel Permeation Chromatography (GPC). Until now, the GPC separation of a sample containing a wide range of molecular weight polymers was performed by one of two strategies. One strategy combines columns with different pore sizes of packing material in series. The other strategy employs a single column with a blend of different pore sizes of packing materials, commonly referred to as a mixed bed. Mixed bed columns do not always provide linear calibration curves, which may result in broad or split peaks. With the introduction of the TSKgel MultiporeH$_{XL}$-M column, a novel strategy was introduced using a single column containing a novel polystyrene packing material with a multi-pore size distribution. FIGURE 14 illustrates the three strategies. The TSKgel Multipore column has several different pore sizes with continuous distribution in every bead. This results in sharper peaks without inflections that may be observed using mixed-bed columns.

The pore size distributions of the TSKgel MultiporeH$_{XL}$-M column and a mixed-bed column are shown in FIGURE 15. The mixed-bed column shows a sharp maximum for pores with a diameter of 0.08 µm, though the overall pore size distribution ranges from 0.006 to 0.6 µm in diameter. In the case of the TSKgel MultiporeH$_{XL}$-M column, the pore size distribution exhibits a wider maximum range from 0.02 to 0.1 µm in diameter. This difference in pore size distribution may explain the reason for the inflection phenomenon. A comparison of calibration curves for polystyrene standards on the TSKgel MultiporeH$_{XL}$-M column, the TSKgel GMH$_{HR}$-H and PLgel Mixed-C, are shown in FIGURE 16. Both the TSKgel GMH$_{HR}$-H and PLgel Mixed-C columns are mixed-bed columns.

FIGURE 14

Strategies for wide range separation using Size Exclusion Chromatography

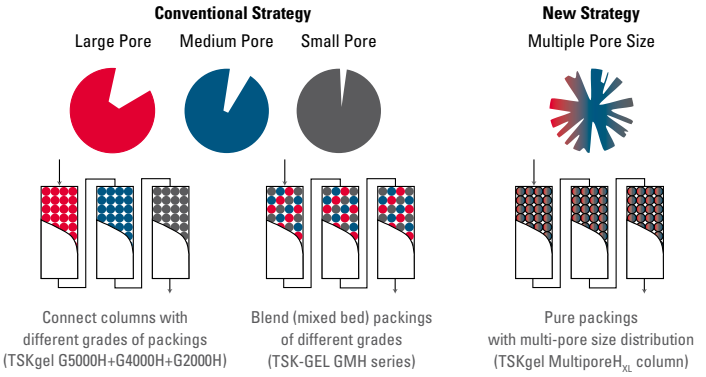


FIGURE 15

Pore size distribution of TSKgel MultiporeH$_{XL}$-M column and a mixed-bed column

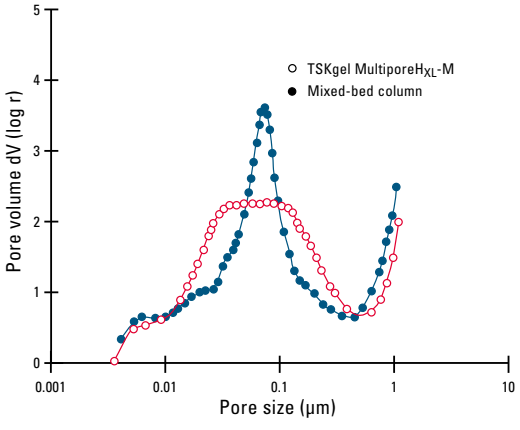
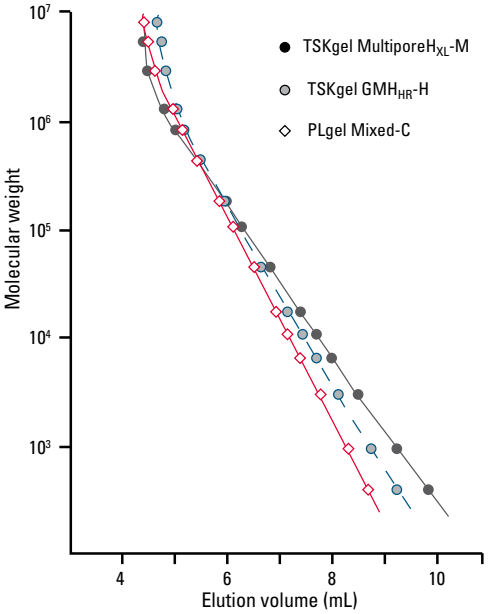


FIGURE 16

Calibration curves for Multipore and mixed-bed columns



Column:	TSKgel Multipore H$_{XL}$-M, 7.8 mm ID x 30 cm L; TSKgel GMH$_{HR}$-H, 7.8 mm ID x 30 cm L; PLgel Mixed-C, 7.5 mm ID x 30cm L
Sample:	polystyrene standards
Elution:	THF
Flow Rate:	1.0 mL/min
Temperature:	40°C
Detection:	UV @ 254 nm

SEC

APPLICATIONS OF TSK-GEL H-TYPE GEL PERMEATION COLUMNS

Phthalate esters

FIGURE 17 demonstrates the high efficiency separation on a TSKgel G1000H$_{XL}$ column for low molecular weight phthalate esters. Resolution was close to baseline, even though the molecular weights of the esters differed by less than 50 Da.

Phenol resin

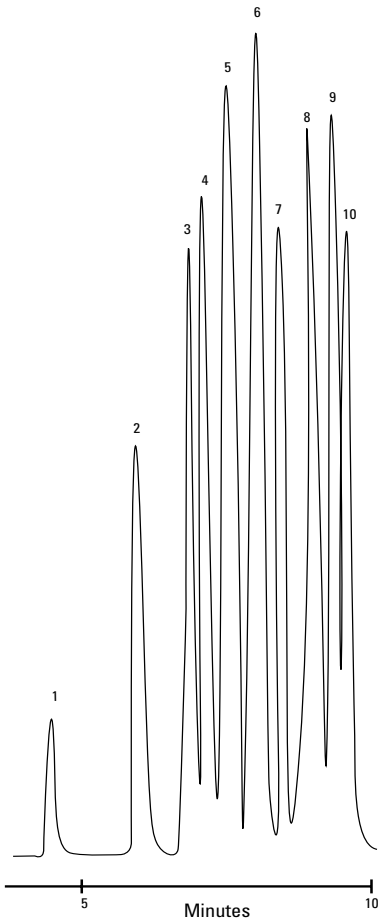
The TSKgel GMH$_{XL}$-L column has been designed to provide a complete profile for high molecular weight samples that contain low molecular weight additives. The calibration curve for this mixed-bed column is shallow in the low molecular weight range of oligomers. Sample adsorption is not observed. For example, the complete profile of a phenol resin, with high resolution of the low molecular weight components, is shown in FIGURE 18. Other applications for the TSKgel GMH$_{XL}$-L column include analyses of paint materials, bond and adhesive components and synthetic polymer additives.

Fatty acids

In FIGURE 19, two TSKgel G2000H$_{XL}$ columns in series separate a mixture of fatty acids ranging from C4 to C30.

FIGURE 17

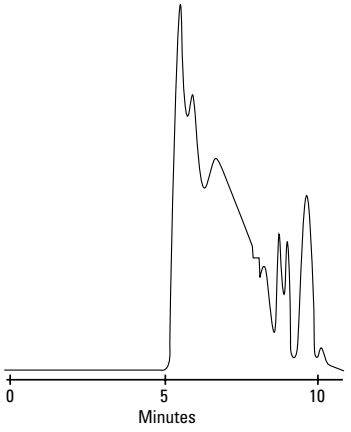
High resolution of phthalate esters on TSKgel G1000H$_{XL}$



Column: TSKgel G1000H$_{XL}$, 7.8mm ID x 30cm
Sample: 1. polystyrene (10,200Da), 2. dioctylphthalate (391Da), 3. dibutylphthalate (278Da), 4.dipropylphthalate (250Da), 5. diethylphthalate (222Da), 6. dimethylphthalate (194Da), 7. *n*-propylbenzene (120Da), 8. ethylbenzene (116Da), 9. toluene (92Da), 10. benzene (78Da)
Elution: THF
Flow Rate: 1.0mL/min
Detection: UV @ 254nm

FIGURE 18

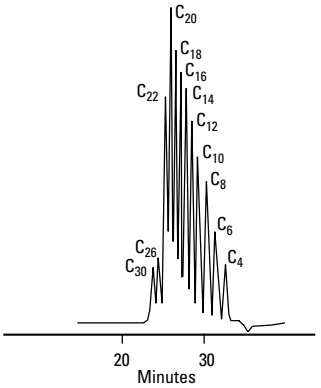
Separation of phenol resin on TSKgel GMH$_{XL}$-L



Column: TSKgel GMH$_{XL}$-L, 7.8mm ID x 30cm
Sample: phenol resin
Elution: THF
Flow Rate: 1.0mL/min
Detection: UV @ 254nm

FIGURE 19

Separation of fatty acids



Column: TSKgel G2000H$_{XL}$, two 7.8mm ID x 30cm in series
Sample: fatty acids
Elution: THF
Flow Rate: 1.0mL/min
Detection: RI

APPLICATIONS OF TSK-GEL H-TYPE GEL PERMEATION COLUMNS

Acrylic polymer

FIGURE 20 shows the separation of an acrylic polymer on the TSKgel MultiporeH$_{XL}$-M column compared with two commercially available mixed-bed columns. The arrows illustrate the inflections seen in the chromatograms from mixed-bed columns and the improvement achieved when using the TSKgel MultiporeH$_{XL}$-M column.

Polymethylmethacrylate

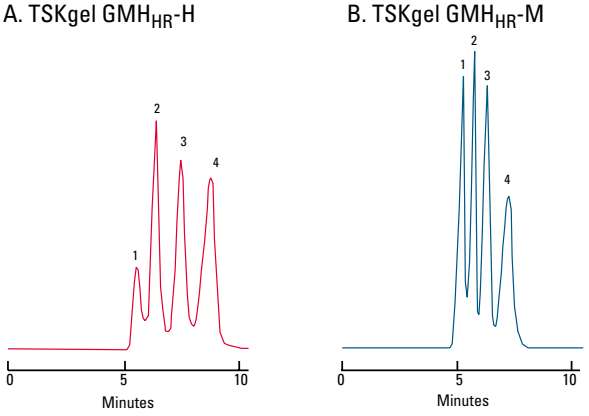
The effect of different pore size distributions in the mixed beds of TSKgel GMH$_{HR}$-H and TSKgel GMH$_{HR}$-M is illustrated in FIGURE 21. The TSKgel GMH$_{HR}$-M produces better resolution in the 8 x 10^5 to 1 x 10^4 Da range.

Epoxy resin

FIGURE 22 demonstrates the excellent fingerprint obtained using small diameter SuperHZM-M mixed bed columns.

FIGURE 21

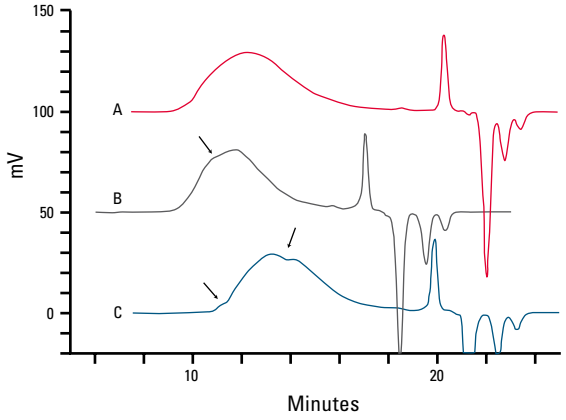
Comparison of TSKgel GMH$_{HR}$-H and -M columns with polymethylmethacrylate standards



Column: A. TSKgel GMH$_{HR}$-H, 7.8 mm ID x 30 cm L;
B. TSKgel GMH$_{HR}$-M, 7.8 mm ID x 30 cm L
Sample: polymethylmethacrylate: 1. 820,000 Da, 2. 67,000 Da,

FIGURE 20

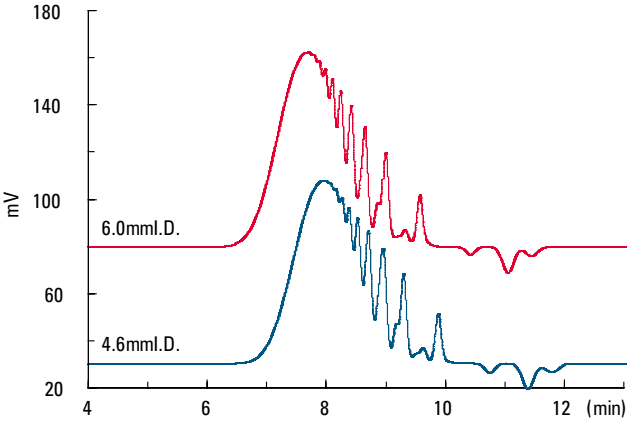
Separation of acrylic resin by SEC on TSKgel MultiporeH$_{XL}$-M and mixed-bed type columns



Column: A. TSKgel MultiporeH$_{XL}$-M, two 7.8 mm ID x 30 cm L columns in series;
B. Competitor P, two 7.5 mm ID x 30 cm L columns in series, mixed-bed type;
C. Competitor S, two 8.0 mm ID x 30 cm L columns in series, mixed-bed type
Sample: acrylic polymer (0.1%, 50 µL)
Elution: THF
Flow Rate: 1.0 mL/min
Temperature: 40°C
Detection: RI

FIGURE 22

Chromatogram of epoxy resin

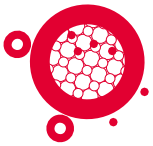


Column: TSKgel Super HZM-M x 2
Eluent: THF
Flow rate: 0.35 mL/min (4.6 mm ID)
0.6 mL/min (6.0 mm ID)
Temperature: 40° C
Detection: RI
Sample: Epoxy resin (10 g/L)
Inj. volume: 5 µL (4.6 mm ID)
9 µL (6.0 mm ID)

SEC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Stainless steel columns								
17352	G1000H_{HR}	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17353	G2000H_{HR}	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17354	G2500H_{HR}	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17355	G3000H_{HR}	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17356	G4000H_{HR}	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17357	G5000H_{HR}	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17358	G6000H_{HR}	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17359	G7000H_{HR}	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17362	GMH_{HR}-L mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
18055	GMH_{HR}-N mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17392	GMH_{HR}-M mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17360	GMH_{HR}-H mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
18393	GMH_{HR}-H(S)HT mixed-bed	7.8	30	13	≥ 8,0000.	5 - 1.0	2.5	20
16131	G1000H_{XL}	7.8	30	5	≥ 16,000	0.5 - 1.0	1.0	50
16134	G2000H_{XL}	7.8	30	5	≥ 16,000	0.5 - 1.0	1.2	50
16135	G2500H_{XL}	7.8	30	5	≥ 16,000	0.5 - 1.0	1.2	50
16136	G3000H_{XL}	7.8	30	6	≥ 16,000	0.5 - 1.0	1.2	35
16137	G4000H_{XL}	7.8	30	6	≥ 16,000	0.5 - 1.0	1.2	35
16138	G5000H_{XL}	7.8	30	9	≥ 14,000	0.5 - 1.0	1.2	15
16139	G6000H_{XL}	7.8	30	9	≥ 14,000	0.5 - 1.0	1.2	15
16140	G7000H_{XL}	7.8	30	9	≥ 14,000	0.5 - 1.0	1.2	15
16141	GMH_{XL} mixed-bed	7.8	30	9	≥ 16,000	0.5 - 1.0	1.2	15
07112	GMH_{XL}-HT	7.8	30	13	≥ 5,5000.	5 - 1.0	1.2	15
16652	GMH_{XL}-L mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	1.2	35
18403	Multipore H_{XL}-M	7.8	30	5	≥ 16,000	0.5 - 1.0	1.0	35
17990	TSKgel SuperH1000	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	60
17991	TSKgel SuperH2000	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	60
17992	TSKgel SuperH2500	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	60
17993	TSKgel SuperH3000	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40
17994	TSKgel SuperH4000	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40
17995	TSKgel SuperH5000	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40
17996	TSKgel SuperH6000	6.0	15	5	≥ 16,000	0.3 - 0.6	0.8	40
17997	TSKgel SuperH7000	6.0	15	5	≥ 16,000	0.3 - 0.6	0.8	40
17998	TSKgel SuperHM-L	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40
17999	TSKgel SuperHM-N	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40
18000	TSKgel SuperHM-M	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40
18001	TSKgel SuperHM-H	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40



ORDERING INFORMATION

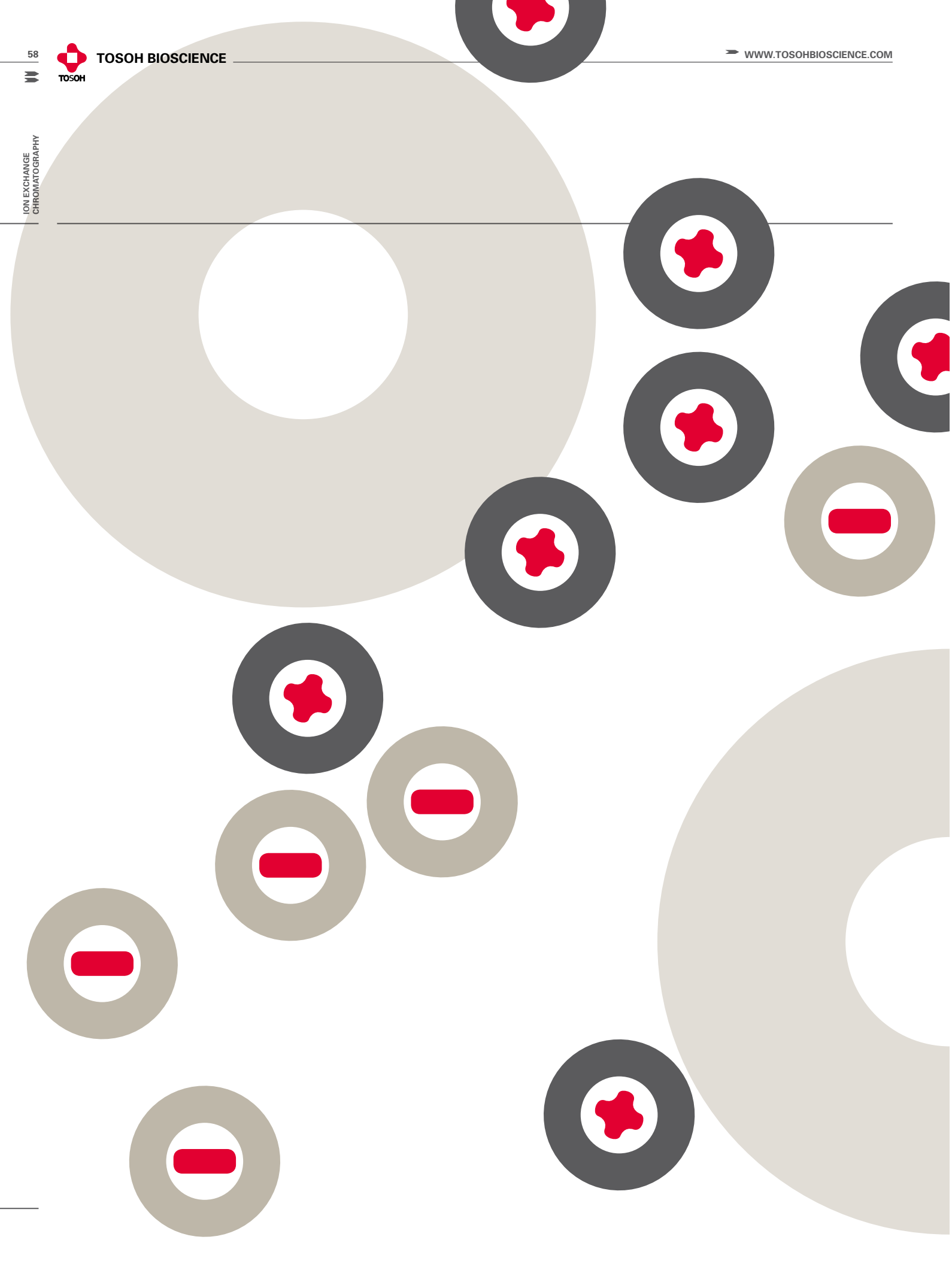
Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Stainless steel columns								
19309	TSKgel SuperHZ1000	4.6	15	3	≥ 16,000	0.15 - 0.35	0.4	56
19302	TSKgel SuperHZ1000	6.0	15	3	≥ 16,000	0.25 - 0.6	0.7	56
19310	TSKgel SuperHZ2000	4.6	15	3	≥ 16,000	0.15 - 0.35	0.4	50
19303	TSKgel SuperHZ2000	6.0	15	3	≥ 16,000	0.25 - 0.6	0.7	50
19311	TSKgel SuperHZ2500	4.6	15	3	≥ 16,000	0.15 - 0.35	0.4	40
19304	TSKgel SuperHZ2500	6.0	15	3	≥ 16,000	0.25 - 0.6	0.7	40
19312	TSKgel SuperHZ3000	4.6	15	3	≥ 16,000	0.15 - 0.35	0.4	30
19305	TSKgel SuperHZ3000	6.0	15	3	≥ 16,000	0.25 - 0.6	0.7	30
19313	TSKgel SuperHZ4000	4.6	15	3	≥ 16,000	0.15 - 0.35	0.4	35
19306	TSKgel SuperHZ4000	6.0	15	3	≥ 16,000	0.25 - 0.6	0.7	35
19960	TSKgel SuperHZM-N	4.6	15	3	≥ 16,000	0.15 - 0.35	0.4	35
19661	TSKgel SuperHZM-N	6.0	15	3	≥ 16,000	0.25 - 0.6	0.7	35
19662	TSKgel SuperHZM-M	4.6	15	3 and 5	≥ 16,000	0.15 - 0.35	0.4	20
19663	TSKgel SuperHZM-M	6.0	15	3 and 5	≥ 16,000	0.25 - 0.6	0.7	20
19664	TSKgel SuperHZM-H	4.6	15	10	≥ 9,000	0.15 - 0.35	0.4	10
19665	TSKgel SuperHZM-H	6.0	15	10	≥ 9,000	0.25 - 0.6	0.7	10
21488	SuperMultiporeHZ-M	4.6	15	4	≥ 16,000			24
21815	SuperMultiporeHZ-N	4.6	15	3	≥ 20,000			40
21885	SuperMultiporeHZ-H	4.6	15	6	≥ 11,000			10

Guard columns					
18404	MultiporeH_{XL}-M Guard	6.0	4.0	5	For P/N 18403
07113	H_{XL} Guard Column	6.0	4.0		For G1000H_{XL} through G4000H_{XL} columns
13727	H_{XL} Guard Column	6.0	4.0		For G5000H_{XL} through GMH$_{XL}$-L mixed-bed columns
17368	H_{HR} Guard Column	6.0	4.0	5	For G1000-4000H_{HR} and GMHhr-L columns
17369	H_{HR} Guard Column	6.0	4.0	5	For G5000-7000H_{HR} and and GMH$_{HR}$-M; -N; -H columns
18002	SuperH Guard Column	4.6	3.5	3	For SuperH1000-4000
18003	SuperH Guard Column	4.6	3.5	3	For SuperH5000-7000 and HM-L;-N;-M;-H columns
19314	SuperHZ Guard Column	4.6	2.0	3	For 4.6 mm ID SuperHZ1000-4000 and HZM-N &-M columns
19668	SuperHZ Guard Column	4.6	2.0	10	For 4.6 mm ID SuperHZM-H columns
19666	SuperHZ Guard Column	4.6	3.5	3	For 6.0 mm ID SuperHZ1000-4000 and HZM-N &-M columns
19667	SuperHZ Guard Column	4.6	3.5	10	For 6.0 mm ID SuperHZM-H columns
21489	SuperMP-M Guard	4.6	2.0	4	For SuperMultipore HZ-M P/N 21488
21816	SuperMP-N Guard	4.6	2.0	3	For SuperMultipore HZ-N P/N 21815
21886	SuperMP-H Guard	4.6	2.0	6	For SuperMultipore HZ-H P/N 21887

SEC

ECOSEC GPC SYSTEM - BASED ON 35 YEARS OF EXPERIENCE IN GPC

EcoSEC is a compact, all-in-one GPC system for fast, high resolution, semi-micro GPC. Comprising a precision solvent delivery system, automatic injector, column oven and a high performance refractive index detector, the design of the system components, their configuration and the optimized flow line provides outstanding performance with minimized dead volume. This makes EcoSEC the ideal instrument to be used in combination with the well respected TSK-GEL semi-micro GPC/SEC columns. In Europe, EcoSEC is offered in cooperation with Polymer Standards Service (PSS), an acknowledged leader in the field of polymer analysis.



IEC
ION EXCHANGE CHROMATOGRAPHY

IEC PRODUCTS

ANION EXCHANGE

TSKgel Q-STAT -NEW-
TSKgel DNA-STAT -NEW-
TSKgel BioAssist Q
TSKgel SuperQ-5PW
TSKgel DEAE-5PW
TSKgel DEAE-NPR
TSKgel DNA-NPR
TSKgel DEAE-2SW
TSKgel DEAE-3SW
TSKgel Sugar AXI
TSKgel Sugar AXG
TSKgel SAX

CATION EXCHANGE

TSKgel SP-STAT -NEW-
TSKgel CM-STAT -NEW-
TSKgel BioAssist S
TSKgel SP-5PW
TSKgel CM-5PW
TSKgel SP-2SW
TSKgel SP-NPR
TSKgel CM-2SW
TSKgel CM-3SW
TSKgel SCX

TOSOH FACT

Tosoh Corporation maintains a large database of HPLC applications utilizing TSK-GEL columns. Sources for this database include articles in journals citing the use of TSK-GEL columns by Tosoh customers as well as technical papers and presentations created by Tosoh scientists.

Tosoh Bioscience offers a large literature library consisting of application notes, instructions manuals, product overviews and separation reports.

Both the literature library and the chromatogram database are available on the website at www.tosohbioscience.com.

INTRODUCTION TO TSK-GEL ION EXCHANGE COLUMNS

Tosoh Bioscience offers a broad line of high efficiency columns for analysis and isolation of biomolecules by anion and cation exchange chromatography. In either mode of Ion Exchange Chromatography (IEC), the product line contains methacrylate-, silica- and polystyrene-based columns. Proteins, peptides, oligonucleotides and other nucleic acid fragments are typical samples that are analyzed or isolated on TSK-GEL ion exchange columns. Most of the available chemistries are offered in analytical as well as semi-preparative column formats. Particle sizes range from 2.5 µm, for fast quality control and process monitoring, to 20 µm and larger particle sizes utilized in process scale separations.

TSK-GEL STAT columns are the latest addition to the IEC column line. They are designed for high effiency separation of biomolecules and low molecular weight compounds. TSK-GEL STAT columns provide superior performance at reduced analysis time. The STAT series encompasses a range of high efficiency anion and cation exchange columns, suitable for various applications from research to quality control.

Also available are a series of ion exchange columns based on a polystyrene matrix. They are most suitable for analyzing small molecular weight sugars, amino acids, individual nucleic acids, and small drug candidates.

Packing Materials and Chemistries

Methacrylate, silica, and polystyrene are used as matrices for the TSK-GEL line of ion exchange columns. The methacrylate backbone chemistry provides a robust, hydrophilic particle that is suitable as a support for high performance analytical and preparative separations of biomolecules.

The polymethacrylate base resin, G5000PW (5PW), is a 10 µm spherical particle with approximately 1000 Å pores. The base resin is derivatized either with diethylaminoethyl (DEAE), sulfopropyl (SP) or carboxymethyl (CM) functionalities to provide a weak anion, a strong cation, and a weak cation exchanger, respectively. While these chemistries result in standard ion exchangers, the chemistry employed in the manufacturing of TSKgel SuperQ-5PW results in a higher capacity strong anion exchanger by introducing polyamine functional groups.

FEATURES	BENEFITS
BioAssist Columns	
High capacity even for larger proteins (1 million Da)sww	Fewer runs to collect required sample amounts
Unique pore structure provides fast mass transfer	Sharper peaks improve analysis and isolation
Biocompatible PEEK column hardware	Less sample loss due to adsorption
Available in analytical and semi-prep formats	Easy scale-up
Polymer-Based Ion Exchange Columns	
Methacrylate backbone	Mechanically and chemically stable (pH 2-12)
	Withstands repeated cleaning with base, and use of organic solvents, denaturants and surfactants
Large pore size (1000 Å) (excl. limit for proteins ~ 5,000,000 Da)	Use same column for most biopolymers
Non porous resin-based (STAT and NPR) columns	Fast QC analysis and process monitoring
Several columns available in 2 mm ID format	Reduced solvent consumption and analysis time
Silica-Based Ion Exchange Columns	
Smaller pore size (2SW = 125 Å and 3SW = 250 Å)	Most suitable for analysing smaller MW samples such as nucleotides, drug candidates, catecholamines and small peptides or proteins

IEC

Due to the higher density of anion exchange sites, TSKgel SuperQ-5PW has a smaller effective pore size than TSKgel DEAE-5PW.

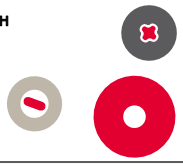
TSK-GEL BioAssist columns are also based on methacrylate particle design technology. TSKgel BioAssist Q contains particles with very large pores (~4000 Å) that are derivatized with a network of polyamine groups. The capacity of TSKgel BioAssist Q has been shown to be high over a wide molecular weight range (up to 1,000,000 Da). TSKgel BioAssist S is packed with particles possessing 1300 Å pores functionalized with sulfopropyl groups. TSKgel BioAssist analytical IEC columns are provided in a 4.6 mm ID x 5 cm L PEEK housing with 7 µm or 10 µm particles for the respective S and Q functionalities. Semi-preparative TSK-GEL BioAssist columns are also available with a 13µm particle size packed in a 10 mm ID x 10 cm L housing. The longer length of the semi-preparative column compensates for the increased particle size, resulting in similar resolution to the analytical column.

The methacrylate chemistry also forms the backbone of non-porous resin columns such as TSK-GEL STAT and NPR columns. Since rate-limiting pore difusion is eliminated with nonporous particles, analysis time is often reduced by as much as 80 % without loss in resolution. Also, recoveries are routinely greater than 90 %. The relatively large particle sizes of nonporous CM-STAT, SP-STAT, Q-STAT, and DNA-STAT columns support fast separations at moderate pressure. Latest surface technology was applied to increase the number of functional groups (carboxymethyl, sulfopropyl or quaternary ammonium group) and reach good sample capacities.

Specific application needs are addressed by offering various column formats and particle sizes: For fast and ultra-fast analysis (e.g. screening or process monitoring) short 3 mm ID columns are packed with 10 µm particles. For high resolution separations longer columns with 4.6 mm ID are packed with 7 µm particles. The DNA-STAT column is packed with smaller particles (5 µm).

TSKgel DEAE-NPR, SP-NPR and DNA-NPR are packed with 2.5 µm particles. High column efficiency coupled with low sample capacity restricts the application of these columns to fast analysis and micro-scale preparative isolation. The DNA-NPR column is a longer version of the DEAE-NPR column that allows improved resolution of oligonucleotides, including those amplified by PCR. Small guard columns are available to protect the DNA-NPR and DEAE-NPR columns.

In the development of new drug candidates, it is often desirable to use the same backbone chemistry throughout the development process. For that reason, the backbone of the 20 µm and 30 µm particle size TSK-GEL PW-type resins and the larger particle size Toyopearl process media are chemically similar to that used in prepacked TSK-GEL PW-type column lines. As a result, TSKgel SuperQ-5PW scales directly to Toyopearl SuperQ-650. Similarly, the TSKgel DEAE-5PW scales directly to TSKgel DEAE-5PW bulk resins, which in turn scales to Toyopearl DEAE-650. The same is true for CM and SP products in the cation exchange column line.



TSK-GEL Anion Exchange Columns

TSK-GEL	Matrix*	Particle Size (µm)	Pore Size (Å)	Functional Group	Counter Ion	Excl. Limit, PEG** (Da)	Capacity (mg BSA/mL)	Small Ion capacity meq/mL	pKa	Column hard-ware***
BioAssist Q	pMA	10, 13	~4000	Polyamine	Cl^-	>5,000,000	70	0.1	9.4	PEEK
SuperQ-5PW	pMA	10,13	1000	Trimethyl-amino	Cl^-	1,000,000	100	> 0.13	12.2	S, G
DEAE-5PW	pMA	10,13, 20	1000	DEAE	Cl^-	1,000,000	30	0.1	11.5	S, G
Q-STAT	pMA	7,10	~ 0	Trimethyl-amino	Cl^-	500	20	0.27	10.5	S
DNA-STAT	pMA	5	~ 0	Trimethyl-amino	Cl^-	500	35	0.27	10.5	S
DEAE-NPR	pMA	2.5	~ 0	DEAE	Cl^-	500	5	> 0.1	11.2	S
DNA-NPR	pMA	2.5	~ 0	Proprietary	ClO_4^-	500	5	> 0.1	11.2	S
DEAE-2SW	Silica	5	125	DEAE	$H_2PO_4^-$	10,000	ND	> 0.3	11.2	S
DEAE-3SW	Silica	10	250	DEAE	Cl^-	30,000	ND	> 0.3	11.2	S
Sugar AXI	PS-DVB	8	60	Trimethyl-amino	HBO_3^-		ND	> 1.2	12.5	S
Sugar AXG	PS-DVB	10	60	Trimethyl-amino	HBO_3^-		ND	> 1.2	12.5	S
SAX	PS-DVB	5	60	Trimethyl-amino	Cl^-		ND	> 1.0	12.5	S

TSK-GEL Cation Exchange Columns

TSK-GEL	Matrix*	Particle Size (µm)	Pore Size (Å)	Functional Group	Counter Ion	Excl. Limit, PEG** (Da)	Capacity (mg BSA/mL)	Small Ion capacity meq/mL	pKa	Column hard-ware***
BioAssist S	pMA	7, 13	~1300	Sulfopropyl	Na^+	~4,000,000	70(1)	0.1	2.4	PEEK
SP-5PW	pMA	10, 13, 20	1000	Sulfopropyl	Na^+	1,000,000	40(2)	> 0.1	2.3	S, G
CM-5PW	pMA	10, 13	1000	Carboxymethyl	Na^+	1,000,000	45(2)	> 0.1	4.2	S, G
SP-STAT	pMA	7, 10	~ 0	Sulfopropyl	Na^+	500	10(3)	> 0.023	4.0	S
CM-STAT	pMA	7, 10	~ 0	Carboxymethyl	Na^+	500	15(3)	> 0.1	4.9	S
SP-NPR	pMA	2.5	~ 0	Sulfopropyl	Na^+	500	5(2)	> 0.1	2.3	S
SP-2SW	Silica	5	125	Sulfopropyl	Na^+	10,000	ND	0.3	2.2	S
CM-2SW	Silica	5	125	Carboxymethyl	Na^+	10,000	110(2)	> 0.3	4.2	S
CM-3SW	Silica	10	250	Carboxymethyl	Na^+	30,000	ND	> 0.3	4.2	S
SCX	PS-DVB	5	60	Sulfonic acid	Na^+, H^+		ND	> 1.5		S

* *pMA = poly methacrylate; PS-DVB = polystyrene-divinylbenzene*

** *Polyethylene glycol*

*** *PEEK = polyethyletherketone, S = stainless steel, G = glass*

(1) γ-globulin; (2) hemoglobin; (3) lysozyme

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Sample Type	MW Range (Da)	TSK-GEL Column	pH Range
Amino Acids, Peptides and Proteins			
Amino acids	< 2000	SAX	1 - 14
		SCX	1 - 14
Peptides and small proteins	< 10,000	Q-STAT	3 - 10
		SP-STAT	3 - 10
		CM-STAT	3 - 10
		SCX	1 - 14
		SP-2SW	2 - 7.5
		CM-2SW	2 - 7.5
		DEAE-2SW	2 - 7.5
Proteins	> 10,000 up to ~ 5,000,000	BioAssist S	2 - 12
		BioAssist Q	2 - 12
		Q-STAT	3 - 10
		SP-5PW	2 - 12
		DEAE-5PW	2 - 12
		CM-5PW	2 - 12
		SP-STAT	3 - 10
		CM-STAT	3 - 10
		SP-NPR	2 - 12
		DEAE-NPR	2 - 12
		SuperQ-5PW	2 - 12
Nucleic Acids			
Purines and pyrimidines		DEAE-2SW	2 - 7.5
		SP-2SW	2 - 7.5
Nucleosides		SP-2SW	2 - 7.5
		DEAE-2SW	2 - 7.5
Nucleotides		Q-/DNA-STAT	3 - 10
		DEAE-2SW	2 - 7.5
Oligonucleotides		Q-/DNA-STAT	3 - 10
		DEAE-5PW	2 - 12
		DEAE-NPR	2 - 12
		DNA-NPR	2 - 12
		SuperQ-5PW	2 - 12
DNA, RNA, and PCR products		Q-/DNA-STAT	3 - 10
		DNA-NPR	2 - 12
		DEAE-NPR	2 - 12
		DEAE-5PW	2 - 12
		DEAE-3SW	2 - 7.5
Other Molecules			
Mono and disaccharides		Sugar AXI, AXG	1 - 14
		SCX	1 - 14
		SAX	1 - 14

TSK-GEL ANION EXCHANGE COLUMNS

HIGHLIGHTS

- TSKgel Q- and DNA-STAT columns provide high efficiency separations at short analysis time.
- TSKgel DNA-NPR columns are ideal for PCR fragment analysis.
- TSKgel SuperQ-5PW columns have higher capacity than TSKgel DEAE-5PW due to novel bonding chemistry, effective pore size is smaller for SuperQ-5PW.
- Pore structure and bonding chemistry of TSKgel BioAssist Q columns provide high capacity for small to very large MW proteins and nucleic acids.
- BioAssist columns are packed in 4.6 mm ID or 10 mm ID PEEK hardware. Other columns are available in glass and stainless steel for analytical, semi-preparative and preparative applications.
- Binding capacity for small to medium size proteins on TSKgel DEAE-3SW is roughly double that of the DEAE-5PW due to the smaller pore size and larger surface area.
- TSKgel DEAE-5PW and DEAE-2SW columns are available in 2 mm ID format for mass spec applications.
- Specialty columns for analysis of mono- and disaccharides and sugar alcohols are also available.

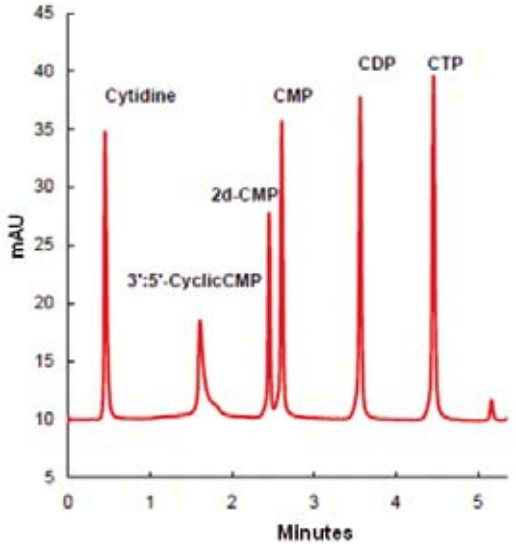
APPLICATIONS

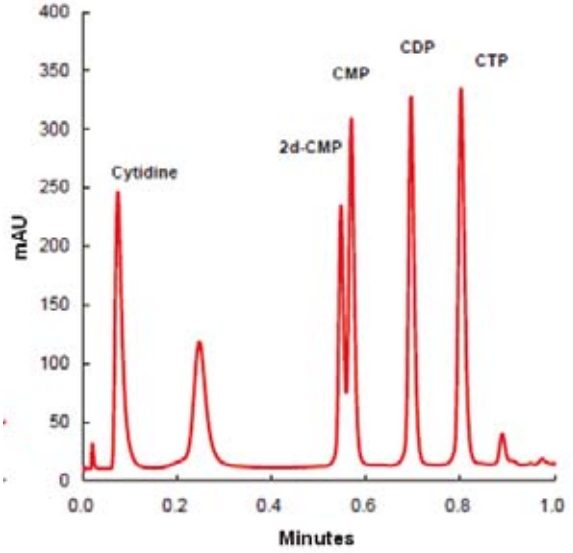
Nonporous TSKgel STAT Anion Exchange Columns

STAT columns are available in various column formats and particle sizes to perfectly match specific application needs. For fast and ultra-fast analysis anion and cation exchange columns in 3 mm ID and 3.5 cm length are packed with 10 µm particles. They are ideally suited for rapid candidate screening or process monitoring. 4.6 mm ID and 10 cm length columns packed with 7 µm particles are designed for high resolution IEC separation for example for the separation of nucleic acids, mAb variants, PEGylated protein or protein aggregates.

FIGURE 1

High resolution versus high throughput analysis of nucleotides





High resolution:

Column:	Prototype Q-STAT 4.6 mm ID x 10 cm L (7 µm)
Eluent:	A) 20 mmol/L Tris-HCl (pH8.5) B) 0.5 mol/L NaCl in A (pH8.5)
Gradient:	0 to 100% B (10 min.)
Flow Rate:	1.5 mL/min.
Detection:	UV @ 260 nm

High throughput:

Column:	Prototype Q-STAT 4.6 mm ID x 3.5 cm L (10 µm)
Eluent:	A) 20 mmol/L Tris-HCl (pH8.5) B) 0.5mol/L NaCl in A (pH8.5)
Gradient:	0 to 100% B (1min.)
Flow Rate:	4.0 mL/min.
Detection:	UV @ 260nm

IEC

The DNA STAT column (4.6 mm ID x 10 cm L) packed with 5 µm Q-type anion exchange resin is ideally suited for the analysis of nucleic acids.

FIGURE 1 compares the high resolution separation of nucleotides on a 10 cm length column to the high throughput separation on a 3.5 cm length column (analysis performed on prototype columns).

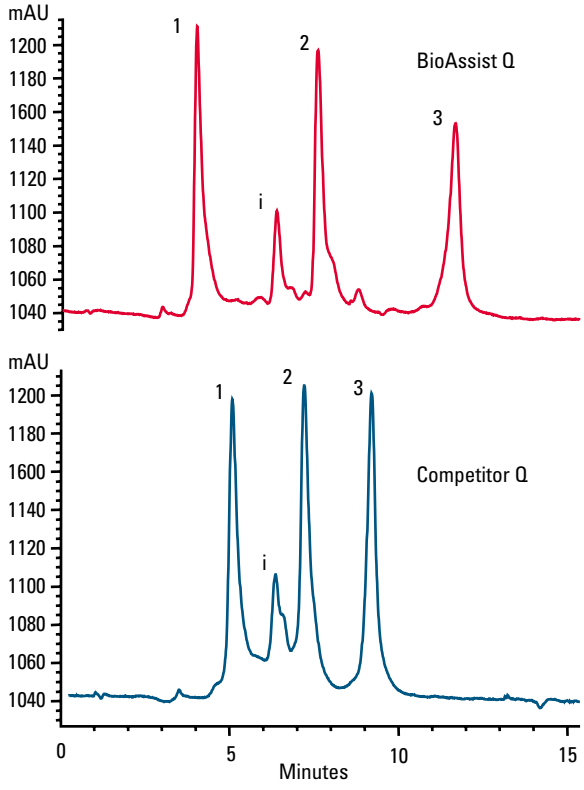
Polymer-based Anion Exchange Columns

BioAssist Q

TSKgel BioAssist Q is suitable for use in systems that are designed for laboratory or semi-preparative applications. FIGURE 2 demonstrates the performance enhancement of TSKgel BioAssist Q over a competitive product when operated side-by-side on an FPLC system. TABLE I shows typical dynamic binding capacities on BioAssist Q relative to competitive products.

FIGURE 2

Performance enhancement on FPLC system



Column: TSKgel BioAssist Q, 4.6 mm ID x 5 cm (PEEK)
Competitor Q, 5.0 mm ID x 5 cm
Elution: 30 min linear gradient from 0 to 1 mol/L NaCl in 20 mmol/L sodium phosphate pH 8.0
Flow Rate: 1.0 mL/min
Detection: UV @ 280 nm
Sample: 1) conalbumin, i) ovalbumin impurity
2) ovalbumin, 3) trypsin inhibitor

TABLE I

Comparison of dynamic binding capacities

	Binding capacity (mg/mL)			
Protein	BioAssist Q	SuperQ -5PW	Conventional Q type product A	Conventional Q type product B
Thyroglobulin	77.4	22.9	20.2	1.8
Monoclonal IgG_1	57.8	43.3	46.7	47.7
Human Serum Albumin	83.1	78.9	48.2	48.8
Trypsin Inhibitor	84.3	92.8	51.8	57.8

Columns: TSKgel BioAssist Q (4.6 mmID x 1 cm L)
TSKgel SuperQ-5PW (4.6 mmID x 1 cm L)
Conventional Q type product A (4.6 mm ID x 1 cm L)
Conventional Q type product B (4.6 mm ID x 1 cm L)
Solvent: 20 mmol/L Tris-HCl buffer, pH 8.0
Flow rate: 0.38 mL/min
Detection: UV (280 nm)
*The capacity was determined at 10% height of the breakthrough curve at UV 280 nm.

SuperQ-5PW and DEAE-5PW

FIGURE 3 shows the analysis of a 16-mer morpholine oligonucleotide on TSKgel SuperQ-5PW column using a NaCl gradient in a 10 mmol/L sodium hydroxide mobile phase.

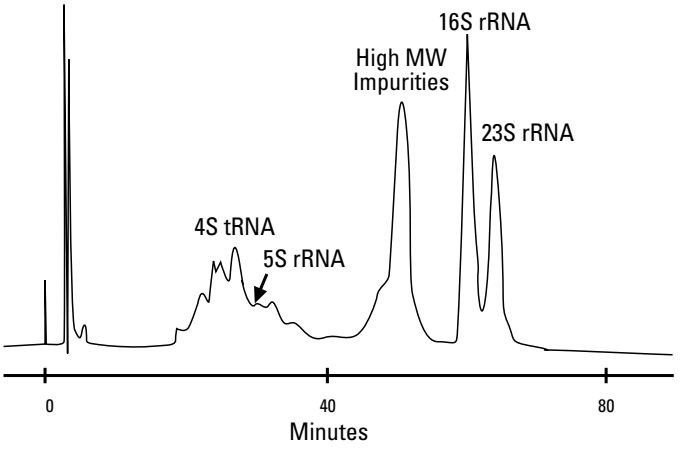
FIGURE 4 shows the fractionation of high molecular weight E. coli RNA on TSKgel DEAE-5PW, effectively utilizing the large 1000 Å pores of this base resin.

DEAE-NPR and DNA-NPR

Because of their small (2.5 µm) particle size, non porous resin (NPR) columns excel in rapid separations of large biomolecules such as DNA digests. A chromatogram of a standard Hae III digest of pBR322 DNA on TSKgel DEAE-NPR, protected by a guard column, is shown in FIGURE 5. To achieve better resolution for PCR fragment analysis we recommend the use of TSK-GEL DNA-NPR columns, which are 7.5 cm long and 4.6 mm wide, providing higher efficiency in a longer column.

FIGURE 4

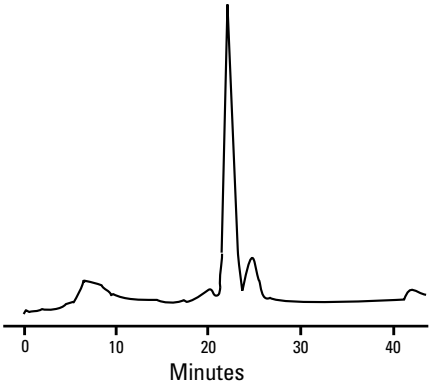
Large pore TSKgel DEAE-5PW resolves high MW RNA



Column:	TSKgel DEAE-5PW, 6mm ID x 15cm
Sample:	total *E. coli* RNA
Elution:	300min linear gradient from 0.3mol/L to 1.0mol/L NaCl in 0.1mol/L Tris-HCl, pH 7.6
Flow Rate:	1.0mL/min
Detection:	UV @ 260nm

FIGURE 3

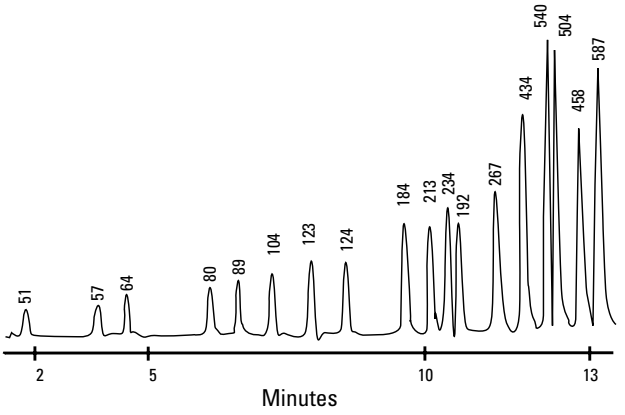
Analysis of synthetic oligonucleotide on TSKgel SuperQ-5PW



Column:	TSKgel SuperQ-5PW, 7.5mm ID x 7.5cm
Sample:	16-mer morpholine oligonucleotide, AAG AAG AAG AGG GGA G
Sample load:	0.5 O.D. (optical density)
Mobile phase:	A: 10mmol/L NaOH B: 10mmol/L NaOH with 1mol/L NaCl
Gradient:	Initial: 0% B 40min: 50% B 41min: 100% B 46min: 100% B
Flow Rate:	1 mL/min
Detection:	UV @ 254nm

FIGURE 5

Higher resolution and faster analysis on TSKgel DEAE-NPR



Column:	TSKgel DEAE-NPR, 4.6mm ID x 3.5cm, with guard column, 4.6mm ID x 0.5cm
Sample:	*Hae* III digest of pBR322 DNA, (base pair number for each peak is indicated)
Buffer A:	0.02mol/L Tris-HCl, pH 9.0
Buffer B:	Buffer A plus 1.0mol/L NaCl
Elution:	15min linear gradient from 48% to 65% buffer B
Flow Rate:	1.5mL/min
Pressure:	2000psi
Temperature:	40° C
Detection:	UV @ 260nm

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Silica-based Anion Exchange Columns

TSK-GEL 2SW-type columns provide high performance separations of small ionic solutes. The increased solubility of the silica backbone above pH 7 limits the use of the TSK-GEL 2SW-type columns to acidic or neutral mobile phases. This restricts method development and requires special cleaning procedures when compared to the more robust TSK-GEL 5PW-type polymer-based columns.

High performance analyses of small anionic species are best performed on small pore silica-based anion exchangers, such as TSKgel DEAE-2SW. This is demonstrated in FIGURE 6.

The 250 Å pore size TSKgel DEAE-3SW column is used for separating peptides, low MW proteins and DNA fragments.

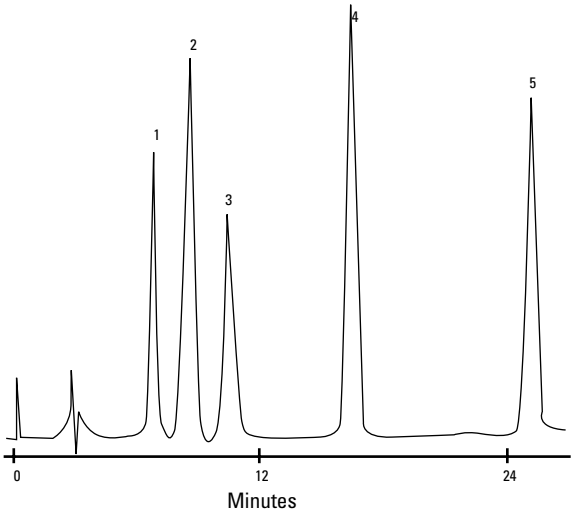
Specialty Columns

Analyses of monosaccharides, disaccharides, and sugar alcohols can be performed on PS-DVB columns, either by isocratic (TSKgel Sugar AXI) or by gradient (TSKgel Sugar AXG) analysis. Saccharides are retained on Sugar AX columns following the formation of negatively charged complexes with boric acid at alkaline pH. FIGURE 7 shows the separation of twelve mono- and di-saccharides.

The strong anion exchange TSKgel SAX column can be used for the separation of isomerized sugars, alcohols, and low molecular weight organic acids.

FIGURE 6

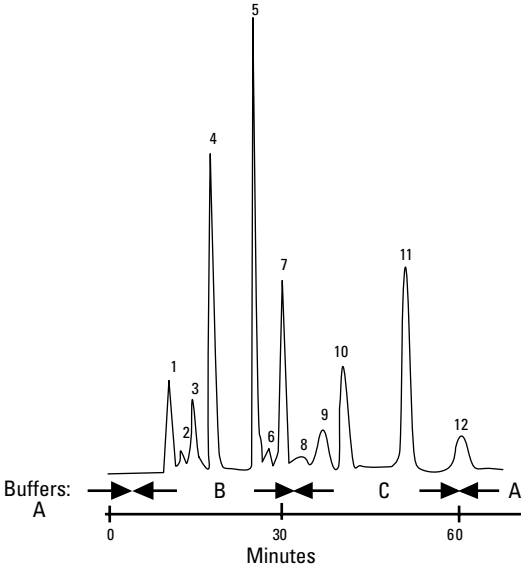
Separation of nucleotides on TSKgel DEAE-2SW



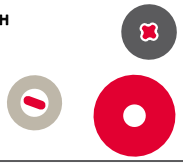
Column:	TSKgel DEAE-2SW, 4.6mm ID x 25cm
Sample:	1. AMP, 2. IMP, 3. GMP, 4.ADP, 5. ATP
Buffer A:	ACN in 0.1mol/L phosphate, pH 3.0, 20/80
Buffer B:	ACN in 0.5mol/L phosphate, pH 3.0, 20/80
Elution:	30min linear gradient from buffer A to B
Flow Rate:	1.0mL/min
Detection:	UV @ 260nm

FIGURE 7

Separation of saccharide mixture on TSKgel Sugar AXG



Column:	TSKgel Sugar AXG, 4.6mm ID x 15cm
Sample:	disaccharides, 25mmol/L; monosaccharides, 50mmol/L: 1. cellobiose, 2. maltose, 3. lactose, 4. rhamnose, 5. lyxose, 6. ribose, 7. mannose, 8. fructose, 9. arabinose, 10. galactose, 11. xylose, 12. glucose
Elution:	step gradient: 6min buffer A, 0.6mol/L boric acid, pH 7.7; then 27min buffer B, 0.7mol/L boric acid, pH 7.25; then 30min buffer C, 0.7mol/L boric acid, pH 8.7
Flow Rate:	0.4mL/min (column and post column reagent solution)
Pressure:	16kg/cm^2
Temperature:	70°C (column), 100°C (post column reactor)
Detection:	fluorescence excitation @ 331nm, emission @ 383nm
PC reagent:	2.5% 2-cyanoacetamide solution



ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Glass columns: polymer-based								
13061	DEAE-5PW Glass, 1000 Å	5.0	5.0	10	≥ 700	0.5 - 0.8	1.0	15
08802	DEAE-5PW Glass, 1000 Å	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.2	10
14016	DEAE-5PW Glass, 1000 Å	20.0	15.0	13	≥ 3,000	4.0 - 6.0	8.0	15
18386	SuperQ-5PW Glass, 1000 Å	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.2	20
PEEK columns: polymer-based								
19685	BioAssist Q, 4000 Å	4.6	5.0	10	≥ 500	0.3 - 1.0	1.2	25
21410	BioAssist Q, 4000 Å	10.0	10.0	13	≥ 500	1.0 - 5.0	7.0	25
Stainless steel columns: polymer-based								
21960	Q-STAT, nonporous -NEW-	3.0	3.5	10	> 200			100
21961	Q-STAT, nonporous -NEW-	4.6	10.0	7	> 2,000			100
21962	DNA-STAT, nonporous -NEW-	4.6	10.0	5	> 4,000			150
13075	DEAE-NPR, nonporous	4.6	3.5	2.5	≥ 1,300	1.0 - 1.5	1.6	200
18249	DNA-NPR, nonporous	4.6	7.5	2.5	≥ 6,000	0.5 - 1.0	1.5	300
18757	DEAE-5PW, 1000 Å	2.0	7.5	10	≥ 1,300	0.05 - 0.10	0.12	15
07164	DEAE-5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	15
07574	DEAE-5PW, 1000 Å	21.5	15.0	13	≥ 3,000	4.0 - 6.0	8.0	25
07930	DEAE-5PW, 1000 Å	55.0	20.0	20	≥ 1,500	20.0 - 40.0	50.0	4
18257	SuperQ-5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	20
18387	SuperQ-5PW, 1000 Å	21.5	15.0	13	≥ 3,000	4.0 - 6.0	8.0	20
08639	Sugar AXI, 60 Å	4.6	15.0	8	≥ 3,700	0.2 - 0.4	0.5	30
08640	Sugar AXG, 60 Å	4.6	15.0	10	≥ 2,700	0.2 - 0.5	0.5	20
07157	SAX	6.0	15.0	5	≥ 2,000	0.5 - 1.0	1.2	150
Stainless steel columns: silica-based								
18761	DEAE-2SW, 125 Å	2.0	25.0	5	≥ 5,000	0.12 - 0.17	0.22	130
07168	DEAE-2SW, 125 Å	4.6	25.0	5	≥ 5,000	0.6 - 0.8	1.0	150
07163	DEAE-3SW, 250 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	20
Guard column products								
17088	DEAE-NPR Guard column	4.6	0.5	5	For P/N 13075			
18253	DNA-NPR Guard column	4.6	0.5	5	For P/N 18249			
18388	SuperQ-5PW Guardgel Kit			20	For P/N 18257			
18389	SuperQ-5PW Guardgel Kit, Glass			20	For P/N 18386			
18390	SuperQ-5PW Guardgel Kit			20	For P/N 18387			
07210	DEAE-5PW Guardgel Kit			20	For P/N 07164			
42152	DEAE-5PW Guard cartridge	2.0	1.0	10	For P/N 18757			
08806	DEAE-5PW Guardgel Kit, Glass			20	For P/Ns 13061 and 08802			
14466	DEAE-5PW Guard column, Glass	20.0	2.0	13	For P/N 14016			
16092	DEAE-5PW Prep Guardgel Kit			20	For P/N 07574			
07928	DEAE-5PW Guard column	45.0	5.0	20	For P/N 07930			
07648	DEAE-SW Guardgel Kit			20	For P/Ns 07168 and 07163			
42154	DEAE-2SW Guard cartridge	2.0	1.0	5	For P/N 18761			
19308	Guard cartridge holder	2.0	1.5		For all 2mm ID guard cartridges			

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TSK-GEL CATION EXCHANGE COLUMNS

HIGHLIGHTS

- TSKgel SP-STAT and CM-STAT nonporous columns provide high efficiency separation at short analysis time.
- Pore structure and bonding chemistry of TSKgel BioAssist S provides high capacity for medium to large MW proteins.
- BioAssist columns are packed in 4.6 mm ID or 10 mm ID PEEK hardware. Other columns are available in glass and stainless steel for analytical, semi-preparative and preparative applications.
- Binding capacity for small to medium size proteins on TSKgel CM-3SW is approximately double that of TSKgel CM-5PW due to the smaller pore size and larger surface area.
- The TSKgel SP-5PW column is available in 2 mm ID format for LC-MS applications.

APPLICATIONS

TSKgel BioAssist S

TSKgel BioAssist S is suitable for use in systems that are designed for HPLC, laboratory, or semi-preparative applications. The large pore size of the TSKgel BioAssist S resin provides high dynamic capacity due to novel bonded phase design (see TABLE II). FIGURE 8 demonstrates these features for the analysis of bromelain, a proteolytic enzyme that is used as a nutritional supplement. Bromelain is a basic glycoprotein with a MW of 33 kDa and a pI of 9.55.

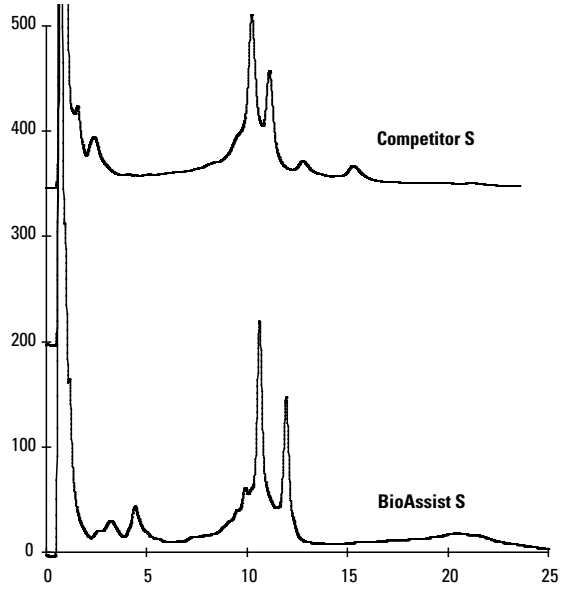
TSKgel SP-5PW and TSKgel CM-5PW

Differences in selectivity between strong (TSKgel SP-5PW) and weak (TSKgel CM-5PW) cation exchangers are demonstrated in FIGURE 9, which is a separation of globular proteins.

The purification of 200mg of crude lipoxidase on a 21.5 mm ID TSKgel SP-5PW column is illustrated in FIGURE 10. Scale-up is simplified as only the particle size changes from 10 µm (7.5 mm ID) to 13 µm (21.5 mm ID) or 20 µm (55 mm ID) columns.

FIGURE 8

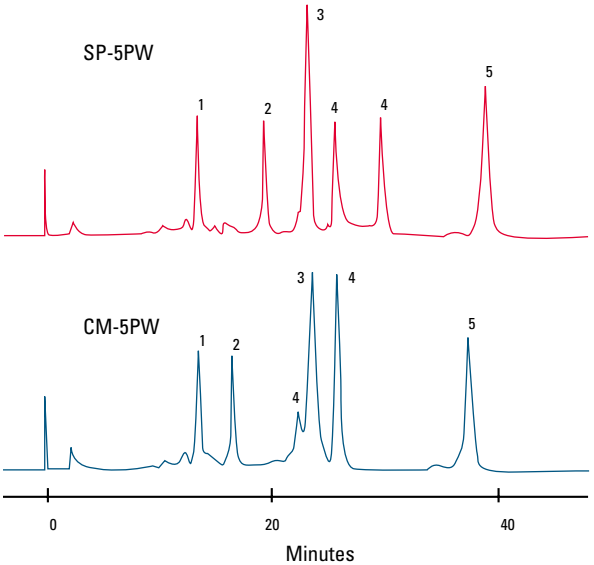
Bromelain Analysis on TSKgel Bioassist S and Competitor S Columns



Columns:	TSKgel BioAssist S, 4.6mm ID x 5cm, PEEK Competitor S 5mm ID x 5cm
Elution:	20 min (TSKgel) or 30 min (Competitor S) linear gradient of NaCl from 0 to 0.5mol/L in 20mmol/L sodium phosphate buffer, pH 7.0
Flow Rate:	0.8mL/min for TSKgel; 1.0mL/min for Competitor S
Detection:	UV @ 280nm
Temperature:	25°C
Sample:	crude bromelain (C4882, Sigma), 1mg in 100µL

FIGURE 9

Selectivity of TSK-GEL strong and weak cation exchangers

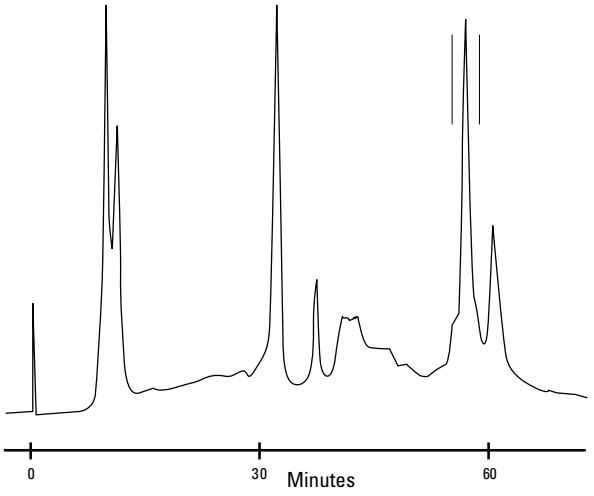


Columns:	TSKgel SP-5PW and TSKgel CM-5PW, 7.5 mm ID x 7.5 cm L
Sample:	1. trypsinogen, 2. ribonuclease A, 3. a-chymotrypsinogen, 4. cytochrome C, 5. lysozyme
Elution:	60 min linear gradient from 0 mol/L to 0.5 mol/L NaCl in 0.02 mol/L phosphate, pH 7.0
Flow Rate:	1.0 mL/min
Detection:	UV @ 280 nm

APPLICATIONS - TSK-GEL CATION EXCHANGE COLUMNS

FIGURE 10

Semi-preparative purification of lipoxidase



Column:	TSKgel SP-5PW, 21.5mm ID x 15cm
Sample:	crude lipoxidase, 200mg
Elution:	120min linear gradient from 0mol/L to 0.5mol/L Na_2SO_4 in 0.02mol/L acetate, pH 4.5
Flow Rate:	4.0mL/min
Detection:	UV @ 280nm
Recovery:	Lipoxidase activity collected between the two vertical lines was 84%

TABLE II

Comparison of dynamic binding capacities

	Binding capacity (mg/mL)*	
Protein	BioAssist S	Conventional S type product
γ-globulin	79	48
Lysozyme	84	63
Cytochrome C	95	43
α-chymotrypsinogen	119	-

Columns:	TSKgel BioAssist Conventional S-type product
Size:	4.6 mm ID x 5 cm L (lysozyme, cytochrome C, α-chymotrypsinogen A) 5.0 mm ID x 1cm L (α-globulin)
Solvent:	20 mmol/L sodium phosphate buffer, pH 6.5 (lysozyme, cytochrome C, α-chymotrypsinogen A) 20 mmol/L sodium phosphate buffer pH 5.0 (α-globulin)
Flow rate:	0.38 mL/min
Temperature:	25°C
Detection:	UV @ 280 nm

*The capacity was determined at 10% height of the breakthrough curve at UV 280nm.

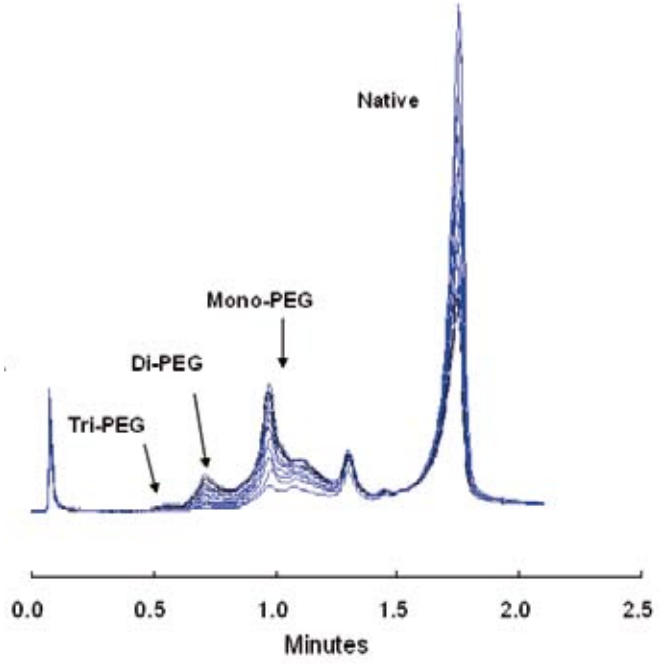
TSKgel SP-STAT, CM-STAT and SP-NPR

Nonporous TSK-GEL STAT columns provide fast, high resolution separations at moderate pressures. FIGURE 11 shows the monitoring of a PEGylation reaction of beta-lactoglobulin on a short SP-STAT column (prototype).

TSKgel SP-NPR columns provide fast separations due to their small (2.5 µm) spherical particles. A purity check of adeno-associated virus, commonly used in gene therapy research, on a TSKgel SP-NPR column is shown in FIGURE 12. This 10 minute HPLC method replaces an existing assay that took two days.

FIGURE 11

Monitoring of PEGylation of ß--lactoglobulin



Column:	Prototype SP-STAT, 4.6 mm ID x 3.5 cm L (10 um)
Eluent:	A: 20 mmol/L Na acetate buffer pH 4.5 B: 0.8 mol/L NaCl in A pH 4.5
Gradient:	0 to 30% B (2 min)
Flow Rate:	4.0 mL/min
Detection:	UV @ 280 nm

Real-time analysis of PEGylation reaction (PEG MW=5000) at 5-minutes intervals

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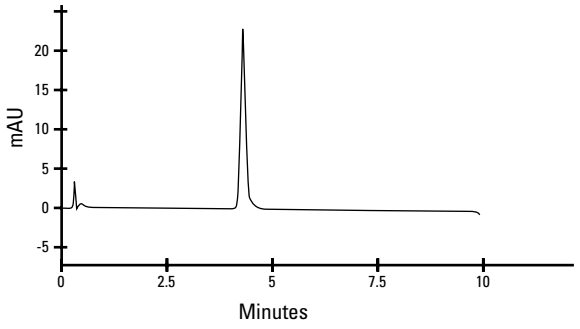
FIGURE 12

Analysis of purified AAV with TSKgel SP-NPR



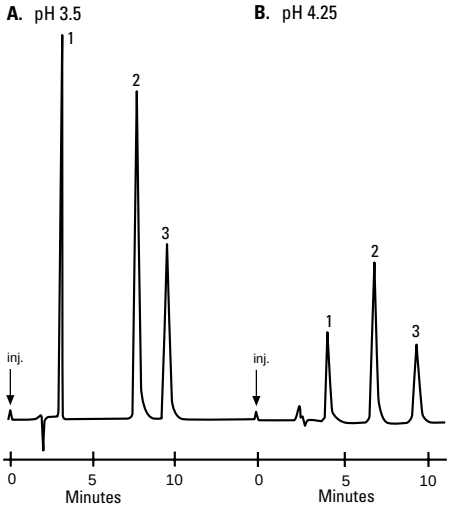
Column: TSKgel SP-NPR, 4.6mm ID x 3.5cm
Sample: purified adeno-associated virus
Elution: A. 50mmol/L HEPES, 1mmol/L EDTA, 5mmol/L MgCl, pH 7.5;
B. 50mmol/L HEPES, 1mmol/L EDTA, 5mmol/L MgCl, pH 7.5 with 0.5mol/L NaCl; linear gradient from 20% to 100% B in 10 column volumes
Flow Rate: 1mL/min
Detection: UV @ 280nm

SKgel SP-2SW, CM-2SW and CM-3SW

Silica-based cation exchangers are typically used in the separation of low molecular weight compounds such as pharmaceuticals, nucleotides, catecholamines, and small peptides. For example, FIGURE 13 shows the separation of nucleosides on the TSKgel SP-2SW column, while FIGURE 14 shows the rapid analysis of the herbicides paraquat and diquat in urine on TSKgel SP-2SW.

FIGURE 13

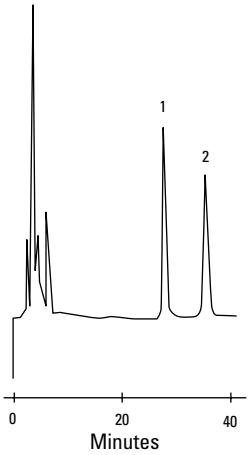
Separation of nucleosides by ion-exchange chromatography on TSKgel SP-2SW



Column: TSKgel SP-2SW 4.6mm ID x 25cm
Sample: Nucleoside Standards: 1) Guanosine, 2) Cytidine, 3) Adenosine
Mobile Phase: A) 0.1 mol/L sodium citrate - phosphoric acid buffer, pH 3.5
B) 0.1 mol/L sodium citrate - acetic acid buffer, pH 4.25
Flow Rate: 0.75 mL/min

FIGURE 14

Rapid Analysis for the Herbicides Paraquat and Diquat



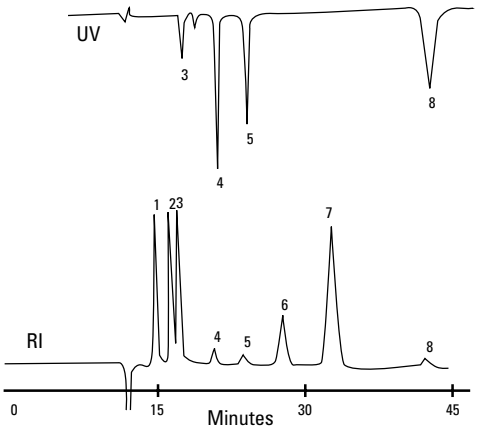
Column: TSKgel SP-2SW, 4.6mm ID x 25cm
Sample: 1. paraquat, 5µg/mL; 2. diquat, 5µg/mL
Elution: 20% ACN in 0.2mol/L phosphate, pH 3.0
Flow Rate: 1.0 mL/min
Detection: UV @ 290nm

Specialty columns

Ion exclusion chromatography can be used as an effective method for separating alcohols. An example of a saccharide, organic acid, and alcohol separation is shown in FIGURE 15 on two TSKgel SCX (H^+) columns in series.

FIGURE 15

Separation of mixture of saccharides, organic acids and alcohols



Column: TSKgel SCX (H^+), two 7.8mm ID x 30cm (in series)
Sample: 1. maltose, 2. glucose, 3. fructose, 4. lactic acid, 5. acetic acid, 6. methanol, 7. ethanol, 8. butyric acid
Elution: 0.05mol/L $HClO_4$
Flow Rate: 0.8mL/min
Detection: UV @ 210nm, Refractive Index

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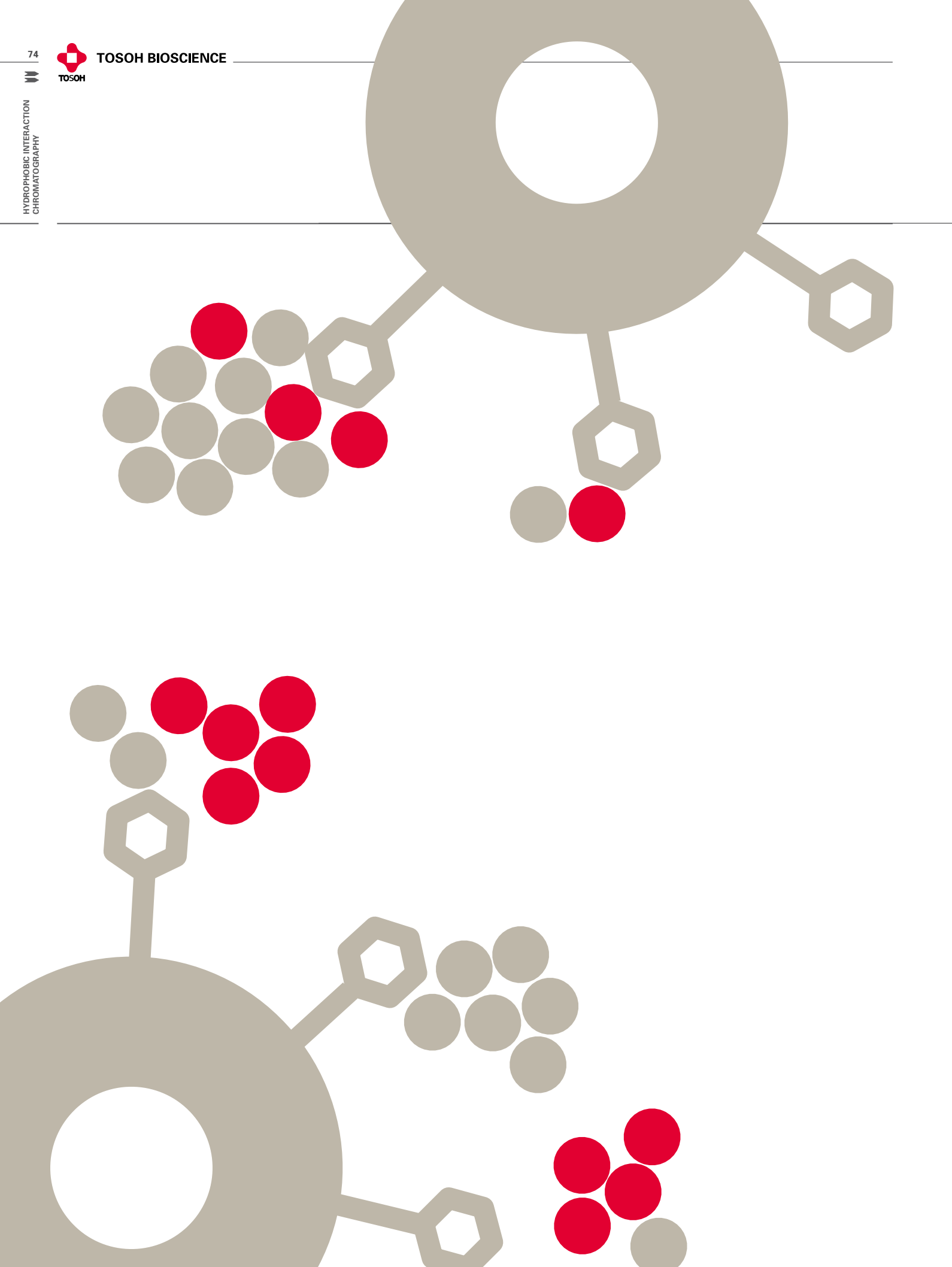
ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Glass columns: polymer-based								
14010	CM-5PW Glass, 1000 Å	5.0	5.0	10	≥ 700	0.5 - 0.8	1.0	15
14011	CM-5PW Glass, 1000 Å	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.2	10
14012	CM-5PW Glass, 1000 Å	20.0	15.0	13	≥ 2,500	4.0 - 6.0	8.0	15
13062	SP-5PW Glass, 1000 Å	5.0	5.0	10	≥ 700	0.5 - 0.8	1.0	15
08803	SP-5PW Glass, 1000 Å	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.2	10
14017	SP-5PW Glass, 1000 Å	20.0	15.0	13	≥ 3,000	4.0 - 6.0	8.0	15
PEEK columns: polymer-based								
19686	BioAssist S, 1300 Å	4.6	5.0	7	≥ 1,500	0.3 - 0.8	1.0	25
21411	BioAssist S, 1300 Å	10.0	10.0	13	≥ 3,000	1.0 - 5.0	7.0	25
Stainless steel columns: polymer-based								
21965	CM-STAT, nonporous -NEW-	3.0	3.5	10	≥ 200			100
21966	CM-STAT, nonporous -NEW-	4.6	10.0	7	≥ 2,000			100
21963	SP-STAT, nonporous -NEW-	3.0	3.5	10	≥ 200			100
21964	SP-STAT, nonporous -NEW-	4.6	10.0	7	≥ 2,000			100
13068	CM-5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	15
14021	CM-5PW, 1000 Å	21.5	15.0	13	≥ 2,500	4.0 - 6.0	8.0	25
18758	SP-5PW, 1000 Å	2.0	7.5	10	≥ 1,300	0.05 - 0.10	0.12	10
07161	SP-5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	15
07575	SP-5PW, 1000 Å	21.5	15.0	13	≥ 3,000	4.0 - 6.0	8.0	25
07934	SP-5PW, 1000 Å	55.0	20.0	20	≥ 1,500	20.0 - 40.0	50.0	4
13076	SP-NPR, nonporous	4.6	3.5	2.5	≥ 1,300	1.0 - 1.5	1.6	200
07156	SCX (Na^+)	6.0	15.0	5	≥ 2,000	0.5 - 1.0	1.2	150
07158	SCX (H^+)	7.8	30.0	5	≥ 12,000	0.5 - 1.0	1.2	50
Stainless steel columns: silica-based								
07165	SP-2SW, 125 Å	4.6	25.0	5	≥ 5,000	0.6 - 0.8	1.0	150
07167	CM-2SW, 125 Å	4.6	25.0	5	≥ 5,000	0.6 - 0.8	1.0	150
07162	CM-3SW, 250 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	20
Guard column products								
13069	CM-5PW Guardgel Kit			10	For P/N 13068			
16094	CM-5PW Prep Guardgel Kit			20	For P/N 14021			
14024	CM-5PW Guardgel Kit, Glass			20	For P/Ns 14010 and 14011			
14468	CM-5PW Guard column, Glass	20.0	2.0	13	For P/N 14012			
07211	SP-5PW Guardgel Kit			20	For P/N 07161			
42153	SP-5PW Guard cartridge	2.0	1.0	10	For P/N 18758			
08807	SP-5PW Guardgel Kit, Glass			20	For P/Ns 13062 and 08803			
14467	SP-5PW Guard column, Glass	20.0	2.0	13	For P/N 14017			
16093	SP-5PW Prep Guardgel Kit			20	For P/N 07575			
07932	SP-5PW Guard column	45.0	5.0	20	For P/N 07934			
07650	CM-SW Guardgel Kit			20	For P/Ns 07167 and 07162			
19308	Guard cartridge holder	2.0	1.5		For all 2mm ID Guard cartridges			



HIC
HYDROPHOBIC INTERACTION CHROMATOGRAPHY

HIC PRODUCTS

- TSKgel Ether-5PW
- TSKgel Phenyl-5PW
- TSKgel Butyl-5PW

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.

INTRODUCTION TO TSK-GEL HIC COLUMNS

Hydrophobic Interaction Chromatography (HIC) is based on the interaction between hydrophobic groups on a protein and a hydrophobic ligand on the solid support. HIC offers a distinct advantage for easily denatured proteins; it can be run using moderate concentrations of ammonium sulfate, which favors the stability of many proteins.

The binding of proteins to a hydrophobic matrix is affected by a number of factors including (1) the type of ligand, (2) the ligand density on the solid support, (3) the backbone material of the matrix, (4) the hydrophobic nature of the protein, and (5) the type of salt used. All of these factors help to make HIC a powerful technique for the separation of biomolecules.

Tosoh Bioscience offers three different HIC column types in analytical format: TSKgel Phenyl-5PW, Ether-5PW and Butyl NPR. TSKgel Phenyl-5PW and Ether-5PW are also available in preparative column formats.

Column Selection

The HIC packing materials are based on the polymeric TSKgel G5000PW size exclusion resin (a hydrophilic gel with an estimated protein exclusion limit of 5,000,000 Da) which is then derivatized with oligoethylene-glycol (Ether-5PW) or phenyl (Phenyl-5PW) groups. Columns, depending on diameter, are packed with 10, 13 or 20 µm particles.

TSKgel Ether-5PW is less hydrophobic than TSKgel Phenyl-5PW. It displays weaker interaction and thus shorter retention times compared to Phenyl-5PW, as shown in FIGURE 1. TSKgel Ether-5PW is the best choice for the separation of very hydrophobic proteins such as membrane proteins or monoclonal antibodies.

The TSKgel Phenyl-5PW columns were the first commercially available, polymer-based columns for high performance HIC. These columns have been instrumental to the increase in popularity of this technique for analytical, preparative, and process scale separations of biopolymers. FIGURE 2 compares the separation of standard proteins on the Ether, Phenyl, and Butyl supports under similar operating conditions.

The base material of TSKgel Butyl-NPR is of the same chemical composition as the G5000PW base material used to prepare Phenyl-5PW and Ether-5PW. The difference between the two packings is that the G5000PW packing is porous, whereas the base material of the TSKgel Butyl-NPR column consists of spherical 2.5 µm nonporous particles. Nonporous resins (NPR) are typically used for high-speed analytical applications. See FIGURE 3 for the structure of the HIC resins.

TSKgel Butyl-NPR is the least hydrophobic among the three TSK-GEL HIC columns and requires a higher salt concentration for binding. TSKgel Butyl-NPR columns provide fast and quantitative HIC, because smaller particles provide higher efficiency. By packing the 2.5 µm nonporous resin particles into shorter columns, typical analysis times are reduced to less than 10 minutes. Pore diffusion is often the rate-limiting step in the overall mass transport of large biomolecules through a porous column. Eliminating the pores provides higher resolution at higher flow rates. Another benefit of NPR resins is excellent mass recovery, allowing quantitation down to nanogram levels. These properties make TSKgel Butyl-NPR the preferred choice for process monitoring and quality control.

TSK-GEL HIC columns are compatible with water-soluble organic solvents at concentration below 50 % (20 % for Butyl-NPR).

FEATURES

- Choice of three hydrophobic ligands (ether, phenyl or butyl)
- Rigid polymeric base resin
- Similar chemistry to Toyopearl resins
- TSKgel Phenyl-5PW offered in PEEK hardware
- Ether and Phenyl available in 2 mm ID format

BENEFITS

- Added flexibility during method development
- Wide pH range (2-12) enabling robust cleaning options
- Seamless scalability from analytical to preparative scaley
- Eliminates undesirable interactions with column hardware
- LC-MS applications

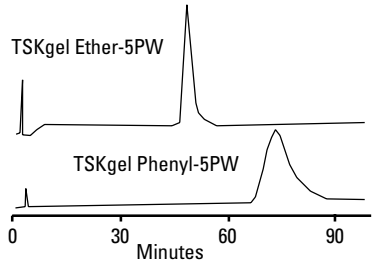
HIC

Column selection for TSK-GEL HIC Columns

Sample	MW range (Da)	TSK-GEL Column
Peptides	< 10,000	Butyl-NPR
Medium to large proteins	> 10,000	Phenyl-5PW Ether-5PW Butyl-NPR
DNA, RNA, and PCR products	> 500,000	Phenyl-5PW Butyl-NPR
Oligonucleotides	> 10,000	Phenyl-5PW Butyl-NPR

FIGURE 1

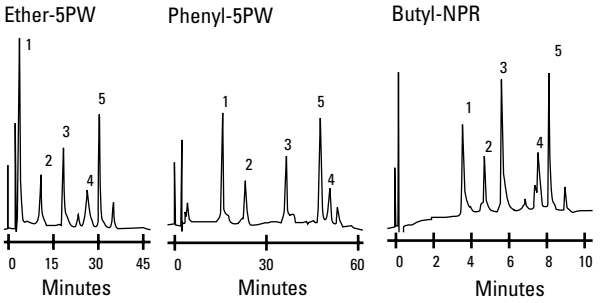
Separation of α-amylase



Column: TSKgel Ether-5PW, 7.5mm ID x 7.5cm
TSKgel Phenyl-5PW, 7.5mm ID x 7.5cm
Sample: α-amylase
Elution: A. 0.1mol/L phosphate buffer (pH 7.0) + 1.1mol/L Na_2SO_4
B. 0.1mol/L phosphate buffer (pH 7.0)
60min linear gradient from A→B(1.1mol/L→0mol/L Na_2SO_4)
Flow Rate: 1.0mL/min
Detection: UV @ 280nm

FIGURE 2

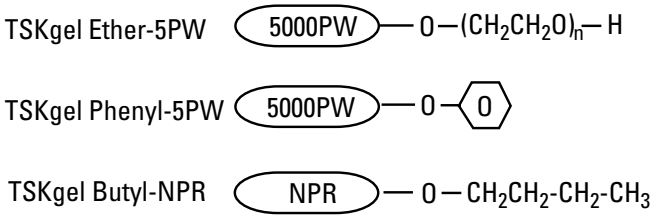
Comparing conventional and nonporous HIC columns



Column: TSKgel Ether-5PW & TSKgel Phenyl-5PW, 7.5mm ID x 7.5cm
TSKgel Butyl-NPR, 4.6mm ID x 3.5cm
Sample: 1. myoglobin, 2. ribonuclease A, 3. lysozyme,
4. α-chymotrypsin, 5. α-chymotrypsinogen
Injection: 5PW-type columns: 100µL (50-100µg);
NPR-type column: 20µL (1.5-40µg)
Elution: 60min linear gradient from 1.8mol/L to 0mol/L $(NH_4)_2SO_4$
in 0.1mol/L phosphate buffer, pH 7.0, for 5PW-type columns;
12min linear gradient from 2.3mol/L to 0mol/L $(NH_4)_2SO_4$
in 0.1mol/L phosphate buffer, pH 7.0 for TSKgel Butyl-NPR
Flow Rate: 1.0mL/min
Detection: UV @ 280nm

FIGURE 3

Structure of TSK-GEL HIC resins



Sample capacity

One definition of sample capacity is the amount of pure compound injected onto the column at which the peak width is 10% larger than the peak width under low loading conditions. Using this definition, the capacity of a 7.5 mm ID x 7.5 cm L TSKgel Phenyl-5PW column varies from 0.1 to 1 mg of protein. Resolution and peak width are dependent on sample loading, as shown in FIGURE 4. Therefore, sample loading should be kept within 0.1-0.5 mg in order to obtain the highest resolution.

Separations on TSKgel Ether-5PW columns usually take 30-60 minutes. 0.5 mg of pure protein can be purified from a 5-10 mg crude protein mixture using a 7.5 mm ID x 7.5 cm L column.

Since almost all of the surface area of a porous particle is inside the pores, the capacity of the 4.6 mm ID x 3.5 cm L TSKgel Butyl-NPR column is significantly less than that for the 7.5 mm ID x 7.5 cm L Phenyl-5PW column. Capacities for the Butyl-NPR column are 100 µg for crude sample and 2 µg for pure sample.

Chemical stability

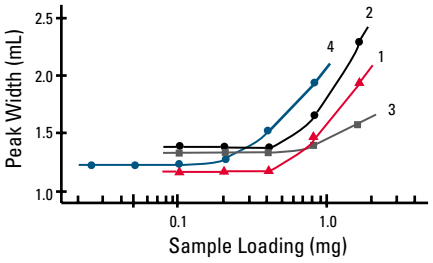
TSK-GEL 5PW-type HIC columns are physically and chemically stable in water-soluble organic solvents (at < 50% methanol, ethanol, ACN, DMF, DMSO or < 30 % chloroform). Change the solvent gradually by reducing the flow rate (preferably with a gradient) because rapid change may cause degradation of column efficiency. Note: When changing to an organic solvent, reduce the salt concentration to prevent precipitation of the salt on the column. Also, chaotropic agents (urea, SDS, etc.) will reduce the adsorption of biomolecules; therefore, use low levels of these agents (<2 mol/L).

The addition of organic solvents or chaotropic agents in the final buffer can improve separations. However, relative elution positions may change. Therefore, add chaotropic agent and organic solvent in small quantities. See FIGURE 5 for the effect of chaotropic agents and organic solvents on the HIC separation of two different samples.

Polymer-based columns are stable when cleaning at alkaline pH. All TSK-GEL HIC columns can be routinely operated from pH 2-12. Table I shows that the phenyl groups on the TSKgel Phenyl-5PW are stable for more than 10 days upon exposure to 0.5 mol/L NaOH or 0.5 mol/L acetic acid.

FIGURE 4

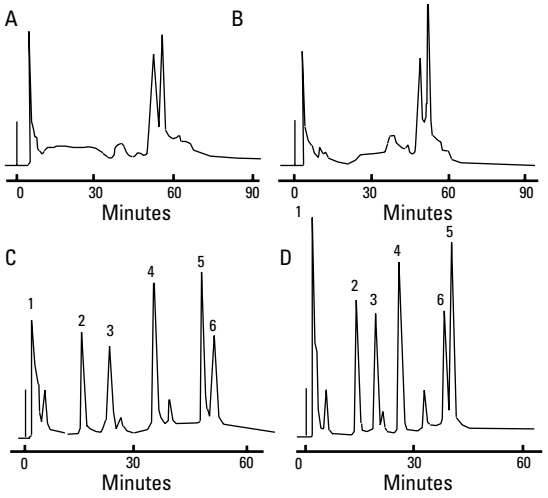
Dependence of peak width on sample loading in the separation of proteins



Column: TSKgel Phenyl-5PW, 7.5 mm ID x 7.5 cm L
Sample: 1. myoglobin; 2. ribonuclease A; 3. ovalbumin; 4. α-chymotrypsin; concentration: 0.025 % to 1.6 %
Elution: 60 min linear gradient of $(NH_4)_2SO_4$ from 1.5 mol/L to 0 mol/L in 0.1 mol/L phosphate buffer (pH 7.0)
Flow Rate: 0.5 mL/min
Temperature: 25 °C
Detection: UV @ 280 nm

FIGURE 5

Effect of urea and isopropanol on the separation of commercial lipoxidase and a standard protein mixture



Column: TSKgel Phenyl-5PW, 7.5mm ID x 7.5cm
Sample: A & B: commercial lipoxidase
C & D: protein mixture:
1. cytochrome C; 2. myoglobin
3. ribonuclease A; 4. lysozyme
5. α-chymotrypsinogen; 6. α-chymotrypsin
Elution: A: 60min linear gradient from 0.1mol/L phosphate buffer containing 1.5mol/L $(NH_4)_2SO_4$ (pH 7.0) to 0.1mol/L phosphate buffer (pH 7.0)
B: 60min linear gradient from 0.1mol/L phosphate buffer containing 1.5mol/L $(NH_4)_2SO_4$ (pH 7.0) to 0.1mol/L phosphate buffer containing 2mol/L urea (pH 7.0)
C: 60min linear gradient from 0.1mol/L phosphate buffer containing 1.8mol/L $(NH_4)_2SO_4$ (pH 7.0) to 0.1 mol/L phosphate buffer (pH 7.0)
D: 60min linear gradient from 0.1mol/L phosphate buffer containing 1.8mol/L $(NH_4)_2SO_4$ (pH 7.0) to 0.1mol/L phosphate buffer (pH 7.0) containing 7% isopropanol
Flow Rate: A & B: 0.5mL/min; C & D: 1.0mL/min
Temperature: 25°C
Detection: UV @ 280nm

TABLE 1

Long-term exposure of TSKgel Phenyl-5PW to acid and base

Acid/base	Phenyl content (mmol/mL - resin)	
	Before exposure	*After 10 days exposure*
0.5 mol/L CH_3COOH	0.105	0.106
0.5 mol/L NaOH	0.105	0.104

HIC

APPLICATIONS OF TSK-GEL ETHER-5PW COLUMNS

HIGHLIGHTS

- Ether, phenyl, and butyl functionalities are available.
- TSKgel Ether-5PW and Phenyl-5PW columns are available in a 2 mm ID format.
- Large 1000 Å pore size of the base matrix accommodates proteins up to 5 x 10^6 Da.
- Polymeric resin is chemically and physically stable to changes in pH and ionic strength and compatible with a variety of organic solvents.
- High binding capacity is achieved for TSK-GEL 5PW-type HIC packing materials.
- Nonporous resins (NPR) allow fast analysis for quality control or process monitoring

Monoclonal Antibodies

Monoclonal antibodies (mAbs) play a part in many research, diagnostic, and therapeutic applications. Monoclonal antibodies are generally the most hydrophobic proteins in ascites fluid and cell culture supernatant. FIGURE 7 shows typical results from the screening of two mAbs.

Antibiotics

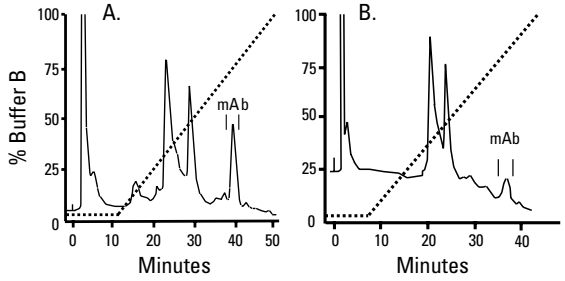
The TSKgel Ether-5PW column was used to determine the relative purity of the antibiotic components C-1027 and C-1027-AG as shown in FIGURE 8. Antibiotic C-1027 is composed of a protein consisting of many hydrophobic and hydroxyamino acids with a non-protein chromophore. Antibiotic C-1027-AG is composed of the hydrophobic and hydroxyamino acids without the chromophore.

Human serum

FIGURE 9 displays the excellent recovery of albumin when 16 mL of human serum was purified on a 55 mm ID preparative TSKgel Ether-5PW column.

FIGURE 7

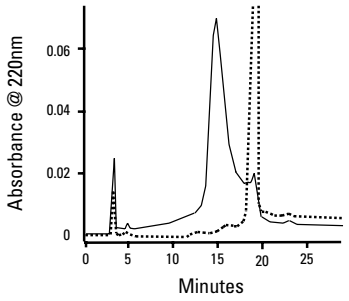
Screening of mouse monoclonal antibodies



Column: TSKgel Ether-5PW, 8.0mm ID x 7.5cm, glass
Sample: A. 20µL unequilibrated mouse IgG$_{2b}$κ ascites
B. 20µL unequilibrated mouse IgMκ ascites
Elution: linear gradient from Buffer A to B as shown
Buffer A: 0.05mol/L sodium phosphate, pH 7.0, 2.0mol/L ammonium sulfate, 1.0mol/L glycine
Buffer B: 0.05mol/L sodium phosphate, pH 7.0, 1.0mol/L glyci
Flow Rate: 1.0mL/min
Detection: UV @ 280nm

FIGURE 8

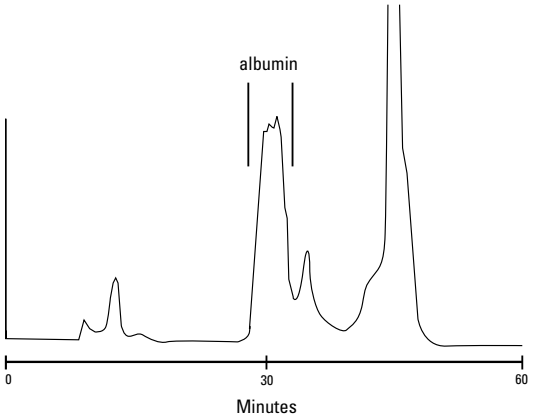
Purification of anti-tumor antibiotic



Column: TSKgel Ether-5PW, 7.5mmID x 7.5cm
Sample: ——— C-1027 ····· C-1027-AG
concentration: 1mg/mL
Injection: 20µL
Elution: linear gradient from 1.5mol/L to 0mol/L $(NH_4)_2SO_4$ in 0.1mol/L phosphate buffer, pH 7.0
Flow Rate: 0.8mL/min
Detection: UV @ 220nm

FIGURE 9

Human serum fractionated on preparative scale



Column: TSKgel Ether-5PW, 55mm ID x 20cm
Sample: 16mL human serum,1.2g total protein
Elution: 36min linear gradient from 1.7mol/L to 0.68mol/L $(NH_4)_2SO_4$ followed by step gradient to 0mol/L $(NH_4)_2SO_4$ in 0.1mol/L phosphate buffer, pH 7.0
Flow Rate: 40mL/min
Detection: UV @ 280nm
Recovery: 92% of the albumin was recovered in the fraction indicated by the vertical lines

APPLICATIONS OF TSK-GEL PHENYL-5PW COLUMNS

Recovery of biological activity for milligram to sub-gram amounts of enzymes eluted from TSKgel Phenyl-5PW columns is shown in the table below. In all cases, at least 80 % of the enzymatic activity was recovered.

TABLE II

Recovery of enzymatic activity from TSKgel Phenyl-5PW at various loadings

Enzyme	Recovery (%)
α-Chymotrypsin, 0.4 mg	92
β-Amylase, 1.3 mg	80
Ferredoxin NADP reductase, 3.0 mg	100
Lactate dehydrogenase, 54 mg	93
Lipoxidase, 1.0 mg	89
Lipoxidase, 200 mg	86
Lysozyme, 0.05 mg	90
Lysozyme, 0.2 mg	90
Phosphoglucose isomerase, 100 mg	96

RNAs

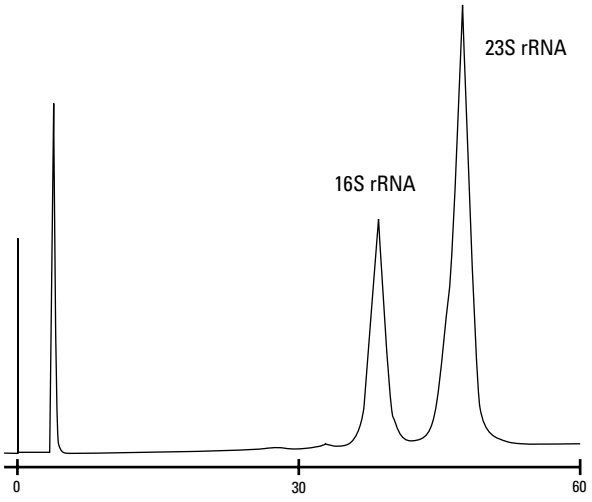
FIGURE 10 illustrates the separation of 16S and 23S ribosomal RNA on a TSKgel Phenyl-5PW column. The approximate molecular weights of these RNAs are 560,000 and 1,100,000 Da, respectively.

Proteins

FIGURE 11 compares the resolution of standard proteins on analytical and preparative TSKgel Phenyl-5PW columns. Different flow rates compensated for the change in particle size and column dimensions. High resolution was obtained on both columns.

FIGURE 10

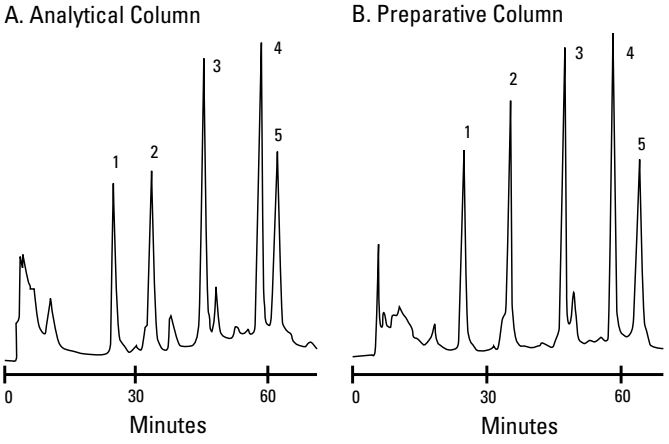
Retain large RNAs on TSKgel Phenyl-5PW



Column: TSKgel Phenyl-5PW, 7.5mm ID x 7.5cm
Sample: 16S and 23S rRNA from *E. coli*, 0.05mg in 0.1mL
Elution: 60min linear gradient from 2mol/L to 0mol/L $(NH_4)_2SO_4$ in 0.1mol/L phosphate buffer, pH 7.0
Flow Rate: 0.5mL/min
Detection: UV @ 280nm

FIGURE 11

Scale up to preparative separations



Column: TSKgel Phenyl-5PW, A.) 7.5mm ID x 7.5cm and B.) 21.5mm ID x 15cm
Sample: 1. myoglobin, 2. ribonuclease A, 3. lysozyme, 4. α-chymotrypsinogen, 5. α-chymotrypsin
Elution: 60min linear gradient from 1.8mol/L to 0mol/L $(NH_4)_2SO_4$ in 0.1mol/L phosphate buffer, pH 7.0
Flow Rate: 0.5mL/min (7.5mm ID) or 4mL/min (21.5mm ID)
Detection: UV @ 280nm

HIC

APPLICATIONS OF TSK-GEL BUTYL-NPR COLUMNS

Proteins

Although loading capacity is limited on NPR columns, small scale separation of proteins is possible.

Almost identical separations were obtained at sample loads from 25 µg up to 100 µg in the separation of a crude sample of phosphoglucose isomerase as shown in FIGURE 12.

FIGURE 13 shows the separation of Fab and Fc fragments of an antibody on TSKgel Butyl-NPR. The appearance of additional Fc fragments is due to the oxidation of methionine residues by 0.10% t-butylhydroperoxide (tBHP). The numbers above the Fc peaks correspond to the number of oxidized residues in each fragment.

Visit our website:

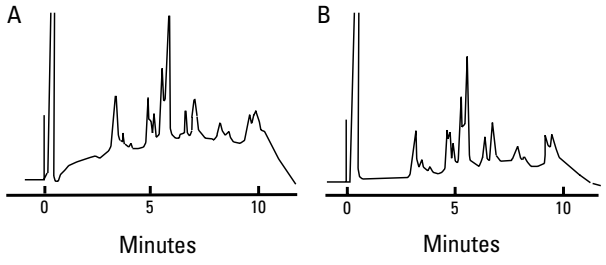
www.tosohbioscience.com for additional applications, product specifications and literature.

Contact our Technical Service specialists to discuss your specific application: +49 (0)711 13257-0 or techsupport.sep@tosoh.com.

Please see next page for ordering information.

FIGURE 12

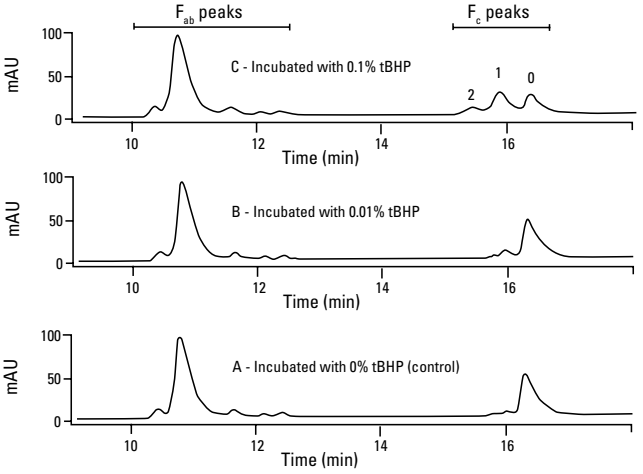
Effect of sample load on the separation of phosphoglucose isomerase



Column:	TSKgel Butyl-NPR, 4.6mm ID x 3.5cm
Sample:	crude sample of phosphoglucose isomerase
Loads:	A. 25µg; B. 100µg
Elution:	10min linear gradient of $(NH_4)_2SO_4$ from 1.8mol/L to 0mol/L in 0.1mol/L phosphate buffer, pH 7.0
Flow Rate:	1.0mL/min
Temperature:	25°C
Detection:	UV @ 280nm

FIGURE 13

Separation of F_{ab} and F_c fragments on TSKgel Butyl-NPR



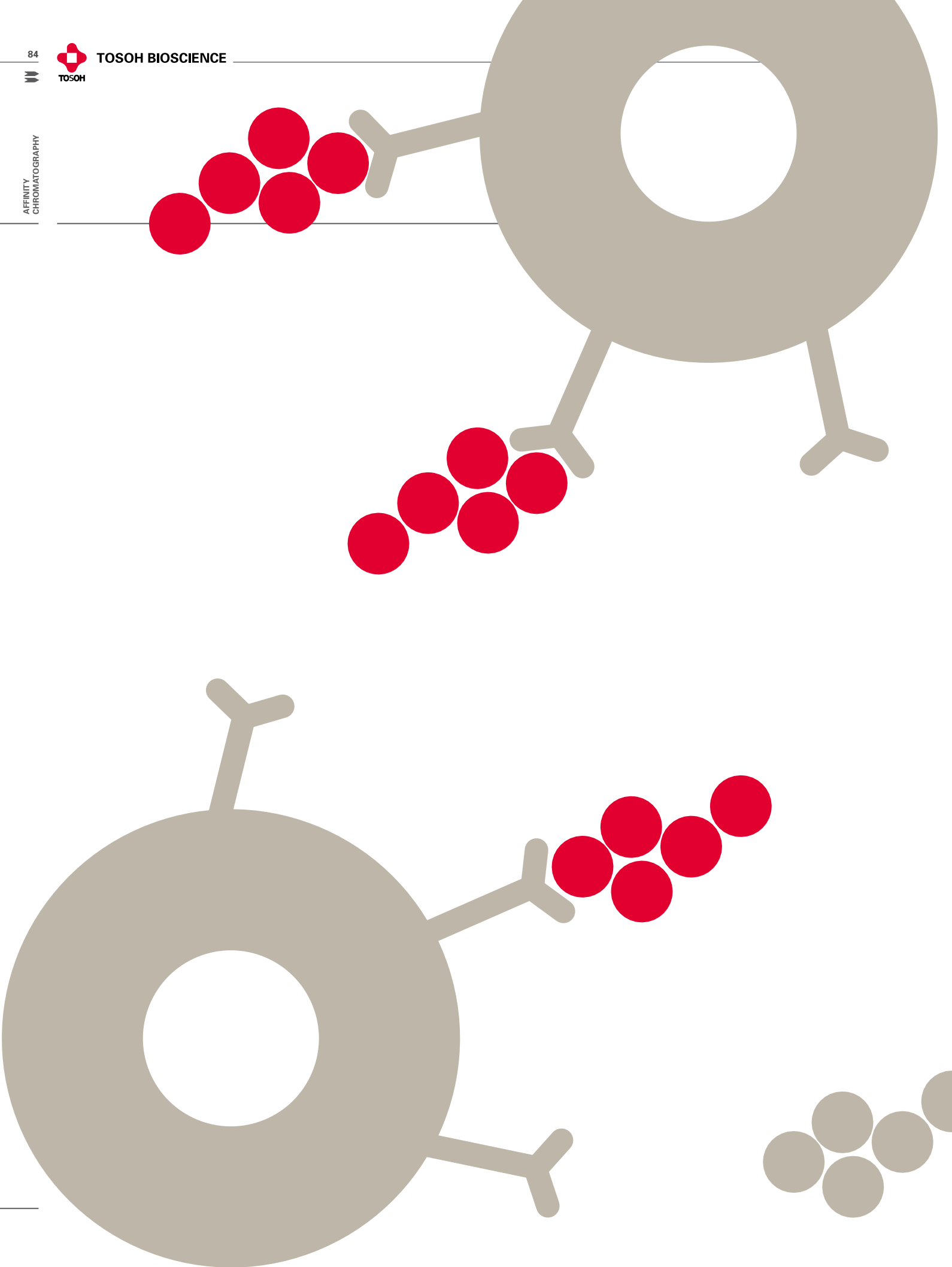
Column:	TSKgel Butyl-NPR, 4.6mm ID x 3.5cm
Elution:	Buffer A: 2mol/L $(NH_4)_2SO_4$, 20mmol/L Tris, pH7 Buffer B: 20mmol/L Tris, pH7
Gradient:	linear from 10%B to 100%B in 34 minutes
Flow rate:	1mL/min
Temperature:	30°C

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max	Maximum Pressure Drop (kg/cm²)
Glass columns								
14013	Ether-5PW Glass, 1000 Å	5.0	5.0	10.0	≥ 600	0.5 - 0.8	1.0	20
14014	Ether-5PW Glass, 1000 Å	8.0	7.5	10.0	≥ 1,000	0.5 - 1.0	1.2	20
14015	Ether-5PW Glass, 1000 Å	20.0	15.0	13.0	≥ 3,000	4.0 - 6.0	8.0	20
13063	Phenyl-5PW Glass, 1000 Å	5.0	5.0	10.0	≥ 600	0.5 - 0.8	1.0	20
08804	Phenyl-5PW Glass, 1000 Å	8.0	7.5	10.0	≥ 1,000	0.5 - 1.0	1.2	20
14018	Phenyl-5PW Glass, 1000 Å	20.0	15.0	13.0	≥ 3,000	4.0 - 6.0	8.0	20
Stainless steel columns								
18760	Ether-5PW, 1000 Å	2.0	7.5	10.0	≥ 1,000	0.05 - 0.10	0.12	6
08641	Ether-5PW, 1000 Å	7.5	7.5	10.0	≥ 1,000	0.5 - 1.0	1.2	20
08642	Ether-5PW, 1000 Å	21.5	15.0	13.0	≥ 3,000	4.0 - 6.0	8.0	20
16255	Ether-5PW, 1000 Å	55.0	20.0	20.0	≥ 1,500	20.0 - 30.0	40.0	5
18759	Phenyl-5PW, 1000 Å	2.0	7.5	10.0	≥ 1,000	0.05 - 0.10	0.12	8
07573	Phenyl-5PW, 1000 Å	7.5	7.5	10.0	≥ 1,000	0.5 - 1.0	1.2	20
07656	Phenyl-5PW, 1000 Å	21.5	15.0	13.0	≥ 3,000	4.0 - 6.0	8.0	20
07938	Phenyl-5PW, 1000 Å	55.0	20.0	20.0	≥ 1,500	20.0 - 40.0	50.0	4
14947	Butyl-NPR, nonporous	4.6	3.5	2.5		0.5 - 1.0	1.2	200
PEEK columns								
20023	BioAssist Phenyl, 1000 Å	7.8	5	10.0	≥ 1,000	0.5 - 1.0	1.2	20

Guard column products		ID (mm)	Length (cm)	Particle Size (µm)	
42156	Ether-5PW Guard cartridge	2.0	1.0	10.0	For P/N 18760
14025	Ether-5PW Guardgel Kit, Glass			20.0	For P/Ns 14013 and 14014
08643	Ether-5PW Guardgel Kit			20.0	For P/N 08641
16091	Ether-5PW Prep Guardgel Kit			20.0	For P/N 08642
14470	Ether-5PW Guard column, Glass	20.0	2.0	13.0	For P/N 14015
16253	Ether-5PW Guard column	45.0	5.0	20.0	For P/N 16255
42155	Phenyl-5PW Guard cartridge	2.0	1.0	10.0	For P/N 18759
08808	Phenyl-5PW Guardgel Kit, Glass			20.0	For P/Ns 08804 and 13063
07652	Phenyl-5PW Guardgel Kit			20.0	For P/N 07573
16095	Phenyl-5PW Prep Guardgel Kit			20.0	For P/N 07656
14469	Phenyl-5PW Guard column, Glass	20.0	2.0	13.0	For P/N 14018
07936	Phenyl-5PW Guard column	45.0	5.0	20.0	For P/N 07938
19308	Guard cartridge holder	2.0	1.5		For all 2 mm ID Guard cartridges

HIC



AFC
AFFINITY CHROMATOGRAPHY

AFC PRODUCTS

- TSKgel ABA-5PW
- TSKgel BORONATE-5PW
- TSKgel CHELATE-5PW
- TSKgel TRESYL-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.

INTRODUCTION TO TSK-GEL AFFINITY CHROMATOGRAPHY COLUMNS

The Tosoh Bioscience TSK-GEL Affinity Chromatography (AFC) column line consists of three group-specific stationary phases: ABA-5PW, Boronate-5PW and Chelate-5PW as well as one activated packing material called Tresyl-5PW. Affinity chromatography offers the highest level of specificity and selectivity in biomolecular separations and purifications. Tosoh Bioscience supplies a full range of products for analytical, preparative and process scale affinity chromatography.

TSK-GEL affinity chromatography columns are based on the well-known G5000PW porous resin, which is the basis for high performance size exclusion chromatography columns. The TSK-GEL 5PW-type resin is a hydrophilic media with 1,000 Å pores and an estimated protein exclusion limit of $5x10^6$ Da. Tosoh Bioscience's process scale affinity media are based on the 65 µm particle size, semi-rigid Toyopearl HW-65 resin. Since analytical and semi-preparative columns are made from the same polymer chemistry as the process scale media, seamless scale-up from lab to process scale is achievable. Consult the chapter on bulk media for more information about resins for packing columns to purify medium to large volume samples.

TABLE I on the next page lists the ligand concentration, adsorption capacity and the test analyte used to determine the capacity of each column type.

Column Selection

TSK-GEL affinity chromatography columns have been developed for purifying peptides, proteins, and nucleic acids. In addition, some columns have been successfully applied to the selective separation of small biomolecules such as nucleosides and catecholamines.

The structures of the functional ligands available from Tosoh Bioscience are shown in FIGURE 1. The choice of a specific ligand is dictated by the expected interaction between the sample and column bonded phase. For example, the TSKgel Chelate-5PW column will bind high concentrations of Zn^{2+} ions. If a given protein is known to bind to Zn^{2+} ions, the Chelate-5PW would be a candidate column for the isolation of that target compound.

Tosoh Bioscience offers AFC columns in both glass and stainless steel formats. Glass columns are available in two formats: 5 mm ID x 5 cm L and 8 mm ID x 7.5 cm L. Stainless steel columns are available as 7.5 mm ID x 7.5 cm L and 6 mm ID x 4 cm L (Tresyl-5PW only). TSKgel Chelate-5PW is also supplied in a semi-preparative size: 21.5 mm ID x 15 cm L. TSKgel BioAssist Chelate is packed in 7.8 mm ID x 5 cm L PEEK hardware. The shipping solvent is distilled water for ABA-5PW and Boronate-5PW. The Chelate-5PW is shipped in 10 mmol/L acetate buffer, pH 4.5, and the Tresyl-5PW column shipping solvent is acetone. Stainless steel or Pyrex frits are employed in the body of the column end-fittings for the metal and glass columns, respectively. The nominal frit size for stainless steel columns is engraved in the end-fittings and all Pyrex® frits

FEATURES	BENEFITS
BioAssist Columns	
High size exclusion limit (>5 x 10^6 Da)	Enhanced access of large proteins to affinity ligands
Small particle size	High efficiency for analytical (10 µm) and semi-preparative (13 µm) affinity applications.
Rigid polymeric base resin	Wide pH range (2-12) of the base resin, enabling robust cleaning options
Stable affinity ligands	Long lifetime, solvent compatibility, autoclavable
Choice of four affinity ligands	Application flexibility, scalability from lab to commercial production.
TSKgel BioAssist Chelate offered in PEEK hardware	Eliminates undesirable interactions with column hardware.

AFC

TABLE 1

Characteristics of TSK-GEL AFC columns

Column packing	Ligand type	Ligand concentration	Adsorption capacity	Sample
ABA-5PW	*p*-aminobenzamidine	not available	3-4 mg/mL resin	trypsin
Boronate-5PW	*m*-aminophenyl-boronate	not available	40 µmol/mL resin	sorbitol
Chelate-5PW	iminodiacetic acid	20 µmol/mL resin	not available	not available
Tresyl-5PW	tresyl	ca. 20 µmol/mL resin	>60 mg/g dry resin (coupling capacity)	soybean trypsin inhibitor

are 10 µm nominal pore size. At the recommended flow rates (see Ordering Information) the pressure drop across a TSK-GEL AFC glass or stainless steel column is less than 20 kg/cm^2.

Separation columns should be protected with a guard column. Tosoh Bioscience offers a unique Guardgel kit consisting of guard column hardware and gel packing, allowing the user to repack the guard column as required. Guardgel kits are available for most affinity columns, both glass and stainless steel.

As with all columns used in gradient elution chromatography, affinity columns should be washed with final elution buffer prior to re-equilibration with initial (binding) buffer.

TSK-GEL ABA-5PW

The p-aminobenzamidine ligand of the ABA-5PW affinity column mimics a serine protease inhibitor. Proteins are loaded onto the column in an alkaline buffer such as Tris and are desorbed by lowering the pH. The column operates in a pH range of 2-9. Given this ability to bind complex proteins, the applications of ABA-5PW are numerous.

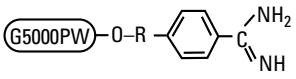
Applications for TSKgel ABA-5PW include: trypsin, urokinase, kallikrein, enterokinase, and blood coagulation factors such as thrombin, factor X, and plasminogen activator.

FIGURE 2 compares the isolation of crude trypsin on a TSKgel ABA-5PW column and a purity check of the collected fraction by reversed phase liquid chromatography.

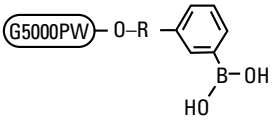
FIGURE 1

TSK-GEL affinity chromatography column packings

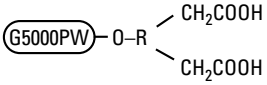
TSKgel ABA-5PW



TSKgel Boronate-5PW



TSKgel Chelate-5PW



TSKgel Tresyl-5PW

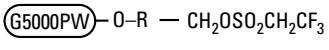
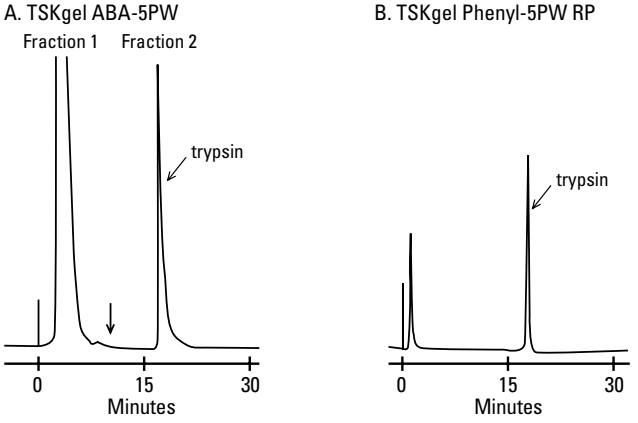


FIGURE 2

Affinity purification of trypsin and purity check by RPC



Column: A. TSKgel ABA-5PW, 7.5mm ID x 7.5cm
B. TSKgel Phenyl-5PW RP, 4.6mm ID x 7.5cm

Sample: A. 10mg crude trypsin
B. fraction 2 from A.

Eluent: A. step gradient at 10min (see arrow on diagram) from 0.05mol/L Tris-HCl, pH 8.0, to 0.05mol/L glycine-HCl, pH 2.8, both in 0.5mol/L NaCl and 2mmol/L $CaCl_2$
B. 2min linear gradient from 5 to 20% acetonitrile in 0.05% TFA followed by 46min linear gradient from 20% to 80% acetonitrile in 0.05% TFA

Flow Rate: 1.0mL/min

Detection: UV @ 280nm

APPLICATIONS OF TSK-GEL AFFINITY CHROMATOGRAPHY COLUMNS

TSK-GEL BORONATE-5PW

Coupling of m-aminophenyl boronate to the TSK-GEL 5PW-type polymeric support results in a ligand capable of forming a tetrahedral boronate anion under alkaline pH conditions. This anionic structure can bind with 1,2 cis-diol groups such as those found in carbohydrates, carbohydrate-containing compounds, and catecholamines. Interaction between the boronate anion and the 1,2 cis-diol groups is enhanced in the presence of Mg^{2+} ions and is inhibited by amine-containing buffers. Adsorption onto the TSKgel Boronate-5PW takes place in basic buffers such as HEPES and morpholine, while desorption takes place in carbohydrate or amine-containing mobile phases like sorbitol or Tris.

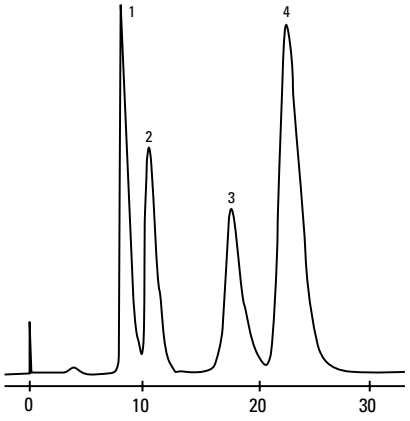
Applications for TSKgel Boronate-5PW include: nucleic acids, nucleotides and nucleosides. This affinity column has also been used to isolate catecholamines and other biomolecules containing the 1,2 cis-diol functionality. FIGURES 3 & 4 demonstrate the applicability of the TSKgel Boronate-5PW affinity chromatography column for the separation of nucleosides and catecholamines.

TSK-GEL CHELATE-5PW

TSKgel Chelate-5PW utilizes the ability of iminodiacetic acid (IDA) to chelate ions such as Zn^{2+}, Ni^{2+} and Cu^{2+}. The column is

FIGURE 3

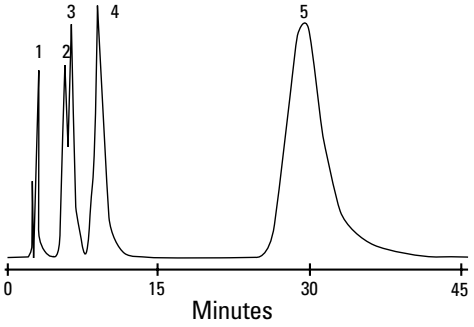
Isocratic separation of nucleosides



Column: TSKgel Boronate-5PW, 7.5mm ID x 7.5cm
Sample: 1. cytidine, 2. uridine, 3. guanosine, 4. adenosine
Elution: 0.1mol/L phosphate buffer, pH 8.0
Flow Rate: 1.0mL/min
Detection: UV @ 280nm

FIGURE 4

Separation of catecholamines on TSKgel Boronate-5PW



Column: TSKgel Boronate-5PW, 7.5mm ID x 7.5cm
Sample: 1. tyrosine, 2. normetanephrine, 3. metanephrine, 4. DOPA, 5. epinephrine
Elution: 0.1mol/L phosphate buffer, pH 6.5
Flow Rate: 1.0mL/min
Detection: UV @ 280nm

AFC

pre-loaded with divalent metal ions by chelation. Peptides and proteins containing histidine residues will normally adsorb to these chelated ions at neutral pH. The retained compounds are then eluted with buffer containing imidazole or glycine.

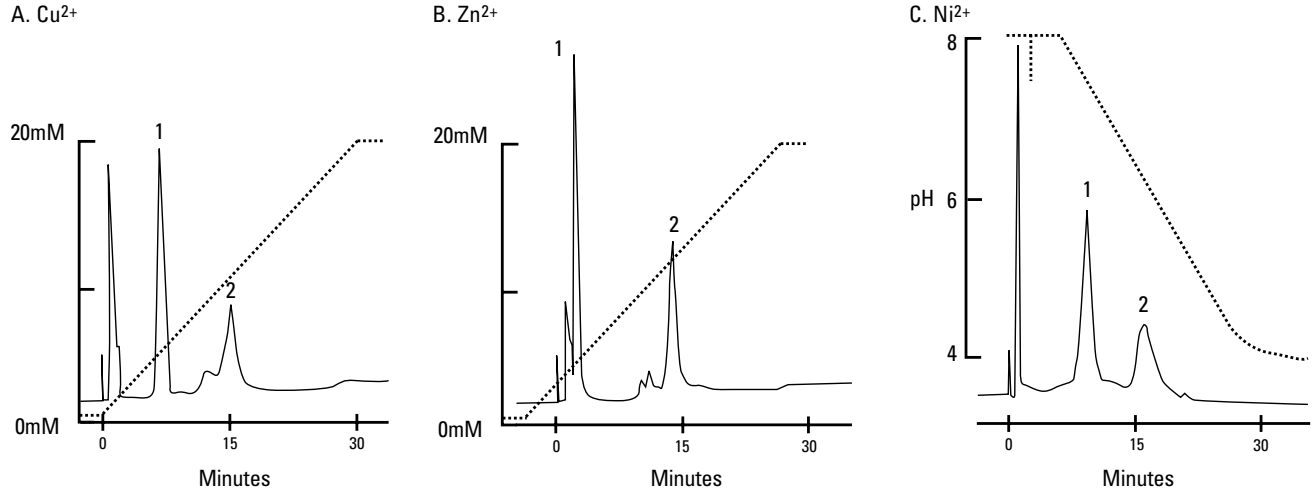
The key to making successful use of this retention mechanism is the proper selection of metal ions for chelation and the elution buffer to desorb the analytes. In general, Cu^{2+} interacts better with protein; however, resolution is usually enhanced with Zn^{2+} ions. A gradient mobile phase containing increasing imidazole or glycine concentrations is used to elute the retained compounds. A decreasing pH gradient can also be used. Glycine, as well as HEPES buffers, will also elute the metallic ion so column regeneration is necessary. Conversely, imidazole in phosphate buffer will extract the metal ions very slowly, avoiding frequent column regeneration.

Applications for TSKgel Chelate-5PW include: immunoglobulins, transferrin, lectins, milk proteins, membrane proteins, and peptides.

In FIGURE 5, the separation of ribonuclease A (bovine) and transferrin (human) are compared on TSKgel Chelate-5PW columns (glass, 5 mm ID x 5 cm L) containing different metal ions.

FIGURE 5

Separation of standard proteins by immobilized metal ion affinity chromatography



Column: TSKgel Chelate-5PW, 5mm ID x 5cm
Metal Ion: A. Cu^{2+}. B. Zn^{2+}, and C. Ni^{2+}
Sample: 1. ribonuclease A (bovine), 2. transferrin (human)
Elution: A. and B.: 30min linear gradient from 1mmol/L to 20mmol/L imidazole in 20mmol/L HEPES-NaOH buffer, pH 8.0, containing 0.5mol/L NaCl
C. 30min linear pH gradient from 20mmol/L HEPES-MES-acetic acid, pH 8.0, to 20mmol/L HEPES-MES-acetic acid, pH 4.0, both in 0.5mol/L NaCl
Flow Rate: 0.8mL/min
Detection: UV @ 280nm

TSK-GEL TRESYL-5PW

Unlike other TSK-GEL affinity columns, the TSKgel Tresyl-5PW (tresyl; 2,2,2-trifluoroethanesulfonyl) requires activation with a user-selected ligand containing amino, thiol, phenol, or imidazole groups. The resulting structure is literally a custom affinity ligand with excellent pH stability and minimal ligand loss due to leaching. TSKgel Tresyl-5PW readily reacts with amino or thiol groups to form stable covalent alkylamines or thioethers.

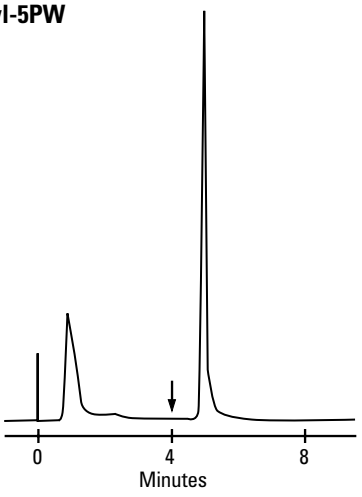
Principal applications for TSKgel Tresyl-5PW include the selective purification of antigens after coupling the appropriate antibody to the solid support. The antibody coupling yield at pH >7.5 is more than 90 %, with the maximum binding occurring at pH 7.5. Antigen adsorption to the antibody ligand is most effective when the antibody concentration is < 2-3 mg/mL of affinity resin. To increase binding capacity, more antibody should be added to the coupling reaction.

However, higher concentrations of antibody can result in steric hindrance, thus lowering the binding capacity of the column. As a general rule, the time required for antibody attachment to the TSKgel Tresyl-5PW column is directly proportional to the antibody concentration. Small amounts of antibody require about 2 hours to complete the cross-linking reaction, whereas it may take 6-7 hours to fully attach an antibody at the concentration of 10 mg/mL-resin.

Examples of the wide range of applications using TSKgel Tresyl-5PW include the binding of such ligands as concanavalin A (a lipoprotein lectin that binds to glycoproteins), numerous antibodies and enzymes. The chromatogram in FIGURE 6 shows the purification of peroxidase by the concanvalin A ligand coupled to the TSKgel Tresyl-5PW affinity support resin.

FIGURE 6

Purification of peroxidase on concanavalin A coupled to TSKgel Tresyl-5PW



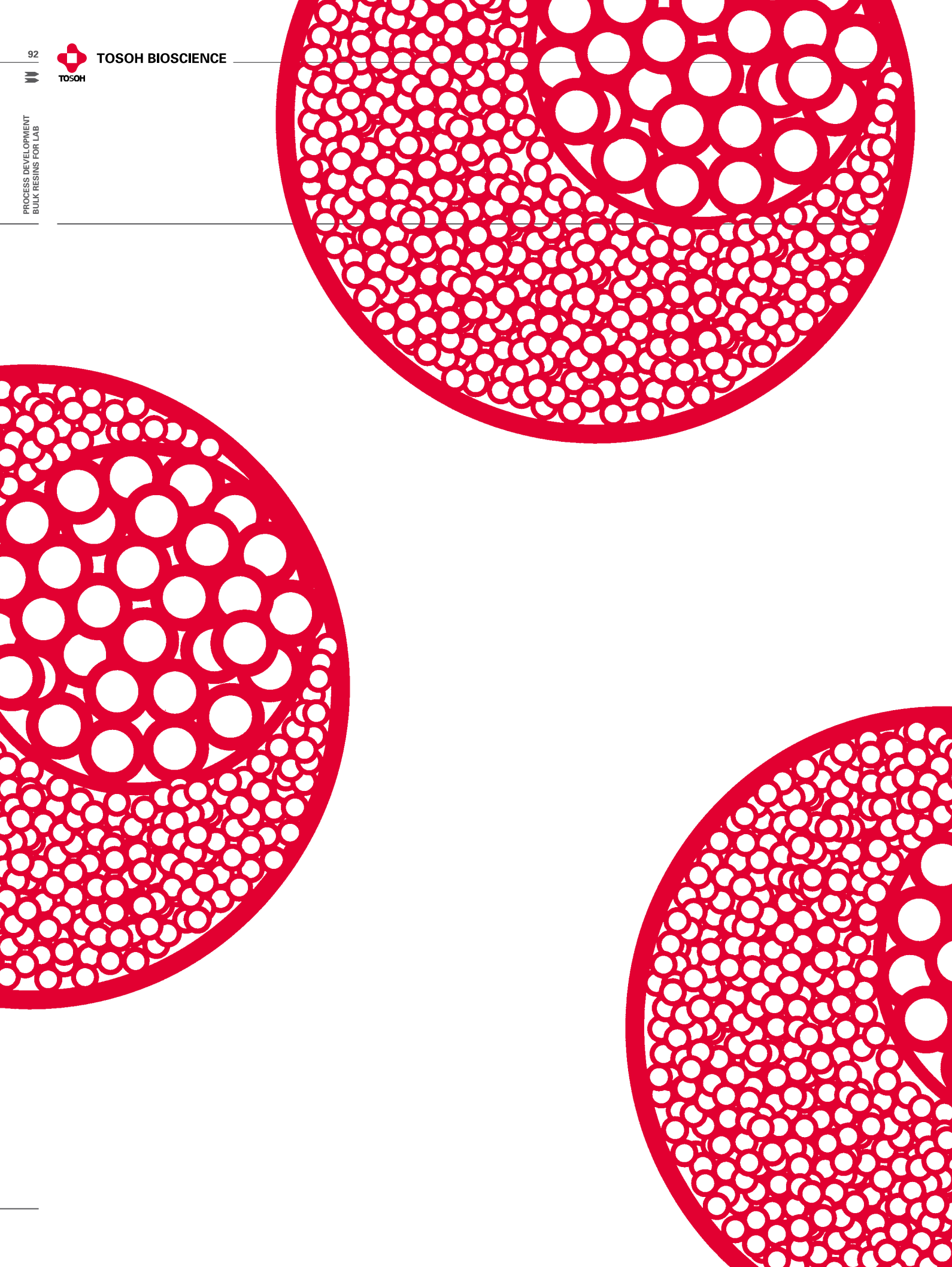
Washing step:	Wash TSKgel Tresyl-5PW, 6mmID x 4cm, with DI water
Ligand solution:	Dissolve 40mg of concanavalin A in 10mL of 0.1mol/L $NaHCO_3$, pH 8.0, containing 0.5mol/L NaCl
Coupling step:	Recycle the ligand solution overnight through the column at 0.2mL/min at 25°C
Blocking step:	Block residual tresyl groups with 0.1mol/L Tris-HCl, pH 8.0, at 1.0mL/min for 1hr at 25°C
Column:	TSKgel Tresyl-5PW modified with concanavalin A
Sample:	Crude peroxidase, 0.5mg
Binding:	0.05mol/L acetate buffer, pH 5.0, containing 0.5mol/L NaCl and 1mmol/L each of $CaCl_2$, $MnCl_2$, and $MgCl_2$
Elution:	Step gradient at 4min (see arrow on diagram) to 25mmol/L α-methyl-D-glucoside in binding buffer
Flow Rate:	1.0mL/min
Detection:	UV @ 403nm

AFC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Glass columns								
14449	Boronate-5PW Glass, 1000 Å	5.0	5.0	10	≥ 500	0.5 - 1.0	1.2	20
14440	Chelate-5PW Glass, 1000 Å	5.0	5.0	10	≥ 500	0.5 - 0.8	1.0	20
14441	Chelate-5PW Glass, 1000 Å	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.2	15
14457	Tresyl-5PW Glass, 1000 Å	5.0	5.0	10	≥ 500	0.2 - 0.8	1.0	10
14458	Tresyl-5PW Glass, 1000 Å	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.2	10
Stainless steel columns								
13067	ABA-5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	10
13066	Boronate -5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	10
08645	Chelate-5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	10
14455	Tresyl-5PW, 1000 Å	6.0	4.0	10		0.2 - 0.5	1.0	10
14456	Tresyl-5PW, 1000 Å	7.5	7.5	10		0.5 - 1.0	1.2	10
PEEK columns								
20022	BioAssist Chelate, 1000 Å	7.8	5.0	10	≥ 800	0.5 - 1.0	1.2	10
Guard column products								
13127	ABA-5PW Guardgel Kit				For P/N 13067			
14451	Boronate-5PW Glass Guardgel Kit			20	For P/N 14450 and 14449			
13125	Boronate-5PW Guardgel Kit				For P/N 13066			
14442	Chelate-5PW Glass Guardgel Kit			20	For P/Ns 14440 and 14441			
08647	Chelate-5PW Guardgel Kit				For P/N 08645			
Bulk packing								
16208	Tresyl-5PW, 2 g dry gel*			10				

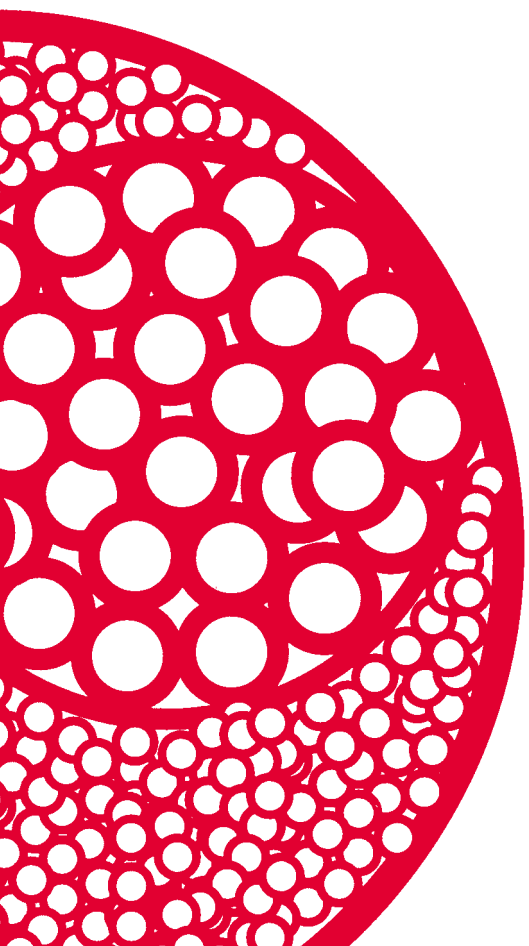
** 1 g is approximately 3.5 mL*



PROCESS DEVELOPMENT PRODUCTS AND BULK RESINS FOR LABORATORY SCALE PURIFICATION

PROCESS DEVELOPMENT & RESINS

- TOYOSCREEN PROCESS DEVELOPMENT COLUMNS
  TOYOPEARL AND TSK-GEL LABPAK
  TOYOPEARL AND TSK-GEL BULK RESINS



TOSOH FACT

Tosoh Bioscience offers a range of technical support services to our TSK-GEL, 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.

TOYOSCREEN PROCESS DEVELOPMENT COLUMNS

ToyoScreen Process Development columns are easy-to-use, pre-packed columns containing Tosoh Bioscience's most popular Toyopearl resins. These columns provide a convenient, low-cost method for the evaluation of Toyopearl ligand chemistries. ToyoScreen Process Development columns are available in packages of 6 x 1 mL and 6 x 5 mL volumes for affinity, ion exchange and hydrophobic interaction chromatography.See the chapter on bulk resins for detailed information on the Toyopearl resins.

Screening

Historically, resin screening was accomplished by manually packing various bulk resins into small columns requiring a significant investment in time and cost. In order to improve the efficiency of resin screening experiments, pre-packed ToyoScreen Process Development columns were developed for the evaluation of different Toyopearl resins.

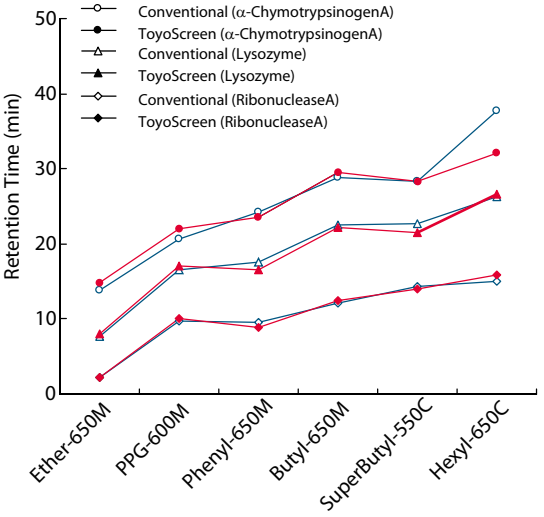
Scalability

Initial results from resin screening and optimization with ToyoScreen columns can accurately predict the separation behavior at larger scales. FIGURE 1 illustrates the similar retention time behavior between 1 mL ToyoScreen columns and conventional 7.5 mm ID x 7.5 cm L analytical columns. Additionally, FIGURE 2 depicts a practical antibody scale up in which conditions were set using a 1 mL ToyoScreen column and applied to a 10 mL semi-preparative column with a different inner diameter and length. Similar resolution results are predicted by the following equation:

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

FIGURE 1

Comparison of selectivity between ToyoScreen and Conventional Column



Columns: ToyoScreen (1 mL), Conventional Column (7.5 mm ID x 7.5 cm L)
Eluent 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)
Flow Rate: 1 mL/min
Gradient: 30 min linear
Inj. Vol.: 50 µL
Samples: Ribonuclease A, Lysozyme, α-Chymotrypsinogen, 1 mg/mL

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

Method Optimization

Besides the determination of what sticks during resin screening experiments, ToyoScreen Process Development columns can be used to quickly establish optimum elution conditions. Varying pH, salt type, salt gradients and flow rate are common experimental parameters explored. The effect of varying salt type and pH are shown in FIGURES 3 & 4 for anti-TSH in cell culture supernatant on ToyoScreen Phenyl-650M.

FEATURES

- Pre-packed columns
- 1 ml and 5 ml columns
- Cartridge design
- Easy connections with ÄKTA, FPLC and HPLC systems
- Offered in mixed or single chemistry six packs optimization

BENEFITS

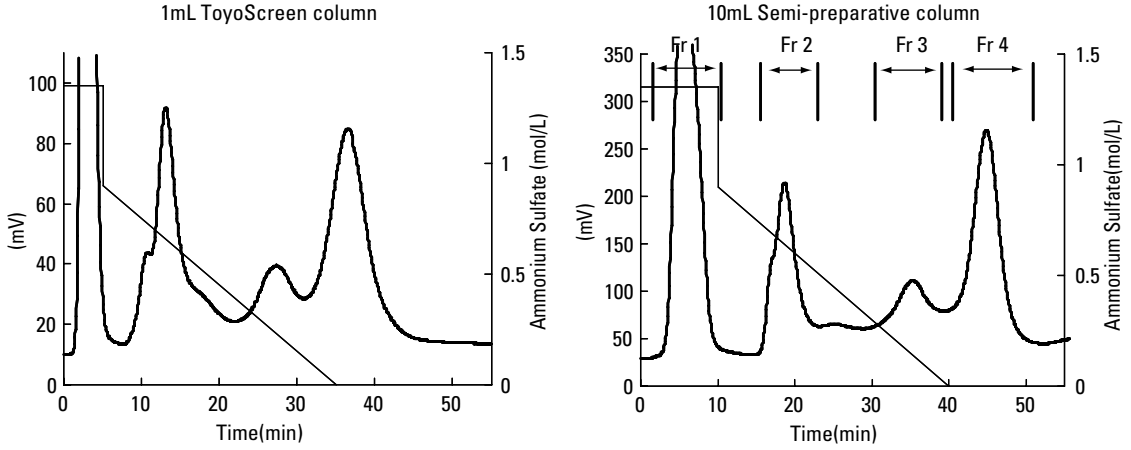
- Easy to set up and screen an entire resin series for a specific chromatographic mode
- For sample limited applications with up to milligram purifications
- Provides low cost, efficient alternative to hand packing with bulk resin
- Seamless integration with any platform
- For cost savings in screening or process experiments

PROCESS DEVELOPMENT

APPLICATIONS - TOYOSCREEN PROCESS DEVELOPMENT COLUMNS

FIGURE 2

Comparison chromatograms between ToyoScreen and Semi-preparative columns



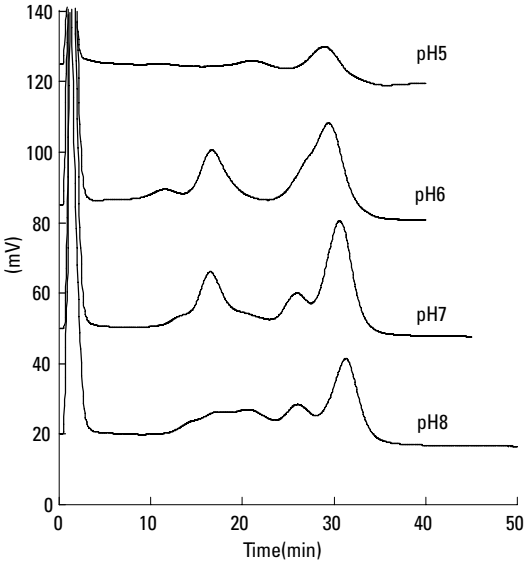
Packing: Toyopearl Phenyl-650M
Eluent: (A) 0.1mol/L phosphate buffer containing 1.8mol/L $(NH_4)_2SO_4$, pH7.0 (B) 0.1mol/L phosphate buffer, pH7.0
Sample: Anti-TSH from cell culture supernatant (x4 diluted)

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

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

FIGURE 3

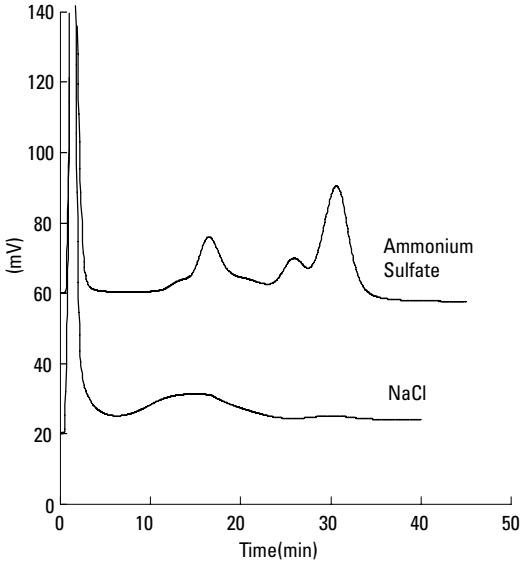
Optimizing eluent pH in HIC



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

FIGURE 4

Optimizing salt conditions in HIC



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

ORDERING INFORMATION

Part #	Description	Package Description
Ion Exchange		
21360	ToyoScreen DEAE-650M, 1 ml	1 ml x 6 ea
21361	ToyoScreen DEAE-650M, 5 ml	5 ml x 6 ea
21362	ToyoScreen SuperQ-650M, 1 ml	1 ml x 6 ea
21363	ToyoScreen SuperQ-650M, 5 ml	5 ml x 6 ea
21364	ToyoScreen QAE-550C, 1 ml	1 ml x 6 ea
21365	ToyoScreen QAE-550C, 5 ml	5 ml x 6 ea
21859	ToyoScreen GigaCap Q-650M, 1 ml -NEW-	1 ml x 6 ea
21860	ToyoScreen GigaCap Q-650M, 5 ml -NEW-	5 ml x 6 ea
21392	ToyoScreen IEC Anion Mix Pack, 1ml	1ml x 3 Grades x 2 ea
21393	ToyoScreen IEC Anion Mix Pack, 5ml	5ml x 3 Grades x 2 ea
21366	ToyoScreen CM-650M, 1ml	1 ml x 6 ea
21367	ToyoScreen CM-650M, 5ml	5 ml x 6 ea
21951	ToyoScreen GigaCap CM 650M, 1 ml -NEW-	1 ml x 6 ea
21952	ToyoScreen GigaCap CM 650M, 5 ml -NEW-	5 ml x 6 ea
21368	ToyoScreen SP-650M, 1ml	1 ml x 6 ea
21369	ToyoScreen SP-650M, 5ml	5 ml x 6 ea
21370	ToyoScreen SP-550C, 1ml	1 ml x 6 ea
21371	ToyoScreen SP-550C, 5ml	5 ml x 6 ea
21868	ToyoScreen GigaCap S-650M, 1 ml -NEW-	1 ml x 6 ea
21869	ToyoScreen GigaCap S 650M, 5 ml -NEW-	5 ml x 6 ea
21392	ToyoScreen IEC Anion Mix Pack, 1 ml	1 ml x 3 Grades x 2 ea
21393	ToyoScreen IEC Anion Mix Pack, 5 ml	5 ml x 3 Grades x 2 ea
21394	ToyoScreen IEC Cation Mix Pack, 1 ml	1 ml x 3 Grades x 2 ea
21395	ToyoScreen IEC Cation Mix Pack, 5 ml	5 ml x 3 Grades x 2 ea
21396	ToyoScreen IEC Mix Pack, 1 ml	1ml x 6 Grades x 1 ea
21397	ToyoScreen IEC Mix Pack, 5 ml	5ml x 6 Grades x 1 ea
Hydrophobic Interaction		
21372	ToyoScreen Ether-650M, 1 ml	1 ml x 6 ea
21373	ToyoScreen Ether-650M, 5 ml	5 ml x 6 ea
21892	ToyoScreen Phenyl-600M, 1 ml -NEW-	1 ml x 6 ea
21893	ToyoScreen Phenyl-600M, 5 ml -NEW-	5 ml x 6 ea
21374	ToyoScreen Phenyl-650M, 1 ml	1 ml x 6 ea
21375	ToyoScreen Phenyl-650M, 5 ml	5 ml x 6 ea
21494	ToyoScreen Butyl-600M, 1 ml -NEW-	1 ml x 6 ea
21495	ToyoScreen Butyl-600M, 5 ml -NEW-	5 ml x 6 ea
21376	ToyoScreen Butyl-650M, 1 ml	1 ml x 6 ea
21377	ToyoScreen Butyl-650M, 5 ml	5 ml x 6 ea
21378	ToyoScreen Hexyl-650C, 1 ml	1 ml x 6 ea
21379	ToyoScreen Hexyl-650C, 5 ml	5 ml x 6 ea

Part #	Description	Package Description
21380	ToyoScreen PPG-600M, 1 ml	1 ml x 6 ea
21381	ToyoScreen PPG-600M, 5 ml	5 ml x 6 ea
21382	ToyoScreen SuperButyl-550C, 1 ml	1 ml x 6 ea
21383	ToyoScreen SuperButyl-550C, 5 ml	5 ml x 6 ea
21398	ToyoScreen HIC Mix Pack, 1 ml	1 ml x 6 Grades x 1 ea
21399	ToyoScreen HIC Mix Pack, 5 ml	5 ml x 6 Grades x 1 ea
Affinity		
21386	ToyoScreen AF-Blue HC-650M, 1 ml	1 ml x 6 ea
21387	ToyoScreen AF-Blue HC-650M, 5 ml	5 ml x 6 ea
21384	ToyoScreen AF-Chelate-650M, 1 ml	1 ml x 6 ea
21385	ToyoScreen AF-Chelate-650M, 5 ml	5 ml x 6 ea
21390	ToyoScreen AF-Heparin HC-650M, 1 ml -NEW-	1 ml x 6 ea
21391	ToyoScreen AF-Heparin HC-650M, 5 ml -NEW-	5 ml x 6 ea
21388	ToyoScreen AF-Red-650M, 1 ml	1 ml x 6 ea
21389	ToyoScreen AF-Red-650M, 5 ml	5 ml x 6 ea
ToyoScreen Accessories		
21400	ToyoScreen column holder	

ToyoScreen columns are cartridge columns. They require a column holder (P/N 21400) to install the column onto the LC system.

PROCESS DEVELOPMENT

TOYOPEARL AND TSK-GEL LABPAK MEDIA

Toyopearl and TSK-GEL LabPak Media products are small package sizes of Toyopearl and TSK-GEL bulk media products. Typically they contain three or four different ligand types offered for a particular chromatography mode.

They are useful for developmental scientists and engineers who wish to familiarize themselves with resin physical properties in different buffer systems:

- slurry and reslurry mechanics
- resin handling during column packing
- mechanical strength relative to agarose
- degree of compressibility

The larger resin amounts in LabPak products allow the packing of wider bore and longer columns than available in the ToyoScreen products. This helps the developmental scientist or engineer to more accurately determine the resin's:

- dynamic binding capacity
- selectivity
- column efficiency
- operating conditions

ORDERING INFORMATION

TSK-GEL LABPAKS

Ion Exchange Chromatography

Part #	Description	Container Size
43380	IEXPAK PW, 20 µm (DEAE-5PW, SP-5PW, SuperQ-5PW)	3 x 25 ml
43280	IEXPAK PW, 30 µm (DEAE-5PW, SP-5PW,SuperQ-5PW)	3 x 25 ml

Hydrophobic Interaction Chromatography

Part #	Description	Container Size
43278	HICPAK PW, 20 µm (Ether-5PW, Phenyl-5PW)	2 x 25 ml
43175	HICPAK PW, 30 µm (Ether-5PW, Phenyl-5PW)	2 x 25 ml

TOYOPEARL LABPAKS

Size Exclusion Chromatography

Part #	Description	Container Size
19820	SECPAK HP, 30 µm (HW-40, 50, 55, 65S)	4 x 150 ml
19821	SECPAK LMW, 45 µm (HW-40, 50, 55F)	3 x 150 ml
19819	SECPAK HMW, 45 µm (HW-55, 65, 75F)	3 x 150 ml

Ion Exchange Chromatography

Part #	Description	Container Size
19817	IEXPAK HP, 35 µm (DEAE-650S, SP-650S,CM-650S, SuperQ-650S)	4 x 25 ml
43210	AIEXPAK, 65/100 µm (DEAE-650M, SuperQ-650M,QAE-550C)	3 x 100 ml
43220	CIEXPAK, 65/100 µm (SP-650M, CM-650M,SP-550C)	3 x 100 ml

Hydrophobic Interaction Chromatography

Part #	Description	Container Size
43150	HICPAK HP, 35 µm (Ether, Phenyl, Butyl-650S)	3 x 25 ml
19806	HICPAK, 65 µm (Ether, Phenyl, Butyl-650M)	3 x 25 ml
43125	HICPAK-C, 100 µm (Phenyl, Butyl, Hexyl-650C)	3 x 25 ml

Affinity Chromatography

Part #	Description	Container Size
43400	AFFIPAK ACT, 65 µm (AF-Epoxy, Tresyl-650M)	2 x 5 g*
43410	AFFIPAK, 65 µm (AF-Amino, Carboxyl, Formyl-650 M)	3 x 10ml

***1 g is approximately 3.5 ml**

INTRODUCTION TO BULK RESINS FOR LABORATORY PURIFICATION

Tosoh Bioscience offers Toyopearl and TSK-GEL resins (media) in bulk quantities (< 1 L) for laboratory-scale applications.

Although the resins can be applied to the purification of small as well as large MW compounds, Toyopearl and TSK-GEL resins are most useful for the separation of peptides, proteins, and oligonucleotides.

The focus of this section is on the use of bulk resins in laboratory applications. Please request the Process Chromatography catalog for information about the use of Toyopearl and TSK-GEL for larger scale separations or visit our website at: www.tosohbioscience.com.

Toyopearl Bulk Resin

Toyopearl resins are hydrophilic, macroporous media for medium pressure liquid chromatographic applications.

The polymethacrylate backbone structure of Toyopearl packings assure excellent pressure/flow characteristics. Toyopearl is mechanically stable up to 3 kg/cm^2, which simplifies column packing by reducing the setup time and improving reproducibility from column to column.

The media is stable over the range of pH 2-12 for normal operating conditions and pH 1-13 for cleaning conditions. In most modes, Toyopearl is available in three grades, S (superfine) for highest performance, F (fine) and M (medium) for economical purification, and C (coarse) and EC (extra coarse) for capture. Consult TABLE 1 for particle sizes associated with the various chemistries and pore sizes.

FEATURES	BENEFITS
chemistries available in Size Exclusion, Ion Exchange, Hydrophobic Interaction and Affinity chromatography	added flexibility during method development
methacrylate backbone has hydrophilic surface properties	less non-specific adsorption
	high recovery of proteins, enzymes, glycoproteins
TSK-GEL and Toyopearl bulk resin product lines feature the same ligand and backbone chemistries from 20 µm to 150 µm particle sizes	simplified scale up from laboratory isolation to process
SEC product line available in 5 pore sizes	suitable for fractionation of large and small biopolymers
IEC, HIC and AFC products are based on 1000 Å, 750 Å and 500 Å pore size particles.	high capacity and efficient chromatography of small protein and large biopolymers due to unrestricted access of available surface area
chemical stability	can clean resins in strong base or acid (pH 1-13)
	compatible with all water soluble organic solvents
	stable in chaotropic agents such as: guanidine chloride, sodium dodecyl sulfate and urea
thermal stability	can be autoclaved at 120°C
	flexible operating temperature (4-60°C)
mechanical stability	linear relationship between flow rate and pressure drop
column bed stability	constant packing volume over a wide range of salt concentrations

BULK RESINS

Toyopearl Bulk Resins

Toyopearl HW-type resins, available in pore sizes ranging from 50 Å to >1000 Å, are employed in size exclusion chromatography (SEC). Toyopearl HW-65 and HW-55 resins are used as starting materials for the production of all other functionalized Toyopearl resins. The large pore size of HW-65 (1000 Å) allows unhindered access of large proteins to the stationary phase, resulting in faster separation and shorter recycling times.

For predictable results during scale up, Toyopearl resins are based on the same chemistry as the prepacked TSK-GEL columns. This allows for seamless scale up from the laboratory to manufacturing.

TSK-GEL Bulk Resins

TSK-GEL resins are larger particle size versions of the chemically equivalent methacrylic packing of analytical-scale TSK-GEL columns used for protein analysis and purification. The TSK-GEL resin product line consists of DEAE-5PW, SuperQ-5PW, SP-5PW resins for ion exchange, Tresyl-5PW resins for afffinity chromatography and Ether-5PW and Phenyl-5PW resins for HIC. All types are available with average particle sizes of 20 µm and 30 µm.

TSK-GEL resins are often employed to simplify scale-up from analytical columns, as only the particle size is different. Their small particle sizes, high degree of cross-linking and high mechanical stability make TSK-GEL resins the preferred choice for high efficiency purifications.

TABLE 1

Characteristics of Toyopearl and TSK-GEL media

Mode	Resin	Grade/particle size (µm)	Pore size (Å)**	MW range Proteins (Da)	Operating pH range	Max. pressure
SEC	Toyopearl HW-40	S (20-40), F (30-60), C (50-100)	50	1×10^2 - 1×10^4	2–12	7 kg/cm²
	Toyopearl HW-50	S (20-40), F (30-60)	125	5×10^2 - 8×10^4	2–12	7 kg/cm²
	Toyopearl HW-55	S (20-40), F (30-60)	500	1×10^3 - 7×10^5	2–12	7 kg/cm²
	Toyopearl HW-65	S (20-40), F (30-60)	1000	4×10^4 - 5×10^6	2–12	7 kg/cm²
	Toyopearl HW-75	S (20-40), F (30-60)	> 1000	5×10^5 - 5×10^7	2–12	7 kg/cm²
IEC	TSKgel SuperQ-5PW	20 and 30	1000	$< 5 \times 10^6$	2–12	20 kg/cm²
	TSKgel DEAE-5PW	20 and 30	1000	$< 5 \times 10^6$	2–12	20 kg/cm²
	TSKgel SP-5PW	20 and 30	1000	$< 5 \times 10^6$	2–12	20 kg/cm²
	Toyopearl SuperQ-650	S (20-50), M (40-90), C (50-150)	1000	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl DEAE-650	S (20-50), M (40-90), C (50-150)	1000	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl GigaCap S-650	M (50-100)	1000	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl SP-650	S (20-50), M (40-90), C (50-150)	1000	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl CM-650	S (20-50), M (40-90), C (50-150)	1000	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl GigaCap S-650	M (50-100)	1000	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl GigaCap CM-650	M (50-100)	1000	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl QAE-550	C (50-150)	500	$< 5 \times 10^5$	2–12	7 kg/cm²
	Toyopearl SP-550	C (50-150)	500	$< 5 \times 10^5$	2–12	7 kg/cm²
	Toyopearl MegaCap II SP-550	EC (100-300)	500	$<5 \times 10^5$	2–12	7 kg/cm²
HIC	TSKgel Ether-5PW	20 and 30	1000	$< 5 \times 10^6$	2–12	20 kg/cm²
	TSKgel Phenyl-5PW	20 and 30	1000	$< 5 \times 10^6$	2–12	20 kg/cm²
	Toyopearl Ether-650	S (20-50), M (40-90)	1000	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl PPG-600	M (40-90)	750	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl Phenyl-600	M (40-90)	750	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl Butyl-600	M (40-90)	750	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl Phenyl-650	S (20-50), M (40-90), C (50-150)	1000	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl Butyl-650	S (20-50), M (40-90), C (50-150)	1000	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl Super Butyl-550	C (50-150)	500	$< 5 \times 10^5$	2–12	7 kg/cm²
	Toyopearl Hexyl-650	C (50-150)	1000	$< 5 \times 10^6$	2–12	7 kg/cm²
AFC	TSKgel Tresyl-5PW	10	1000	$< 5 \times 10^6$	2–12	10 kg/cm²
	Toyopearl AF-Chelate-650	M (40-90)	1000	$< 5 \times 10^6$	2–12	7 kg/cm²
	Toyopearl AF-Tresyl-650	M (40-90)	1000	$< 5 \times 10^6$	N/A	7 kg/cm²
	Toyopearl AF-Epoxy-650	M (40-90)	1000	$< 5 \times 10^6$	N/A	7 kg/cm²
	Toyopearl AF-Formyl-650	M (40-90)	1000	$< 5 \times 10^6$	6-9	7 kg/cm²
	Toyopearl AF-Amino-650	M (40-90)	1000	$< 5 \times 10^6$	2-12	7 kg/cm²
	Toyopearl AF-Carboxy-650	M (40-90)	1000	$< 5 \times 10^6$	2-12	7 kg/cm²
	Toyopearl AF-Red-650	M (40-90)	1000	$< 5 \times 10^6$	4-9	7 kg/cm²
	Toyopearl AF-Blue HC-650	M (40-90)	1000	$< 5 \times 10^6$	4-9	7 kg/cm²
	Toyopearl AF-Heparin HC-650	M (40-90)	1000	$< 5 \times 10^6$	5-10	7 kg/cm²

** nominal values

TOYOPEARL BULK RESINS FOR SEC

HIGHLIGHTS

- Pore sizes ranging from 50 Å to >1000 Å
- Three particle sizes (S, F, C)
- HW-40 is ideal for desalting applications
- Easy to pack in semi-preparative and process scale columns

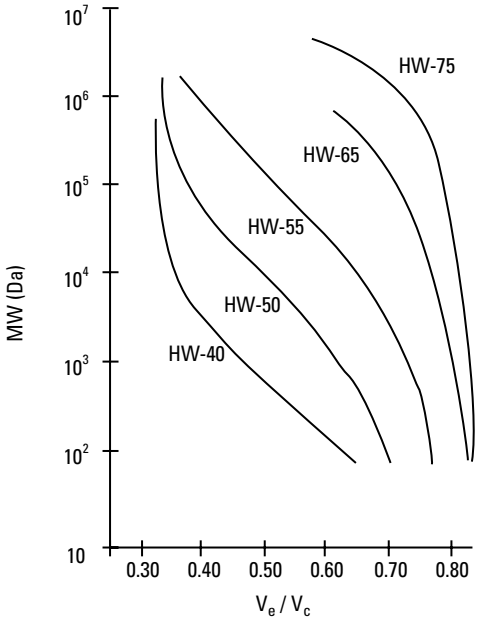
Size exclusion chromatography (SEC) is a common technique for separating molecules based on their apparent molecular weight. For nearly twenty-five years, Toyopearl SEC bulk resins, with their macroporous packings, have been used for laboratory and production-scale biochromatography.

Toyopearl SEC resins are semi-rigid, spherical polymethacrylate beads. The resins have hydrophilic surfaces due to the presence of ether and hydroxyl groups. The numerous surface hydroxyl groups provide attachment points for other functional groups and ligands. Toyopearl HW-65 resins are used as the principle base matrix for the Toyopearl ion exchange, hydrophobic interaction and affinity resins. TABLE II provides an overview of the Toyopearl SEC resin product line including corresponding molecular weight ranges of common target samples. Calibration curves of the Toyopearl HW-type resins determined with globular proteins are presented in FIGURE 5.

Ordering information for quantities <1 L is provided at the end of this section. For larger quantities, please contact customer service at +49 (0)711 13257 0. LABPAK kits are also available in popular combinations of Toyopearl media. See the page 97 for additional information.

FIGURE 5

Calibration curves for globular proteins on Toyopearl HW-type resins



Column: 22mm ID x 30cm
Sample: protein standards
Elution: 0.06mol/L phosphate buffer, pH 7, in 0.06mol/L KCl
Legend: V_e=elution volume, V_c=column volume

Applications: proteins, peptides, amino acids, nucleic acids, and small molecular weight molecules. Please visit our website: www.tosohbioscience.com for extensive data on applications.

TABLE II

Properties and molecular weight separation ranges for Toyopearl HW-type resins (HW = Hydrophilic, Water-compatible polymeric base resins)

Toyopearl Resin	Particle Size (µm)	Pore Size (Å)	Molecular weight of sample (Da) PEG and PEO	Dextrans	Globular proteins
HW-40S HW-40F HW-40C	20 - 40 30 - 60 50 - 100	50 50 50	1×10^2 - 3×10^3	1×10^2 - 7×10^3	1×10^2 - 1×10^4
HW-50S HW-50F	20 - 40 30 - 60	125 125	1×10^2 - 1.8×10^4	5×10^2 - 2×10^4	5×10^2 - 8×10^4
HW-55S HW-55F	20 - 40 30 - 60	500 500	1×10^2 - 1.5×10^5	1×10^3 - 2×10^5	1×10^3 - 7×10^5
HW-65S HW-65F	20 - 40 30 - 60	1000 1000	5×10^2 - 1×10^6	1×10^4 - 1×10^6	4×10^4 - 5×10^6
HW-75F	30 - 60	>1000	4×10^3 - 5×10^6	1×10^5 - 1×10^7	5×10^5 - 5×10^7

BULK RESINS

TOYOPEARL AND TSK-GEL BULK RESINS FOR IEC

HIGHLIGHTS

- -NEW- Toyopearl GigaCapS®-650M, CM-650M and Q-650M resins are high capacity ion exchange resins featuring high dynamic binding capacities for both small proteins like insulin and larger proteins like monoclonal antibodies.
- Weak and strong anion and cation exchangers are offered in both product lines.
- Standard 1,000 Å pore size for large biopolymers and 500 Å pore size packing for optimal binding capacity are available.
- High efficiency TSK-GEL resins scale up directly from TSK-GEL analytical columns.

For separating mixtures of biomolecules, Ion Exchange Chromatography (IEC) is known for its high resolution and high capacity. It is very effective in the initial capture step of a chromatography process. IEC is also useful for further purification and/or polishing. It can complement other chromatographic techniques in the design of an economical downstream purification process. IEC is often used as a purification step before HIC, SEC, and RPC. IEC will also purify and concentrate the target molecule in one step when the sample is dilute. This also allows it to be used as a concentration step after SEC.

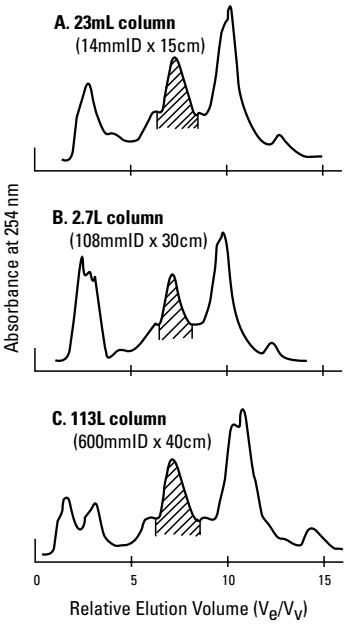
A 5000-fold scale-up of a α-galactosidase enzyme purification was accomplished using Toyopearl DEAE-650M. The chromatograms in FIGURE 6 demonstrate the excellent scale up characteristics of Toyopearl ion exchange media. Gradient slope and particle diameter remained unchanged. Linear velocity was reduced by 15% in the largest scale separation, and resolution actually improved relative to the smallest scale separation. This may be partly attributed to increased bed height and the slower linear velocity. Although the column volume was increased in part by increasing the bed height, the principal change in column volume was a result of the greater column diameter (1.4 cm to 60 cm L). This example illustrates how Toyopearl media can be conveniently scaled up from laboratory to production scale applications using the same particle size if desired.

Because the correct choice of an ion exchange resin can have a considerable impact on the economy of a process, Tosoh Bioscience provides many product options in both Toyopearl and TSK-GEL IEC bulk polymeric media. See TABLE III for a complete listing of the particle sizes.

Ordering information for quantities < 1 L is provided at the end of this section.

FIGURE 6

Process scale-up purification of β-galactosidase with Toyopearl DEAE-650M



Column: Toyopearl DEAE-650M
Sample: 1% β-galactosidase: A. 8mL; B. 1L; C. 40L
Elution: linear gradient from 0.03 to 0.10mol/L NaCl in 0.014mol/L Tris-HCl (pH7.7)
Flow rate: A. 1.0mL/min; B. 60mL/min; C. 1.6L/min
Linear velocity: A. 39cm/h; B. 40cm/h; C. 34cm/h
Detection: UV @ 254nm

TABLE III

Toyopearl and TSK-GEL Ion Exchange Resins

Description	Type*	Part. Size (µm)
Anion Exchange		
TSKgel DEAE-5PW	W	20, 30
TSKgel SuperQ-5PW	S	20, 30
Toyopearl DEAE-650	W	35, 65, 100
Toyopearl SuperQ-650	S	35, 65, 100
Toyopearl QAE-550	S	100
Toyopearl GigaCap Q-650M	S	75
Cation Exchange		
TSKgel SP-5PW	S	20, 30
Toyopearl CM-650	W	35, 65, 100
Toyopearl GigaCap CM-650M	W	75
Toyopearl SP-550	S	100
Toyopearl SP-650	S	35, 65, 100
Toyopearl MegaCap II SP-550EC	S	100-300
Toyopearl GigaCap S-650M	S	75

**W = Weak; S = Strong*

TOYOPEARL AND TSK-GEL BULK RESINS FOR HIC

HIGHLIGHTS

- A wide range of hydrophobicities is suitable for most proteins.
- Standard 1,000 Å pore size is available for large biopolymers, and three Butyl pore sizes (500 Å, 750 Å and 1,000 Å) are available.
- Toyopearl "600M" series of HIC resins with optimized pore size of 750 Å for antibody separation. NEW Toyopearl Phenyl-600M and Butyl-600M with highest DBCs for IgG.
- Seamless scale up from high efficiency TSK-GEL 5PW-type analytical columns is possible.

Hydrophobic Interaction Chromatography (HIC) has become a popular mode of chromatography for the purification of biopolymers at analytical as well as preparative scale. This is accomplished by the interaction of hydrophobic ligands on the base matrix with the hydrophobic areas located on the surface of proteins. HIC is an excellent complement to size exclusion and ion exchange chromatography in difficult separations, particularly those where the contaminants are of similar pI or molecular weight. It is often preferred over reversed phase chromatography when preservation of biological activity of the protein is of utmost importance.

Tosoh Bioscience offers both the TSK-GEL and Toyopearl resin product lines for HIC. See TABLE IV for a complete listing of functionalities. Each product line has similar backbone chemistry. TSK-GEL 5PW-type resins possess a higher degree of cross-linking than the corresponding Toyopearl resins. Additionally, choices in particle size are offered to match the desired resolution and throughput. HIC bulk media is offered in quantities < 1 L and in a combination of resins with varying functionalities as LABPAK kits. Additionally, HIC media are available in ToyoScreen process development columns for convenient scouting and methods development.

Ordering information for quantities < 1 L is provided at the end of this section.

Applications: proteins with similar chemical or structural properties, plasmids and monoclonal antibodies. See FIGURE 7 for separation of large glycoprotein from crude extract on Toyopearl Butyl-650S. Please visit our website: www.tosohbioscience.com for extensive application data.

TABLE IV

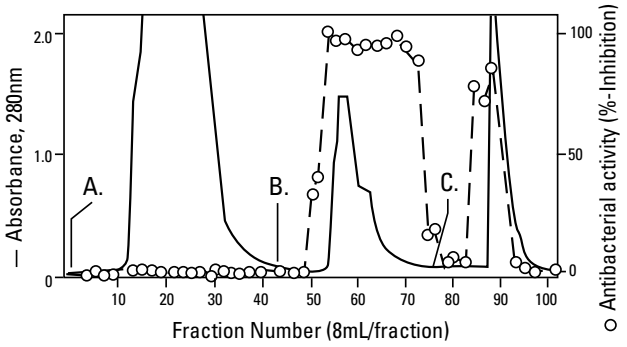
Toyopearl and TSK-GEL HIC Resins

Description	Strength*	Part. Size Grades (µm)
TSKgel Ether-5PW	1	20, 30
Toyopearl Ether-650	1	35, 65
Toyopearl PPG-600	2	65, 100
TSKgel Phenyl-5PW	3	20, 30
Toyopearl Phenyl-600	4	65
Toyopearl Phenyl-650	3	35, 65, 100
Toyopearl Butyl-600	4	65
Toyopearl Butyl-650	4	35, 65, 100
Toyopearl SuperButyl-550	4	100
Toyopearl Hexyl-650	5	100

* Relative scale: 1 = least hydrophobic, 5 = most hydrophobic.

FIGURE 7

Large glycoprotein purified on Toyopearl Butyl-650S



Column: Toyopearl Butyl-650S, 22mm ID x 26cm
Sample: crude protein from sea hare *Aplysia kurodai*
Elution: multi-step $(NH_4)_2SO_4$ in 50mmol/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 $(NH_4)_2SO_4$

BULK RESINS

TOYOPEARL RESINS FOR AFC

HIGHLIGHTS

- Active, reactive and group specific resins
- Provided in standard 1000 Å pore size for high capacity of large biopolymers.
- The chemical stability of the final product depends on the ligand.
- Toyopearl AF-Blue HC-650 is available for albumin and interferon applications with the lowest leaching blue.
- Toyopearl AF-Heparin HC-650 high capacity resin exhibits an Antithrombin III dynamic capacity of 4 mg/ml.

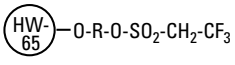
Toyopearl AF-Heparin HC-650 high capacity resin exhibits an Antithrombin III dynamic binding capacity of 4 mg/mL.
Toyopearl media for Affinity Chromatography (AFC) are based on Toyopearl HW-65 resin and functionalized with either chemically active groups or group-specific ligands. Resins with activated functional groups are ready for direct coupling of a protein or other ligand, while resins with reactive groups employ coupling or reductive amination to achieve covalent bonding. The 1000 Å pore size common to all Toyopearl affinity resins accommodates proteins up to 5,000,000 Da. In general, Toyopearl AF-Tresyl-650M and Toyopearl AF-Formyl-650M are recommended for coupling proteins, while Toyopearl AF-Epoxy-650M is suited for coupling low molecular weight ligands. Toyopearl AF-Amino-650M and Toyopearl AF-Carboxy-650M may be used in either application. Toyopearl AF-Heparin HC-650 interacts with a wide range of biomolecules including plasma components, lipoprotein lipase, collagenase, and DNA polymerase. The structures of Toyopearl activated and reactive ligands are given in FIGURE 8, while the structures of Toyopearl group-specific ligands are listed in FIGURE 9.
LABPAK kits are also available, which group popular combinations of chemically active functionalized Toyopearl media. Some Affinity media are available in ToyoScreen process development columns for convenient scouting and methods development.
Ordering information for quantities < 1 L is provided at the end of this section.

Applications: bacteria, proteins, ligands, saccharides and synthetic oligonucleotides. Please visit our website: www.tosohbioscience.com for extensive application data.

FIGURE 8

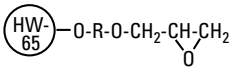
Activated and reactive Toyopearl affinity resins

Toyopearl AF-Tresyl-650M (1)



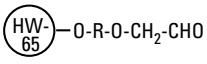
Ligand Density: 80 µmol/g (dry)

Toyopearl AF-Epoxy-650M (1)



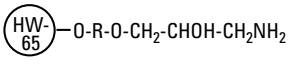
Ligand Density: 800 µmol/g (dry)

Toyopearl AF-Formyl-650M (2)



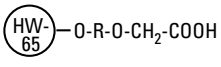
Ligand Density: 60 µeq/mL

Toyopearl AF-Amino-650M (3)



Ligand Density: 100 µeq/mL

Toyopearl AF-Carboxy-650M (3)



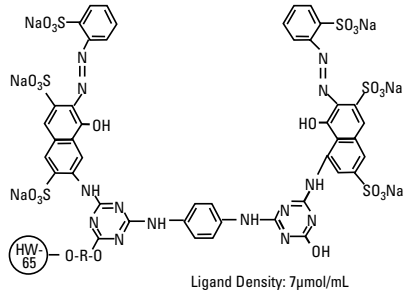
Ligand Density: 100 µeq/mL

(1) Provided as dry, free-flowing powder.
One gram of dry powder produces about 3.5 mL of hydrated resin.
(2) Provided as aqueous slurry, containing 1% gluteraldehyde.
(3) Provided as aqueous slurry, containing 20% ethanol.

FIGURE 9

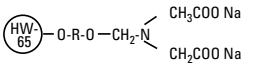
Group-specific Toyopearl affinity resins

Toyopearl AF-Red-650 (1)



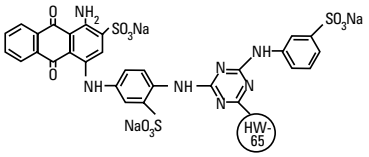
Ligand Density: 7µmol/mL

Toyopearl AF-Chelate-650 (2)

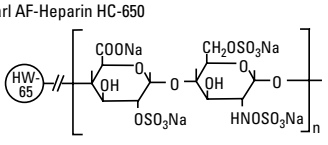


Ligand Density: 20µmol/mL

Toyopearl AF-Blue HC-650 (1)



Toyopearl AF-Heparin HC-650



Approximate Ligand Density: 5mg/mL

(1) Provided as an aqueous slurry containing 20% ethanol, v/v in 1mol/L NaCl.
(2) Provided as an aqueous slurry containing 20% ethanol.

ORDERING INFORMATION

A. Size Exclusion Chromatography

Toyopearl Bulk Resins

Part #	Description	Container Size
19809	HW-40S, 30 µm	150 ml
07451	HW-40S, 30 µm	250 ml
19808	HW-40F, 45 µm	150 ml
07448	HW-40F, 45 µm	500 ml
19807	HW-40C, 75 µm	150 ml
07449	HW-40C, 75 µm	500 ml
19811	HW-50S, 30µm	150 ml
07455	HW-50S, 30µm	250 ml
19810	HW-50F, 45 µm	150 ml
07453	HW-50F, 45 µm	500 ml
19813	HW-55S, 30 µm	150 ml
07459	HW-55S, 30 µm	250 ml
19812	HW-55F, 45 µm	150 ml
07457	HW-55F, 45 µm	500 ml
19815	HW-65S, 30 µm	150 ml
07467	HW-65S, 30 µm	250 ml
19814	HW-65F, 45 µm	150 ml
07465	HW-65F, 45 µm	500 ml
21481	HW-65C, 75 µm	150ml
07466	HW-65C, 75 µm	500ml
19816	HW-75F, 45 µm	150 ml
07469	HW-75F, 45 µm	500 ml

B. Anion Exchange Chromatography

TSK-GEL Bulk Resins

Part #	Description	Container Size
43381	DEAE-5PW (20)	25 ml
14710	DEAE-5PW (20)	250 ml
43281	DEAE-5PW (30)	25 ml
14712	DEAE-5PW (30)	250 ml
43383	SuperQ-5PW (20)	25 ml
18535	SuperQ-5PW (20)	250 ml
43283	SuperQ-5PW (30)	25 ml
18536	SuperQ-5PW (30)	250 ml

Toyopearl Bulk Resins

Part #	Description	Container Size
19823	SuperQ-650S, 35 µm	25 ml
17223	SuperQ-650S, 35 µm	250 ml
43205	SuperQ-650M, 65 µm	100 ml
17227	SuperQ-650M, 65 µm	250 ml
43275	SuperQ-650C, 100 µm	100 ml
17231	SuperQ-650C, 100 µm	250 ml
43271	QAE-550C, 100 µm	100 ml
14026	QAE-550C, 100 µm	250 ml
19804	DEAE-650S, 35 µm	25 ml
07472	DEAE-650S, 35 µm	250 ml
43201	DEAE-650M, 65 µm	100 ml
07473	DEAE-650M, 65 µm	250 ml
07988	DEAE-650C, 100 µm	250 ml
21854	GigaCap Q-650M, 75 µm -NEW-	100 ml
21866	GigaCap Q-650M, 75 µm -NEW-	250 ml

C. Cation Exchange Chromatography

TSK-GEL Bulk Resins

Part #	Description	Container Size
43382	SP-5PW (20)	25 ml
14714	SP-5PW (20)	250 ml
43282	SP-5PW (30)	25 ml
14716	SP-5PW (30)	250 ml

Toyopearl Bulk Resins

Part #	Description	Container Size
19803	CM-650S, 35 µm	25 ml
07474	CM-650S, 35 µm	250 ml
43203	CM-650M, 65 µm	100 ml
07475	CM-650M, 65 µm	250 ml
07991	CM-650C, 100 µm	250 ml
19822	SP-650S, 35 µm	25 ml
08437	SP-650S, 35 µm	250 ml
43202	SP-650M, 65 µm	100 ml
07997	SP-650M, 65 µm	250 ml
07994	SP-650C, 100 µm	250 ml
43272	SP-550C, 100 µm	100 ml
14028	SP-550C, 100 µm	250 ml
21804	Toyopearl MegaCap II SP-550EC, > 100 µm	100 ml
21805	Toyopearl MegaCap II SP-550EC, > 100 µm	250 ml
21833	GigaCap S-650M, 75 µm -NEW-	100 ml
21834	GigaCap S-650M, 75 µm -NEW-	250 ml
21946	GigaCap CM-650M, 75 µm -NEW-	100 ml
21947	GigaCap CM-650M, 75 µm -NEW-	250 ml

BULK RESINS

ORDERING INFORMATION

D. Hydrophobic Interaction Chromatography

TSK-GEL Bulk Resins

Part #	Description	Container Size
43276	Ether-5PW (20)	25 ml
16052	Ether-5PW (20)	250 ml
43176	Ether-5PW (30)	25 ml
16050	Ether-5PW (30)	250 ml
43277	Phenyl-5PW (20)	25 ml
14718	Phenyl-5PW (20)	250 ml
43177	Phenyl-5PW (30)	25 ml
14720	Phenyl-5PW (30)	250 ml

Toyopearl Bulk Resins

Part #	Description	Container Size
19955	SuperButyl-550C, 100 µm	25 ml
19956	SuperButyl-550C, 100 µm	100 ml
21448	Butyl-600M, 65 µm - NEW-	25 ml
21449	Butyl-600M, 65 µm - NEW-	100 ml
43153	Butyl-650S, 35 µm	25 ml
07476	Butyl-650S, 35 µm	100 ml
19802	Butyl-650M, 65 µm	25 ml
07477	Butyl-650M, 65 µm	100 ml
43127	Butyl-650C, 100 µm	25 ml
07478	Butyl-650C, 100 µm	100 ml
43151	Ether-650S, 35 µm	25 ml
16172	Ether-650S, 35 µm	100 ml
19805	Ether-650M , 65 µm	25 ml
16173	Ether-650M , 65 µm	100 ml
44465	Hexyl-650C, 100 µm	25 ml
19026	Hexyl-650C, 100 µm	100 ml
21887	Phenyl-600M, 65 µm -NEW-	25 ml
21888	Phenyl-600M, 65 µm -NEW-	100 ml
43152	Phenyl-650S, 35 µm	25 ml
14477	Phenyl-650S, 35 µm	100 ml
19818	Phenyl-650M, 65 µm	25 ml
14478	Phenyl-650M, 65 µm	100 ml
43126	Phenyl-650C, 100 µm	25 ml
14479	Phenyl-650C, 100 µm	100 ml
21301	PPG-600M, 65 µm	25 ml
21302	PPG-600M, 65 µm	100 ml

E. Affinity Chromatography

TSK-GEL Bulk Resins

Part #	Description	Container Size
16208	Tresyl-5PW (10)	2 g*

Toyopearl Bulk Resins

Part #	Description	Container Size
43411	AF-Amino-650M, 65 µm	10 ml
08002	AF-Amino-650M, 65 µm	25 ml
08039	AF-Amino-650M, 65 µm	100 ml
19688	AF-Blue HC-650M,65 µm	25 ml
19689	AF-Blue HC-650M, 65 µm	100 ml
43412	AF-Carboxy-650M, 65 µm	10 ml
08006	AF-Carboxy-650M, 65 µm	25 ml
08041	AF-Carboxy-650M, 65 µm	100 ml
14475	AF-Chelate-650M, 65 µm	25 ml
19800	AF-Chelate-650M, 65 µm	100 ml
43402	AF-Epoxy-650M, 65 µm	5 g*
08000	AF-Epoxy-650M, 65 µm	10 g*
08038	AF-Epoxy-650M, 65 µm	100 g*
43413	AF-Formyl-650M, 65 µm	10 ml
08004	AF-Formyl-650M, 65 µm	25 ml
08040	AF-Formyl-650M, 65 µm	100 ml
20030	AF-Heparin-HC650M, 65 µm -NEW-	10 ml
20031	AF-Heparin-HC650M, 65 µm -NEW-	100 ml
08651	AF-Red-650 ml, 65 µm	25 ml
19801	AF-Red-650 ml, 65 µm	100 ml
14471	AF-Tresyl-650M, 65 µm	5 g*
14472	AF-Tresyl-650M, 65 µm	100 g*

*1 g is approximately 3.5 ml

APPENDIX A

About TSK-GEL Columns, their Maintenance and Scale Up

Tosoh Corporation closely monitors all stages of the manufacturing process for chromatographic media that is used to pack TSK-GEL columns. Packing materials are produced in large gel batches which must pass stringent quality control specifications for particle size distribution, pore size distribution, pore volume, and surface area. After producing the particles, each lot is then used to prepare multiple batches of bonded phase by attaching the appropriate ligand. Each gel lot is again tested to ensure that it meets the specifications for parameters such as ligand density, retention, selectivity, etc.

TSK-GEL columns are designed for general purpose HPLC or FPLC applications. They are not guaranteed to work for specific customer applications. Suitability of a column has to be determined by the end user. Good Laboratory Practice (GLP) demands that a rugged method must be developed by testing at least three different gel lots to understand the type of variability in retention and selectivity that may be encountered with future columns.

Tosoh Bioscience recommends that shipments are inspected for the presence of the Inspection Data sheet, Operating Conditions and Specifications (OCS) sheet, and column appearance. After review of the shipping contents, the column should be tested within 30 days according to the conditions listed in the Inspection Data sheet to confirm that the column meets the specifications listed in the OCS sheet.

Troubleshooting column problems

Listed below are the five most common causes of poor column performance and the precautions that must be taken to prevent these problems:

1. Void or dead space at the column inlet or channeling of the packing

Sudden pressure surges and higher than recommended flow rates can compress the column packing, which can result in a void or a channel, especially with large pore size columns such as TSKgel G4000SW and TSKgel G4000SWXL. We recommend using an injector that ensures continuous flow onto the column during injection, i.e., no pressure pulse due to interrupted flow, and installation of a pulse dampener to suppress the sudden pressure surges encountered with quick-return pumps.

Bulk packing material is available to refill voids in some of the analytical and semi-preparative columns. We highly recommend the use of a guard column to protect your analytical column from pressure surges and to prevent irreversibly binding impurities from reaching the analytical column. A guard column also helps to neutralize the pH of the sample solvent if it is different from that of the mobile phase. The pH of the sample will be equilibrated with the mobile phase before it reaches the analytical column. This is particularly important in the silica-based SW-type columns because this silica-type is not stable at a pH higher than 7.5.

2. Air in Column

The column should be tightly capped when not in use to prevent air from entering it. Air dissolved in the mobile phase must be removed before it can enter the column. This is particularly important for polymer-based columns. Air can be removed by sparging with helium, mobile phase filtration or other degassing procedures. If air does enter the column, follow the rehydration procedure described on page 107.

3. Column contamination or incomplete sample recovery

Cleaning conditions for all column types are provided on the OCS sheets that are shipped with each column. Cleaning solvents are discussed in the cleaning section below.

4. Frit plugging and high pressure

Solvents and samples should be filtered through at least a 0.45 µm filter to prevent clogging the column frits. If the frit becomes partially plugged, the result may be split peaks or high pressure. The entire end-fitting can be removed and sonicated in 6 M nitric acid. Rinse the end-fitting thoroughly after cleaning. (Be careful not to disturb the packing.) Alternatively, this end-fitting can be replaced. Installing a membrane filter prior to the injector is recommended to prevent particles created by pump seal wear from reaching the analytical column. Consult the Accessories chapter for these and other hardware products.

5. Peak splitting

Column overload, whether in volume or concentration, can cause peak splitting and poor resolution. Consult the sample capacity information for each column type to determine the appropriate concentration and volume of analyte.

Cleaning

Columns should be cleaned at regular intervals. The frequency depends on the purity of the samples. Occasionally, samples are run which adsorb onto the packing material. If one of the performance characteristics (asymmetry factor, retention time, theoretical plates, or resolution) changes by 10% or more, it is prudent to clean the column.

A Data Inspection sheet and an Operating Conditions and Specifications (OCS) sheet accompanies all TSK-GEL columns. The Data Inspection sheet identifies the testing method that was used to verify the column's performance. The column's specifications are listed on the OCS sheet. However, a well resolved sample component could be used to monitor the column. Establish that the column is performing properly using the standard test probes listed on the Data Inspection sheet. Calculate the asymmetry factor, theoretical plates and resolution of one or more of the sample components. Note the retention time. This becomes the baseline test mix which provides a basis for comparison.

Basic rules for cleaning TSK-GEL columns - all types

1. Clean the column in the reverse flow direction.
2. During cleaning, do not connect the column to the detector.
3. Run the column at half the maximum flow rate making sure to monitor the pressure.
4. If cleaning with a high or low pH solution, make certain that the rest of the chromatographic system (pump, pump seals, injector, etc.) is compatible.
5. Use at least 5 column volumes (CV) of each cleaning solution and rinse with 5 CV of ultra pure water between each cleaning step.
6. Equilibrate with 5 CV of the mobile phase for the method.

Each type of TSK-GEL column has a recommended set of cleaning solutions specific to the column, as described below and on the OCS sheet. Choose a cleaning solution based upon the column and sample

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type. In general low pH salt solution will remove basic proteins, and organics will remove hydrophobic proteins. Chaotropic agents will remove strongly adsorbed materials (e.g. hydrogen bonded). For columns or column types not listed below, please contact Tosoh Bioscience Technical Service Specialists at +49 (0)711 13257-0.

Cleaning Solutions

Size Exclusion, TSK-GEL SW and SW_{XL} types

1. Concentrated salt (e.g. 0.5 mol/L Na_2SO_4) at low pH (e.g. pH 3.0)
2. Water soluble organic (MeOH, ACN, EtOH, 10 % - 20 %) in aqueous buffer
3. Note: Chaotropic agents are difficult to remove. They require rinsing with 20 to 40 CV of 20% ACN. Therefore they should be used only when the previous cleaning solutions are not effective.
   Buffered solutions of SDS (0.1 %), urea (8 mol/L), or guanidin (6 M)

Size Exclusion, TSK-GEL PW and PW_{XL} types

1. High concentration salt (e.g. 0.5 mol/L - 1.0 mol/L Na_2SO_4) in aqueous buffer
2. Buffered solutions at low pH (e.g. 2 - 3) or high pH (e.g. 11 - 12)
3. Water soluble organic (MeOH, ACN, EtOH, 10% - 20%) in aqueous buffer
4. Note: Chaotropic agents are difficult to remove. They require rinsing with 20 to 40 CV of 20% ACN. Therefore they should be used only when the previous cleaning solutions are not effective.
   Buffered solutions of SDS (0.1 %), urea (8 mol/L), or guanidine (6 mol/L).

Ion Exchange, TSK-GEL SW-type

1. High concentration salt (e.g. 0.5 mol/L - 1.0 mol/L Na_2SO_4) in aqueous buffer
2. Buffered solutions at low pH (e.g. 2 - 3)
3. Water soluble organic (MeOH, ACN, EtOH, 10% - 20%) in aqueous buffer
4. Note: Chaotropic agents are difficult to remove. They require rinsing with 20 to 40 CV of 20% ACN. Therefore they should be used only when the previous cleaning solutions are not effective.
   Urea (8 mol/L) or non-ionic surfactant in buffer solution.

Ion Exchange, TSK-GEL PW-type

1. Inject up to 1 CV in 250 µL increments of 0.1 mol/L - 0.2 mol/L NaOH on analytical columns. Inject proportionally larger volumes on semi-preparative columns.
2. 20 % - 40 % aqueous acetic acid* (Since acid can precipitate protein it should be used after other cleaning methods.)
3. Water soluble organic (MeOH, ACN, EtOH, 10% - 20%) in aqueous buffer
4. Note: Chaotropic agents are difficult to remove. They require rinsing with 20 to 40 CV of 20% ACN. Therefore they should be used only when the previous cleaning solutions are not effective.
   Urea (8 mol/L) or non-ionic surfactant in buffer solution.

Note: Rinse Ion Exchange columns with 5 CV of the appropriate solution to restore the correct counter-ion before equilibrating with loading buffer.

Hydrophobic Interaction, TSK-GEL PW-type

1. 0.1 mol/L - 0.2 mol/L NaOH*
2. 20 % - 40 % aqueous acetic acid* (Since acid can precipitate protein it should be used after other cleaning methods.)

Reversed Phase, Silica-based

1. 100% acetonitrile or methanol
2. Gradient from 10% - 100% acetonitrile in 0.05% trifluoro- acetic acid

Reversed Phase, Polymer-based

1. 100 % acetonitrile or methanol
2. 0.1 mol/L - 0.2 mol/L NaOH*
3. 20 % - 40 % aqueous acetic acid* (Since acid can precipitate protein it should be used after other cleaning methods.)

HILIC, TSK-GEL SW-type

1. Water
2. 45 % acetonitrile or acetone
3. 0.1 % triethylamine in at least 75 % acetonitrile
4. 50 mmol/L phosphate buffer pH 6.0 in 50 % acetonitrile

Affinity Columns, TSK-GEL PW-type

Consult the OCS sheet of the specific column type for cleaning directions.

**Inject up to 1 CV in 250 µL increments of solutions 2 & 3 on analytical columns. Inject proportionally larger volumes on semi-preparative columns.*

Guarding your column

GLP procedures demand that the separation column be protected by a guard column. Tosoh Bioscience supplies an assortment of packed guard columns and guardgel kits. Guardgel kits contain the hardware and the gel packing material to fill a guard column using an aspirator. For those columns where a guard column is not available, Tosoh Bioscience recommends the use of an in-line filter with a 0.5 µm cut-off to avoid frequent plugging of the 1.0 µm pores in the column frit. A pre-injector membrane filter is also recommended to prevent particles generated by pump seal wear from reaching the column. Consult the Standards and Accessories section for these and other hardware products.

Rehydration

Dehydration of TSK-GEL liquid chromatography columns can occur during long-term storage or from improper use. Dehydration can also occur if the plugs are not tightened or if air inadvertently is pumped into the column during use. It is easier to detect dehydration in glass columns because the dry packing will appear to pull away from the column walls. This condition can be remedied by using the following procedure:

1. Connect the column to your LC system in the reverse flow direction.
2. Do not connect the column to the detector.

3. Pump a filtered mobile phase of 20 % methanol in ultrapure water over the column at half of the recommended maximum flow rate.

   Note: reversed phase columns require 60 % methanol.

4. Continue this procedure until the column has been rehydrated. Rehydration can take several hours, depending on the column size.
5. Connect the column to the LC system in the proper flow direction.
6. Rinse with 3 column volumes (CV) of ultra pure water to remove the organic if it is not part of the normal mobile phase.
7. Equilibrate with loading buffer (usually 3-5 CV).
8. Perform the recommended QC tests to ensure that the column is performing properly. Evaluation methods are available from the Technical Service Department of Tosoh Bioscience.

Column Storage

When the column will be used the next day, allow it to run overnight at a low flow rate in a buffer that does not contain a halide salt. When the column will not be used for more than a day, clean it first, then flush salt from the column and store in 0.05 % sodium azide or 20 % ethanol. Seal tightly to prevent the column from drying out.

Scaling Up for Size Exclusion Chromatography

Tosoh Bioscience offers semi-preparative (21.5 mm ID), preparative (55 mm ID), and larger ID stainless steel columns packed with TSK-GEL SW-type or PW-type resin for seamless scale-up to commercial production of therapeutic proteins and other biopharmaceuticals. These packing materials have a larger particle size that is appropriate for use in process scale equipment. The packing materials, however, have the same pore size and provide the same selectivity as the corresponding TSK-GEL analytical column. The column volume (CV) of the preparative column that is needed to produce the required amount of product (per injection) is given by the relationship:

$(CV)pc / (CV)ac = (\text{mg product})pc / (\text{mg product})ac$

in which pc and ac refer to the preparative and analytical column respectively. The volume of a column is equal to $^1/4\ \pi\ (ID)^2L$, in which ID is the internal diameter and L the length of the column. In scaling up, column length (L) is usually kept constant. If so, to achieve a 100-fold increase in product per run, the ID of the prep column should be 10 times larger than that of the analytical column. As noted, the particle size in the preparative column is usually larger, and one should select a larger ID column than predicted by the above equation. As a rule of thumb, a 2-fold increase in particle size reduces resolution and thus output by the square root of 2.

Since scale-up from analytical columns is relatively straightforward, preparative TSK-GEL SW columns may be an economical route for the rapid production of biomolecules for clinical testing. See the SEC section of this catalog for more information and request a copy of the process media catalog. For more detailed analysis of your scale-up requirements, please contact Tosoh Bioscience's Technical Service Specialists .

Scaling Up for Hydrophobic Interaction and Ion Exchange Chromatography

Tosoh Bioscience provides various ID preparative columns for hydrophobic interaction (HIC) and ion exchange (IEC) chromatography. As shown above, to calculate the sample capacity of a larger column, multiply the capacity obtained on a 7.5 mm ID column by the ratio of the column volumes. The table below lists the column volumes for TSK-GEL HIC and IEC columns and their ratios relative to the 7.5 mm ID x 7.5 cm L column.

Dimensions

(mm ID x cm L)	Volume (mL)	Volume Ratio*
5 x 5	1.0	0.3
7.5 x 7.5	3.3	1.0
8.0 x 7.5	3.8	1.2
20 x 15	47.1	14.3
21.5 x 15	54.4	16.4
55 x 20	474.9	143.6
108 x 20	1831.2	554.8

*** Relative to 7.5 mm ID x 7.5 cm L column**

Based on a 1 mg capacity for a 7.5 mm ID x 7.5 cm L column, the capacity for a 55 mm ID x 20 cm L column is expected to be about 150 mg. Much larger amounts of crude sample can be injected as long as impurities do not co-elute from the column with the compound of interest.

Beware of extra-column band broadening

In recent years Tosoh has introduced several high efficiency column types with small internal diameters. Examples are:

- 1 mm ID, 2 mm ID and 4.6 mm ID x 30 cm L TSKgel SuperSW3000,
- 4.6 mm ID and 6 mm ID x 15 cm L TSK-GEL SuperAW columns.

It is well known that when the column diameter decreases, peak volumes decrease by the square of the ratio of column diameter. In contrast, a decrease in column length results in a proportional decrease in peak volume. Thus, when changing column dimensions from 7.8 mm ID x 30 cm L to 6 mm ID x 15 cm L results in a reduction of peak volume by a factor of $(7.8/6)^2*(30/15) = 3.4$. Similarly, the reductions in peak volume are 5.8 when going from 7.8 mm ID x 30 cm L to 4.6 mm ID x 15 cm L, and 21.1 when replacing a 4.6 mm ID x 30 cm L column by one that is 1 mm ID x 30 cm L. Such large reductions in peak volume require that the HPLC system is optimized with respect to external factors that contribute to the sample band broadening that takes place inside the column. Neglecting to optimize the HPLC system can seriously detract from the true column efficiency, which ultimately can result in unacceptable analysis results.

Main contributors to extra-column band broadening are capillary tubing that connect the column to the injector and the detector, injection volume, detector cell volume, detector time constant, and others.

Separation Report 95 discusses some of the variables to check when working with a smaller ID column, in this case the use of a 4.6 mm ID x 30 cm L TSKgel SuperSW3000 column (4 micron) compared to a 7.8 mm ID x 30 cm L TSKgel G3000SW$_{XL}$column (5 micron). You can download this and other separation reports from our website: www.tosohbioscience.com

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APPENDIX B

Recommended standards for quality control of TSK-GEL Columns

Standard	Approximate* Molecular Weight (Da)
Adenylate Kinase	6,000
Alcohol Dehydrogenase	150,000
Aldolase	158,000
β-Amylase	200,000
Blue Dextran	2,000,000
Bombesin	1,620
Bovine Serum Albumin (BSA)	67,000
Carbonic Anhydrase	29,000
α-Chymotrypsin	25,200
α-Chymotrypsinogen	25,700
Conalbumin	70,000
Cytidine	243
Cytidine-5′-monophosphate	323
Cytochrome C	12,400
D-Mannitol	182
Dopamine HCl	190
Enolase	67,000
Ethylene glycol	62
Ferretin	460,000
γ-Globulin	150,000
Glutamate Dehydrogenase	55,000
Glycine Monomer	246
IgG	160,000
IgM	900,000
Insulin	6,000
Lactate Dehydrogenase	36,500
Lysozyme	14,500
Myoglobin	16,900
Ovalbumin	43,000
p-Aminobenzoic Acid	137
Peroxidase	40,200
Phosphorylase B	94,000
Polyethylene Glycol Kit	1.1K, 1.5K, 3.7K, 10.9K, 19.7K
Polyethylene Oxide Kit	18K, 39K, 86K, 145, 252K, 594K, 996K
Polystyrene Kit	530, 950, 2.8K, 6.2K, 10.3K, 15.7K, 43.9K, 102K, 186K, 422K, 775K, 1260K
Pyruvate kinase	58,000
Ribonuclease A	12,600
Thyroglobulin	660,000
Transferrin	80,000
Trypsin	23,300
Trypsin Inhibitor	20,000
Trypsinogen	24,000
Tryptamine•HCl	24,000
Uric Acid	168

* exact molecular weight will depend on the species

APPENDIX C

United States Pharmacopeia (USP) specifications and corresponding Tosoh Bioscience columns

L1 - Octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter, or a monolithic rod.
Recommendations: TSK-GEL ODS-100V, ODS-100Z, ODS-100S, Super-ODS, ODS-80TM, ODS-80TS, ODS-120A, ODS-120T
See: Reversed Phase section

L7 - Octylsilane chemically bonded to totally porous silica particles, 1.5 to 10 µm in diameter.
Recommendations: TSK-GEL Super-Octyl, Octyl-80TS
See: Reversed Phase section

L-9 - Irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10 µm in diameter.
Recommendations: TSK-GEL SP-2SW
See: Ion Exchange section

L10 - Nitrile groups chemically bonded to porous silica particles, 3 to 10 µm in diameter.
Recommendations: TSK-GEL CN-80TS
See: Reversed Phase section

L11 - Phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter.
Recommendations: TSK-GEL Super-Phenyl
See: Reversed Phase section

L13 - Trimethylsilane chemically bonded to porous silica particles, 3 to 10 µm in diameter.
Recommendations: TSK-GEL TMS-250
See: Reversed Phase section

L20 - Dihydroxypropane groups chemically bonded to porous silica particles, 5 to 10 µm in diameter.
Recommendations: TSK-GEL QC-PAK 200 and 300, SW_{XL} series, SW series
See: Size Exclusion section

L21 - A rigid, spherical styrene-divinylbenzene copolymer, 5 to 10 µm in diameter
Recommendations: TSK-GEL H_{XL} and H_{HR} series
See: Size Exclusion section

L22 - A cation-exchange resin made of porous polystyrene gel with sulfonic acid groups, about 10 µm in size
Recommendations: TSK-GEL SCX
See: Ion Exchange section

L23 - An anion-exchange resin made of porous polymethacrylate or polymethacrylate gel with quartenary ammonium groups, about 10 µm in size.
Recommendations: TSK-GEL SuperQ-5PW, BioAssist Q, IC-Anion PW
See: Ion Exchange section

L24- A semi-rigid hydrophilic gel consisting of vinyl polymers with numerous hydroxyl groups in the matrix surface, 32 to 63 µm in diameter.
Recommendations: Toyopearl HW-type
See: Size Exclusion in the Bulk Resin section

L25- Packing having the capacity to separate compounds with a molecular weight range from 100-5000 (as determined by polyethylene oxide), applied to neutral, anionic, and cationic water-soluble polymers.
Recommendations: TSK-GEL G2500PW, G2500PW_{XL}, Alpha-2500, SuperAW2500
See: Size Exclusion section

L33- Packing having the capacity to separate dextrans by molecular size over a range of 4,000 to 500,000 daltons. It is spherical, silica-based, and processed to provide pH stability.
Recommendations: TSK-GEL SuperSW, SW_{XL}, QC-PAK, and SW series
See: Size Exclusion section

L37- Packing having the capacity to separate proteins by molecular size over a range of 2,000 to 40,000 daltons. It is a polymethacrylate gel.
Recommendations: TSK-GEL G3000PW_{XL}, G3000PW
See: Size Exclusion section

L38- A methacrylate-based size-exclusion packing for water-soluble samples
Recommendations: TSK-GEL PW_{XL}, PW, Alpha, and SuperAW series
See: Size Exclusion section

L39- A hydrophilic polyhydroxymethacrylate gel of totally porous spherical resin.
Recommendations: TSK-GEL PW, PW_{XL}, Alpha, and SuperAW series
See: Size Exclusion section

L52- A strong cation exchange resin made of porous silica with sulfopropyl groups, 5 to 10 µm in diameter.
Recommendations: TSK-GEL IC-Cation-SW, SP-2SW
See: Ion Exchange section

L58- Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the sodium form, about 7 to 11 µm diameter.
Recommendations: TSK-GEL SCX (Na^+)
See: Ion Exchange section

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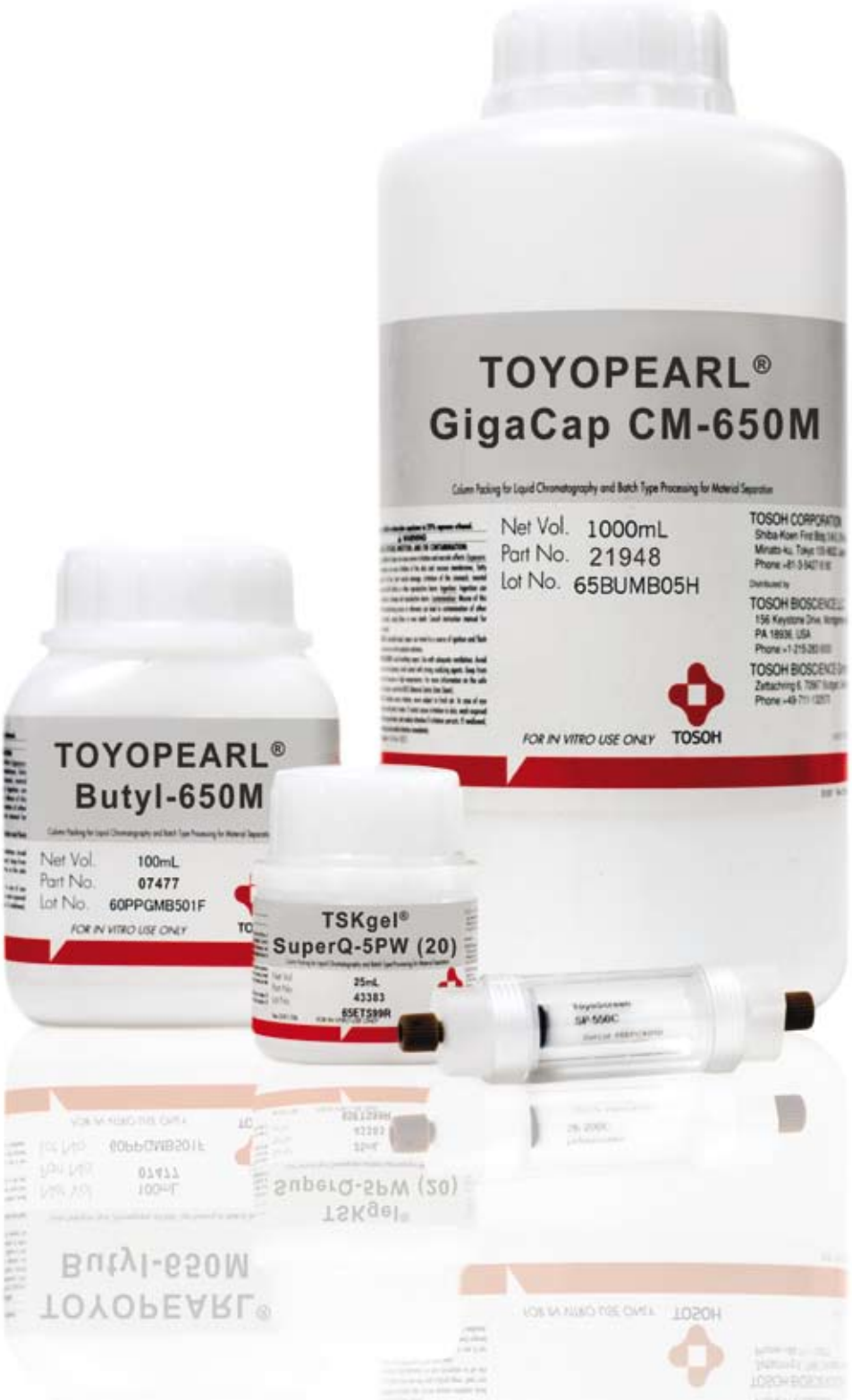
L59- Packing having the capacity to separate proteins by molecular weight over the range of 10 to 500 kDa. It is spherical (10 µm), silica-based, and processed to provide hydrophilic characteristics and pH stability.
Recommendations: TSK-GEL G2000SW, G3000SW and G4000SW
See: Size Exclusion section

L60- Spherical, porous silica gel, 10 µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and endcapped.
Recommendations: TSK-GEL Amide-80
See: HILIC section



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