TOSOH

TSKgel U/HPLC Columns

Experts in Chromatography

2019 Product Guide

TSKgel® U/HPLC Columns • TOYOPEARL® Bulk Resin • TSKgel Bulk Resin • Ca⁺⁺Pure-HA® Resin • EcoSEC® GPC Systems

TOSOH BIOSCIENCE

TSKgel Columns

The Gold Standard in Biomolecule Analysis

TSKgel U/HPLC Columns

For Every Mode of Chromatography

Hydrophilic Interaction

Reversed Phase

Affinity

Hydrophobic Interaction

Ion Exchange

Size Exclusion

Discover the extensive family of TSKgel U/HPLC columns. Our portfolio includes a variety of particle sizes and coulmn designs to meet all of your bioseparation needs.

Get Started

Additional resources are available for helping you implement TSKgel U/HPLC columns into your laboratory:

Web
Visit tosohbioscience.com for videos, product information and ordering.

Email
Our technical service staff is ready to answer questions: techservice.tbl@tosoh.com

In Person
A technical seminar can be arranged on-site or via the web. Request via seminars@tosoh.com

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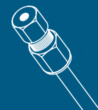
TSKgel®
TSKgel UP-SW3000
4.6 mm ID x 30 cm
TSKgel®
TSKgel UP-SW3000
4.6 mm ID x 15 cm
TSKgel guardcolumn UP-SW
4.6 mm ID x 2 cm
TSKgel guardcolumn UP-SW DC
4.6 mm ID x 2 cm



Silica-based for protein analysis:

TSKgel UP-SW3000
TSKgel SW mAb
TSKgel SW
TSKgel SWXL
TSKgel SuperSW

Polymer-based for desalting:

TSKgel BioAssist DS Columns

Polymethacrylate-based for water-soluble polymers analysis:

TSKgel PW
TSKgel PWXL
TSKgel PWXL-CP
TSKgel SuperMultiporePW

Polymethacrylate-based for polar organic-soluble polymers analysis:

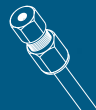
TSKgel Alpha
TSKgel SuperAW

Polystyrene-divinylbenzene-based for organic-soluble polymers analysis:

TSKgel HXL
TSKgel HHR
TSKgel SuperH
TSKgel SuperHZ
TSKgel SuperMultiporeHZ

Size Exclusion Tips:

- TSKgel size exclusion columns are offered in glass, PEEK (polyetheretherketone), and stainless steel (SS) hardware. SS or Pyrex® frits are embedded in the body of the column end-fittings of metal and glass columns, respectively. The nominal frit size for SS columns is engraved in the end-fittings; Pyrex frits in the glass columns have a 10 µm nominal pore size.
- Halide salts corrode stainless steel tubing, fitting, and frits. Do not store SS columns in a mobile phase containing NaCl and, where possible, use another salt in the operating buffer. Chlorotrifluorethylene and tetrafluorethylene are the materials in the glass column fittings that come into contact with the mobile phase and sample.
- Good laboratory procedures demand that the analytical column be protected by a guard column. Packed guard columns are available for use with TSKgel size exclusion columns.
- It is important to employ an HPLC system that is optimized with regards to extra-column band broadening to take full advantage of the high column efficiency that can be obtained on TSKgel UP-SW, SuperSW, SuperH and SuperHZ columns. Components such as connecting tubing, injector, injection volume, detector cell volume, and detector time constant may require optimization.
- TSKgel size exclusion columns are supplied with an Inspection Data Sheet, which includes a QC chromatogram and test data, and an OCS Sheet summarizing the recommended operating conditions for optimum column performance.
- A separate TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems, can be downloaded from the Tosoh Bioscience LLC website (www.tosohbioscience.com).



About: Size Exclusion Chromatography

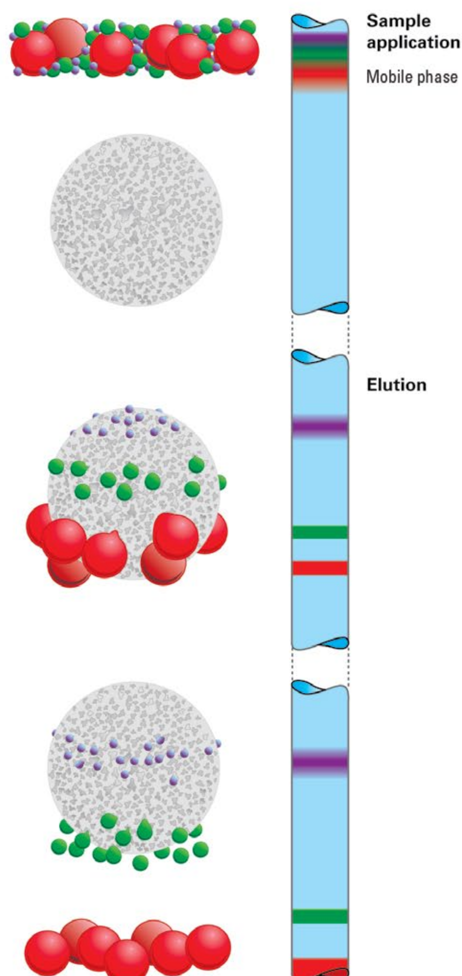
Size Exclusion Chromatography (SEC) separates molecules based on their size, or more precisely, their hydrodynamic volume. It is based on the discrimination of individual sample components by the pores of the packing material. Large sample molecules cannot or can only partially penetrate the pores and elute from the column first, whereas smaller molecules can access all or a larger number of pores and elute later. SEC is the only mode of chromatography that does not involve interaction with a stationary phase by means of adsorption or partitioning of the solutes.

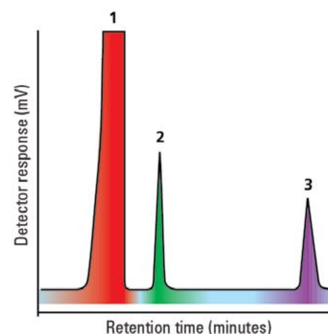
The terms SEC, GFC (gel filtration chromatography) and GPC (gel permeation chromatography) all refer to the same chromatographic technique. In GFC an aqueous mobile phase is used, while an organic mobile phase is employed in GPC. The general term SEC covers both uses.
SEC is the dominant mode of separation for natural and synthetic polymers:

- GFC is the term used for the size-based separation of water-soluble polymers, for example biopolymers or natural polymers.
- GPC is the term used for the size-based separation of polymers soluble in organic solvents.

Size exclusion chromatography columns are traditionally packed with porous polystyrene divinylbenzene (PS-DVB) or silica particles. PS-DVB columns are commonly used for the analysis of synthetic polymers in organic solvents, while silica-based columns are used for the separation of biopolymers.

Figure 1: Size Exclusion Chromatography





TSKgel Gel Filtration Chromatography Columns: GFC

The principal feature of GFC is its gentle non-interaction with the sample, enabling high retention of biomolecular enzymatic activity while separating multimers that are not easily distinguished by other chromatographic methods. SEC has limited peak capacity, however, requiring that the molar mass of the biomolecules differ by at least two-fold. GFC is popular among biochemists for the isolation of protein fractions or for the removal of aggregates in a final polishing step in biotechnology production. GFC is also frequently used for desalting a (protein) sample solution, often to prepare the sample for elution by another chromatographic mode.

- TSKgel columns for GFC analysis consist of the TSKgel SW and PW series column lines. The main criterion in choosing between these TSKgel columns is the molar mass of the sample and its solubility. The fact that the TSKgel SW columns are based on silica and the TSKgel PW columns are derived from a hydrophilic polymer network has less impact on the separation than the particle and pore size differences between the column lines.

- Due to higher resolving power, the TSKgel SW series columns are suitable for the separation of the monodisperse biopolymers such as proteins and nucleic acids. TSKgel UP-SW3000 columns are for the analysis of antibody therapeutics using both HPLC and UHPLC systems. The TSKgel SW mAb columns within the TSKgel SW series are designed specifically for the analysis of monoclonal antibodies. TSKgel PW series columns are commonly used for the separation of synthetic water-soluble polymers because they exhibit a much larger separation range, better linearity of calibration curves, and less adsorption than the TSKgel SW columns. While a TSKgel SW column is typically the first column to try for biopolymers, TSKgel PW columns have demonstrated good results for smaller peptides (<1,000 Da), protein aggregates, DNA fragments, and viruses.

TSKgel Gel Permeation Chromatography Columns: GPC

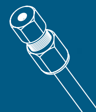
GPC plays an important role in the characterization of polar organic-soluble and organic-soluble polymers in consumer, chemical, and petrochemical industries. GPC is often used to determine the relative molar mass of polymer samples as well as the distribution of molar masses.

- TSKgel Alpha and SuperAW columns were developed for the GPC analysis of polymers of intermediate polarity. The TSKgel Alpha columns are compatible with a wide range of solvents. TSKgel SuperAW columns are based on the same chemistry as TSKgel Alpha columns but have smaller particle sizes and shorter, narrower column dimensions for high throughput applications.

- For the GPC analysis of organic-soluble polymers, Tosoh developed TSKgel H series columns. Each line of columns within the TSKgel H series differs in degree of inertness and operating temperature range.

Table 1: All TSKgel SEC columns share these features and benefits

Features	Benefits
Rigid hydrophilic and hydrophobic packings	Minimal swelling and excellent physical strength; Low adsorption resulting in high mass recovery
Four series of SEC columns with different ranges of solvent compatibility	Suitable for both types of size exclusion, aqueous (GFC) and non-aqueous (GPC)
Easy scale up	Analytical and preparative pre-packed SEC columns



About: TSKgel SW Series Size Exclusion Columns

TSKgel UP-SW3000 columns are the latest addition to the popular TSKgel SW column series, the gold standard for QC analysis of antibody therapeutics. TSKgel UP-SW3000 columns feature the same pore size as the well-established TSKgel G3000SWXL columns. Hence methods developed using TSKgel G3000SWXL columns can easily be transferred to TSKgel UP-SW3000 columns on conventional HPLC systems as well as on UHPLC systems.

The TSKgel SW mAb columns meet the growing demand for the higher resolution and high throughput separation of monoclonal antibody (mAb) monomer and dimer/fragment, as well as higher resolution of mAb aggregates. While mAbs can be analyzed using many different modes of HPLC, size exclusion is best for aggregation, dimer, and fragmentation, making it the best method for heterogeneity studies.

TSKgel SW series SEC columns contain a large pore volume per unit column volume. This is critical in SEC, because the more pore volume per unit column volume, the better two proteins of different molar mass are separated. TSKgel UP-SW3000, SW mAb, SW, SWXL, and SuperSW columns are based on highly porous silica particles, the surface of which has been shielded from interacting with proteins by derivatization with ligands containing diol functional groups. TSKgel SW series columns stand out from other silica- or polymer-based high performance size exclusion columns by virtue of their large pore volumes and low residual adsorption.

TSKgel UP-SW3000, SW mAb, SW, SWXL, and SuperSW columns are stable from pH 2.5 to 7.5 and can be used in 100% aqueous conditions. The different pore sizes of the TSKgel SW series columns result in different exclusion limits for globular proteins, polyethylene oxides and dextrans, as summarized in Table 2. Furthermore, different particle sizes, column dimensions and housing materials are available for each of the TSKgel SW series columns.

The column internal diameter of TSKgel SuperSW columns has been reduced from 7.8 mm ID to 4.6 mm ID to provide higher sensitivity in sample-limited cases and to cut down on solvent use. It is important to employ an HPLC system that is optimized with regards to extra-column band broadening to take full advantage of the high column efficiency that can be obtained on these columns.

TSKgel BioAssist columns are available within the TSKgel SWXL line. These columns are made of PEEK housing material to reduce sample adsorption to stainless steel or glass. Also available within the TSKgel G2000SWXL and G3000SWXL line are QC-PAK columns. These columns are 15 cm in length with 5 µm particles and offer the same resolution in half the time as the 30 cm, 10 µm TSKgel G2000SW and G3000SW columns.

TSKgel BioAssist DS desalting columns are designed to reduce the concentration of salt and buffer of protein or polynucleotide sample solutions at semi-preparative scale. Packed with 15 µm polyacrylamide beads in PEEK hardware, TSKgel BioAssist DS columns show excellent desalting performance.

Recommendations for TSKgel SW series selection:

- Samples of known molar mass
  - Calibration curves for each TSKgel SW series column are provided in this HPLC Column Product Guide. Each curve represents a series of various standards (protein, PEO, or globular proteins, for example) with known molar masses. The molar mass range of the compound to be analyzed should be within the linear range of the calibration curve and similar to the chemical composition and architecture of the standards used to construct the calibration curve.
- Samples of unknown molar mass
  - Use the TSKgel QC-PAK GFC300 column to develop the method (scouting) and the TSKgel G3000SWXL column to obtain the highest resolution.
  - If the protein of interest elutes near the exclusion volume, then a TSKgel G4000SWXL column is the logical next step. Conversely, if the protein of interest elutes near the end of the chromatogram, try a TSKgel G2000SWXL column.
- Proteins (general)
  - Choose one of the TSKgel SWXL columns using the calibration curves to select the appropriate pore size based on knowledge or estimate of protein molar mass.
- Monoclonal antibodies
  - TSKgel SW mAb columns are ideal for the analysis of monoclonal antibodies. Alternatives include the TSKgel UP-SW3000, G3000SWXL and SuperSW3000 columns when sample is limited or the components of interest are present at very low concentrations.
- Peptides
  - TSKgel G2000SWXL columns are the first selection for the analysis of peptides.
  - TSKgel SuperSW2000 columns are utilized when sample is limited or the components of interest are present at very low concentration.
- Other
  - Use TSKgel SW columns when not sample limited or when larger amounts of sample need to be isolated.

Table 2: Properties and separation ranges of TSKgel UP-SW3000, SW mAb, SW, SW_{XL}, SuperSW, and BioAssist DS columns

			Molar mass of samples (Da)		
TSKgel column	**Particle size**	**Pore size**	**Globular proteins**	**Dextrans**	**Polyethylene glycols & oxides**
G2000SW	10 µm and 13 µm	12.5 nm	5,000 – 1.5×10^5	1,000 – 3×10^4	500 – 1.5×10^4
G3000SW	10 µm and 13 µm	25 nm	1×10^4 – 5×10^5	2,000 – 7×10^4	1,000 – 3.5×10^4
G4000SW	13 µm and 17 µm	45 nm	2×10^4 – 7×10^6	4,000 – 5×10^5	2,000 – 2.5×10^5
G2000SW_{XL}, BioAssist G2SW_{XL}, QC-PAK GFC 200	5 µm	12.5 nm	5,000 – 1.5×10^5	1,000 – 3×10^4	500 – 1.5×10^4
G3000SW_{XL}, BioAssist G3SW_{XL}, QC-PAK GFC 300	5 µm	25 nm	1×10^4 – 5×10^5	2,000 – 7×10^4	100 – 3.5×10^4
G4000SW_{XL}, BioAssist G4SW_{XL}	8 µm	45 nm	2×10^4 – 7×10^6	4,000 – 5×10^5	2,000 – 2.5×10^5
SuperSW2000	4 µm	12.5 nm	5,000 – 1.5×10^5	1,000 – 3×10^4	500 – 1.5×10^4
SuperSW3000	4 µm	25 nm	1×10^4 – 5×10^5	2,000 – 7×10^4	1,000 – 3.5×10^4
BioAssist DS	15 µm	Excludes 2,500 Da PEG	–	–	–
SuperSW mAb HR	4 µm	25 nm	1×10^4 – 5×10^5	–	–
SuperSW mAb HTP	4 µm	25 nm	1×10^4 – 5×10^5	–	–
UltraSW Aggregate	3 µm	30 nm	1×10^4 – 2×10^6	–	–
UP-SW3000	2 µm	25 nm	1×10^4 – 5×10^5	–	–

About: TSKgel UP-SW3000 U/HPLC Size Exclusion Columns

TSKgel UP-SW3000 columns packed with 2 µm silica based particles are the latest addition to the popular TSKgel SW series, the gold standard for QC analysis of antibody therapeutics. These new silica-based UHPLC/HPLC columns are based on the same proven proprietary surface technology of the renowned TSKgel SW series. The surface of the particles has been shielded from interacting with proteins by derivatization with ligands containing diol functional groups.

TSKgel UP-SW3000 columns feature the same pore size as the well-established TSKgel G3000SWxl columns. Hence methods developed using TSKgel G3000SWxl columns can easily be transferred to TSKgel UP-SW3000 columns on conventional HPLC systems as well as on UHPLC systems. TSKgel UP-SW3000 columns are available in 4.6 mm ID with 15 or 30 cm length. The 15 cm column offers a shortened analysis time with improved efficiency versus the TSKgel G3000SWxl column. The 30 cm column delivers dramatically increased peak parameters such as efficiency, asymmetry, and resolution between fragments, monomers, and aggregates compared to the TSKgel G3000SWxl column.

The lifetime of the TSKgel UP-SW3000 columns are superior and can be maintained and further improved when using the corresponding guard columns. A “direct connect” (DC) guard column allows the user to minimize extra column dead volume.

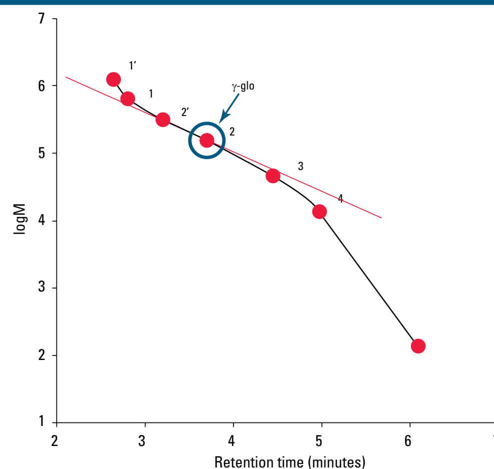
Attributes and Applications

Table 3 lists the product attributes of TSKgel UP-SW3000 columns. Figure 2 shows the protein standard calibration curve data that was generated using the TSKgel UP-SW3000 column. The column was run with a simple aqueous mobile phase (sodium phosphate buffer, pH 6.8) as typically reported in literature for SEC separations. The data demonstrates that the TSKgel UP-SW3000 column has a broad and linear resolving range of molecular weights. The shallow slope around the molecular weight of γ-globulin suggests that the particles of the column have an optimized pore size for the separation of proteins with a molecular weight of approximately 150 kDa.

Table 3: Product attributes

Attribute	Value
Base material	Silica
Particle size (mean)	2 µm
Pore size (mean)	25 nm
Functional group	Diol
pH stability	2.5-7.5
Calibration range (proteins)	10 - 500 kDa

Figure 2: Standard calibration curve of QC protein standard mixture generated by TSKgel UP-SW3000, 4.6 mm ID × 30 cm column

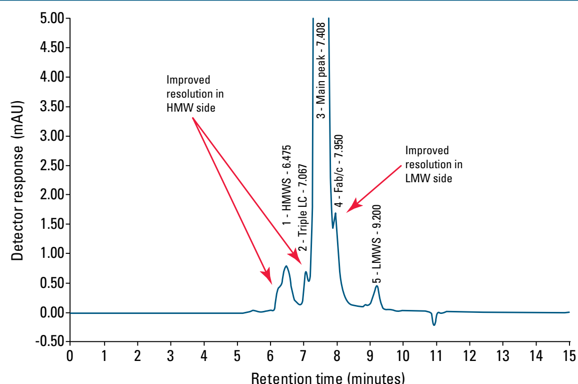


Column:	**TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 15 cm**
Mobile phase:	100 mmol/L phosphate buffer, pH 6.7 + 100 mmol/L Na_2SO_4 + 0.05% NaN_3
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Inj. vol.:	5 µL
Samples:	1′. thyroglobulin dimer, 1. thyroglobulin, 640 kDa 2′. γ-globulin dimer , 2. γ-globulin, 155 kDa 3. ovalbumin, 47 kDa, 4. ribonuclease A, 13,700 Da 5. p-amino benzoic acid, 137 Da

Superior Resolution for mAb Analysis

Figure 3 demonstrates the advantages of the TSKgel UP-SW3000 column for mAb analysis. The TSKgel UP-SW3000 column offers high resolution of both the high molecular weight (HMW) species and the Fab/c on the low molecular weight side. In addition, the analysis was completed in half the run time compared to a traditional 30 cm SEC column.

Figure 3: mAb analysis using TSKgel UP-SW3000 column

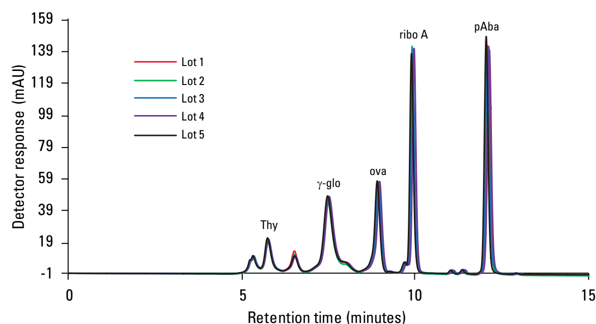


Column:	**TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm**
Instrument:	Dionex UltiMate® 3000RS UHPLC System
Mobile phase:	0.2 mol/L potassium phosphate/0.25 mol/L KCl, pH 6.2
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	40 °C
Injection vol.:	10 µL

Reproducibility

TSKgel UP-SW3000 columns offer superior reproducibility injection-to-injection, from column-to-column within the same lot and from lot-to-lot. A QC standard protein mixture of three consecutive injections was analyzed, yielding low percent relative standard deviation (%RSD) for retention time and theoretical plate count for all peaks, as shown in Figure 4 below.

Figure 4: TSKgel UP-SW3000 lot-to-lot reproducibility

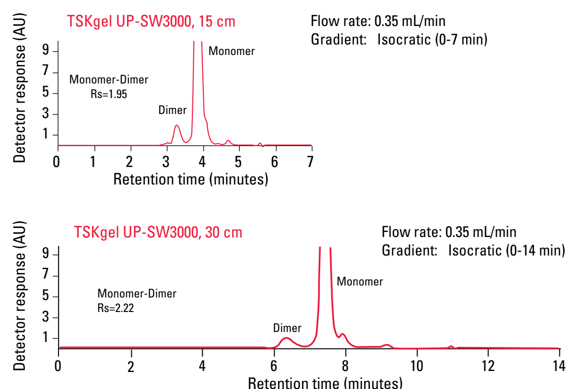


Column:	**TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm**
Instrument:	Thermo Fisher/Dionex Ultimate 3000 UHPLC System
Mobile phase:	100 mmol/L sodium phosphate buffer, pH 6.7, + 100mmol/L Na_2SO_4 + 0.05% NaN_3
Gradient:	Isocratic
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	5 µL
Samples:	QC standard protein test mixture: thyroglobulin, dimer thyroglobulin, 640 kDa, 0.5 g/L γ-globulin, dimer γ-globulin, 155 kDa, 1 g/L ovalbumin, 47 kDa, 1 g/L ribonuclease A , 13,700 Da, 1.5 g/L p-aminobenzoic acid, 137 Da, 0.01 g/L

Advantages of 15 cm TSKgel UP-SW3000 Column

Figure 5 compares the separation profile of a mAb on a 30 cm and a 15 cm length TSKgel UP-SW3000 column operated under the same mobile phase conditions and flow rate. The results indicate that the 15 cm TSKgel UP-SW3000 column provides a similar profile to the 30 cm column with 50% less run time and 50% lower backpressure at a typical flow rate of 0.35 mL/min (see Figure 6). The resolution between dimer and monomer is slightly less with the 15 cm column but it is still above the resolution guidelines from the USP monogram (1.2 resolution is acceptable). In addition, when the 15 cm column is operated at the typical flow rate of 0.35 mL/min, the backpressure is only 11 MPa. Therefore, these columns can be used with both HPLC and UHPLC systems.

Figure 5: Comparison of mAb aggregates analysis between TSKgel UP-SW3000, 15 cm and 30 cm columns



Columns:	**TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 15 cm** **TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm**
Mobile phase:	100 mmol/L sodium phosphate buffer, pH 6.8, + 100 mmol/L sodium sulfate + 0.05% sodium azide
Gradient:	Isocratic
Flow rate:	as indicated in each chromatogram
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	mAb (0.4 mg/mL)

Figure 6 demonstrates the rapid aggregate determination of a mAb using a TSKgel UP-SW3000, 4.6 mm ID × 15 cm column operated at 0.5 mL/min. The figure shows that the analysis was completed in only 4 minutes, nearly a 4 times faster run time than the 30 cm length column (compare the run time of Figure 5, bottom panel to this figure) and nearly 8 times faster than a traditional SEC column run time of 30 minutes at 1 mL/min (data not shown). The resolution profile of the aggregates and monomer of mAb (Rs = 1.97) is still maintained at the acceptable range in the USP guideline.

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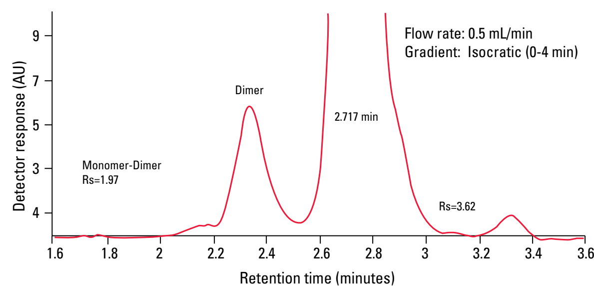
technical service: 800-366-4875

Figure 6: Fast analysis of mAb sample using TSKgel UP-SW3000, 4.6 mm ID × 15 cm column



Columns:	**TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 15 cm**
Mobile phase:	100 mmol/L sodium phosphate buffer, pH 6.8, + 100 mmol/L sodium sulfate + 0.05% sodium azide
Gradient:	Isocratic
Flow rate:	as indicated in each chromatogram
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	mAb (0.4 mg/mL)

Easy Method Transfer: From HPLC to HPLC and UHPLC Systems

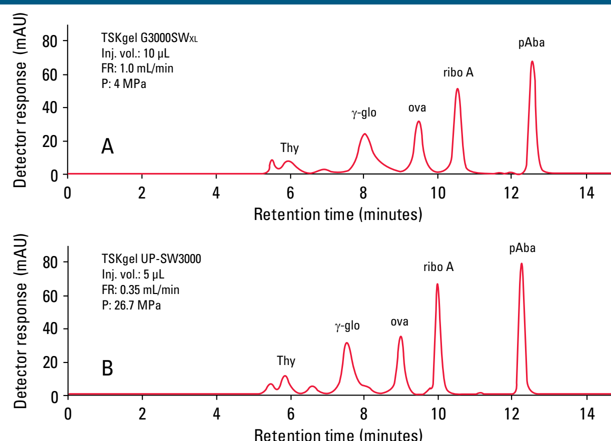
TSKgel UP-SW3000 columns feature the same pore size as the well-established TSKgel G3000SWXL columns. Hence methods developed using TSKgel G3000SWXL columns can easily be transferred to TSKgel UP-SW3000 columns on conventional HPLC systems as well as on UHPLC systems.

The TSKgel UP-SW3000 column offers several advantages versus the TSKgel G3000SWXL column, as shown in Figure 7 comparing the analysis of QC protein standards at the same concentrations. The TSKgel UP-SW3000 column offers higher sensitivity, with better peak shape, higher resolution and slightly shorter retention time. No change in the mobile phase composition is required; only an adjustment to a lower flow rate is necessary.

A method developed on a conventional HPLC system using a TSKgel UP-SW3000, 2 µm column is smoothly transferrable to a UHPLC system. Two separation profiles of a QC protein standard mixture using a TSKgel UP-SW3000 column from a HPLC and a UHPLC system are overlaid and shown in Figure 8. The two overlaid chromatograms clearly show that the method is robust, reproducible and transferrable.

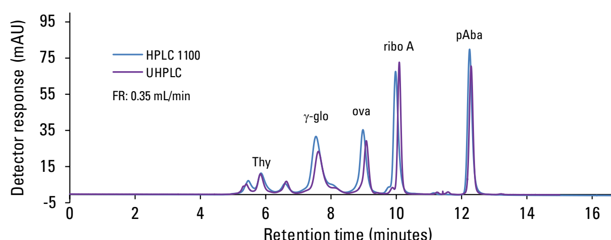
The analysis was carried out under identical chromatographic conditions at a flow rate of 0.35 mL/min. The two sets of three consecutive runs were carried out with the following criteria: two different instruments (Agilent 1100 HPLC and Thermo Fisher/Dionex Ultimate 3000 UHPLC), on two different days, using two columns from two different lots, using two different batches of QC buffer, and two different preparations of the QC protein standard mixture.

Figure 7: Analysis of QC protein standards using TSKgel G3000SWXL and UP-SW3000 columns on a conventional HPLC system



Columns:	**A. TSKgel G3000SWXL 5 µm, 7.8 mm ID × 30 cm** **B. TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm**
Mobile phase:	100 mmol/L sodium phosphate buffer, pH 6.7 + 100 mmol/L Na_2SO_4 + 0.05% NaN_3
Flow rate:	A. 1.0 mL/min B. 0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Inj. vol.:	A. 10 µL B. 5 µL
Samples:	1'. thyroglobulin, dimer 1. thyroglobulin, 640 kDa 2'. γ-globulin, dimer 2. γ-globulin, 155 kDa 3. ovalbumin, 47 kDa 4. ribonuclease A, 13,700 Da 5. p-amino benzoic acid, 137 Da

Figure 8: Analysis of QC protein standards using a TSKgel UP-SW3000 column on a HPLC and UHPLC system



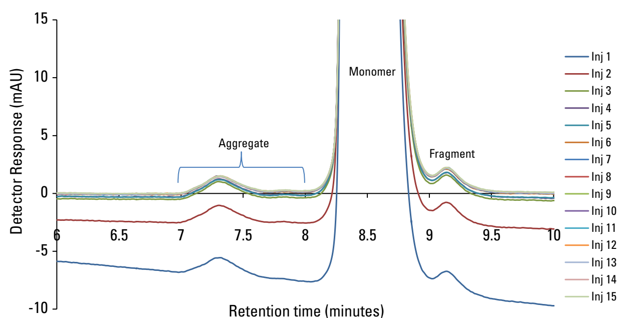
Column:	**TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm**
Mobile phase:	100 mmol/L sodium phosphate buffer, pH 6.7 + 100 mmol/L Na_2SO_4 + 0.05% NaN_3
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Inj. vol.:	5 µL
Samples:	1'. thyroglobulin, dimer 1. thyroglobulin, 640 kDa 2'. γ-globulin, dimer 2. γ-globulin, 155 kDa 3. ovalbumin, 47 kDa 4. ribonuclease A, 13,700 Da 5. p-amino benzoic acid, 137 Da

Usage of Isopropyl Alcohol for mAb Separation

Figure 9 shows the overlay of 15 injections of the USP mAb standard sample onto a TSKgel UP-SW3000 column with the addition of 15% IPA. These injections are performed after the column is subjected to 15 injections of the USP mAb standard sample with sodium phosphate buffer, pH 6.7, without IPA. The baseline of the first injection (as shown in blue) indicates that the column takes only one to two injections to be stabilized. After that all subsequent injections are overlaid perfectly.

At 0.3 mL/min, the pressure of the column is only at 22 MPa with the IPA added. It is still far below the allowance of the maximum pressure of 34 MPa of the column's rating. With this low operating pressure, the TSKgel UP-SW3000 column can be operated with both HPLC and UHPLC systems. As the chromatograms indicate, all runs are completed within 15 minutes.

Figure 9: Separation of USP mAb standard using TSKgel UP-SW3000 column with sodium phosphate buffer, pH 6.7, plus 15% IPA added



Column:	**TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm**
Instrument:	UltiMate 3000 UHPLC system run by Chromeleon® (ver 7.2)
Mobile phase:	15% IPA in 100 mmol/L KH_2PO_4/ Na_2HPO_4, pH 6.7, 100 mmol/L Na_2SO_4, 0.05% NaN_3
Flow rate:	0.30 mL/min
Detection:	UV @ 280 nm
Temperature:	30 °C
Pressure:	22 MPa (maximum column pressure is 34 MPa)
Injection vol.:	5 µL, 4 mg/mL
Sample:	USP mAb standard

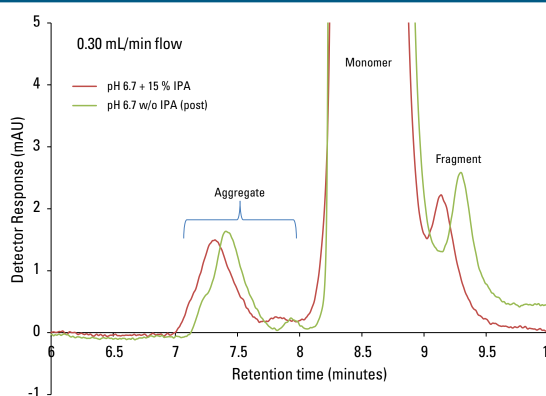
Table 4 lists the calculated data from the monomer and dimer peaks of the 15 injections from Figure 8 with the %RSD of retention time and percent relative area. As shown, the %RSD is below the allowance from the USP monograph guidance.

Table 4: %RSD of monomer and dimer peak of 15 injections: separation of USP mAb standard with sodium phosphate buffer, pH 6.7, plus 15% IPA added

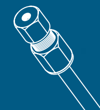
	Monomer peak		Dimer peak	
Injection	Ret. time (min)	Area (mAU*min)	Ret. time (min)	Area (mAU*min)
1	8.340	97.110	7.417	0.470
2	8.340	98.280	7.410	0.460
3	8.340	98.420	7.410	0.470
4	8.340	98.400	7.407	0.490
5	8.340	98.440	7.417	0.470
6	8.340	97.940	7.413	0.500
7	8.337	98.010	7.420	0.470
8	8.337	98.030	7.437	0.470
9	8.337	98.110	7.407	0.470
10	8.337	98.110	7.423	0.470
11	8.337	98.120	7.413	0.460
12	8.337	98.220	7.417	0.470
13	8.337	98.130	7.420	0.480
14	8.337	98.220	7.413	0.460
15	8.337	98.260	7.413	0.450
Average	**8.338**	**98.120**	**7.416**	**0.471**
Std Dev	**0.002**	**0.317**	**0.008**	**0.012**
%RSD	**0.018**	**0.323**	**0.102**	**2.598**

Figure 10 is an overlay of injections with and without IPA added to the mobile phase. The overlay indicates the similarities of peak retention times, peak width and peak height of dimer, monomer, aggregate and fragment peaks between the two different conditions.

Figure 10: Separation of USP mAb standard using TSKgel UP-SW3000 column with sodium phosphate buffer, pH 6.7, with and without 15% IPA added



Column:	**TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm**
Instrument:	UltiMate 3000 UHPLC system run by Chromeleon® (ver 7.2)
Mobile phase:	15% IPA in 100 mmol/L KH_2PO_4/ Na_2HPO_4, pH 6.7, 100 mmol/L Na_2SO_4, 0.05% NaN_3
Flow rate:	0.30 mL/min
Detection:	UV @ 280 nm
Temperature:	30 °C
Pressure:	22 MPa (maximum column pressure is 34 MPa)
Injection vol.:	5 µL, 4 mg/mL
Sample:	USP mAb standard

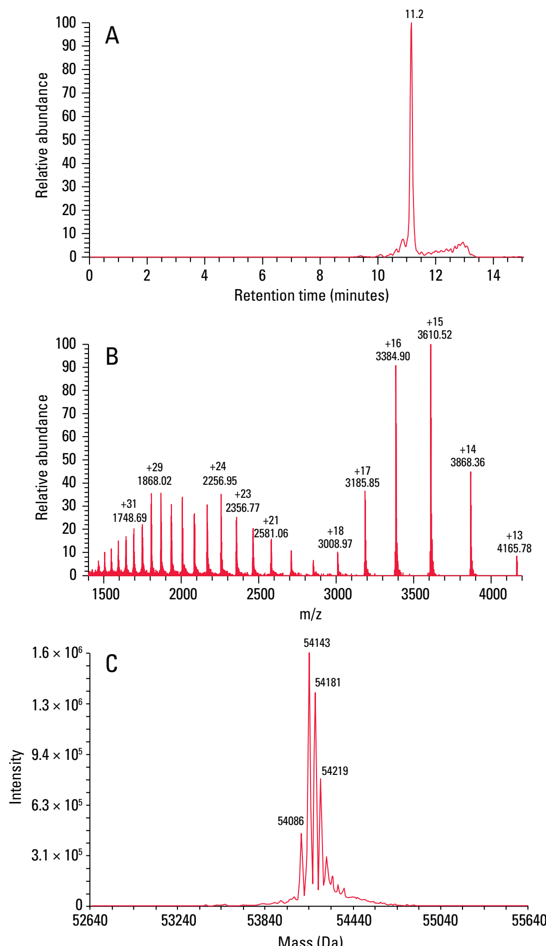


SEC/MS Analysis of a Bispecific T Cell Engager (BiTE®)

The TSKgel UP-SW3000, 2 µm SEC column can be used for accurate molar mass determination by SEC/MS. A MS compatible mobile phase under non-denaturing condition was successfully used with the TSKgel UP-SW3000 column. No signs of particle shedding or sample carryover, which may interfere with MS signal response, were noted with the TSKgel UP-SW3000 column.

A ~55 kDa BiTE and ~150 kDa parent mAbs (data not shown) were subsequently injected onto a TSKgel UP-SW3000 column coupled to a Q Exactive Plus mass spectrometer for molar mass determination. Figure 11 shows the (*A*) total ion chromatogram, (*B*) mass spectrum and (*C*) deconvoluted mass spectrum of the BiTE. A main peak can be seen at m/z 54,143; adjacent peaks at m/z 54,181, 54,219 and 54,086 correspond to different salt adducts.

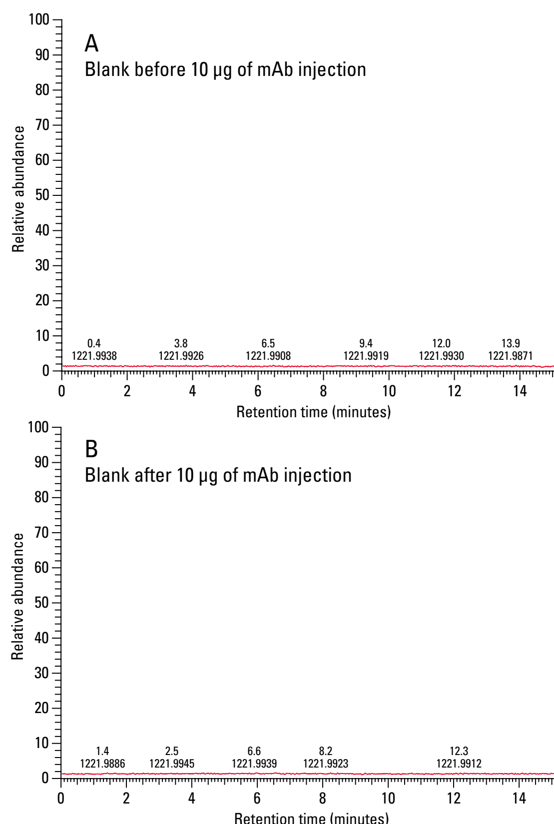
Figure 11: SEC/MS analysis of the BiTE. Accurate molar mass of the BiTE was identified as 54.1 kDa via SEC/MS.



Column:	**TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm**
HPLC Instrument:	Nexera® XR UHPLC system
MS Instrument:	Q Exactive™ Plus
Mobile phase:	20 mmol/L ammonium acetate, 10 mmol/L ammonium bicarbonate; pH 7.2
Gradient:	isocratic
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	30 °C
Injection vol.:	5.0 µL
Samples:	BiTE, 0.3 mg/mL (Creative Biolabs) parent mAb shown, 0.5 mg/mL (Creative Biolabs)
Ionization mode:	Electrospray ionization, positive mode
MS mode:	Scanning, m/z 800-6000

Prior to analysis, a blank injection was run in order to assess column particle shedding. Figure 12A shows the total ion chromatogram of a blank injection that was run on a new TSKgel UP-SW3000 column. MS data indicates that there is no shedding from the TSKgel UP-SW3000 column prior to sample injection. Additionally, a blank injection was run between each of the sample injections in order to monitor sample carryover. Figure 12B shows the total ion chromatogram of a blank injection run between the BiTE and parent mAb. No evidence of carryover can be seen in the run after sample injection. The lack of shedding and carryover indicate that the TSKgel UP-SW3000 column is suitable for use with MS.

Figure 12A and 12B:Column Shedding and Carryover Analysis. No shedding or carryover was observed via MS total ion chromatogram.



About: TSKgel SW mAb Size Exclusion Columns

TSKgel SW mAb columns are the newest innovation in size exclusion technology from Tosoh. This line of columns consists of three specialized columns designed for the separation and analysis of monoclonal antibodies (mAb).

Compared to competitive columns, these new stainless steel, silica-based TSKgel columns offer reduced lot-to-lot variation, long column life, reduction of unspecified adsorption, and superior recovery of aggregates.

These columns are available within the TSKgel SW mAb column line:

- TSKgel SuperSW mAb HR
- TSKgel SuperSW mAb HTP
- TSKgel UltraSW Aggregate

TSKgel SuperSW mAb HR and SuperSW mAb HTP both contain 4 µm particles. The HR designation represents the high resolution analysis of mAb monomer, dimer, and fragments, while the HTP stands for "high throughput" due to the smaller dimensions (4.6 mm ID × 15 cm). The TSKgel SuperSW mAb HTP column is compatible with both HPLC and UHPLC systems. The TSKgel UltraSW Aggregate column is a smaller particle size, 3 µm, and offers high resolution separation of mAb multimers and aggregates.

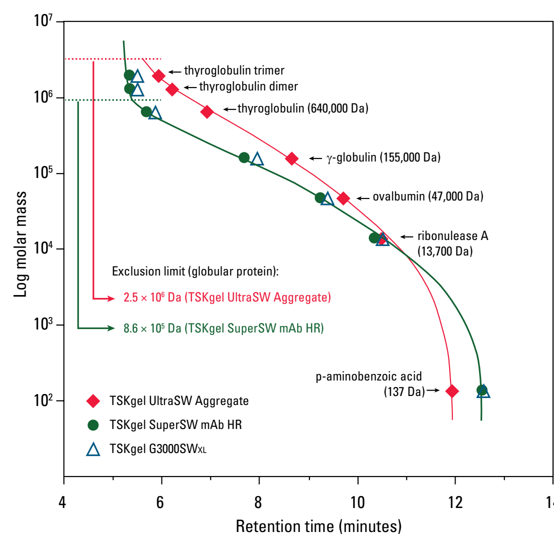
Attributes and Applications

Table 5 shows a summary of the product attributes for the TSKgel SW mAb columns. These columns utilize a unique pore-controlled technology, which produces a shallow calibration curve in the molar mass region of a typical monoclonal antibody. As shown in Figure 13, the calibration curve for the TSKgel SuperSW mAb HR column is similar to that of the TSKgel G3000SWXL column curve and has a shallower slope than the TSKgel UltraSW Aggregate column around the molar mass range of gamma-globulin. This shallow calibration curve produces high resolution separations. The TSKgel UltraSW Aggregrate calibration curve shows a separation range up to around 2 million Da, which implies better resolution of aggregate/multimer of a mAb.

Table 5: Product attributes

TSKgel column	SuperSW mAb HR	SuperSW mAb HTP	UltraSW Aggregate
Base material	Silica		
Particle size (mean)	4 µm	4 µm	3 µm
Pore size (mean)	25 nm	25 nm	30 nm
Functional group	Diol		
pH stability	2.5-7.5		
Calibration range	1×10^4 - 5×10^5 Da (globular proteins)	1×10^4 - 5×10^5 Da (globular proteins)	1×10^4 - 2×10^6 Da (globular proteins)

Figure 13: Protein calibration curves for TSKgel SW mAb columns

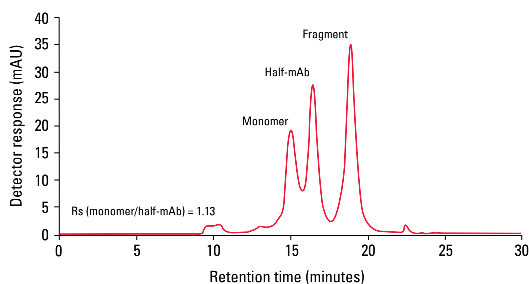


mAb Monomer from its Half-Body

Recent research has shown an interest in mAb half-bodies as therapeutic vectors as they can be further targeted for conjugation, enzyme labeling, or antibody immobilization. Monoclonal antibody half-bodies can be generated through the genetic engineering of cells or by selective reduction of hinge-region disulfide bonds present in the mAb by mild reducing agents, such as TCEP [tris(2carboxyethyl) phosphine]. A mAb half-body was generated through protein reduction using TCEP and subsequently identified by gel electrophoresis.

Figure 14 illustrates the separation of human IgG monomer, half-body (70 kDa) and fragment (1/3 mAb) using a TSKgel SuperSW mAb HR column. High resolution (Rs = 1.13) of the IgG monomer and half-body species was achieved.

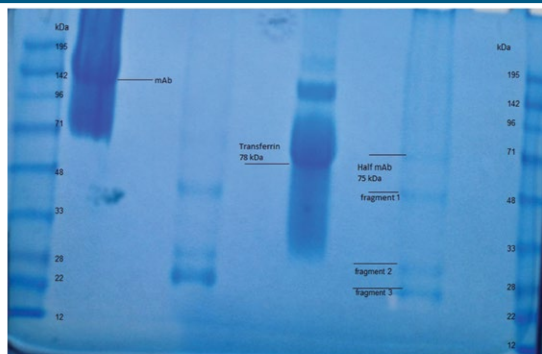
Figure 14: Separation of human IgG monomer, half-body, and fragments using a TSKgel SuperSW mAb HR column



Column:	**TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.1 mol/L phosphate/0.1 mol/L sulfate buffer + 0.05% NaN_3
Flow rate:	0.5 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	human IgG (4.6 g/L) from Sigma Aldrich

SDS-PAGE was used to confirm the identity of the mAb monomer, half-body and fragment collected from the SEC separation on the TSKgel SuperSW mAb HR column. The monoclonal antibody, half mAb and the fragment are clearly identified with the SDS-PAGE molar mass marker as well as transferrin (78 kDa) (Figure 15). This clearly shows that the half mAb could be generated using the TCEP reduction method and separated using the TSKgel SuperSW mAb HR column.

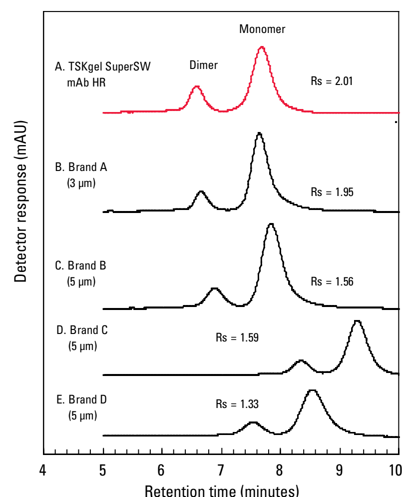
Figure 15: SDS-PAGE gel of human IgG monomer, half-body and fragments separated using a TSKgel SuperSW mAb HR column



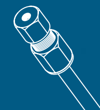
mAb Monomer and Dimer

Figure 16 demonstrates the superior resolution of the TSKgel SuperSW mAb HR column compared to four competitive columns in the analysis of a mAb monomer and dimer. TSKgel SuperSW mAb HR shows excellent resolution of gamma-globulin dimer and monomer.

Figure 16: Comparison of resolution of mAb monomer and dimer



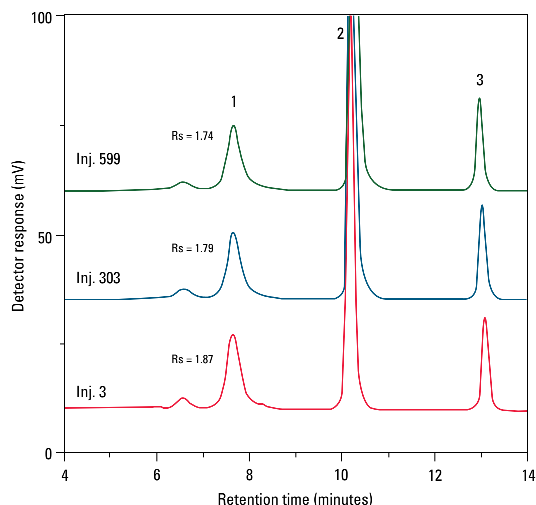
Columns:	**A. TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm**
	B. Brand A, 3 µm, 7.8 mm ID × 30 cm
	C. Brand B, 5 µm, 7.8 mm ID × 30 cm
	D. Brand C, 5 µm, 8.0 mm ID × 30 cm
	E. Brand D, 5 µm, 8.0 mm ID × 30 cm
Mobile phase:	200 mmol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	IgG (human polyclonal), 1.0 g/L



Durability

Figure 17 demonstrates the good durability of the TSKgel SuperSW mAb HR column through the reproducibility of Rs for a γ-globulin sample injection.

Figure 17: High durability of TSKgel SuperSW mAb HR column

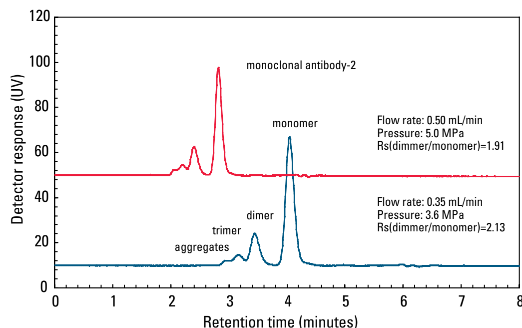


Column:	**TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate:	0.8 mL/min
Detection:	UV @ 280 nm
Injection vol.:	10 µL
Samples:	1. γ-Globulin 2. Cytochrome C 3. DNP-L-Alanine

Therapeutic mAb

A shorter column length allows the TSKgel SuperSW mAb HTP column to provide fast and efficient run times in the high resolution separation of a mAb monomer and dimer. Figure 18 shows no loss in resolution in the analysis of a therapeutic mAb at a 0.50 mL/min flow rate and an increased pressure of 5.0 MPa.

Figure 18: High speed separation of therapeutic mAb



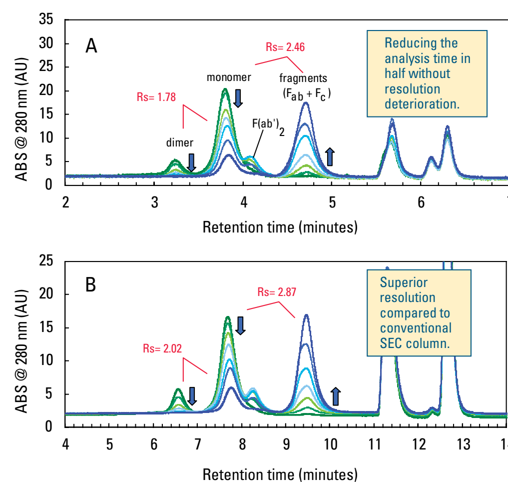
Column:	**TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm**
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate:	0.50 mL/min, 0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Sample:	monoclonal antibody-2 (mouse-human chimeric IgG, Erbitux®), 5 µL

Papain Digested IgG

IgG monomer, dimer, and fragments from IgG digested by papain over a 24 hour period were analyzed using the TSKgel SuperSW mAb HR and SuperSW mAb HTP columns (Figure 19). The results exhibit the superior resolving power of these columns for monomer/fragment and monomer/dimer separation. The TSKgel SuperSW mAb HTP column shows no deterioration in resolution while decreasing the analysis time in half.

The results also show that the TSKgel SuperSW mAb HR column has superior performance of mAb separation in comparison to the TSKgel G3000SWXL column. While TSKgel G3000SWXL has set the standard for the separation of general proteins for more than 25 years, the new TSKgel SuperSW mAb HR column is more specifically suited for the analysis of mAb, as seen in the results of the analysis of IgG.

Figure 19: Analysis of IgG monomer, dimer and fragments

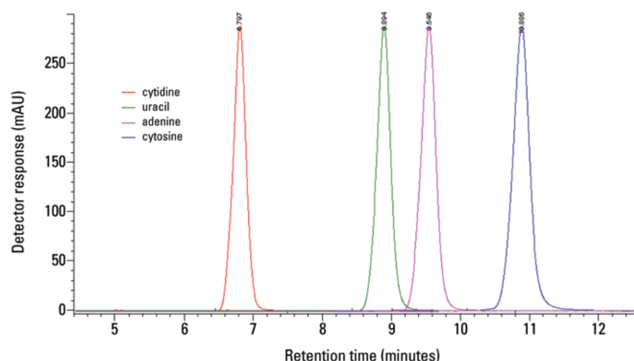


Columns:	**A. TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm** **B. TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm**
Mobile phase:	200 mmol/L phosphate buffer + 0.05% NaN_3, pH 6.7
Flow rate:	A: 0.35 mL/min; B: 1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	A: 5 µL; B: 10 µL
Sample:	10 g/L IgG digested with papain for 0-24 hr

Nucleobases Analyzed in HILIC Mode

Figure 20 illustrates the separation of 4 nucleobases using the TSKgel SuperSW mAb HTP column in HILIC mode with CH_3CN as mobile phase A, and 15 mmol/L ammonium bicarbonate, pH 7.4, as mobile phase B. It is important to note that the order of elution of the analytes does not correlate with their molecular mass (as in SEC separations), but instead is based on their relative hydrophilicity.

Figure 20: Separation of four nucleobases using TSKgel SuperSW mAb HTP column in HILIC mode at pH 7.4

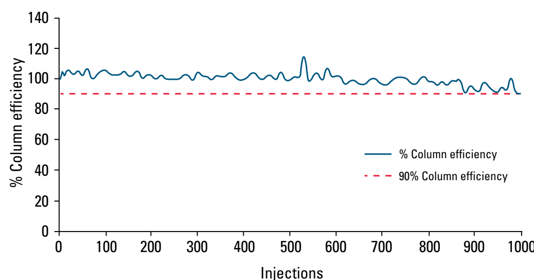


Column:	**TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm**
Mobile phase:	A: acetonitrile B: 15 mmol/L ammonium bicarbonate, pH 7.4
Gradient:	isocratic
Flow rate:	0.4 mL/min
Detection:	UV @ 280 nm
Injection vol.:	1 µL
Temperature:	ambient
Samples:	uracil (1.5 g/L), adenine (1.5 g/L), cytosine (1.5 g/L), cytidine (1.5 g/L) from Sigma Aldrich

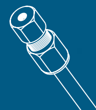
Column Lifetime

The TSKgel SuperSW mAb HTP column demonstrates highly reproducible performance over a significant number of injections of protein standard, as shown in Figure 21. The column yielded less than a 10% loss in column efficiency over 1,000 consecutive injections. Additionally, the packing integrity of the column is extremely high since even in the reverse flow orientation a nearly identical chromatographic trace to that of normal flow orientation was obtained (data not shown). During this study a guard column was not used, the analytical column was not cleaned/back flushed, and the mobile phase and sample were not filtered, to give additional stress to the analytical column. Implementation of such protective measures can be expected to yield extended column lifetime.

Figure 21: Performance stability of the TSKgel SuperSW mAb HTP column over 1000 consecutive injections of protein standard



Column:	**TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm**
Mobile phase:	100 mmol/L phosphate/100 mmol/L sodium sulfate, pH 6.7 + 0.05 % NaN_3
Gradient:	isocratic
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	5 µL (21.1 µg total protein load)
Samples:	protein standard: thyroglobulin, 0.58 g/L, γ-globulin, 1.02 g/L, ovalbumin, 1.08 g/L, ribonuclease, 1.53 g/L, PABA, 0.01 g/L

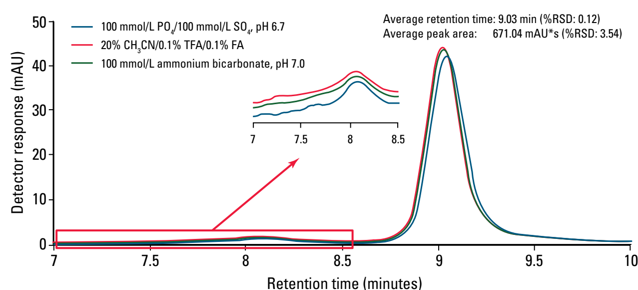


Monoclonal Antibody Aggregate Analysis using MS-Friendly Mobile Phases

Conventional SEC separations make use of relatively high ionic strength mobile phase compositions in an effort to minimize ionic interactions between the analyte and stationary phase. Due to the substantial amount of salt present in the mobile phase, on-line interfacing with mass spectrometry is not feasible due to the inevitable contamination of the MS ion source by the mobile phase salts. In order to make SEC-MS an applicable technique, volatile, MS-friendly mobile phase compositions must be implemented to avoid damage to the MS system.

A mAb 1 antibody was subjected to thermal stress for forced aggregation to evaluate various mobile phase compositions – volatile and salt-based. As shown in Figure 22, aggregates of mAb 1 are clearly separated from the monomeric species using all three mobile phase compositions. Results for critical peak parameters of the mAb 1 monomer are highly reproducible regardless of the mobile phase composition.

Figure 22: Separation of forced aggregated mAb 1 using volatile and salt-based mobile phase compositions on the TSKgel UltraSW Aggregate column

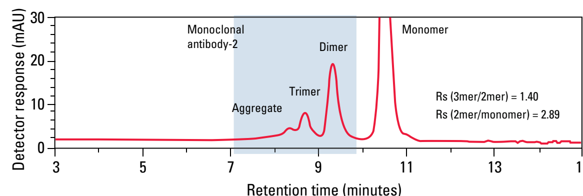


Column:	**TSKgel UltraSW Aggregate, 3 µm, 7.8 mm ID × 30 cm**
Mobile phase:	100 mmol/L PO_4/100 mmol/L SO_4, pH 6.7 20% CH_3CN/0.1% TFA/0.1% FA 100 mmol/L ammonium bicarbonate, pH 7.0
Gradient:	isocratic
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	TBL mAb 1 (4.0 g/L)

Mouse-Human Chimeric IgG

Figure 23 shows the analysis of a mouse-human chimeric IgG using the TSKgel UltraSW Aggregate column. Superior resolution of the mAb trimer and dimer is obtained. The smaller particle size (3 µm) and higher molar mass exclusion limit (2,500 kDa, globular proteins) of the TSKgel UltraSW Aggregate column, compared to the TSKgel SuperSW mAb HR and HTP columns, allows for high resolution separation of mAb multimers and aggregates.

Figure 23: Separation of mAb trimer and dimer

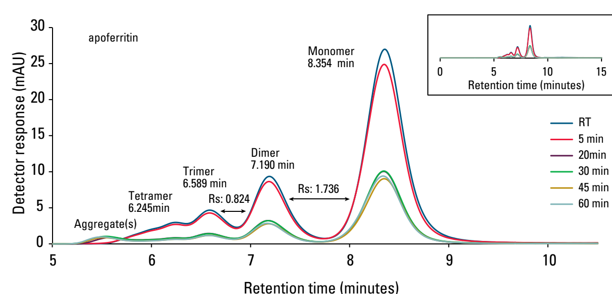


Column:	**TSKgel UltraSW Aggregate, 3 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate:	0.8 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Sample:	monoclonal antibody-2 (mouse-human chimeric IgG, Erbitux), 10 µL

Metalloprotein

The analysis of a heat denatured, large hydrophobic metalloprotein, apoferritin, is shown in Figure 24. A set of six, 0.3 mL HPLC vials each containing 100 μL stock solution of apoferritin was used for protein thermal denaturation. Thermal denaturation was carried out at 60 °C using an electric heating block. Individual sample vials were tightly capped and exposed to the heat for 5, 20, 30, 45, and 60 minutes. Samples were analyzed using a TSKgel UltraSW Aggregate column at the end of each incubation time period. The TSKgel Ultra SW Aggregate column yielded high resolution between the monomer and dimer. The trimer, tetramer and higher order aggregates of apoferritin were well separated.

Figure 24: Analysis of heat induced forced denatured, large hydrophobic metalloprotein, apoferritin



Protein	Molecular weight (kDa)			
	Monomer	Dimer	Trimer	Tetramer
ferritin and apoferritin	450	900	1350	1800

Column: **TSKgel UltraSW Aggregate, 3 μm, 7.8 mm ID × 30 cm**
Mobile phase: 50 mmol/L potassium phosphate (monobasic), 50 mmol/L sodium phosphate (dibasic), 100 mmol/L sodium sulfate, 0.05% NaN_3, pH 6.7
Flow rate: 1.0 mL/min
Detection: UV @ 280 nm
Temperature: 30 °C
Injection vol.: 10 μL
Samples: ferritin – Sigma, 4.7 g/L, in saline (0.9% NaCl in water) solution, stored at 2-8 °C
apoferritin – Sigma, 5.0 g/L, in 50% glycerol and 0.075 mol/L sodium chloride, stored at -20 °C

About: TSKgel SW Size Exclusion Columns

TSKgel SW columns, introduced in 1977, were the first of a long line of high performance Gel Filtration columns that have become synonymous with isolating proteins and analyzing protein molar masses in the emerging field of biotechnology.

TSKgel SW columns are based on highly porous silica particles, the surface of which has been shielded from interacting with proteins by derivatization with ligands containing diol functional groups. TSKgel SW columns stand out from other silica- or polymer-based high performance size exclusion columns by virtue of their large pore volumes.

Particles having three different pore sizes are available packed as:

- TSKgel G2000SW
- TSKgel G3000SW
- TSKgel G4000SW

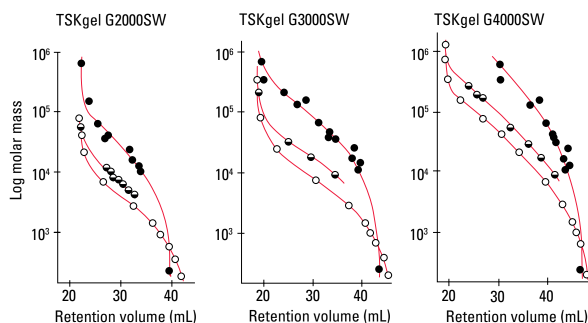
Attributes and Applications

Table 6 shows a summary of the product attributes for each of the TSKgel SW columns. The TSKgel G2000SW column provides excellent separation of peptides and proteins with molar masses up to 1.0×10^5 Da. TSKgel G3000SW columns are the best choice for separation of proteins and other biomolecules with molar masses up to 5.0×10^5 Da, while TSKgel G4000SW columns are preferred for proteins and other biomolecules of even higher molar masses. Figure 25 shows the calibration curves for globular proteins, polyethylene oxides and dextrans for each of the three TSKgel SW columns.

Table 6: Product attributes

TSKgel column	G2000SW	G3000SW	G4000SW
Base material	Silica		
Particle size (mean)	10 µm and 13 µm	10 µm and 13 µm	13 µm and 17 µm
Pore size (mean)	12.5 nm	25 nm	45 nm
Functional group	Diol		
pH stability	2.5-7.5		
Calibration range	5,000 - 1.0×10^5 Da (globular proteins)	1.0×10^4 - 5.0×10^5 Da (globular proteins)	2.0×10^4 - 7.0×10^6 Da (globular proteins)

Figure 25: Calibration curves for globular proteins, polyethylene oxides and dextrans for TSKgel SW columns

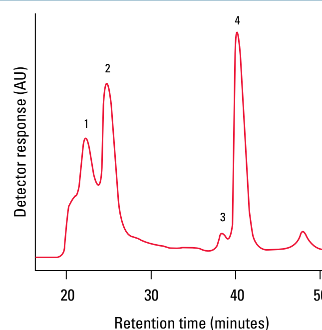


Column: **TSKgel SW columns, 7.5 mm ID × 60 cm × 2**
Mobile phase: dextrans and polyethylene oxides: distilled water; proteins: 0.3 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.0
Flow rate: 1.0 mL/min
Detection: UV @ 220 nm and RI
Samples: ● proteins, ○ polyethylene oxides, ◒ dextrans

Separation of *E. coli* RNA

Separation of four *E. coli* RNAs, shown in Figure 26, confirms the high performance of a TSKgel G4000SW column for samples with a wide molar mass range. The sample consists of 4S tRNA (2.5×10^4 Da), 5S rRNA (3.9×10^4 Da), 16S rRNA (5.6×10^5 Da), and 23S rRNA (1.1×10^6 Da). All four polynucleotides are within the molar mass range recommended for this TSKgel SW column. The chromatogram demonstrates a superior separation with the TSKgel G4000SW column.

Figure 26: Separation of total E. coli RNA

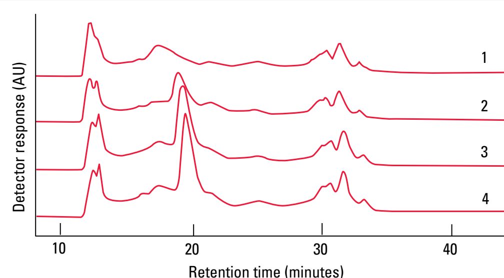


Columns: **TSKgel G4000SW, 13 µm, 7.5 mm ID × 30 cm × 2**
TSKgel G4000SW, 17µm, 7.5 mm ID × 30 cm × 2
Mobile phase: 0.13 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.0, + 1 mmol/L EDTA
Flow rate: 1.0 mL/min
Detection: UV @ 260 nm
Injection vol.: 5 µg
Sample: 0.1 mL of 1:10 diluted solution of total *E. coli* RNA:
1. 23s rRNA (1.1×10^6 Da)
2. 16s rRNA (5.6×10^5 Da)
3. 5s rRNA (3.9×10^4 Da)
4. 4s rRNA (2.5×10^4 Da)

Membrane Protein

Surfactants are routinely used for the isolation of proteins from membranes. Although this is an efficient method for solubilization, the presence of detergents affects the performance of chromatographic separations. A TSKgel G3000SW column was used to study the effect of different concentrations of the non-ionic surfactant octaethyleneglycol dodecylether on the analysis of membrane proteins from a crude extract from rat liver microsome. Figure 27 demonstrates that as the concentration of the surfactant increases to 0.05%, the main peak becomes sharper and recovery increases (chromatogram #4). Caution: we recommend that columns that have been used with a surfactant-containing mobile phase are dedicated for that particular use.

Figure 27: Analysis of membrane protein with differing surfactant concentrations in the mobile phase

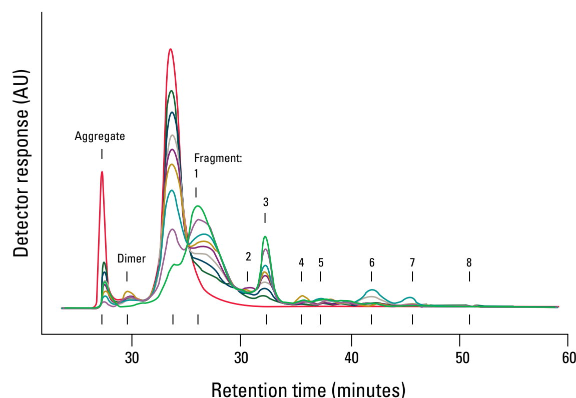


Column:	**TSKgel G3000SW, 10 µm, 7.5 mm ID × 60 cm**
Mobile phase:	(0.2 mol/L sodium chloride + 20% glycerol + octaethylene glycol dodecylether) in 50 mmol/L phosphate buffer, pH 7.0 Note: concentration of surfactant: 1. 0.005% 2. 0.01% 3. 0.025% 4. 0.05%
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Sample:	membrane protein from a crude extract from rat liver microsome

Degradation Products of IgG

High speed is important when analyzing the rate of chemical alteration of proteins (denaturation, condensation, degradation, etc.). Tomono et al[1] tracked the course of enzyme digestion of commercial IgG by pepsin using a TSKgel G3000SW column (Figure 28).

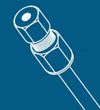
Figure 28: Tracking changes over time



Column:	**TSKgel G3000SW, 10 µm, 7.5 mm ID × 60 cm**
Mobile phase:	0.1 mol/L acetate buffer, pH 5.0 + 0.1 mol/L sodium sulfate
Samples*:	100 µL solutions produced by digestion of IgG (20 g/L) by pepsin after 0, 2, 4, 6, 8, 10, 15, 30 and 60 minutes

*Courtesy of Tsugikazu Tomono, Director of Japanese Red Cross Central Blood Center

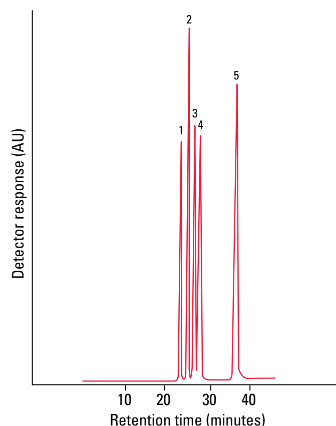
1. a) T. Tomono, T. Suzuki, and E. Tokunaga, Anal, Biochem., 123, 394 (1982)
b) T. Tomono, T.Suzuki, and E. Tokunaga, Bio, Bio. Phys. Acta., 660, 186 (1981)



Nucleic Acid

Figure 29 shows the separation of nucleic acid bases and nucleosides using a TSKgel G2000SW column.

Figure 29: Separation of nucleic acid

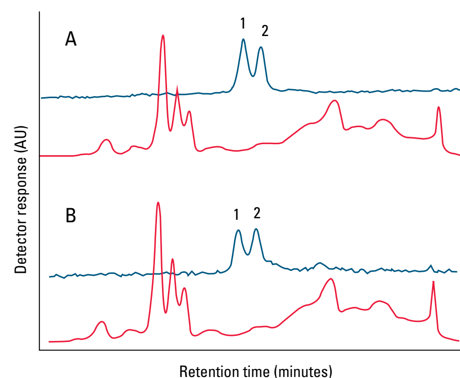


Column:	**TSKgel G2000SW, 10 µm, 7.5 mm ID × 60 cm**
Mobile phase:	acetic acid/triethylamine/H_2O = 3/3/94
Flow rate:	0.74 mL/min
Detection:	UV @ 260 nm
Samples:	1. uridine
	2. uracil
	3. thymine
	4. adenosine
	5. adenine

Metallothionein

Suzuki et al have conducted detailed studies involving the quantitative analysis of metallothionein. In these studies, the liver and kidney of cadmium-administered rats were used as samples, and the SEC columns were directly coupled to an atomic absorption detector. Metallothionein was separated into two isozymes. Presumably, the cation exchange capacity of residual silanol groups on the TSKgel SW packing material played a role in this isozyme separation. Representative chromatograms are shown in Figure 30.

Figure 30: Analysis of metallothionein



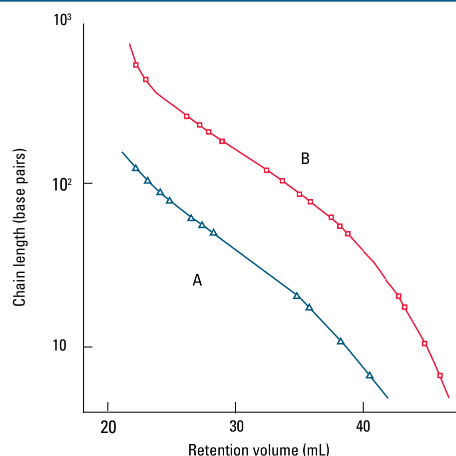
Column:	**TSKgel G3000SW, 13 µm, 21.5 mm ID × 60 cm**
Mobile phase:	50 mmol/L Tris-HCl buffer
Detection:	atomic absorption (Cd, Zn) + UV @ 280 nm
	A: Cd
	B: Zn
Samples*:	rat liver supernatant
	1.metallothionein I
	2.metallothionein II

*Courtesy of Professor Kazuo Suzuki of the National Institute for Environmental Studies

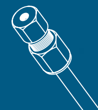
DNA Fragments

DNA fragments smaller than 300 bases have been separated into discrete peaks using the TSKgel G3000SW and G4000SW columns. Recovery of the fragments from these columns was greater than 90%. A plot of chain length versus elution volume for double-stranded DNA is shown in Figure 31.

Figure 31: Double stranded DNA fragments



Columns:	**A: TSKgel G3000SW, 10 µm, 7.5 mm ID × 60 cm × 2** **B: TSKgel G4000SW, 13 µm, 7.5 mm ID × 60 cm × 2**
Mobile phase:	0.05 mol/L Tris-HCl, 0.2 mol/L NaCl, 1 mmol/L EDTA, pH 7.5
Flow rate:	A: 1 mL/min, B: 0.33 mL/min
Detection:	UV @ 260 nm
Temperature:	25 °C
Sample:	Hae III-cleaved pBR322 DNA
Sample load:	13 µg in 50 µL



About: TSKgel SWXL Size Exclusion Columns

TSKgel SWXL columns, introduced in 1987, are packed with 5 or 8 µm particles to improve sample resolution or to reduce analysis time (over TSKgel SW columns). Like the TSKgel SW columns, TSKgel SWXL columns feature highly porous silica particles, the surface of which has been shielded from interacting with proteins by derivatization with ligands containing diol functional groups. TSKgel SWXL columns stand out from other silica- or polymer-based high performance size exclusion columns by virtue of their large pore volumes.

These columns are available within the TSKgel SWXL column line:

- TSKgel G2000SWXL
- TSKgel G3000SWXL
- TSKgel G4000SWXL
- TSKgel BioAssist G2SWXL
- TSKgel BioAssist G3SWXL
- TSKgel BioAssist G4SWXL
- TSKgel QC-PAK GFC 200
- TSKgel QC-PAK GFC 300

The TSKgel BioAssist columns are made of PEEK housing material to reduce sample adsorption to stainless steel or glass. QC-PAK columns are 15 cm in length with 5 µm particles and offer the same resolution in half the time as the 30 cm, 10 µm TSKgel G2000SW and G3000SW columns.

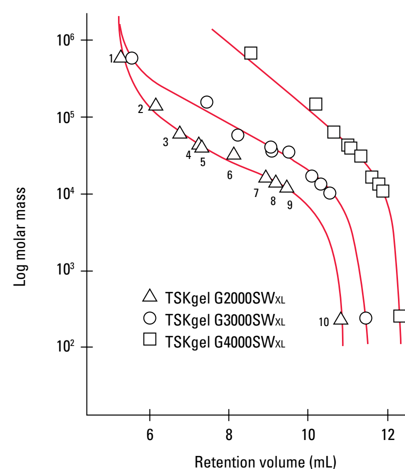
Attributes and Applications

Table 7 shows a summary of the product attributes for each of the TSKgel SWXL columns. TSKgel SWXL columns are commonly used in the quality control of monoclonal antibodies and other biopharmaceutical products. TSKgel G2000SWXL columns are an excellent choice for small proteins and peptide separations. Proteins and large peptides are separated well on TSKgel 3000SWXL columns, while TSKgel G4000SWXL provides the largest exclusion limit and the widest fractionation range. It is an excellent choice for pegylated proteins or glycosylated biomolecules. Figure 32 shows the calibration curves for globular proteins, polyethylene oxides, and dextrans for each of the three TSKgel SWXL columns.

Table 7: Product attributes

TSKgel column	G2000SWXL	G3000SWXL	G4000SWXL
Base material	Silica		
Particle size (mean)	5 µm	5 µm	8 µm
Pore size (mean)	12.5 nm	25 nm	45 nm
Functional group	Diol		
pH stability	2.5-7.5		
Calibration range	5,000 - 1.5 × 10^5 Da (globular proteins)	1.0 × 10^4 - 5.0 × 10^5 Da (globular proteins)	2.0 × 10^4 - 7.0 × 10^6 Da (globular proteins)

Figure 32: Calibration curves for proteins for TSKgel SWXL columns



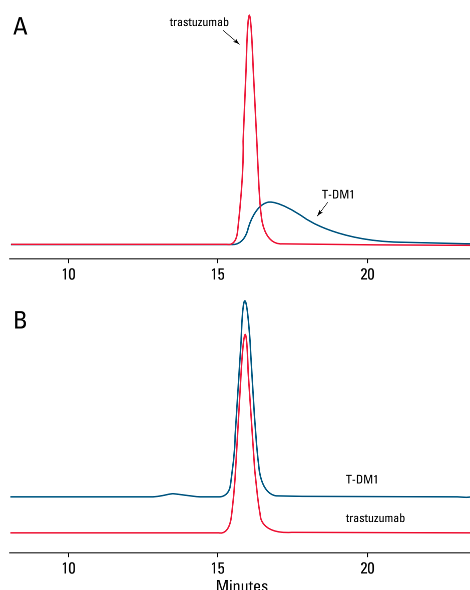
Column: **TSKgel SWXL columns, 7.8 mm ID × 30 cm**
Mobile phase: 0.3 mol/L NaCl in 0.1 mol/L sodium phosphate buffer, pH 7.0
Detection: UV @ 220 nm
Samples:
1. thyroglobulin (6.6 × 10^5 Da)
2. IgG (1.56 × 10^5 Da)
3. bovine serum albumin (6.7 × 10^4 Da)
4. ovalbumin (4.3 × 10^4 Da)
5. peroxidase (4.02 × 10^4 Da)
6. β-lactoglobulin (3.5 × 10^4 Da)
7. myoglobin (1.69 × 10^4 Da)
8. ribonuclease A (1.37 × 10^4 Da)
9. cytochrome C (1.24 × 10^4 Da)
10. glycine tetramer (246 Da)

Size Variant Analysis of Conjugates

A sample of both conjugated (T-DM1) and unconjugated (Trastuzumab) monoclonal antibody was analyzed on a TSKgel G3000SWXL column eluted isocratically with a phosphate-buffered saline mobile phase. The use of an inorganic mobile phase for unconjugated mAb analysis showed no change in the expected results. With the analysis of the conjugated mAb (ADC) in an inorganic mobile phase, poor peak shape (greatly increased tailing) and incomplete resolution of aggre¬gates from the monomeric conjugated antibody were observed (see A in Figure 33).

Addition of an organic modifier to the mobile phase, in this case 15% 2-propanol, restored peak shape and resolution of the conjugated mAb analyzed on a TSKgel G3000SWXL column (B in Figure 33). These results indicate that the attached hydrophobic drugs lead to non-specific interaction between the ADC and the column stationary phase. The addition of organic solvents to the mobile phase can be used to overcome non-specific interactions between the ADC and the column stationary phase.

Figure 33: Size variant analysis of conjugates using a TSKgel G3000SWXL column with mobile phase 0.2 mol/L KPi and 0.25 mol/L KCl (A) and 85% KPi/KCl, 15% 2-propanol (B)



Column:	**TSKgel G3000SWXL, 7.8 mm ID × 30 cm**
Mobile phase:	A: 0.2 mol/L KPi and 0.25 mol/L KCl, pH 6.95
	B: 85% KPi/KCl + 15% 2-propanol
Flow rate:	0.5 mL/min
Detection:	UV @ 280 nm

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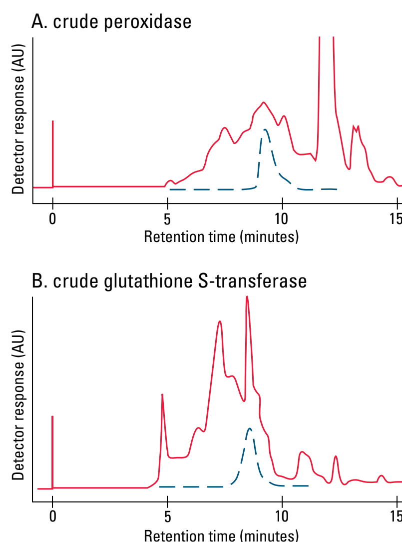
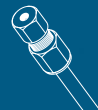
Enzymes

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 (Figure 34A) and glutathione S-transferase (Figure 34B) were separated in only 15 minutes on a TSKgel G3000SWXL column and activity recovery was 98% and 89%, respectively. The elution profiles of the separations show that all of the activity eluted in a narrow band of about 1.5 mL.

Figure 34A and 34B: Analysis of crude protein samples



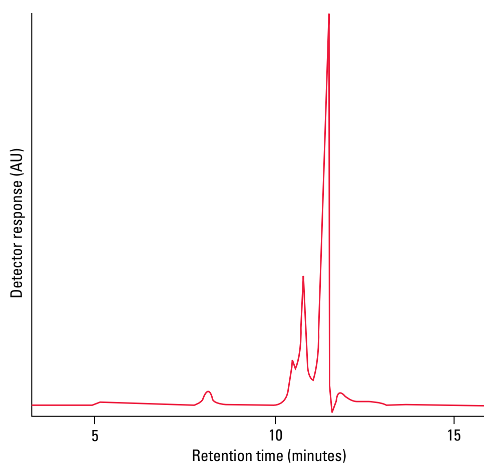
Column:	**TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.3 mol/L NaCl in 0.05 mol/L phosphate buffer, pH 7.0
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm (solid line) and enzyme assay tests (dashed line)
Recovery:	enzymatic activity recovered was 98% in A and 89% in B
Samples:	A. crude peroxidase from Japanese radish, 0.15 mg in 0.1 mL B. crude glutathione S-transferase from guinea pig liver extract, 0.7 mg in 0.1 mL



Rat Liver Extract

The separation of a crude extract of rat liver using a TSKgel G2000SWxl column is displayed in Figure 35.

Figure 35: Separation of crude extract of rat liver (10 μL)

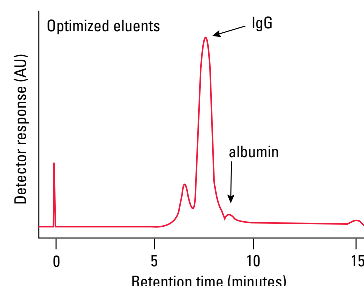


Column:	**TSKgel G2000SWxl, 5 μm, 7.8 mm ID × 30 cm**
Mobile phase:	0.05 mol/L phosphate buffer, pH 7.0 + 0.3 mol/L NaCl
Flow rate:	1 mL/min
Detection:	UV @ 220 nm
Temperature:	25 °C

IgG

A therapeutic solution of intravenous IgG may contain albumin as a stabilizer, and both proteins must be quantified following manufacture. Although literature reports describe the separation of these two proteins by many other chromatographic methods, long analysis times and complex gradient elutions are required. A method developed on a TSKgel G3000SWxl column provides quantitative separation of the two proteins in 15 minutes with a simple, isocratic elution system. As shown in Figure 36, human albumin can be separated from a 20-fold excess of IgG and quantified using an optimized elution buffer. This simple separation method can be applied to the isolation of other IgGs, such as monoclonal antibodies in ascites fluid.

Figure 36: QC test for albumin

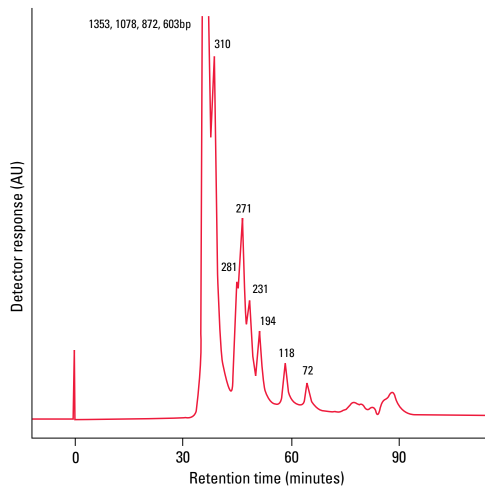


Column:	**TSKgel G3000SWxl, 5 μm, 7.8 mm ID × 30 cm**
Mobile phase:	0.1 mol/L Na_2SO_4 in 0.05 mol/L sodium phosphate buffer, pH 5.0
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Sample:	5 μL of venilon, containing 237.5 mg of IgG and 12.5 mg of albumin

DNA Digest

Figure 37 shows the separation of øX174 RF DNA-Hae III digest using a TSKgel G4000SWXL column.

Figure 37: Separation of ϕX174 RF DNA-Hae III digest (4.5 µg/50 µL)

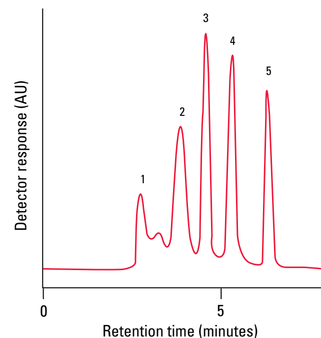


Column:	**TSKgel G4000SWXL, 8 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.05 mol/L phosphate buffer, pH 7.0 + 0.3 mol/L NaCl + 1 mmol/L EDTA
Flow rate:	0.15 mL/min
Detection:	UV @ 260 nm
Temperature:	25 °C

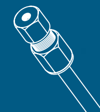
Reduced Analysis Times

For preliminary research or reducing quality control testing time, the 15 cm long TSKgel QC-PAK columns provide analysis times half as long as those on standard 30 cm columns, while retaining baseline resolution of protein mixtures (Figure 38).

Figure 38: Analysis of various proteins



Column:	**TSKgel QC-PAK GFC 300, 5 µm, 8 mm ID × 15 cm**
Mobile phase:	0.1 mol/L Na_2SO_4 in 0.1 mol/L phosphate buffer, pH 7.0 and 0.05% NaN_3
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Samples:	1. thyroglobulin 2. IgG 3. ovalbumin 4. ribonuclease 5. p-aminobenzoic acid

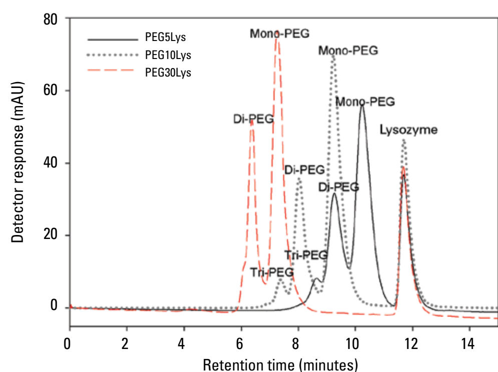


Characterization Studies of PEGylated Lysozyme

Chemical modification of therapeutic proteins in order to enhance their biological activity is of increasing interest. One of the most frequently used protein modification methods, PEGylation, changes the biochemical and physicochemical properties of the protein, which can result in several important benefits, among them more effective target delivery, slower in vivo clearance, and reduced toxicity and immunogenicity of therapeutic proteins. After PEGylation reaction the mixture has to be purified in order to remove non-reacted protein and undesired reaction products.

A TSKgel G3000SWXL column was used for the characterization of PEGylated lysozyme, as shown in Figure 39. A random PEGylation of lysozyme using methoxy PEG aldehyde of sizes 5 kDa, 10 kDa and 30 kDa was performed. The retention volumes of PEGylated lysozymes were used to assign the peaks based on a standard calibration curve. As a result of PEGylation, a large increase in the size of lysozyme by size exclusion chromatography was observed. The SEC elution position of lysozyme modified with a 30 kDa PEG was equivalent to that of a 450 kDa globular protein. There was a linear correlation between the theoretical molar mass of PEGylated protein and the molar mass calculated from SEC. This result illustrates the strong effect that PEG has on the hydrodynamic radius of the resulting PEGylated protein.

Figure 39: SEC analysis of reaction mixtures

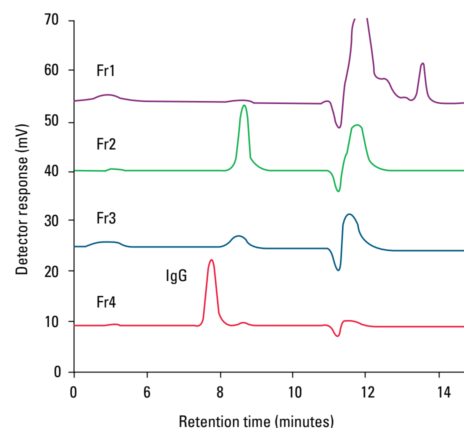


Column:	**TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.1 mol/L phosphate buffer, 0.1 mol/L Na_2SO_4, pH 6.7
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Injection vol.:	20 µL
Sample:	5, 10, 30 kDa methoxy PEG aldehyde

Purity of an Antibody

When the analysis of proteins needs to be performed in a metal free environment, the TSKgel BioAssist columns can be used. These columns offer TSKgel SWXL packings in PEEK housings featuring the same performance as with stainless steel columns. Figure 40 demonstrates the purity of an antibody from a cell culture supernatant (Anti TSH). The chromatograms represent the fractions collected from a HIC purification step.

Figure 40: Purity of an antibody



Column:	**TSKgel BioAssist G3SWXL, 5 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.3 mol/L phosphate buffer, pH 7.0
Flow rate:	1.0 mL/min
Injection vol.:	50 µL

About: TSKgel SuperSW Size Exclusion Columns

TSKgel SuperSW columns, introduced in 1997, contain smaller particles than TSKgel SWXL columns; 4 µm versus 5 µm. In addition, the column internal diameter has been reduced from 7.8 mm ID to 4.6 mm ID to provide higher sensitivity in sample-limited cases and to cut down on solvent use.

It is important to employ an HPLC system that is optimized with regards to extra-column band broadening to take full advantage of the high column efficiency that can be obtained on TSKgel SuperSW columns. See Table 9 for recommendations on minimizing the dead volume in the HPLC system.

The following two columns are available within the TSKgel SuperSW column line:

- TSKgel SuperSW2000
- TSKgel SuperSW3000

Attributes and Applications

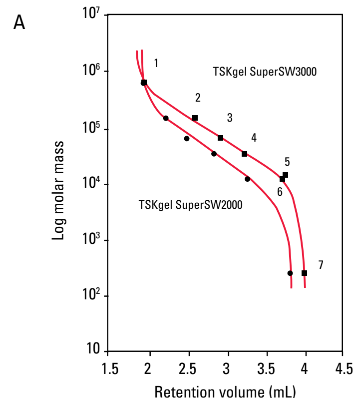
Table 8 shows a summary of the product attributes for each of the TSKgel SuperSW columns. The 12.5 nm pore size of the TSKgel SuperSW2000 columns results in a fractionation range up to 1.5 × 10^5 Da for globular proteins. The TSKgel SuperSW3000 columns have a fractionation range up to 5.0 × 10^5 Da for globular proteins due to its 25 nm pore size. Since both columns have a 4.6 mm inner diameter, they are ideal for sample-limited applications. Figure 41A and 41B show the calibration curves for protein, polyethylene oxides and glycols for the TSKgel SuperSW columns.

Table 8: Product attributes

TSKgel column	SuperSW2000	SuperSW3000
Base material	Silica	
Particle size (mean)	4 µm	4 µm
Pore size (mean)	12.5 nm	25 nm
Functional group	Diol	
pH stability	2.5-7.5	
Calibration range	5,000 - 1.5 × 10^5 Da (globular proteins)	1.0 × 10^4 - 5.0 × 10^5 Da (globular proteins)

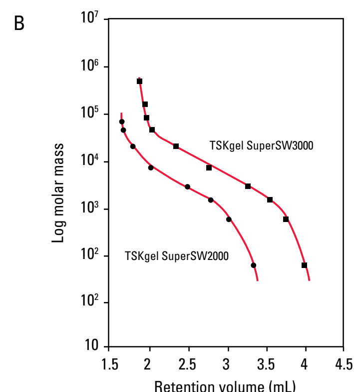
Figure 41A and 41B: Calibration curves for proteins and polyethylene oxides and glycols for TSKgel SuperSW columns

A



Column: **TSKgel SuperSW columns, 4.6 mm ID × 30 cm**
Mobile phase: 0.2 mol/L phosphate buffer, pH 6.7
Flow rate: 0.35 mL/min
Detection: UV @ 280 nm
Samples: standard proteins (5 µL, 0.1 g/L each)
1. thyroglobulin
2. γ-globulin
3. bovine serum albumin
4. β-lactoglobulin
5. lysozyme
6. cytochrome C
7. glycine tetramer

B



Column: **TSKgel SuperSW columns, 4.6 mm ID × 30 cm**
Mobile phase: 0.05% sodium azide aqueous solution
Flow rate: 0.35 mL/min
Detection: RI
Temperature: 25 °C
Samples: polyethylene oxides (PEO) standards
polyethylene glycols (PEG) standards, (5 µL)

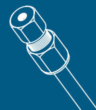


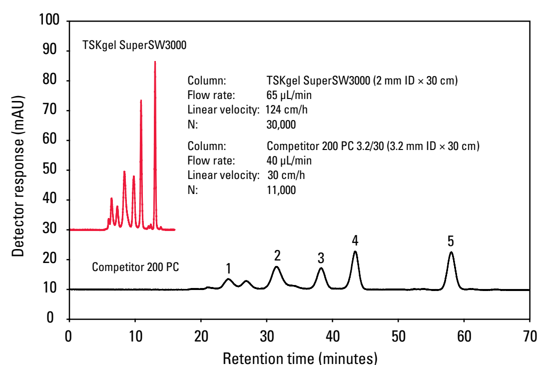
Table 9: Operating instructions when using TSKgel SuperSW columns

In general:
• Suppress peak broadening in connecting tubing between injector, guard column, analytical column, and detector. • Prevent the sample volume from causing extra-column band broadening due to volume overloading. You can test this by injecting half the sample volume and measuring peak efficiency.
Tubing:
• Use 0.004” or 0.005” ID (0.100 mm or 0.125 mm) tubing, when available, and as short a length as is practical. • Sections requiring 0.004” or 0.005” ID tubing o Between injection valve and guard column, and between guard column outlet and column o Between column outlet and detector inlet
Pumping system:
• The pump(s) should work well at low flow rates as the recommended flow rate range is 0.1-0.35 mL/min.
Injector:
• A low dispersion injector (such as Rheodyne 8125) is recommended.
Guard column:
• We recommend that you install a guard column (part no. 18762) to protect your TSKgel SuperSW column.
Detector:
• When working with a UV detector, install a micro flow cell or a low dead volume-type cell. Low dead volume-type cells are effective in high-sensitivity analysis. (Use of a standard cell is also possible. However, theoretical plates will be approximately 80% of those obtained with a micro flow cell.)
Sample:
• Sample injection volume should be 1-10 μL. Sample load should be 100 μg or smaller.

Trace Levels of Proteins

Figure 42 shows a comparative separation of several standard proteins at low level concentrations on a 2 mm ID TSKgel SuperSW3000 column and on a competitive GFC column. As the results reveal, the TSKgel SuperSW3000 column is an excellent choice for the rapid analysis of proteins at trace levels, showing improved peak shape and superior resolution.

Figure 42: Analysis of standard proteins at low level concentrations



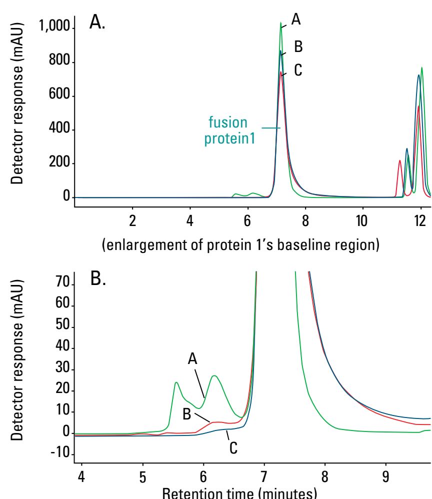
Columns:	**TSKgel SuperSW3000, 4 μm, 2 mm ID × 30 cm** Competitor 200 PC 3.2/30, 13 μm, 3.2 mm ID × 30 cm
Mobile phase:	0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3, pH 6.7
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	0.2 μL
Samples:	1. thyroglobulin (1.0 g/L) 2. β-globulin (2.0 g/L) 3. ovalbumin (2.0 g/L) 4. ribonuclease A (3.0 g/L) 5. p-aminobenzoic acid (0.02 g/L)

Antibody-Based Fusion Protein and Aggregates

During method development, many variables are examined to ensure method robustness. Factors such as elution profile, peak shape, and recovery are required to be consistent by GMP/GLP protocols. During a method re-qualification at Lexigen Pharmaceuticals, several variables were investigated to eliminate non-specific binding and increase the robustness of an established antibody separation method using a TSKgel SuperSW3000 column.

As shown in Figure 43A, excessive peak tailing of “fusion protein 1” is evident with the use of 0.2 mol/L NaCl (chromatogram c in the figure). Additionally, the expected protein dimer and trimer aggregates are not visible in the chromatogram. By switching from 0.2 mol/L sodium chloride to 0.2 mol/L of the more chaotropic sodium perchlorate salt, together with a two-fold reduction in the buffer concentration, less peak tailing and distinct peaks for the dimer and trimer species of mAb1 resulted (chromatogram b in the figure). Doubling the perchlorate concentration to 0.4 mol/L provided further improvement in the peak shape of fusion protein 1 and associated aggregate species (chromatogram a in the figure). Figure 43B is an enlargement of fusion protein 1’s baseline region, showing an improved peak shape of the dimer and trimer aggregates with the use of 0.4 mol/L $NaClO_4$.

Figure 43A and 43B: Overlays of monoclonal antibody separation

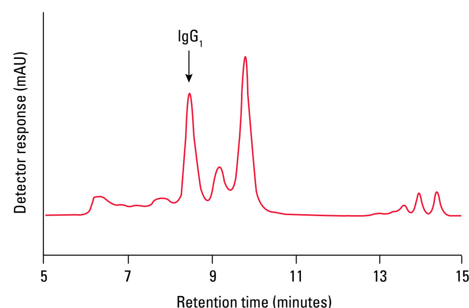


Column:	**TSKgel SuperSW3000, 4 µm, 4.6 mm ID × 30 cm**
Mobile phase:	A: 0.4 mol/L $NaClO_4$, 0.05 mol/L NaH_2PO_4,
	B: 0.2 mol/L $NaClO_4$, 0.05 mol/L NaH_2PO_4,
	C: 0.2 mol/L NaCl, 0.1 mol/L NaH_2PO_4
Flow rate:	0.35 mL/min
Detection:	UV @ 214 nm
Injection vol.:	5 µL
Samples:	monoclonal antibodies

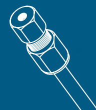
IgG_1

The TSKgel Super SW3000 provides an excellent high resolution separation of IgG_1 from mouse ascites fluid as can be seen in Figure 44.

Figure 44: Separation of monoclonal antibody



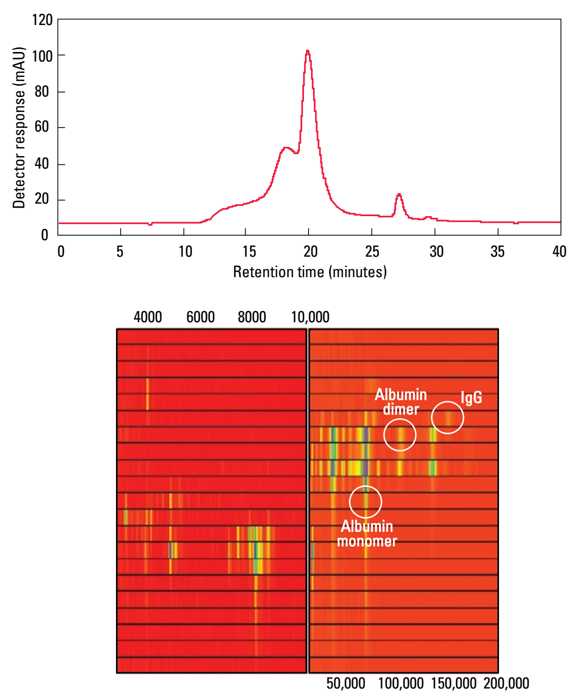
Column:	**TSKgel SuperSW3000, 4 µm, 4.6 mm ID × 30 cm**
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.7
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm, micro flow cell
Sample:	mouse ascites (5 µL)



Human Serum Proteins

A 1 mm ID TSKgel SuperSW3000 column was used to analyze proteins in human serum. A fraction of interest was then analyzed by off-line SELDI/TOF/MS to establish the presence of BSA aggregates and IgG. Figure 45 demonstrates the applicability of TSKgel SuperSW3000 columns for the trace analysis of biological components by LC/MS analysis.

Figure 45: Analysis of proteins in human serum



Fraction of interest analyzed by off-line SELDI/TOF/MS to establish presence of BSA aggregates and IgG.

Column:	**TSKgel SuperSW3000, 4 µm, 1 mm ID × 30 cm**
Mobile phase:	50 mmol/L NaH_2PO_4 + 0.5 mol/L NaCl, pH 7.0
Flow rate:	8 µL/min
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	serum (x 10), 1 µL

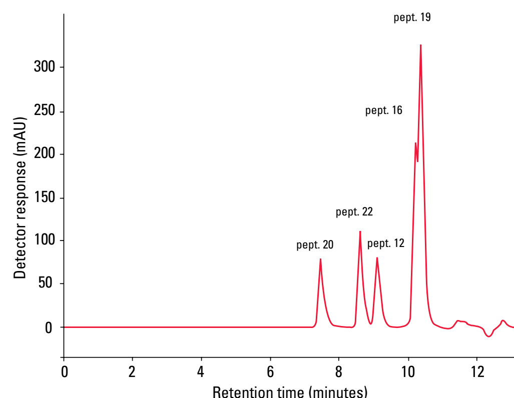
Fraction (1 mL) was directly loaded to SELDI chip H50. The chip was washed and desalted then applied to MS.

This data is courtesy of Dr. Majima, Protenova.

Peptide Mixture

Figure 46 demonstrates that very small molecules can be separated efficiently on a TSKgel SuperSW2000 column under non-SEC conditions. Although the peptides 16 and 19 do not elute according to their molar mass, a separation was possible with only one amino acid difference (based on different interaction with the gel surface).

Figure 46: Analysis of peptides



Column:	**TSKgel SuperSW2000, 4 µm, 4.6 mm ID × 30 cm**
Mobile phase:	0.1% TFA in 45% aq. ACN
Flow rate:	0.35 mL/min
Detection:	UV @ 210 nm
Injection vol:	3 µL
Samples:	Peptide P12: Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu Peptide P16: Trp-Gly-Gly-Tyr Peptide P19: Gly-Trp-Gly Peptide P20: H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val- Gly-Lys-Gly-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-OH Peptide P22: H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH

About: TSKgel BioAssist DS Size Exclusion Columns

TSKgel BioAssist DS columns are designed for the desalting and buffer exchange of proteins and polynucleotides at analytical and semi-preparative scale. Packed with 15 µm polyacrylamide beads in PEEK hardware, TSKgel BioAssist DS columns show excellent desalting performance.

The novel* hydrophilic highly cross-linked polyacrylamide beads exhibit superior mechanical strength compared with conventional hydrophilic polyacrylamide beads and cross-linked dextran beads. This increase in strength is what allows the use of the small spherical 15 µm beads.

*US patent number 7,659,348

Attributes and Applications

Table 10 summarizes the product attributes of the TSKgel BioAssist DS columns. TSKgel BioAssist DS columns can be operated in standard HPLC systems to quickly and efficiently reduce salt and/or buffer concentrations of collected protein or nucleic acid fractions.

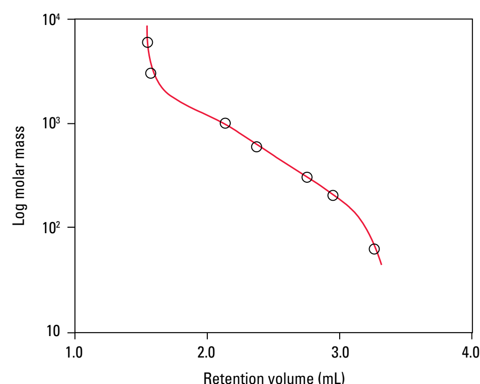
Table 10: Product attributes

Attribute	Value
Base material	urea cross-linked polyacrylamide
Particle size	15 µm
Pore size	excludes 2,500 Da PEG
Particle porosity	ca. 60%
Mechanical strength	<4 MPa

Calibration Curve

Figure 47 shows the calibration curve of a 6 mm ID × 15 cm TSKgel BioAssist DS column using polyethylene glycol standards and a water mobile phase. As is desirable in SEC, the pore volume of BioAssist DS columns is larger than the volume in between the particles. The molar mass cut-off (exclusion limit) for PEGs is about 2,500 Da. Results similar to those shown in Figure 47 can be obtained on the commercially available 4.6 mm ID × 15 cm and 10 mm ID × 15 cm TSKgel BioAssist DS columns.

Figure 47: Calibration curve of TSKgel BioAssist DS desalting columns



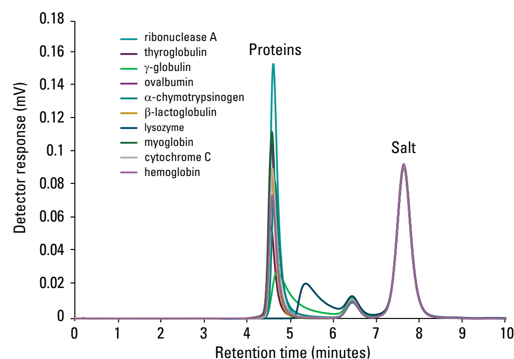
Column: **Custom TSKgel BioAssist DS column, 6 mm ID × 15 cm**
Mobile phase: distilled H_2O
Flow rate: 0.5 mL/min
Sample: ethylene glycol, PEGs

Desalting of Large Protein Sample Loads

Figure 48 demonstrates the rapid and reproducible desalting of a large number of proteins (see Table 11) at semi-preparative scale using a TSKgel BioAssist DS, 10 mm ID × 15 cm column. In this application, the salt concentration of the proteins was reduced 10-fold from 0.1 to 0.01 mol/L. The reproducibility of the separation was determined by measuring the plate number of the ribonuclease A peak for four injections of various sample loads. The % RSD value (n=4) was less than 5% for a 1.5 mg injection. At this load, the resolution between ribonuclease A and the salt peak was larger than 6. At 1.95 mg load of ribonuclease A, the resolution between the protein and salt peak was 4.3. Note that the analysis is complete within 10 minutes.

In a similar study performed on a 4.6 mm ID × 15 cm TSKgel BioAssist DS column, the resolution for a 1.95 mg load of ribonuclease A was larger than 2 at the high flow rate of 0.8 mL/min.

Figure 48: Desalting of proteins

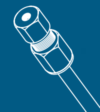


Column: **TSKgel BioAssist DS, 15 µm, 10 mm ID × 15 cm**
Mobile phase: 0.1 mol/L KH_2PO_4/K_2HPO_4, pH 6.7, 0.1 mol/L Na_2SO_4 + 0.005% NaN_3
Proteins in 0.1 mol/L phosphate buffer, pH 6.7
Flow rate: 0.8 mL/min (4.6 mm ID) and 1.0 mL/min (10 mm ID)
Detection: UV @ 80 nm and RI
Temperature: 25 °C
Injection vol.: 10 µL

Table 11: Proteins

Protein	*MM* (kDa)	Concentration* (g/L approx.)
ribonuclease A	14.7	19.5
thyroglobulin	670	11.3
γ-globulin	150	14.5
ovalbumin	45	13.1
α-chymotrypsinogen	25.6	13.1
β-lactoglobulin	18.4	10.8
lysozyme	14.7	11.6
myoglobin	16.7	14.5
cytochrome C	12.3	11.0
hemoglobin	68	11.9

*in 100 mmol/L phosphate buffer, pH 6.7



About: TSKgel PW Series Size Exclusion Columns

TSKgel PW and PWXL columns are recommended for analyses of water-soluble polymers and are prepared from hydrophilic polymethacrylate resin. TSKgel PWXL-CP columns are prepared from the same base resin as the TSKgel PWXL columns and were specifically developed for the analysis of water-soluble cationic polymers. TSKgel SuperMultiporePW columns are packed with particles containing a wide range of pore sizes for the analysis of water-soluble polymers with a wide molar mass range.

Stable from pH 2 to 12, TSKgel PW series columns can be used in mobile phases of water or buffer (up to 20% methanol/80% aqueous) and can tolerate temperatures up to 80 ˚C (50 ˚C for TSKgel G-DNA-PW column).

- Use TSKgel PW columns when analysis time is not critical, when sample mass is not limited, to collect fractions, or to obtain maximum number of plates (at the expense of analysis time). Particle sizes range from 12 µm for the smaller pore size columns (>10 nm - 20 nm) to 17 µm for the larger pore size columns (20 nm - >100 nm).

  The TSKgel GMPW column, within the TSKgel PW column line, is a mixed bed column containing a mixture of different pore sizes that has an extended linear calibration range, suitable for samples with a broad MM distribution as well as unknown samples.

  A TSKgel G6000PW column is available in PEEK column hardware, TSKgel BioAssist G6PW, when ultra-low sample adsorption is required, such as in virus analysis.

- Use higher efficiency TSKgel PWXL columns for optimal resolution, to reduce analysis time or in sample-limited applications. TSKgel PWXL columns have smaller particle sizes than TSKgel PW columns, resulting in improved resolution.

  The TSKgel PWXL product line also offers specialty columns for analyzing carbohydrate oligomers (TSKgel G-Oligo-PW) and DNA and RNA fragments of 500-5000 base pairs (TSKgel G-DNA-PW). TSKgel GMPWXL is a mixed bed scouting column for aqueous water-soluble linear polymers. Its pore volume is accessible to polymers ranging from molar masses of 500 up to 8.0×10^6 Da.

- Cationic groups were introduced on the surface of the TSKgel PWXL-CP packing material to prevent adsorption of cationic polymers and allow elution under low salt conditions. These columns show high theoretical plate numbers, linear calibration curves and excellent durability. The base resin is the same as that used in the TSKgel PWXL columns.

  Three columns are available within the TSKgel PWXL-CP line, each with a different particle size, separation range and exclusion limit, allowing polymers within a wide molar mass range to be separated and characterized.

- A wide molar mass range can be analyzed with the three different TSKgel SuperMultiporePW columns, from high mass water-soluble polymers to oligomers. The packing material in the TSKgel SuperMultiporePW columns is more hydrophilic than that of TSKgel PWXL columns, which further reduces the chance of adsorption of hydrophilic polymers.

The range of pore sizes in which TSKgel PW and TSKgel PWXL columns are available permits a wide spectrum of water-soluble substances to be analyzed. The properties and molar mass separation ranges for all TSKgel PW series columns are summarized in Table 12.

The mechanism of 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 all TSKgel PW series packings can cause changes in elution order from that of an ideal system. Fortunately, the mobile phase composition can vary greatly with TSKgel PW series columns to be compatible with a wide range of neutral, polar, anionic, and cationic samples. Table 13 lists appropriate mobile phases for GFC of major polymer types on TSKgel PW series columns.

For some nonionic, nonpolar polymers, such as polyethylene glycols, ideal size exclusion behavior can be obtained by using distilled water as the mobile phase. 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 to the aqueous eluent. Generally, a salt concentration of 0.1 mol/L to 0.5 mol/L is needed to overcome undesirable ionic interactions.

TSKgel PW 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 Table 13 below. All TSKgel PW series packings are compatible with 20% aqueous solutions of methanol, ethanol, propanol, acetonitrile, dimethylformamide, dimethyl sulfoxide, formic acid, and acetic acid. In addition, these columns can be operated in 50% aqueous acetone.

Table 12: Properties and separation ranges of TSKgel PW, PW_{XL}, PW_{XL}-CP, and SuperMultiporePW columns

			Molar mass of samples (Da)
TSKgel column	**Particle size**	**Pore size**	**Polyethylene glycols & oxides**
SuperMultiporePW-N	4 µm	20 nm	300 – 5×10^4
SuperMultiporePW-M	5 µm	100 nm	500 – 1×10^6
SuperMultiporePW-H	8 µm	>100 nm	1,000 – 1×10^7
G2000PW	12 µm	12.5 nm	<3,000
G2500PW	12 µm and 17 µm	12.5 nm	<3,000
G3000PW	12 µm and 17 µm	20 nm	$<5 \times 10^4$
G4000PW	17 µm	50 nm	$<3 \times 10^5$
G5000PW	17 µm	100 nm	$<1 \times 10^6$
G6000PW BioAssist G6PW	17 µm	>100 nm	$<8 \times 10^6$
GMPW	17 µm	mixed pore sizes	1,000 – 8×10^6
G2500PW_{XL}	7 µm	12.5 nm	<3,000
G3000PW_{XL}	7 µm	20 nm	$<5 \times 10^4$
G4000PW_{XL}	10 µm	50 nm	$<3 \times 10^5$
G5000PW_{XL}	10 µm	100 nm	$<1 \times 10^6$
G6000PW_{XL}	13 µm	>100 nm	$<8 \times 10^6$
G-DNA-PW	10 µm	>100 nm	$<8 \times 10^6$
GMPW_{XL}	13 µm	mixed pore sizes	1,000 – 8×10^6
SuperOligoPW	3 µm	12.5 nm	100 – 3,000
G-Oligo-PW	7 µm	12.5 nm	<3,000
G3000PW_{XL}-CP	7 µm	20 nm	200 – 5×10^4
G5000PW_{XL}-CP	10 µm	100 nm	400 – 5×10^5
G6000PW_{XL}-CP	13 µm	>100 nm	1,000 – 1×10^7

Columns: TSKgel PW columns, 7.5 mm ID × 60 cm
TSKgel PW_{XL}, G-Oligo-PW and G-DNA-PW columns, 7.8 mm ID × 30 cm
TSKgel SuperMultiporePW and SuperOligoPW columns, 6.0 mm ID × 15 cm
Mobile phase: polyethylene glycols and oxides (PEOs): distilled water
Flow rate: 1.0 mL/min, except for TSKgel SuperMultiporePW and SuperOligoPW columns: 0.6 mL/min

Call customer service: 866-527-3587,
technical service: 800-366-4875

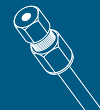


Table 13: Recommended mobile phases for GFC of water-soluble polymers on TSKgel PW, PW_{XL}, PW_{XL}-CP, and SuperMultiporePW columns

Type of polymer	Typical sample	Suitable mobile phase
Nonionic hydrophilic	polyethylene glycol	Distilled water
	soluble starch, methyl cellulose, pullulan	0.01 mol/L NaOH
	dextran, hydroxyethyl cellulose	20% DMSO (dimethyl sulfoxide)
	polyvinyl alcohol, polyacrylamide	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% CH_3CN in 0.1 mol/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% CH_3CN in 0.1 mol/L $NaNO_3$)
Cationic hydrophilic	glycol chitosan, DEAE-dextran, poly(ethylene imine), poly(trimethylaminoethyl methacrylate) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L Na_2SO_4 or 0.8 mol/L $NaNO_3$
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% CH_3CN in 0.1 mol/L $NaNO_3$ or 35-45% CH_3CN in 0.1% TFA)

About: TSKgel PW Size Exclusion Columns

TSKgel PW columns are composed of spherical, hydrophilic polymethacrylate beads. Particle sizes range from 12 µm for the smaller pore size columns to 17 µm for the larger pore size columns. Stable from pH 2 to 12, TSKgel PW columns can be used in mobile phases of water or buffer (up to 20% methanol/80% aqueous) and can tolerate temperatures up to 80 °C.

The TSKgel PW column line consists of the following columns:

- TSKgel G2000PW
- TSKgel G2500PW
- TSKgel G3000PW
- TSKgel G4000PW
- TSKgel G5000PW
- TSKgel G6000PW
- TSKgel GMPW

The mixed bed column, TSKgel GMPW, has an extended linear calibration range, suitable for samples with a broad molar mass distribution, as well as for unknown samples. The pore volume can be accessed by polymers ranging in molar mass from 500 to 8.0×10^6 Da. By quickly categorizing the molar mass profile of an unknown sample, the column enables a fast selection of the best TSKgel PW column for routine analysis.

Attributes and Applications

Product attributes of all eight TSKgel PW columns are shown in Table 14. All TSKgel PW columns have a base material of hydroxylated polymethacrylate, can be used in a maximum of 20% organic, and are shipped in water. The main application area for TSKgel PW columns is the analysis of water-soluble polymers, such as celluloses, acrylamides, glycols, dextrans, polyvinylalcohol, and oligosaccharides. TSKgel G2000PW, the larger particle size equivalent of TSKgel G-Oligo-PW, is most suitable for semi-preparative and preparative isolation of oligosaccharides. Representative application examples for the PW columns are illustrated in Table 15. The calibration curve for polyethylene glycol and oxides for the TSKgel PW columns is shown in Figure 49.

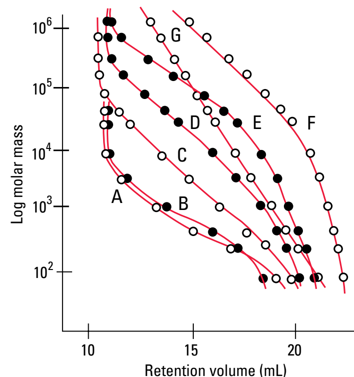
Table 14: Product attributes

TSKgel column	Particle size (mean)	Pore size (mean)	Calibration range
G2000PW	12 µm	12.5 nm	Up to 3,000 Da (polyethylene glycols and oxides)
G2500PW	12 µm and 17 µm	12.5 nm	Up to 3,000 Da (polyethylene glycols and oxides)
G3000PW	12 µm and 17 µm	20 nm	Up to 5.0×10^4 Da (polyethylene glycols and oxides)
G4000PW	17 µm	50 nm	Up to 3.0×10^5 Da (polyethylene glycols and oxides)
G5000PW	17 µm	100 nm	Up to 1.0×10^6 Da (polyethylene glycols and oxides)
G6000PW	17 µm	>100 nm	Up to 8.0×10^6 Da (polyethylene glycols and oxides)
GMPW	17 µm	mixed pore sizes	1,000 - 8.0×10^6 Da (polyethylene glycols and oxides)

Table 15: Representative application examples for TSKgel PW columns

Classification	Examples
1. Synthetic polymers • Nonionic • Cationic • Anionic	• PEG, polyglycerin, polyacrylamide • Polyethyleneimine, polyvinylpyrolidine • Poly (sodium acrylate), Poly (sodium styrene sulfonate)
2. Polysaccharides and derivatives	• Standard dextran, clinical dextran, pullulan, inulin, heparin, chitosan • Carboxymethylcellulose
3. Very large biopolymers • Polynucleotides • Viruses • Proteins	• DNA fragments • TMV, SBMV, TBSV • Lipoprotein (VLDL, LDL), apoferritin, gelatin, sea worm chlorocruorin
4. Small molecules • Oligomers • Others	• oligosaccharides (dextran hydrolysate, cyclodoxtrin • hydrolysate), cyclodextrins • oligopeptides • oligonucleotides

Figure 49: Polyethylene glycol and oxide calibration curves for TSKgel PW columns

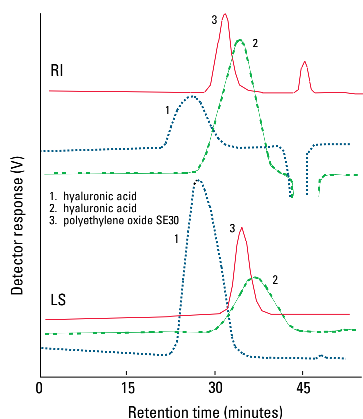


Column:	**A. G2000PW B. G2500PW C. G3000PW D. G4000PW E. G5000PW F. G6000PW G. GMPW all 7.5 mm ID × 60 cm**
Mobile phase:	distilled H_2O
Flow rate:	1.0 mL/min
Detection:	RI

Oligosaccharides

TSKgel PW columns are recommended for polysaccharide analysis due to their ability to separate a wide molar mass distribution. An effective separation of the anionic hydrophilic glucosaminoglycan, hyaluronic acid, is shown in Figure 50 on a TSKgel G6000PW and TSKgel G4000PW column in series with a 0.2 mol/L sodium chloride mobile phase. To obtain shorter analysis time and similar resolution, we recommend using TSKgel G3000PWxL and G4000PWxL columns in series.

Figure 50: Analysis of polysaccharides

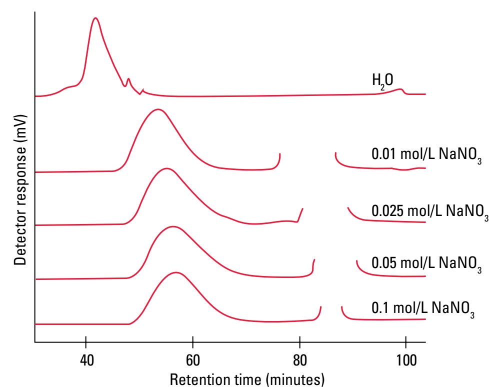


Columns:	**TSKgel G6000PW + G4000PW, two 7.5 mm ID × 60 cm columns in series**
Mobile phase:	H_2O with 0.2 mol/L NaCl
Flow rate:	0.9 mL/min
Temperature:	40 °C
Sample:	hyaluronic acid, polyethylene oxide

Polymers

Sodium polyacrylate, an anionic polymer, is effectively separated on two TSKgel GMPW columns in Figure 51. The addition of 0.01 mol/L $NaNO_3$ results in normal elution and peak shape overcoming the ionic repulsion between the anionic sample and the resin.

Figure 51: Effect of ionic strength on the elution of anionic polymers



Column:	**TSKgel GMPW, 17 µm, 7.5 mm ID × 60 cm × 2**
Mobile phase:	H_2O with 0.01 mol/L, 0.025 mol/L, 0.05 mol/L or 0.1 mol/L $NaNO_3$
Flow rate:	0.5 mL/min
Detection:	RI
Sample:	0.5 mL of 0.05-0.1% of the sodium salt of polyacrylic acid, an anionic polymer

About: TSKgel PWXL Size Exclusion Columns

TSKgel PWXL columns are composed of spherical, hydrophilic polymethacrylate beads. The smaller particle size of TSKgel PWXL columns provide 1.7x higher resolution than their TSKgel PW columns counterpart, making TSKgel PWXL columns more suitable for analytical purposes. Four specialty columns are included in the TSKgel PWXL column line.

The TSKgel G-DNA-PW column is designed for the separation of large polynucleotides such as DNA and RNA fragments of 500 - 5,000 base pairs. This column is a smaller particle size version of the TSKgel G6000PWXL column. The TSKgel G-Oligo-PW column is designed for high resolution separations of aqueous nonionic and cationic oligomers, and oligosaccharides such as hydrolyzed cyclodextrins. Because of the presence of cationic groups on the gel matrix, this column is not suitable for separating anionic polymers. The TSKgel G-Oligo-PW column has a PEG and PEO calibration curve identical to that of the TSKgel G2500PWXL column. The mixed-mode column, TSKgel GMPWXL, has an extended linear calibration range, suitable for samples with a broad MM distribution and unknowns.

The TSKgel SuperOligoPW column is designed for the determination of molar mass of aqueous oligomers, particularly oligosaccharides, and low molar mass aqueous polymers. The combination of the decreased particle size and semi-micro dimensions of the TSKgel SuperOligoPW column enables high speed separation with high resolution and lowered solvent consumption. Since the packing material in the TSKgel SuperOligoPW columns is more hydrophilic compared with TSKgel G-Oligo-PW columns, an even wider range of water-soluble polymers can be analyzed without the need to add organic solvent to the eluent.

The following TSKgel PWXL columns are offered:

- TSKgel G2500PWXL
- TSKgel G3000PWXL
- TSKgel G4000PWXL
- TSKgel G5000PWXL
- TSKgel G6000PWXL
- TSKgel G-DNA-PW
- TSKgel GMPWXL
- TSKgel G-Oligo-PW
- TSKgel SuperOligoPW

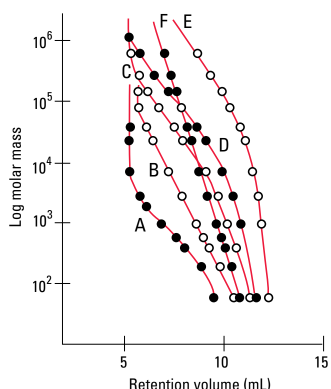
Attributes and Applications

The main application area for TSKgel PWXL columns is the analysis of water-soluble polymers, such as celluloses, acrylamides, glycols, dextrans, polyvinylalcohol, and oligosaccharides. Because of the presence of cationic groups on the base bead of TSKgel G2500PWXL, this column is not suited for separating anionic polymers. Product attributes of all of the TSKgel PWXL columns are shown in Table 16. All TSKgel PWXL columns have a base material of hydroxylated polymethacrylate, can be used in a maximum of 20% organic and are shipped in water. Figures 52 - 56 show the calibration curves for all of the TSKgel PWXL columns.

Table 16: Product attributes

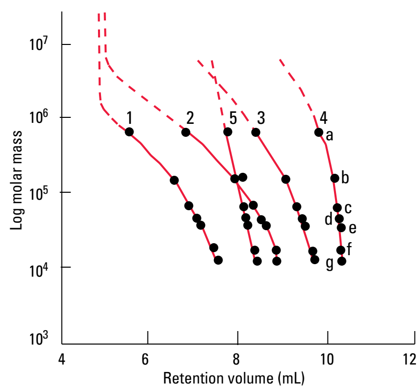
TSKgel column	Particle size (mean)	Pore size (mean)	Calibration range
G2500PWXL	7 µm	12.5 nm	<3,000 Da (polyethylene glycols and oxides)
G3000PWXL	7 µm	20 nm	<4.0×10^4 Da (polyethylene glycols and oxides)
G4000PWXL	10 µm	50 nm	2,000 - 3.0×10^5 Da (polyethylene glycols and oxides)
G5000PWXL	10 µm	100 nm	4,000 - 8.0×10^5 Da (polyethylene glycols and oxides)
G6000PWXL	13 µm	>100 nm	4.0×10^4 - 8.0×10^6 Da (polyethylene glycols and oxides)
G-DNA-PW	10 µm	>100 nm	4.0×10^4 - 8.0×10^6 Da (polyethylene glycols and oxides)
GMPWXL	13 µm	mixed pore sizes	1,000 - 8.0×10^6 Da (polyethylene glycols and oxides)
G-Oligo-PW	7 µm	12.5 nm	Up to 3,000 Da (polyethylene glycols and oxides)
SuperOligoPW	3 µm	12.5 nm	Up to 3,000 Da (PEO,PEG/H_2O)

Figure 52: Polyethylene glycol and oxide calibration curves for TSKgel PWXL columns



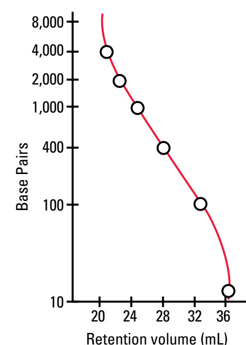
Column:	**A. G2500PWXL B. G3000PWXL C. G4000PWXL D. G5000PWXL E. G6000PWXL F. GMPWXL all 7.8 mm ID × 30 cm**
Mobile phase:	distilled H_2O
Flow rate:	1.0 mL/min
Detection:	RI

Figure 53: Protein calibration curves for TSKgel PWXL columns



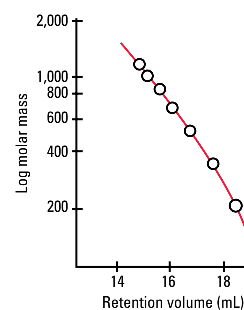
Column:	**1. TSKgel G3000PWXL** **2. TSKgel G4000PWXL** **3. TSKgel G5000PWXL** **4. TSKgel G6000PWXL** **5. TSKgel GMPWXL** **all 7.8 mm ID × 30 cm**
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.8
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Samples:	a. thyroglobulin (6.6×10^5 Da) b. γ-globulin (1.5×10^5 Da) c. albumin (6.7×10^4 Da) d. ovalbumin (4.3×10^4 Da) e. β-lactoglobulin (3.6×10^4 Da) f. myoglobin (1.69×10^4 Da) g. cytochrome C (1.24×10^4 Da)

Figure 54: Double stranded DNA calibration curves for TSKgel G-DNA-PW column



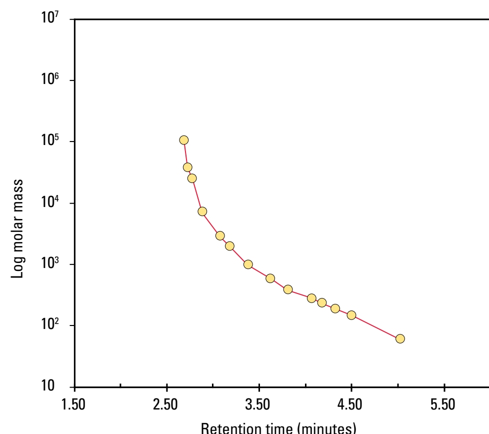
Column:	**TSKgel G-DNA-PW, 10 µm, 7.8 mm ID × 30 cm × 4**
Mobile phase:	H_2O with 0.3 mol/L NaCl in 0.1 mol/LTris-HCl, pH 7.5, + 1 mmol/L EDTA
Flow rate:	0.15 mL/min
Detection:	UV @ 260 nm
Sample:	*Eco* RI and *Bst* NI-cleaved pBR322 DNA, void volume determined with λ-DNA

Figure 55: Oligosaccharide calibration curves for TSKgel G-Oligo-PW column



Column:	**TSKgel G-Oligo-PW, 7 µm, 7.8 mm ID × 30 cm × 2**
Mobile phase:	distilled H_2O
Flow rate:	1.0 mL/min
Detection:	UV @ 260 nm
Sample:	hydrolyzed β-cyclodextrin

Figure 56: Polyethylene glycol, oxide and ethylene glycol calibration curve for TSKgel SuperOligoPW column

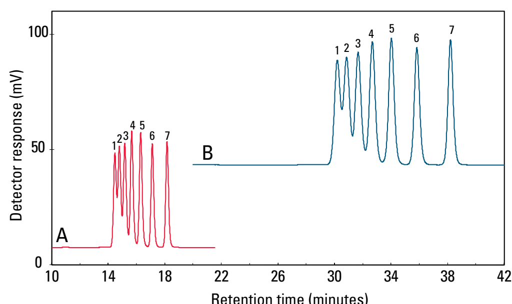


Column:	**TSKgel SuperOligoPW, 6.0 mm ID × 15 cm**
Mobile phase:	H_2O
Flow rate:	0.60 mL/min
Detection:	RI
Temperature:	25 °C
Samples:	PEO, PEG and ethylene glycol

Oligosaccharides

Figure 57 demonstrates the high speed analysis of maltose oligomers using a TSKgel SuperOligoPW column compared to a TSKgel G-Oligo-PW column. The faster analysis time is due to the semi-micro dimensions (6.0 mm ID × 15 cm) and the small particle size (3 µm) of the TSKgel SuperOligoPW column compared to the 7.8 mm ID × 30 cm size and 7 µm particle size of the TSKgel G-Oligo-PW column.

Figure 57: Analysis of maltose oligomers

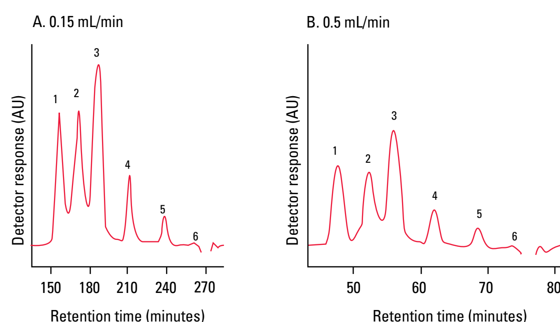


Columns:	**A. TSKgel SuperOligoPW, 3 µm, 6.0 mm ID × 15 cm × 4** **B. TSKgel G-Oligo-PW, 7 µm, 7.8 mm ID × 30 cm × 4**
Mobile phase:	H_2O
Flow rate:	A: 0.6 mL/min B: 1.0 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol.:	A: 10 µL B: 50 µL
Samples:	1. maltoheptose 2. maltohexose 3. maltopentose 4. maltotetraose 5. maltotriose 6. maltose 7. glucose

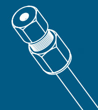
Large DNA Fragments

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. Figure 58A shows the elution of double stranded DNA fragments, obtained from pBR322 DNA cleaved by both EcoRI and BstNI, on four TSKgel G-DNA-PW columns in series. The eluted peaks were collected and subjected to polyacrylamide gel electrophoresis, which showed almost complete separation of the 1060, 1857, and 4362 base pair fragments. Although lower flow rates typically yield better separations of most fragments, the resolution of the 1857 and 4362 base pair fragments was slightly greater at the higher flow rate, as shown in Figure 58B.

Figure 58A and 58B: Analysis of large DNA fragments



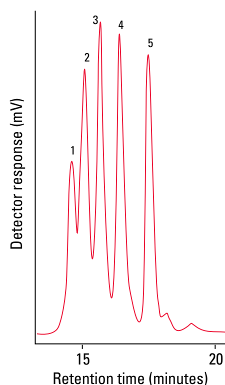
Column:	**TSKgel G-DNA-PW, 10 µm, 7.8 mm ID × 30 cm × 4**
Mobile phase:	H_2O with 0.3 mol/L NaCl in 0.1 mol/LTris-HCl, pH 7.5, + 1 mmol/L EDTA
Flow Rate:	A. 0.15 mL/min B. 0.5 mL/min
Detection:	UV @ 260 nm
Samples:	60 µL of Eco RI and Bst NI - cleaved pBR322 DNA, base pairs: 1. 4362 2. 1857 3. 1060 & 928 4. 383 5. 121 6. 13



Oligomers

The TSKgel G-Oligo-PW column is designed for high resolution separations of nonionic and cationic oligomers. Figure 59 demonstrates excellent resolution of chito-oligosaccharides obtained by using the smaller, 6 µm particle size packing in the TSKgel G-Oligo-PW column.

Figure 59: Analysis of large chitooligosaccharides

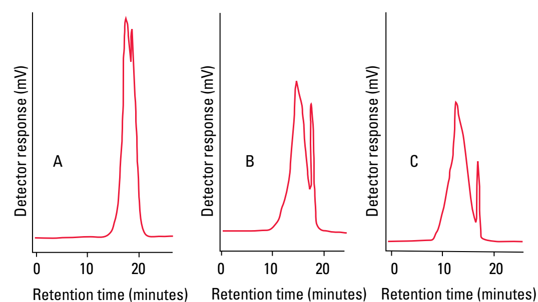


Column:	**TSKgel G-Oligo-PW, 7 µm, 7.8 mm ID × 30 cm × 2**
Mobile phase:	distilled H_2O
Flow rate:	1.0 mL/min
Detection:	RI
Samples:	1. chitohexaose 2. chitopentaose 3. chitotetraose 4. chitotriose 5. chitobiose

Complex Polymers

An example on the influence of pore size on the separation of complex polymers is shown in Figure 60. While on the large pore TSKgel G6000PWXL column, gelatin elutes in one narrow peak, on the G4000PWXL column the peak is much broader and the shoulder nearly separated from the main peak. This allows better determination of M_w/M_n and M_z/M_w.

Figure 60: Separation of gelatin

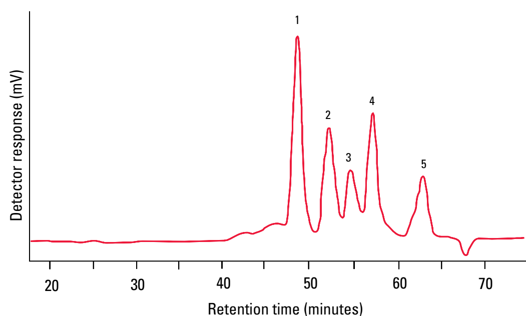


Columns:	**A. TSKgel G6000PWXL B. TSKgel G5000PWXL C. TSKgel G4000PWXL; all 7.8 mm ID × 30 cm**
Mobile phase:	0.2 mol/L phoshpate buffer, pH 6.0
Flow rate:	1.0 mL/min
Detection:	RI
Sample:	gelatin

Small Peptides

Figure 61 demonstrates that the separation of small peptides is possible on a TSKgel G3000PWXL column under denaturing conditions. Using an aqueous eluent containing 45% acetonitrile and 0.1% trifluoroacetic acid, the peptides were retained on the column using a size exclusion mechanism. An advantage of this method is that the eluent is volatile.

Figure 61: Analysis of small peptides

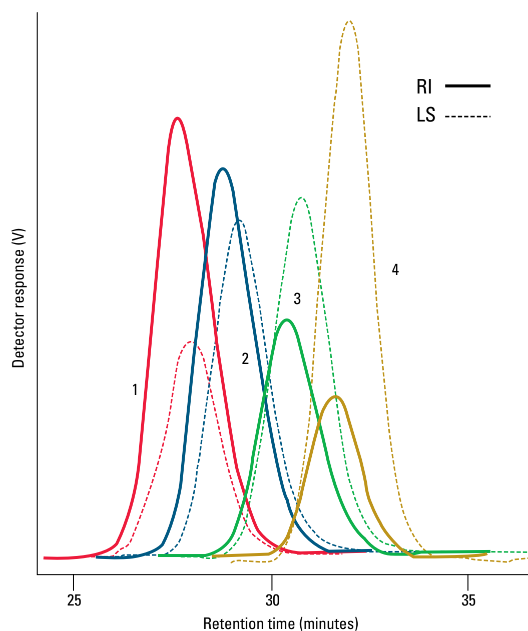


Column:	**TSKgel G3000PWXL, 6 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.1% TFA / 45% CH_3CN
Flow rate:	1.0 mL/min
Samples:	peptides
	1. aprotinin
	2. insulin β-chain
	3. α-MSH
	4. bradykinin potentiator C
	5. glutathione

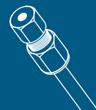
Molar Mass

Pullulan standard samples with a narrow molar mass distribution are commercially available. The molar mass of pullulan was analyzed by GFC/LALLS using a TSKgel GMPWXL column (Figure 62).

Figure 62: Analysis of pullulan



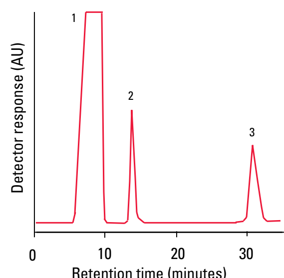
Column:	**TSKgel GMPWXL, 13 µm, 7.8 mm ID × 30 cm × 4**
Mobile phase:	0.1 mol/L sodium chloride
Flow rate:	1.0 mL/min
Temperature:	40 °C
Detection:	RI
	LS
Injection vol:	500 µL
Samples:	1. pullulan P400
	2. pullulan P200
	3. pullulan P100
	4. pullulan P50



Nucleic Acids

Desalting of nucleosides can be accomplished using the TSKgel G2500PWXL as depicted in Figure 63. Clearly, adenosine elutes after the void volume in the un-buffered water mobile phase.

Figure 63: Desalting of nucelosides

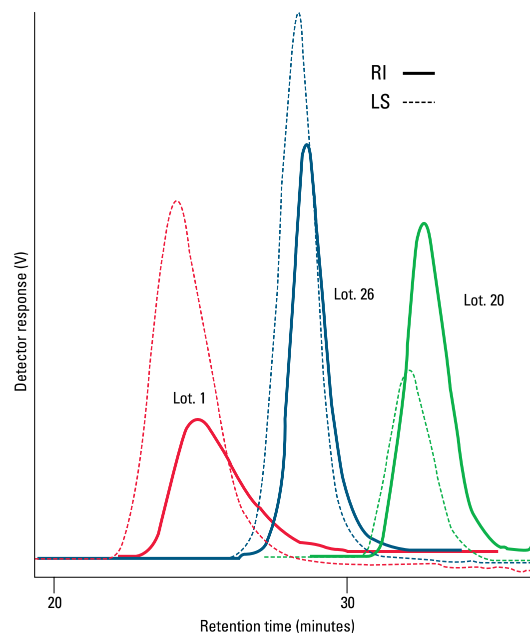


Column:	**TSKgel G2500PWXL, 7 µm, 7.8 mm ID × 30 cm**
Mobile phase:	distilled H_2O
Flow rate:	1.0 mL/min
Detection:	UV @ 260 nm
Samples:	1. 0.5 mol/L NaCl 2. uridine 3. adenosine

Sodium Polystrene

Separation of sodium polystyrene sulfonate standards by GFC requires the addition of at least 10% acetonitrile or methanol to a 0.2 mol/L Na_2SO_4 mobile phase. Figure 64 shows chromatograms for sodium polystyrene sulfonate standards using a TSKgel GMPWXL column. Peak shapes for sodium polystyrene sulfonate samples obtained by adding 10% acetonitrile to a 0.2 mol/L Na_2SO_4 mobile phase remained constant upon addition of more acetonitrile.

Figure 64: Separation of sodium polystyrene sulfonate standards



Column:	**TSKgel GMPWXL, 13 µm, 7.8 mm ID × 30 cm × 4**
Mobile phase:	ACN/0.2 mol/L sodium sulfate = 10/90
Flow rate:	1.0 mL/min
Detection:	RI LS
Temperature:	40 °C
Injection vol:	500 µL
Sample:	sodium poly(styrene sulfonates)

About: TSKgel PWXL-CP Size Exclusion Columns

TSKgel PWXL-CP columns were specifically developed for the analysis of water-soluble cationic polymers. Composed of polymethacrylate beads, cationic groups are introduced on the surface of the TSKgel PWXL-CP packing material to prevent adsorption of cationic polymers and allow elution under low salt conditions. These columns show high theoretical plate numbers, linear calibration curves, and high durability because the base resin is the same as that used in the TSKgel PWXL columns.

Three columns are available within the TSKgel PWXL-CP series, each with a different particle size, separation range, and exclusion limit, allowing polymers within a wide molar mass range to be separated and characterized.

- TSKgel G3000PWXL-CP
- TSKgel G5000PWXL-CP
- TSKgel G6000PWXL-CP

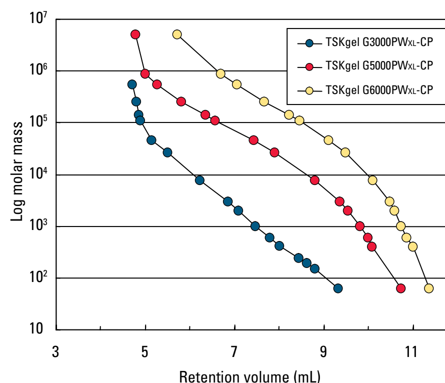
Attributes and Applications:

Table 17 shows the product attributes for each of the three TSKgel PWXL-CP columns. Figure 65 shows calibration curves produced with standard polyethylene oxide and polyethylene glycol in a 0.1 mol/L aqueous solution of sodium nitrate.

Table 17: Product attributes

TSKgel column	G3000PWXL-CP	G5000PWXL-CP	G6000PWXL-CP
Base material	polymethacrylate	polymethacrylate	polymethacrylate
Particle size	7 µm	10 µm	13 µm
Pore size	20 nm	100 nm	>100 nm
Exclusion limit	1.0×10^5 Da	1.0×10^6 Da	2.0×10^7 Da
Separation range (PEO, PEG)	200 ~ 5.0×10^4 Da	400 ~ 5.0×10^5 Da	1,000 ~ 1.0×10^7 Da
Theoretical plates	16,000	10,000	7,000

Figure 65: Polyethylene glycol and oxide calibration curves for TSKgel PWXL-CP columns

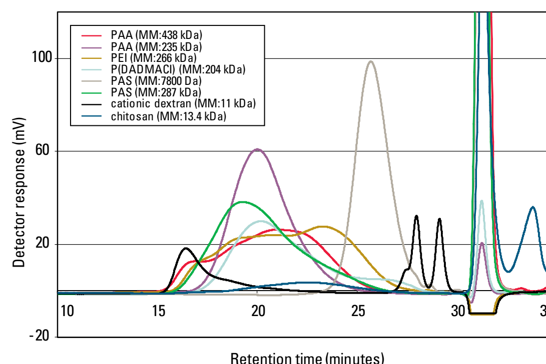


Columns: **TSKgel G3000PWXL-CP, 7 µm, 7.8 mm ID × 30 cm**
TSKgel G5000PWXL-CP, 10 µm, 7.8 mm ID × 30 cm
TSKgel G6000PWXL-CP, 13 µm, 7.8 mm ID × 30 cm
Mobile phase: H_2O with 0.1 mol/L $NaNO_3$
Flow Rate: 1 mL/min
Detection: RI
Temperature: 25 °C
Samples: polyethylene oxides (PEO) standards
polyethylene glycols (PEG) standards

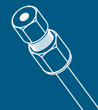
Cationic Polymers

Various cationic polymers with different functional groups and molar masses were injected on the three TSKgel PWXL-CP columns (TSKgel G6000PWXL-CP, G5000PWXL-CP, and G3000PWXL-CP) connected in series. Figure 66 demonstrates that these SEC columns can be utilized for the analysis of a wide variety of cationic polymers.

Figure 66: Analysis of cationic polymers



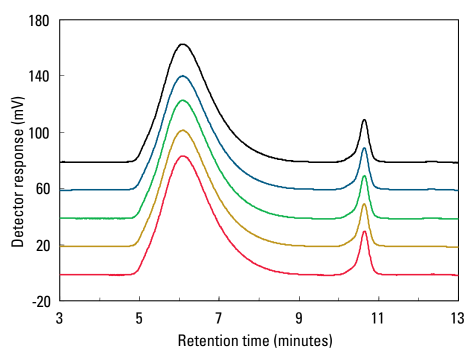
Columns: **TSKgel G3000PWXL-CP, 7 µm, 7.8 mm ID × 30 cm**
TSKgel G5000PWXL-CP, 10 µm, 7.8 mm ID × 30 cm
TSKgel G6000PWXL-CP, 13 µm, 7.8 mm ID × 30 cm
Mobile phase: H_2O with 0.1 mol/L $NaNO_3$
Flow Rate: 1 mL/min
Detection: RI
Temperature: 25 °C
Sample Load: 3 g/L, 100 µL



PAA

The TSKgel PWXL-CP columns eliminate ionic adsorption onto the particle by incorporating a cationic functionality on the particle surface. This is demonstrated in Figure 67 below. PAA [poly(acrylic acid)] was injected onto a TSKgel G5000PWXL-CP column. Each chromatogram, from the first injection (red) to the fifth injection (black), showed similar elution profiles without any adsorption of the polymer.

Figure 67: Analysis of PAA

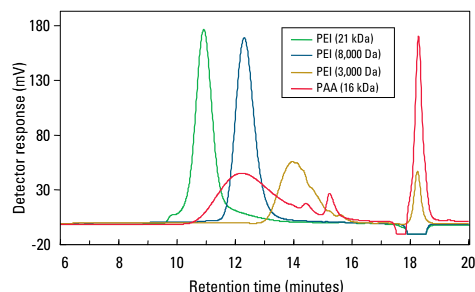


Column:	**TSKgel G5000PWXL-CP, 10 µm, 7.8 mm ID × 30 cm**
Mobile phase:	H_2O with 0.1 mol/L $NaNO_3$
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	25 °C
Sample:	polyallylamine-HCl (PAA)
Sample load:	3 g/L, 100 µL

Small Molar Mass Cationic Polymers

Small molar mass cationic polymers were analyzed on two TSKgel G3000PWXL-CP columns in series. As Figure 68 shows, these narrow molar mass cationic polymers eluted in order of their molar masses.

Figure 68: Elution profiles of PAA and PEI polymers



Column:	**TSKgel G3000PWXL-CP, 7 µm, 7.8 mm ID × 30 cm × 2**
Mobile phase:	H_2O with 0.1 mol/L $NaNO_3$
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	25 °C
Samples:	polyethyleneimine (PEI) polyallylamine-HCl (PAA)

About: TSKgel SuperMultiporePW Size Exclusion Columns

The innovative multi-pore particle synthesis technology*, pioneered by Tosoh scientists, is incorporated into TSKgel SuperMultiporePW columns for water-soluble polymer analysis. Three semi-micro columns varying in linear range are available within this series, enabling high speed and high resolution analysis with lowered solvent consumption. The base material of each TSKgel SuperMultiporePW column is polymethacrylate.

A wide molar mass range can be analyzed with the three different TSKgel SuperMultiporePW columns, from high molar mass water-soluble polymers to oligomers. The packing material in the TSKgel SuperMultiporePW columns is more hydrophilic than that of TSKgel PWXL series columns, which further reduces the chance of adsorption of hydrophilic polymers.

- TSKgel SuperMultiporePW-N
- TSKgel SuperMultiporePW-M
- TSKgel SuperMultiporePW-H

*Using this proprietary technology, Tosoh can manufacture particles, each containing a broad range of pore sizes. This innovative approach essentially creates a linear calibration curve within each particle. As a result, columns with an extended linear calibration curve can now be prepared without mixing particles of different pore sizes.

Attributes and Applications:

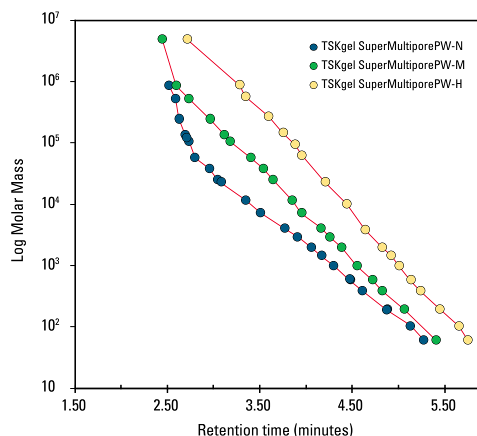
Table 18 shows the product attributes for each of the three TSKgel SuperMultiporePW columns. Figure 69 shows polyethylene glycol, oxide and ethylene glycol calibration curves for each of the TSKgel SuperMultiporePW columns.

Table 18: Product attributes

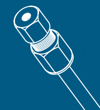
TSKgel column	SuperMultipore PW-N	SuperMultipore PW-M	SuperMultipore PW-H
Base material	polymethacrylate		
Particle size	4 µm*	5 µm*	8 µm*
Pore size	20 nm	100 nm	>100 nm
Exclusion limit (PEO, PEG/H_2O)	1.0×10^5 - 1.5×10^5 Da	6.0×10^5 - 1.5×10^6 Da	-
Separation range	300 ~ 5.0×10^4 Da	500 ~ 1.0×10^6 Da	1,000 ~ 1.0×10^7 Da
Theoretical plates/15cm column	>16,000	>12,000	>7,000

* Particle size distribution is monodisperse.

Figure 69: Polyethylene glycol, oxide, and ethylene glycol calibration curves for TSKgel SuperMultiporePW columns



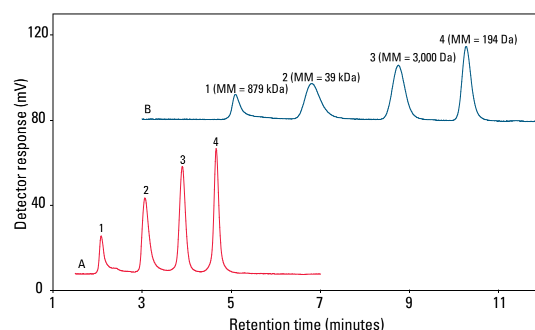
Columns:	**TSKgel SuperMultiporePW-N, 6.0 mm ID × 15 cm** **TSKgel SuperMultiporePW-M, 6.0 mm ID × 15 cm** **TSKgel SuperMultiporePW-H, 6.0 mm ID × 15 cm**
Mobile phase:	H_2O
Flow rate:	0.60 mL/min
Detection:	RI
Temperature:	25 °C
Samples:	polyethylene oxides (PEO) standards polyethylene glycols (PEG) standards ethylene glycol (EG) standards



Comparison with Conventional GPC Columns

A mixture of polyethylene oxide (PEO) and polyethylene glycol (PEG) was analyzed on a semi-micro TSKgel SuperMultiporePW-M column and on conventional-sized TSKgel G3000PWXL and TSKgel G5000PWXL columns in series. As shown in Figure 70, the analysis using the TSKgel SuperMultiporePW-M column was completed in ½ the time and with higher resolution than the analysis performed using the TSKgel G3000PWXL and TSKgel G5000PWXL columns. This is due to the semi-micro dimensions (6.0 mm ID × 15 cm) and the smaller particle size (4 µm) of the TSKgel SuperMultiporePW-M column compared to the 7.8 mm ID × 30 cm size and 7 and 10 µm particle size of the TSKgel G3000PWXL and TSKgel G5000PWXL columns respectively.

Figure 70: Comparison of analysis



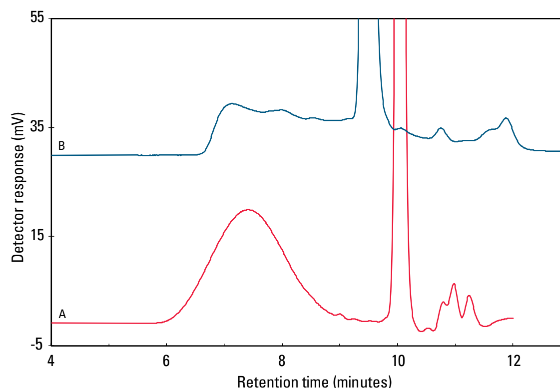
Resolution	TSKgel PWXL	TSKgel SuperMultiporePW-M
Peak 1/Peak 2	3.45	4.25
Peak 2/Peak 3	3.29	3.17
Peak 3/Peak 4	3.30	3.39

Columns: A: **TSKgel SuperMultiporePW-M, 6.0 mm ID × 15 cm**
B: TSKgel G5000PWXL + G3000PWXL, each 6.0 mm ID × 15 cm
Mobile phase: H_2O
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 25 °C
Injection vol.: A: 20 µL B: 100 µL
Samples: mixture of PEO and PEG

PVP

Figure 71 demonstrates the lower hydrophobicity of the TSKgel SuperMultiporePW columns compared to the conventional TSKgel PWXL columns. Hydrophobic interaction causes partial adsorption of PVP-15 polymer on the TSKgel G3000PWXL and TSKgel G2500PWXL columns, while the absence of adsorption on the TSKgel SuperMultiporePW-N column suggests that the internal particle surface is more hydrophilic than the conventional columns.

Figure 71: Analysis of a PVP-15 polymer



Columns: **A. TSKgel SuperMultiporePW-N, 6.0 mm ID × 15 cm × 2**
B. TSKgel G3000PWXL+G2500PWXL, 6.0 mm ID × 15 cm × 2
Mobile phase: 100 mmol/L $NaNO_3$
Flow Rate: 0.60 mL/min
Detection: RI
Temperature: 40 °C
Injection vol.: 20 µL
Samples: PVP(K-15)

About: TSKgel Alpha and SuperAW Size Exclusion Columns

TSKgel Alpha and SuperAW columns were developed for the GPC analysis of polymers of intermediate polarity. As in the TSKgel PW and PW_{XL} columns, the particles in these TSKgel columns have a hydroxylated methacrylate polymer backbone, but they differ in that they are crosslinked to a higher degree to minimize swelling in polar organic solvents (methanol, acetonitrile, DMSO, isopropanol, THF, and HFIP). The TSKgel Alpha and SuperAW columns provide accurate molar mass determination and exhibit normal retention of polystyrene polymers in dimethyl formamide (DMF) solvent. Unlike TSKgel PW columns, which are stable to a 50% organic mixed with water at most, TSKgel SuperAW and Alpha columns are stable in a wide variety of organic solvents at concentrations up to 100%. TSKgel Alpha and SuperAW columns are offered in 5 discrete exclusion ranges and as a mixed bed column. Both column types can accommodate polymer standards up to several million Dalton molar mass.

- Use TSKgel Alpha columns when throughput is not critical, when sample mass is not limited, to collect fractions, and to obtain maximum number of plates (at the expense of analysis time). The main application area for TSKgel Alpha columns is the analysis of polymers that are soluble in polar organic solvents. Examples include cellulose derivatives, polyimide, and sodium dodecylsulfate (all in 10 mmol/L LiBr in DMF), cleansing gel in methanol, and degree of saponification of polyvinylalcohol in hexafluoroisopropanol (HFIP).

  The TSKgel Alpha Series consists of six columns with three particle sizes: 7, 10, and 13 µm. These columns span a wide molar mass separation range, from 100 to more than 1×10^6 Da, when using polyethylene oxide (PEO) as a molar mass standard. There is one mixed bed column within the TSKgel Alpha line, TSKgel Alpha-M, which has an extended linear calibration range and is suitable for samples with a broad molar mass distribution, as well as samples with unknown molar mass.

TSKgel Alpha columns include:

- TSKgel Alpha-2500
- TSKgel Alpha-3000
- TSKgel Alpha-4000
- TSKgel Alpha-5000
- TSKgel Alpha-6000
- TSKgel Alpha-M

- Use TSKgel SuperAW columns for high throughput applications, to reduce solvent consumption and to reduce solvent disposal cost. TSKgel SuperAW columns contains a similar chemistry as the TSKgel Alpha columns but offer the benefit of smaller particle sizes (4, 6, 7, and 9 µm), smaller column dimensions, and equivalent resolution. Reductions in analysis time and mobile phase consumption make TSKgel SuperAW columns ideal for high throughput applications.

  The TSKgel SuperAW column line consists of five columns and a mixed bed column. These high efficiency columns are only available in 6.0 mm ID × 15 cm dimensions.

TSKgel SuperAW columns include:

- TSKgel SuperAW2500
- TSKgel SuperAW3000
- TSKgel SuperAW4000
- TSKgel SuperAW5000
- TSKgel SuperAW6000
- TSKgel SuperAWM-H

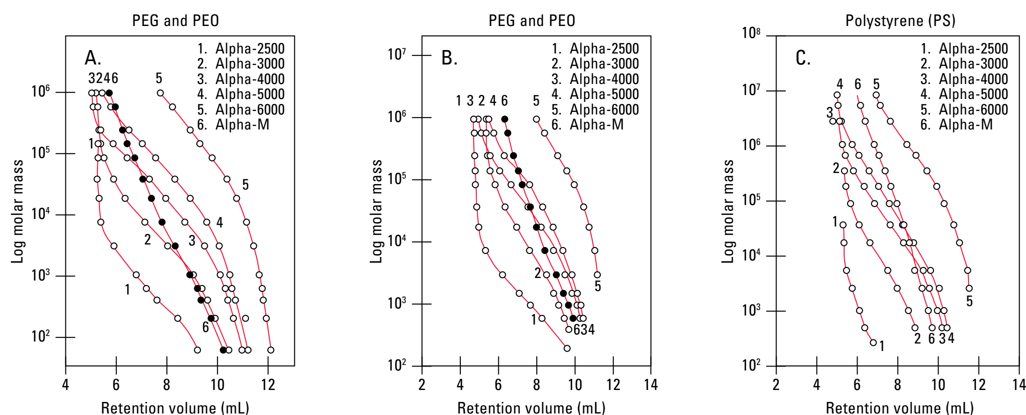
Attributes and Applications:

Product attributes of the TSKgel Alpha and SuperAW columns are shown in Table 19. These columns are for the analysis of polymers that are soluble in methanol, acetonitrile, DMSO, isopropanol, or THF and can also be used for water-soluble polymers. Figures 72-73 show the calibration curves for the TSKgel Alpha and SuperAW columns. Unlike TSKgel PW/PW_{XL} columns, some of which are stable up to 50% organic mixed with water, TSKgel SuperAW and Alpha columns are stable in a wide variety of organic solvents at concentrations up to 100%. As shown in Figure 74, efficiency of all TSKgel SuperAW columns is maintained when changing solvents from water via acetonitrile, DMF, DMSO, THF to HFIP. Suitable solvents for TSKgel Alpha columns are shown in Figure 75.

Table 19: Product attributes

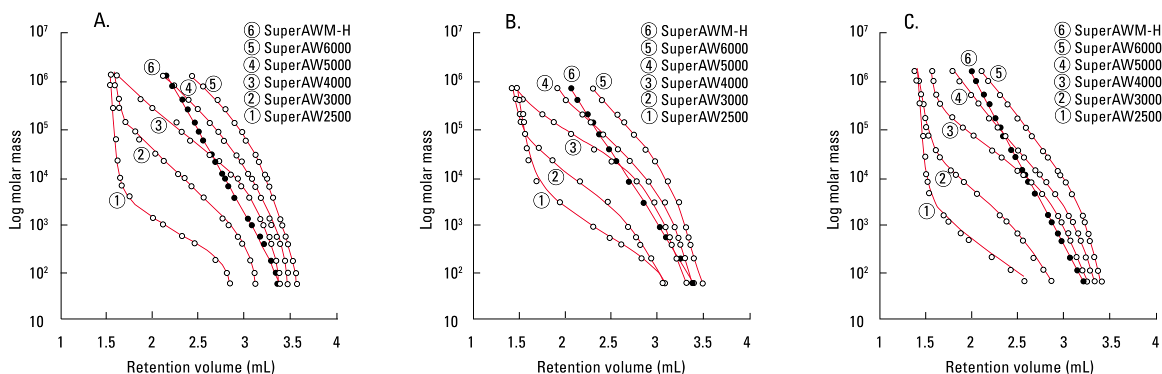
			Exclusion limit (Da) for various standards & eluents		
TSKgel column	**Particle size**	**Pore size**	**PEO in H_2O**	**PS in DMF with 10 mmol/L LiBr**	**PEG in MeOH with 10 mmol/L LiBr**
Alpha-2500	7 µm	2.5 nm	5,000	1×10^4	1×10^4
Alpha-3000	7 µm	15 nm	9×10^4	1×10^5	6×10^4
Alpha-4000	10 µm	45 nm	4×10^5	1×10^6	3×10^6
Alpha-5000	10 µm	100 nm	1×10^6	7×10^6	$>3 \times 10^5$
Alpha-6000	13 µm	>100 nm	$>1 \times 10^7$	$>1 \times 10^7$	$>3 \times 10^5$
Alpha-M	13 µm	mixed bed	$>1 \times 10^7$	$>1 \times 10^7$	$>3 \times 10^5$
SuperAW2500	4 µm	2.5 nm	5,000	1×10^4	1×10^4
SuperAW3000	4 µm	15 nm	9×10^4	1×10^5	6×10^4
SuperAW4000	6 µm	45 nm	4×10^5	1×10^6	3×10^6
SuperAW5000	7 µm	100 nm	1×10^6	7×10^6	$>3 \times 10^5$
SuperAW6000	9 µm	>100 nm	$>1 \times 10^7$	$>1 \times 10^7$	$>3 \times 10^5$
SuperAWM-H	9 µm	mixed bed	$>1 \times 10^7$	$>1 \times 10^7$	$>3 \times 10^5$

Figure 72: Polyethylene oxide, polyethylene glycol, and polystyrene calibration curves for TSKgel Alpha columns



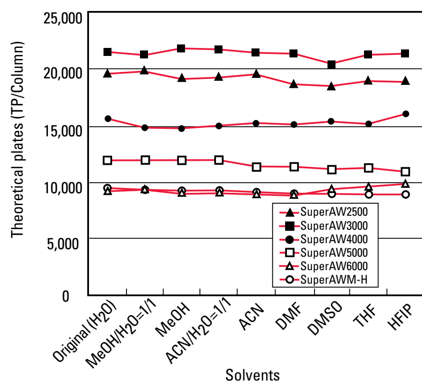
Column:	**TSKgel Alpha columns, 7.8 mm ID × 30 cm**
Mobile phase:	A. H_2O B. MeOH with 10 mmol/L LiBr C. DMF with 10 mmol/L LiBr
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	A. 25 °C B. 25 °C C. 40 °C
Samples:	A, B: polyethylene glycol (PEG) and polyethylene oxide (PEO) standards C. polystyrene (PS) standards

Figure 73: Polyethylene oxide, polyethylene glycol, and ethylene glycol calibration curves for TSKgel SuperAW columns



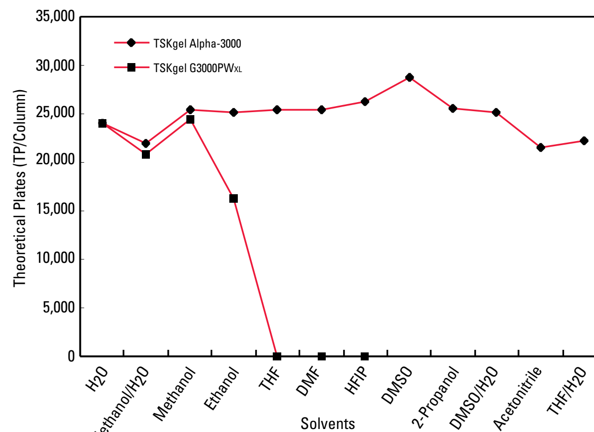
Column: **TSKgel SuperAW columns, 6.0 mm ID × 15 cm**
Mobile phase: A. H_2O
B. MeOH with 10 mmol/L LiBr
C. DMF with 10 mmol/L LiBr
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 25 °C
Samples: polyethylene oxide (PEO), polyethylene glycol (PEG), and ethylene glycol (EG) standards

Figure 74: Column efficiency of TSKgel SuperAW columns



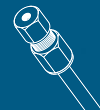
Column: **TSKgel SuperAW columns, 6.0 mm ID × 15 cm**
Mobile phase: H_2O
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 25 °C
Injection vol.: 5 µL (2.5 g/L)
Sample: ethylene glycol

Figure 75: Solvent compatibility for TSKgel Alpha-3000 for organic solvents



Conditions of solvent change
Flow Rate: 1.0 mL/min
Temperature: 25 °C
Time for purge: 8 h

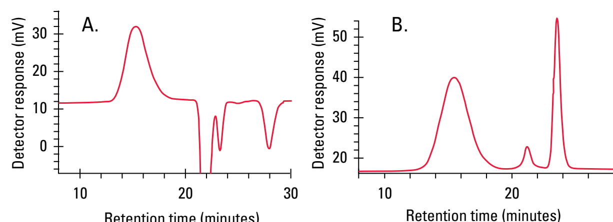
Conditions for TP measurement
Flow Rate: 1.0 mL/min
Detection: RI
Temperature: 25 °C
Sample: ethylene glycol



Cellulose Derivatives

The versatility of using TSKgel Alpha columns with various polar solvents is illustrated in Figure 76 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.

Figure 76: Analysis of cellulose derivatives

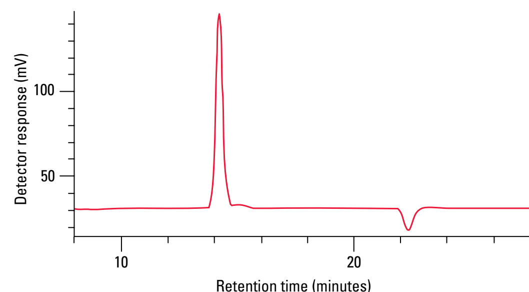


Column:	**TSKgel Alpha-M, 13 µm, 7.8 mm ID × 30 cm**
Mobile phase:	A. DMF with 10 mmol/L LiBr B. MeOH with 10 mmol/L LiBr
Flow rate:	0.5 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol:	50 µL
Samples:	A. ethyl cellulose, 0.1% B. ethyl hydroxyethyl cellulose, 0.1%

Glyceryl tri(2-ethylhexanoate)

Glyceryl tri(2-ethylhexanoate) is used as a plastic lubricant and as a cosmetic base. The analysis of this compound using a TSKgel Alpha-2500 column is shown in Figure 78.

Figure 78: Analysis of glyceryl tri(2-ethylhexanoate)

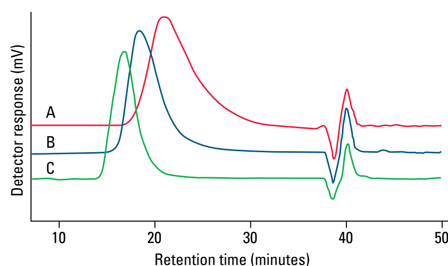


Column:	**TSKgel Alpha-2500, 7 µm, 7.8 mm ID × 30 cm**
Mobile phase:	MeOH
Flow rate:	0.5 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol:	50 µL
Sample:	glyceryl tri-2-ethylhexanoate
Sample load:	0.10%

Polyvinylalcohol Characterization

The separation of polyvinylalcohol with different degrees of saponification is shown in Figure 77. This separation was performed with a TSKgel Alpha-5000 and a TSKgel Alpha-3000 column in series using a hexafluoroisopropanol (HFIP) mobile phase.

Figure 77: Analysis of polyvinylalcohol with different degrees of saponification

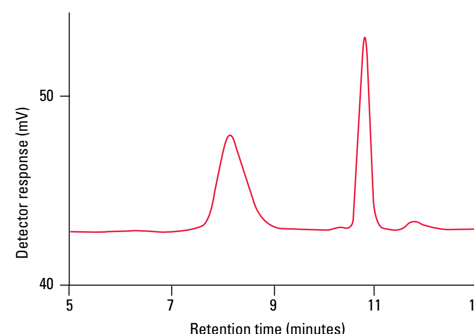


Column:	**TSKgel Alpha-5000 and Alpha-3000, 7.8 mm ID × 30 cm in series**
Mobile phase:	hexafluoroisopropanol (HFIP)
Flow rate:	0.5 mL/min
Detection:	RI
Temperature:	40 °C
Samples:	degree of saponification of polyvinyl alcohol: A. 75% B. 88% C. 100%

Sodium Chondroitin Sulfate

Figure 79 demonstrates the successful analysis of sodium chondroitin sulfate on a TSKgel SuperAWM-H column.

Figure 79: Analysis of sodium chondroitin sulfate

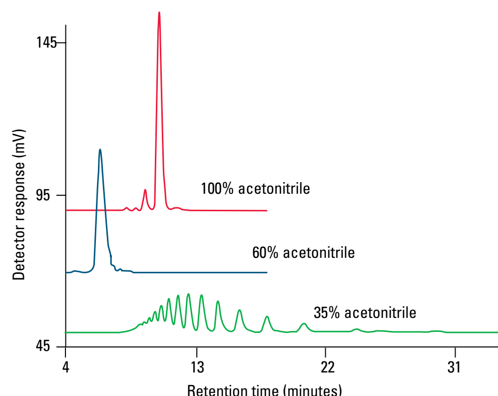


Column:	**TSKgel SuperAWM-H, 9 µm, 6.0 mm ID × 15 cm × 2**
Mobile phase:	H_2O with 0.2 mol/L sodium nitrate
Flow rate:	0.6 mL/min
Detection:	RI
Temperature:	25 °C
Injection vol.:	5 µL (2.5 g/L)
Sample:	chondroitin sulfate

Applications in Non-SEC Mode

Since TSKgel SuperAW columns have excellent solvent compatibility, it is possible to obtain different chromatograms by changing the mobile phase composition of a sample. An example of this is shown in Figure 80 for the analysis of a surfactant using a TSKgel SuperAW2500 column. The sample is separated based on the molecular size (SEC mode) when using a mobile phase with a 60% acetonitrile solution, while it is retained in other mobile phase compositions (separated by a non-SEC mode). Thus it is possible to set up the mobile phase conditions to suit the purpose of measurement (molar mass measurement, assay, separation) in one column.

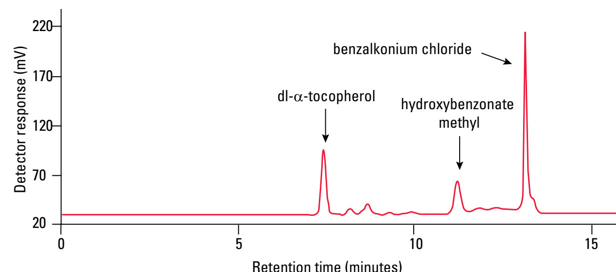
Figure 80: Analysis of a surfactant



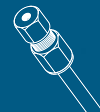
Column:	**TSKgel SuperAW2500, 7 µm, 6.0 mm ID × 15 cm**
Mobile phase:	ACN, ACN solution
Flow rate:	0.6 mL/min
Detection:	UV @ 280 nm
Temperature:	40 °C
Injection vol.:	20 µL

An application of measuring a formulated drug is shown in Figure 81. Additives are retained on the TSKgel SuperAW2500 column and separated. The effectiveness of this column for the separation of samples containing low molar mass to high molar mass components is apparent.

Figure 81: Analysis of medicated cream



Column:	**TSKgel SuperAW2500, 7 µm, 6.0 mm ID × 15 cm × 2**
Mobile phase:	ethanol
Flow rate:	0.6 mL/min
Detection:	UV @ 275 nm
Temperature:	40 °C
Injection vol.:	10 µL



About: TSKgel H Series Size Exclusion Columns

TSKgel H series columns are recommended for the analysis of organic-soluble polymers and are packed with spherical particles composed of polystyrene crosslinked with divinylbenzene (PS-DVB). This series includes TSKgel HXL, HHR, SuperH, Super HZ, and SuperMultiporeHZ columns. Each line of columns within this series differs in degree of inertness and operating temperature range.

The Super prefix designates short (15 cm) columns packed with particles as small as 3 µm. The smaller particle allows for equivalent resolution to conventional TSKgel HXL columns, with 50% reduction in analysis time due to the shorter column length. The TSKgel Super series columns are an excellent choice for high throughput polymer analysis.

- The TSKgel HXL columns are conventional GPC columns of 7.8 mm ID × 30 cm. The column line consists of eight columns with different pore sizes, TSKgel G1000HXL through TSKgel G7000HXL, and three columns with an extended linear range of the calibration curve, TSKgel GMHXL, TSKgel GMHXL-L and TSKgel MultiporeHXL-M. The 5 µm particles in the TSKgel MultiporeHXL-M column contain a broad range of pore sizes. This innovative approach essentially creates a linear calibration curve within each particle. As a result, columns with an extended linear calibration curve can now be prepared without mixing particles of different pore sizes.

  The main characteristics of TSKgel HXL columns are: ultra-low sample adsorption, i.e., the columns show true size exclusion behavior for most polymers, limited solvent range, and a maximum operating temperature of 60 °C for TSKgel G1000HXL - G3000HXL, and 80 °C for the remaining columns in the TSKgel HXL column line.

- The TSKgel HHR column line consists of eight conventional GPC columns of 7.8 mm ID × 30 cm with different pore sizes, TSKgel G1000HHR through TSKgel G7000HHR, and seven mixed bed columns, in which particles with different pore sizes are blended to provide an extended linear calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel GMHHR-L, GMHHR-N, GMHHR-M, to GMHHR-H. The main characteristic of these TSKgel HHR columns is a broad solvent range.

  In addition, four TSKgel HHR mixed bed columns are available for ultra-high temperature analysis. The maximum operating temperature of these columns is 220 °C.

- The TSKgel SuperH column line consists of eight columns of 6.0 mm ID × 15 cm with different pore sizes, TSKgel SuperH1000 through TSKgel SuperH7000, and four mixed bed columns with an extended linear range of the calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel SuperHM-L, SuperHM-N, SuperHM-M, to SuperHM-H. TSKgel SuperH columns are high efficiency/high throughput versions of the conventional TSKgel HHR columns. Both column types are based on the same bead chemistry.

  The main characteristics of TSKgel SuperH columns are: a maximum operating temperature of 140 °C and the ability to use a broad range of solvents.

- The TSKgel SuperHZ column line consists of five columns of 4.6 mm ID × 15 cm and 6.0 mm ID × 15 cm with different pore sizes, TSKgel SuperHZ1000 through TSKgel SuperHZ4000, and three columns with an extended linear range of the calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel SuperHZM-L, SuperHZM-N to SuperHZM-H.

  The main characteristics of TSKgel SuperHZ columns are: developed for high throughput, high efficiency GPC applications such as those encountered in combinatorial chemistry experiments, ultra-low sample adsorption, limited solvent range, and a maximum operating temperature of 60 °C for TSKgel SuperHZ1000 - SuperHZ3000 and 80 °C for the remaining columns in the TSKgel SuperHZ line.

- The TSKgel SuperMultiporeHZ column line consists of three columns of 4.6 mm ID × 15 cm with particles sizes of 3, 4 and 6 µm. The particles in TSKgel SuperMultiporeHZ columns are monodisperse in size and exhibit a broad range of pore sizes. Each particle, by design, has an extended linear calibration curve, thereby greatly diminishing chromatograms with inflection points.

A comparison of TSKgel H series columns is detailed in Table 20. The cross-linking of the polystyrene particles in TSKgel H series columns ensures minimal shrinking and swelling of the column bed when the organic solvent is changed according to the solvent recommendations outlined in Table 21. Suggested flow rates for solvent exchange in TSKgel SuperH and HHR columns are outlined in Table 22. Table 23 lists the recommended solvents by application for TSKgel H series columns.

Table 20: Comparison of TSKgel H series columns

TSKgel series	SuperMultiporeHZ	SuperHZ	SuperH	HXL	HHR
Application focus	Ultra-high performance with a low dead volume and a wide pore distribution in each particle for superior linearity	High throughput polymer analysis with ultra-low polymer adsorption, limited solvent compatibility range	High throughput polymer analysis with expanded solvent compatibility range	Conventional polymer analysis with ultra-low polymer adsorption, limited solvent compatibility range	Conventional polymer analysis with expanded solvent compatibility range
Particle size	3 µm, 4 µm, and 6 µm, depending on pore size	3 µm, 5 µm, and 10 µm, depending on pore size	3 µm and 5 µm, depending on pore size	5 µm, 6 µm, 9 µm, and 13 µm, depending on pore size	5 µm, 13 um, 20 µm, and 30 µm
Particle matrix	Polystyrene divinylbenzene (PS-DVB)	Polystyrene divinylbenzene (PS-DVB)	Polystyrene divinylbenzene (PS-DVB)	Polystyrene divinylbenzene (PS-DVB)	Polystyrene divinylbenzene (PS-DVB)
Number of solvent substitutions	None	One time only	Several[1]	One time only	Several[1]

[1] After switching to a very polar solvent such as acetone, switching back to a nonpolar solvent is not recommended.

Table 21: Solvent compatibility for TSKgel H series columns

TSKgel series	Shipping solvent*	Can be replaced with:
SuperHZ[1]	Tetrahydrofuran[3,4]	benzene, chloroform, toluene, xylene, dichloromethane, dichloroethane
	Dimethylformamide*, cyclohexane*	
SuperHZ and HXL[1]	Tetrahydrofuran[3,4]	benzene, chloroform, toluene, xylene, dichloromethane, dichloroethane
	Acetone*	carbon tetrachloride[5], *o*-dichlorobenzene, dimethylformamide, dimethyl sulfoxide, 1,4-dioxane, ethylacetate, FC-113, hexafluoroisopropanol/chloroform, methyl ethyl ketone, quinoline, cyclohexane, N-methylpyrrolidone
	Chloroform*	*m*-cresol/chloroform, up to 10% hexafluoroisopropanol/chloroform
	Dimethylformamide*	dimethyl sulfoxide, dioxane, tetrahydrofuran, toluene
SuperH and HHR[2]	Tetrahydrofuran[3]	acetone, ethanol, quinoline, benzene, *o*-dichlorobenzene, ethyl acetate, dodecane, FC-113, carbon tetrachloride[5], dichloromethane, dichloroethane, trichloroethane, *n*-hexane, cyclohexane, xylene, chloroform, 1,4-dioxane, hexafluoroisopropanol, toluene, 1-chloronaphthalene, N,N-dimethylacetoacetamide, methyl ethyl ketone, trichlorobenzene, *m*-cresol/chloroform, dimethylformamide, *o*-chlorophenol/chloroform, dimethyl sulfoxide, pyridine, N-methylpyrrolidone, hexafluoroisopropanol/chloroform
SuperMultiporeHZ	Tetrahydrofuran[3]	Cannot be replaced. TSKgel SuperMultiporeHZ columns can be used only in tetrahydrofuran

[1] In case of TSKgel SuperHZ and HXL, keep flow rate as mentioned below during solvent change. Solvent can be changed one way/one time only.
TSKgel HXL: below <0.5 mL/min
TSKgel SuperHZ (4.6 mm ID): below <0.15 mL/min
TSKgel SuperHZ (6.0 mm ID): below <0.3 mL/min

[2] In case of TSKgel SuperH and HHR, see Table 22 below for appropriate flow rates for solvent exchange. After switching to a very polar solvent, switching to a nonpolar solvent is not recommended.

[3] All TSKgel HXL, HHR, SuperHZ, SuperH, SuperMultipore, and GMH analytical columns are shipped containing tetrahydrofuran (THF), except the TSKgel high temperature columns, which contain *o*-dichlorobenzene (ODCB).

[4] THF in TSKgel SuperHZ1000 and G1000HXL columns cannot be replaced with dichloromethane or dichloroethane.

[5] Prolonged exposure to carbon tetrachloride can corrode the stainless steel parts of a column and an HPLC system.

* TSKgel H series columns may be specially ordered with this shipping solvent.

Please note: 100% methanol cannot be used with TSKgel H series columns; use this solvent with TSKgel SW or Alpha columns.

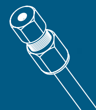


Table 22: Recommended flow rates (mL/min) for TSKgel SuperH and HHR columns

Solvent	TSKgel SuperH, 6.0 mm ID × 15 cm	TSKgel HHR, 7.8 mm ID × 30 cm
n-Hexane	0.5	0.9
methyl ethyl ketone	0.4	0.7
dichloromethane, ethyl acetate	0.35	0.6
toluene, chloroform	0.3	0.5
dimethylformamide	0.2	0.4
carbon tetrachloride, pyridine	0.15	0.3
dimethyl sulfoxide, dioxane, ethanol, N-methylpyrrolidone, *o*-dichlorobenzene	0.1	0.2
quinoline, hexafluoroisopropanol, 1-chloronaphthalene	0.05	0.1

Table 23: Recommended solvents by application for TSKgel H series columns

Recommended solvent	Application
THF	polystyrene, epoxy resin, phenoxy resin, polycarbonate, polyisoprene, polyvinyl acetate, polyvinyl chloride, monoglycerides, fatty acids, polybutadiene, poly(methyl methacrylate), poly(styrene-butadiene), poly(styrene-acrylonitrile)
N,N-Dimethylformamide (DMF) + 5 mmol/L LiBr	polyvinyl chloride, polyvinyl fluoride, urea resins, polyurethane, polystyrene, polyester, polyimido ether, polyimido ester, polyphenol (aqueous solution), polyacrylonitrile
o-Dichlorobenzene (ODCB)	polyethylene, polypropylene
Chloroform	polycarboxylic ether, acrylic resin, epoxy resin, polystyrene
m-Cresol/Chloroform	nylon, polyester, polyamide, poly (ethylene terephthalate)
Toluene	polybutadiene, polysiloxane

About: TSKgel HXL Size Exclusion Columns

TSKgel HXL columns are conventional GPC columns of 7.8 mm ID × 30 cm containing 5, 6, 9, or 13 µm particles composed of PS-DVB. The TSKgel HXL column lines consists of eight columns with different pore sizes, TSKgel G1000HXL through TSKgel G7000HXL, and three columns with an extended linear range of the calibration curve, TSKgel GMHXL, TSKgel GMHXL-L and TSKgel MultiporeHXL-M.

The TSKgel HXL column line consists of the following columns:

- TSKgel G1000HXL
- TSKgel G2000HXL
- TSKgel G2500HXL
- TSKgel G3000HXL
- TSKgel G4000HXL
- TSKgel G5000HXL
- TSKgel G6000HXL
- TSKgel G7000HXL
- TSKgel GMHXL mixed bed
- TSKgel GMHXL-L mixed bed
- TSKgel MultiporeHXL-M

Three of the linear columns are mixed bed columns, in which particles with different pore sizes are blended to provide an extended linear calibration curve. The remaining column is a multi-pore column, in which each particle contains a range of pore sizes that provide a linear calibration curve. The innovative multi-pore approach, pioneered by Tosoh, is a synthetic chemistry answer to the question of how to obtain a column with an extended linear calibration curve, while mixed bed columns represent a mechanical way of obtaining a linear calibration curve. In general, Multipore columns have a smoother, more linear, calibration curve.

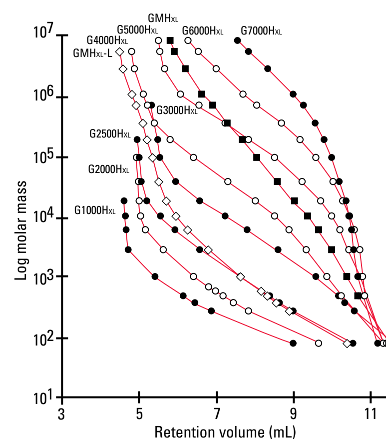
Attributes and Applications:

Product attributes of all of the TSKgel HXL columns are shown in Table 24. These columns are for the use of conventional polymer analysis and show ultra-low polymer absorption, i.e., the columns show true size exclusion behavior for most polymers. TSKgel HXL columns are shipped in THF, with the exception of the TSKgel GMHXL-HT column, which is shipped in *o*-dichlorobenzene. These columns can be exchanged for a limited number of organic solvents. See the table within the TSKgel H series column overview for a listing of these solvents. Figures 82-83 show the calibration curves for the TSKgel HXL columns.

Table 24: Product attributes

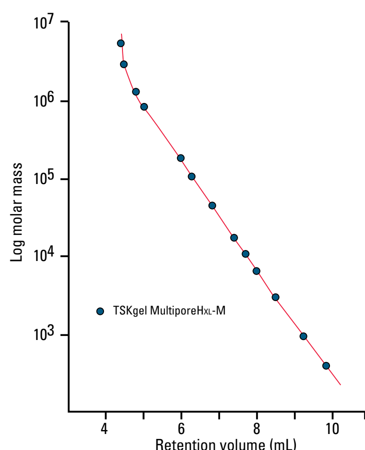
TSKgel column	Particle size	Pore size	Exclusion limit	Max. temp.
G1000HXL	5 µm	1.5 nm	1,000 Da	60 °C
G2000HXL	5 µm	2 nm	1.0×10^4 Da	60 °C
G2500HXL	5 µm	3 nm	2.0×10^4 Da	60 °C
G3000HXL	5 µm	7.5 nm	6.0×10^4 Da	60 °C
G4000HXL	5 µm	20 nm	4.0×10^5 Da	80 °C
G5000HXL	9 µm	65 nm	4.0×10^6 Da	80 °C
G6000HXL	9 µm	>65 nm	4.0×10^7 Da	80 °C
G7000HXL	9 µm	>65 nm	4.0×10^8 Da	80 °C
GMHXL	9 µm	mixed pore sizes	4.0×10^8 Da	80 °C
GMHXL-L	5 µm	mixed pore sizes	4.0×10^6 Da	80 °C
MultiporeHXL-M	5 µm	broad distribution of pore size in each particle	2.0×10^6 Da	60 °C

Figure 82: Calibration curves of TSKgel HXL columns



Column: **TSKgel HXL columns, 7.8 mm ID × 30 cm**
Mobile phase: THF
Flow rate: 1.0 mL/min
Detection: UV @ 254 nm
Temperature: 25 °C
Sample: polystyrene standards

Figure 83: Calibration curve of TSKgel MultiporeHXL-M column

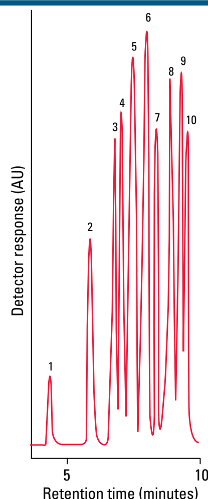


Columns:	**TSKgel MultiporeHXL-M, 5 µm, 7.8 mm ID × 30 cm**
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	40 °C
Sample:	polystyrene standards

Phthalate Esters

Figure 84 demonstrates the high efficiency separation on a TSKgel G1000HXL column for low molar mass phthalate esters. Resolution was close to baseline even though the molar masses of the esters differed by less than 50 Da.

Figure 84: High resolution of phthalate esters

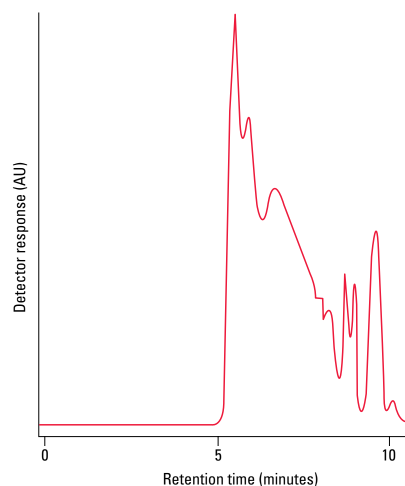


Column:	**TSKgel G1000HXL, 5 µm, 7.8 mm ID × 30 cm**
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Samples:	1. polystyrene (1.0 × 10^4 Da) 2. dioctylphthalate (391 Da) 3. dibutylphthalate (278 Da) 4. diprophylphthalate (250 Da) 5. diethylphthalate (222 Da) 6. dimethylphthalate (194 Da) 7. n-propylbenzene (120 Da) 8. ethylbenzene (116 Da) 9. toluene (92 Da) 10. benzene (78 Da)

Phenol Resin

The TSKgel GMHXL-L column has been designed to provide a complete profile for high molar mass samples that contain low molar mass additives. The calibration curve for this mixed bed column is shallow in the low molar mass range of oligomers. Sample adsorption is not observed. For example, the complete profile of a phenol resin, with high resolution of the low molar mass components, is shown in Figure 85. Other applications for the TSKgel GMHXL-L column include analyses of paint materials, bond and adhesive components and synthetic polymer additives.

Figure 85: Separation of phenol resin

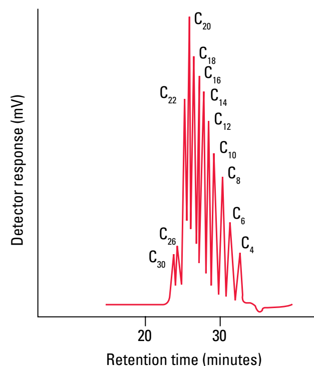


Column:	**TSKgel GMHXL-L, 5 µm, 7.8 mm ID × 30 cm**
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Sample:	phenol resin

Fatty Acids

In Figure 86, two TSKgel G2000HXL columns in series separate a mixture of fatty acids ranging from C4 to C30.

Figure 86: Separation of fatty acids

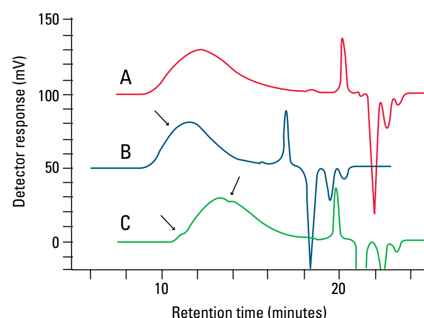


Column:	**TSKgel G2000HXL, 5 µm, 7.8 mm ID × 30 cm × 3**
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	RI
Sample:	fatty acids

Acrylic Polymer

Figure 87 shows the separation of an acrylic polymer on the TSKgel MultiporeHXL-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 MultiporeHXL-M column.

Figure 87: Separation of acrylic resin

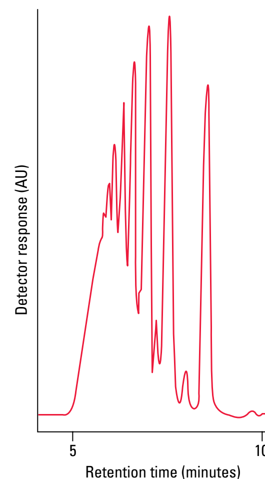


Columns:	**A. TSKgel MultiporeHXL-M, 5 µm, 7.8 mm ID × 30 cm × 2 in series**
	B. Competitor P, 7.5 mm ID × 30 cm × 2 in series, mixed bed type
	C. Competitor S, 8.0 mm ID × 30 cm × 2 in series, mixed bed type
Mobile phase:	THF
Flow rate:	1.0 mL/min
Temperature:	40 °C
Detection:	RI
Sample:	acrylic polymer (0.1%, 50 µL)

Epoxy Resin

The analysis of a commercial epoxy resin, Epikote 1001, using a TSKgel G2500HXL column is shown in Figure 88.

Figure 88: Separation of epoxy resin

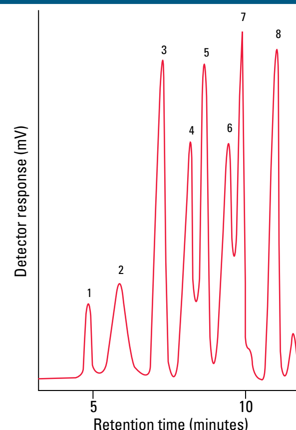


Column:	**TSKgel G2500HXL, 5 µm, 7.8 mm ID × 30 cm**
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Sample:	Epikote 1001 epoxy resin

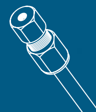
Polystyrene

For polymer mixtures that contain low and high molar mass compounds, a TSKgel G4000HXL column provides high resolution of samples ranging in size from benzene to 1.126×10^6 Da as shown in Figure 89.

Figure 89: Separation of polystyrene standards



Column:	**TSKgel G4000HXL, 5 µm, 7.8 mm ID × 30 cm**
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Samples:	polystyrene standards 1. 1.126×10^6 Da 2. 1.86×10^5 Da 3. 4.28×10^4 Da 4. 1.67×10^4 Da 5. 1.02×10^4 Da 6. 2,800 Da 7. 890 Da 8. benzene, 78 Da



About: TSKgel HHR Size Exclusion Columns

TSKgel HHR columns are conventional GPC columns with dimensions of 7.8 mm ID × 30 cm containing spherical particles composed of PS-DVB. The TSKgel HHR column line consists of eight columns with different pore sizes, TSKgel G1000HHR through TSKgel G7000HHR, and ten columns with an extended linear range of the calibration curve.

The TSKgel HHR column line consists of the following columns:

- TSKgel G1000HHR
- TSKgel G2000HHR
- TSKgel G2500HHR
- TSKgel G3000HHR
- TSKgel G4000HHR
- TSKgel G5000HHR
- TSKgel G6000HHR
- TSKgel G7000HHR
- TSKgel GMHHR-H mixed bed
- TSKgel GMHHR-L mixed bed
- TSKgel GMHHR-M mixed bed
- TSKgel GMHHR-N mixed bed
- TSKgel G2000HHR (20) HT
- TSKgel GMHHR-H (S) HT mixed bed
- TSKgel GMHHR-H HT mixed bed
- TSKgel G2000HHR (20) HT2
- TSKgel GMHHR-H (S) HT2 mixed bed
- TSKgel GMHHR-H HT2 mixed bed

The linear, or mixed bed columns, contain particles with different pore sizes that are blended to provide an extended linear calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel GMHHR-L, GMHHR-N, GMHHR-M, to GMHHR-H. All of the TSKgel high temperature mixed bed columns are shipped in ODCB (*o*-dichlorobenzene).

The TSKgel HHR HT2 mixed bed columns are available for ultra-high temperature analysis. Packed with PS-DVB beads, the maximum operating temperature of these columns is 220 °C.

The issue of shearing that occurs with the analysis of ultra-high molar mass polymers is overcome by the TSKgel GMHHR-M (S), TSKgel GMHHR-H (S), GMHHR-H (S) HT and GMHHR-H (S) HT2 columns. The (S) is a reference to this shearing effect.

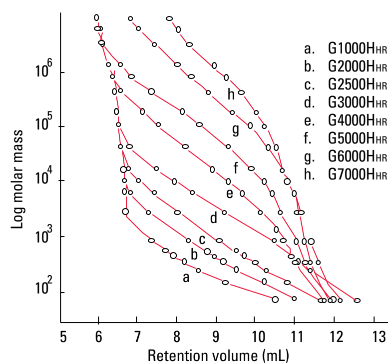
Attributes and Applications:

The product attributes for all of the TSKgel HHR columns is shown in Table 25. TSKgel HHR columns have a broad solvent range and are shipped in THF, except for the high temperature mixed bed columns, which are shipped in ODCB (*o*-dichlorobenzene). THF can be exchanged for a wide variety of organic solvents. See the table within the TSKgel H series column overview for a listing of these solvents. Figures 90-94 show the calibration curves for the TSKgel HHR columns.

Table 25: Product attributes

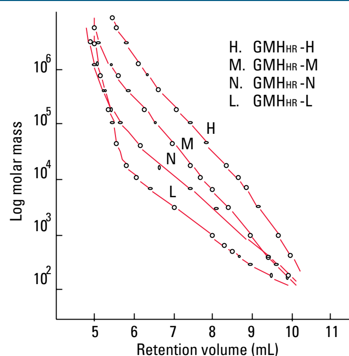
TSKgel column	Particle size	Pore size	Exclusion limit	Max. temp.
G1000HHR	5 µm	1.5 nm	1,000 Da	140 °C
G2000HHR	5 µm	2 nm	1.0×10^4 Da	140 °C
G2500HHR	5 µm	3 nm	2.0×10^4 Da	140 °C
G3000HHR	5 µm	7.5 nm	6.0×10^4 Da	140 °C
G4000HHR	5 µm	20 nm	4.0×10^5 Da	140 °C
G5000HHR	5 µm	65 nm	4.0×10^6 Da	140 °C
G6000HHR	5 µm	>65 nm	4.0×10^7 Da	140 °C
G7000HHR	5 µm	>65 nm	4.0×10^8 Da	140 °C
GMHHR-H	5 µm, 13 µm, 20 µm, 30 µm	mixed pore sizes	4.0×10^8 Da	80 °C
GMHHR-H (S) HT	13 µm	mixed pore sizes	4.0×10^8 Da	140 °C
TSKgel G2000HHR (20) HT	20 µm	2 nm	1×10^4 Da	140 °C
GMHHR-H (20) HT	20 µm	mixed pore sizes	4×10^8 Da	140 °C
GMHHR-H (30) HT	30 µm	mixed pore sizes	4×10^8 Da	140 °C
GMHHR-H HT	5 µm	mixed pore sizes	4×10^8 Da	140 °C
GMHHR-L	5 µm	mixed pore sizes	4.0×10^6 Da	80 °C
GMHHR-M	5 µm, 13 µm	mixed pore sizes	4.0×10^6 Da	80 °C
GMHHR-N	5 µm	mixed pore sizes	4.0×10^5 Da	80 °C
TSKgel G2000HHR (20) HT2	20 µm	2 nm	1×10^4 Da	220 °C
GMHHR-H (20) HT2	20 µm	mixed pore sizes	4×10^8 Da	220 °C
GMHHR-H (30) HT2	30 µm	mixed pore sizes	4×10^8 Da	220 °C
GMHHR-H (S) HT2	13 µm	mixed pore sizes	4×10^8 Da	220 °C

Figure 90: Calibration curves of TSKgel HHR columns



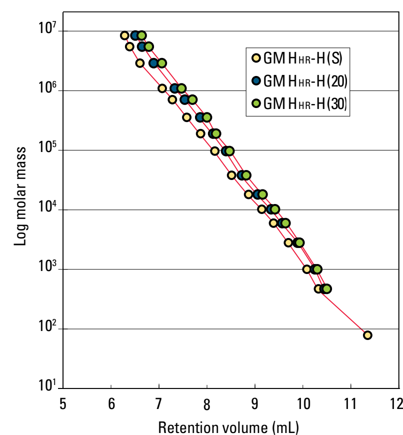
Column:	**TSKgel HHR columns, 7.8 mm ID × 30 cm**
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	polystyrene standards

Figure 91: Calibration curves of TSKgel HHR mixed bed columns



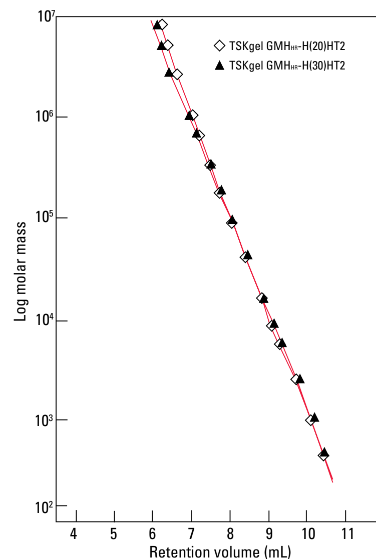
Columns:	**TSKgel HHR columns, 7.8 mm ID × 30 cm**
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	polystyrene standards

Figure 92: Calibration curves of TSKgel HHR columns



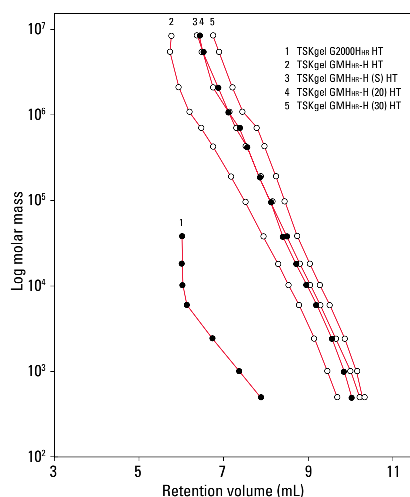
Columns:	**TSKgel GMHHR-H (S), 13 µm, 7.8 mm ID × 30 cm** **TSKgel GMHHR-H (20), 20 µm, 7.8 mm ID × 30 cm** **TSKgel GMHHR-H (30), 30 µm, 7.8 mm ID × 30 cm**
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Sample:	polystyrene standards

Figure 93: Calibration curves of TSKgel GMHHR-H HT2 columns



Columns:	**TSKgel GMHHR-H (20) HT2, 20 µm, 7.8 mm ID × 30 cm** **TSKgel GMHHR-H (30) HT2, 30 µm, 7.8 mm ID × 30 cm**
Mobile phase:	ODCB with 0.05% BHT
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	135 °C
Sample:	polystyrene standards

Figure 94: Calibration curves of TSKgel HT columns

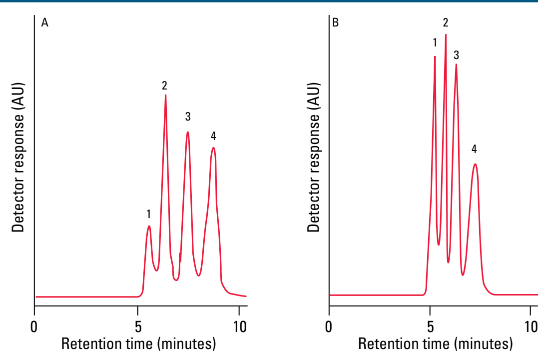


Columns:	**TSKgel G2000HHR (20) HT, 20 µm, 7.8 mm ID × 30 cm**
	TSKgel GMHHR-H HT, 5 µm, 7.8 mm ID × 30 cm
	TSKgel GMHHR-H (S) HT, 13 µm, 7.8 mm ID × 30 cm
	TSKgel GMHHR-H (20) HT, 20 µm, 7.8 mm ID × 30 cm
	TSKgel GMHHR-H (30) HT, 30 µm, 7.8 mm ID × 30 cm
Mobile phase:	ODCB with 0.05% BHT
Flow rate:	1.0 mL/min
Detection:	RI (EcoSEC High Temperature GPC System)
Temperature:	135 °C
Injection vol.:	300 µL
Sample:	polystyrene

Polymethyl Methacrylate

The effect of different pore size distributions in the mixed beds of TSKgel GMHHR-H and TSKgel GMHHR-M is illustrated in Figure 95. The TSKgel GMHHR-M produces sharper polymethyl methacrylate peaks in the 8.0×10^5 to 1.0×10^4 Da range.

Figure 95: Comparison of standard polymethyl methacrylate mixture

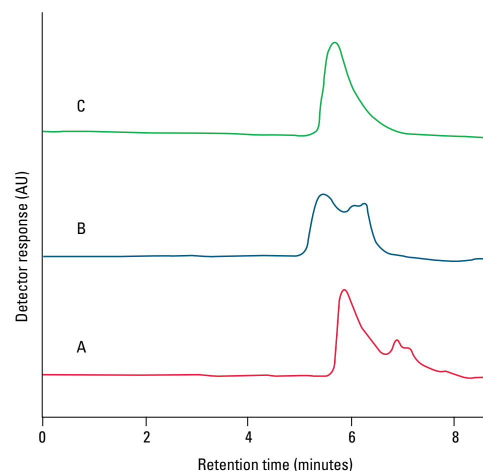


Columns:	**A. TSKgel GMHHR-H, 5 µm, 7.8 mm ID × 30 cm**
	B. TSKgel GMHHR-M, 5 µm, 7.8 mm ID × 30 cm
Mobile phase:	5 mmol/L sodium trifluoroacetate in HFIP
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Temperature:	40 °C
Sample:	standard polymethylmethacrylate
	1. 8.2×10^5 Da
	2. 6.7×10^4 Da
	3. 1.02×10^4 Da
	4. 1,950 Da

Shear Degradation

Shear degradation is observed especially when ultra-high molar mass compounds are analyzed. It tends to occur when analysis is carried out at high flow rates using a micro-particle size packing material. Figure 96 demonstrates the relationship between shear degradation and particle size of the packing material, when TSKgel GMH columns were used. When the flow rate is 1.0 mL/min, normal elution of an ultra-high molar mass sample (2.06×10^7 Da) is only possible with the TSKgel GMHHR-H (S) column, which has a large particle size. However, with the TSKgel GMHXL and GMHHR-H columns, shear degradation does take place and new peaks appear in the chromatogram on the smaller molar mass side.

Figure 96: Shear degradation comparison



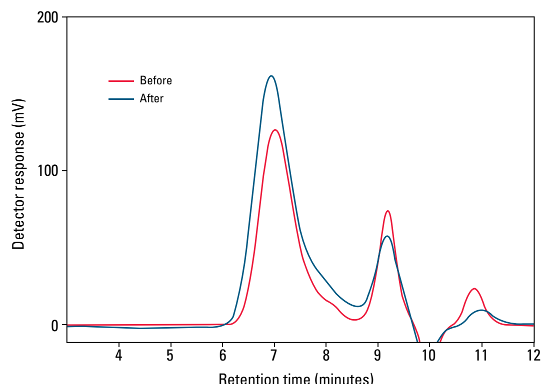
Columns:	**A: TSKgel GMHHR-H, 5 µm, 7.8 mm ID × 30 cm**
	B: TSKgel GMHXL, 9 µm, 7.8 mm ID × 30 cm
	C: TSKgel GMHHR-H (S), 13 µm, 7.8 mm ID × 30 cm
Mobile phase:	THF
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Sample:	polystyrene standard F2000 (2.06×10^7 Da)
	20 µL (0.025%)

Column Durability at 220 °C

Column durability in high temperature GPC polymer analysis is essential as these columns are continuously exposed to harsh organic solvents, extremely elevated temperatures and temperature cycling as GPC systems are turned on and off. The durability of a high temperature GPC column directly influences the quality, applicability and selectivity, or resolution, of the GPC column, thus the accuracy of the molar mass averages obtained. As a high temperature GPC column begins to fail or lose resolution due to the extreme experimental conditions required for high temperature GPC polymer analysis, the number- and z-average molar mass values obtained become inflated and the GPC elution profile begins to shift due to a decrease in multiple factors that affect the ability of the columns to separate species varying in hydrodynamic volume.

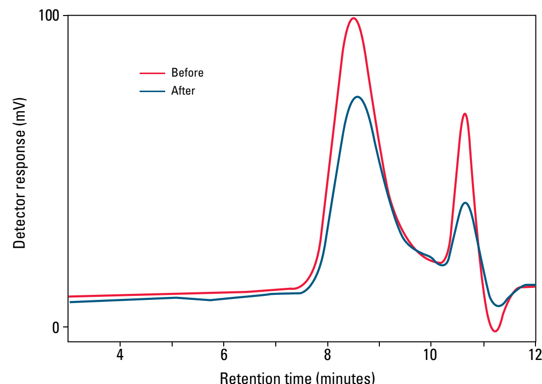
A durability and stability study of a TSKgel GMHHR-H (S) HT high temperature GPC column was performed and the results were compared to another commercially available column for polymer analysis at 220 °C. The deterioration of the commercially available high temperature GPC column is observed in the GPC elution profiles, Figure 97, as the resolution between the sample and solvent peaks decreases after the column is exposed to temperature cycling. The GPC elution profiles obtained for the TSKgel GMHHR-H (S) HT column before and after temperature cycling remain superimposable, Figure 98.

Figure 97: GPC elution profile for a polymer before and after temperature cycling obtained using a commercially available high temperature GPC column



Column:	Commercially available high temperature GPC column, 13 µm, 7.8 mm ID × 30 cm
Mobile phase:	1-chloronaphthalene
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	220 °C
Injection vol.:	200 µL
Sample:	synthetic polymer

Figure 98: GPC elution profile for a polymer before and after temperature cycling obtained using a TSKgel GMHHR-H (S) HT column

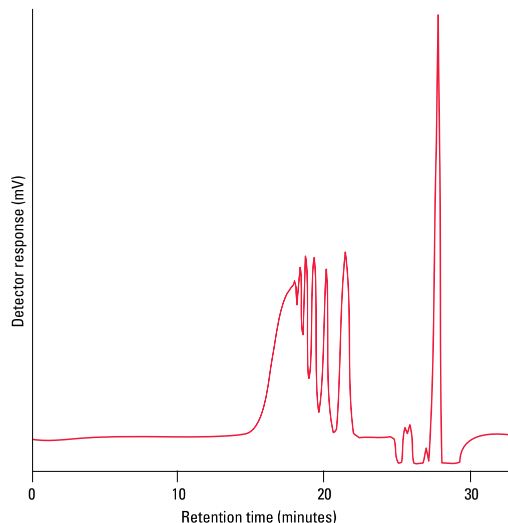


Column:	**TSKgel GMHHR-H (S) HT, 13 µm, 7.8 mm ID × 30 cm**
Mobile phase:	1-chloronaphthalene
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	220 °C
Injection vol.:	200 µL
Sample:	synthetic polymer

Dextran T-40 Hydrolysate

The analysis of dextran T-40 hydrolysate is shown using TSKgel G3000HHR and G2500HHR columns in series in Figure 99 below.

Figure 99: Dextran T-40 hydrolysate analysis

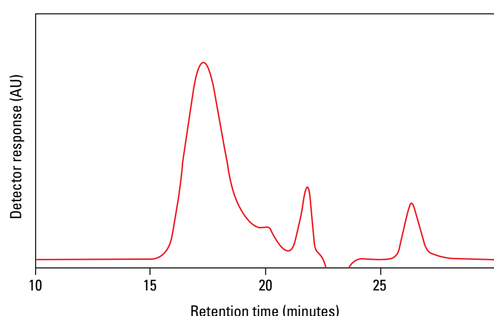


Columns:	**TSKgel G3000HHR, 5 µm, 7.8 mm ID × 30 cm**
	TSKgel G2500HHR, 5 µm, 7.8 mm ID × 30 cm
Mobile phase:	10 mmol/L lithium chloride in N-methylpyrrolidone
Flow rate:	0.75 mL/min
Detection:	RI
Temperature:	80 °C
Sample:	dextran T-40 hydrolysate

Polyphenylene Sulfide

The analysis of PPS (polyphenylene sulfide) is shown using two TSKgel GMHHR-H (S) HT2 columns in series in Figure 100 below.

Figure 100: Polyphenylene sulfide analysis

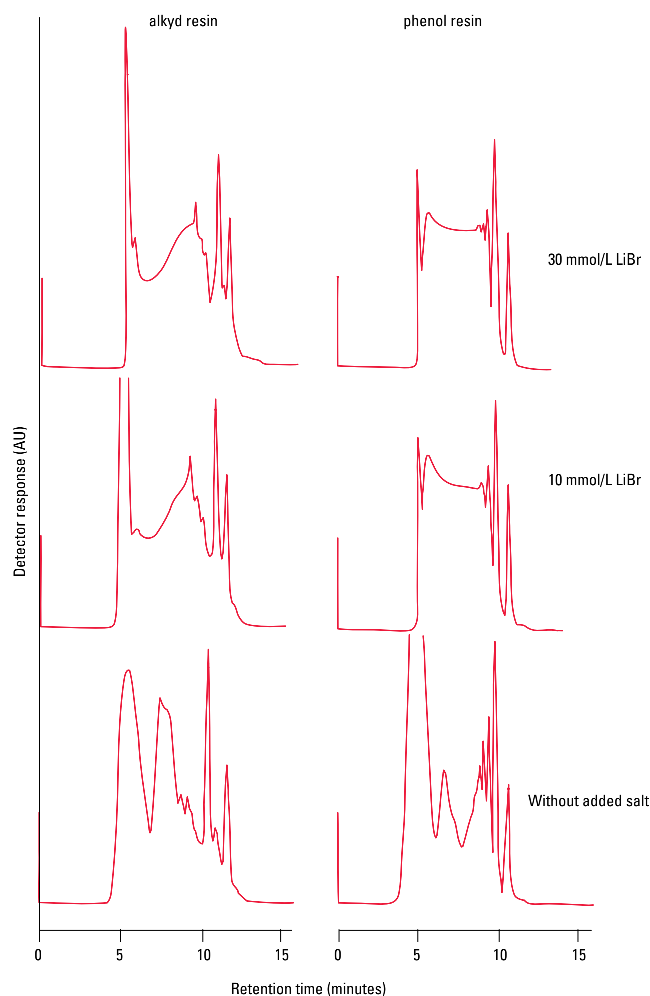


Column:	**TSKgel GMHHR-H (S) HT2, 13 µm, 7.8 mm ID × 30 cm × 2**
Mobile phase:	1-chloronapthalane
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	220 °C
Injection vol.:	300µL
Sample:	PPS (polyphenylene sulfide), 2 g/L

Effect of Adding Salt

Using the TSKgel G3000HHR column, Figure 101 shows the elution behavior of alkyd resin and phenol resin analyzed using a DMF solvent, as well as the effects of adding LiBr to DMF. With the DMF solvent, both resins eluted abnormally early from the column due to a static electric interaction. However, by adding LiBr to the DMF solvent, a normal chromatogram is obtained. Normal elution behavior of alkyd resins is possible when the LiBr concentration is about 30 mmol/L, and with phenol resins, when the concentration of the salt is around 10 mmol/L.

Figure 101: Separation of alkyd resin and phenol resin



Column:	**TSKgel G3000HHR, 5 µm, 7.8 mm ID × 30 cm**
Mobile phase:	DMF (containing LiBr)
Flow rate:	1.0 mL/min
Detection:	UV @ 270 nm
Temperature:	25 °C
Samples:	alkyd resin, phenol resin

About: TSKgel SuperH Size Exclusion Columns

TSKgel SuperH columns are conventional GPC columns with dimensions of 6.0 mm ID × 15 cm containing spherical particles composed of PS-DVB. The TSKgel SuperH column line consists of eight columns with different pore sizes, TSKgel SuperH1000 through TSKgel SuperH7000, and four columns with an extended linear range of the calibration curve.

TSKgel SuperH columns are high efficiency/high throughput versions of the conventional TSKgel HHR columns. Both column types are based on the same bead chemistry.

The TSKgel SuperH line consists of the following columns:

- TSKgel SuperH1000
- TSKgel SuperH2000
- TSKgel SuperH2500
- TSKgel SuperH3000
- TSKgel SuperH4000
- TSKgel SuperH5000
- TSKgel SuperH6000
- TSKgel SuperH7000
- TSKgel SuperHM-H mixed bed
- TSKgel SuperHM-L mixed bed
- TSKgel SuperHM-M mixed bed
- TSKgel SuperHM-N mixed bed

The TSKgel SuperH product line contains four linear or mixed bed columns, in which particles with different pore sizes are blended to provide an extended linear calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel SuperHM-L, SuperHM-M, SuperHM-N, to SuperHM-H.

The volume of a 6 mm ID × 15 cm TSKgel SuperH column is 3.4 times smaller than that of a conventional 7.8 mm ID × 30 cm column. As a result, peak volumes will be proportionally smaller on TSKgel SuperH columns compared to a corresponding TSKgel HHR column. Thus, your HPLC system may require optimization of components that can give rise to extra-column band broadening, such as connecting tubing, injector, injection volume, detector cell volume, and detector time constant.

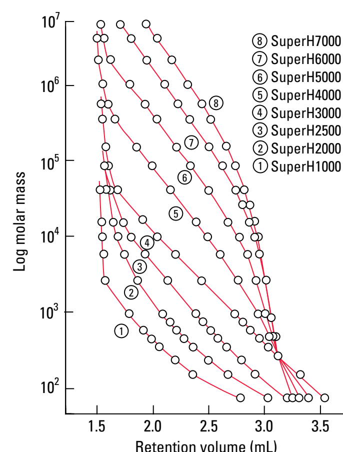
Attributes and Applications:

Table 26 shows product attributes of TSKgel SuperH columns. The maximum operating temperature for TSKgel SuperH columns is 140 °C. All TSKgel SuperH columns are shipped in THF, which can be exchanged for a wide variety of organic solvents. See the table within the TSKgel H series column overview for a listing of these solvents. Figures 102 and 103 show the calibration curves for the TSKgel SuperH columns.

Table 26: Product attributes

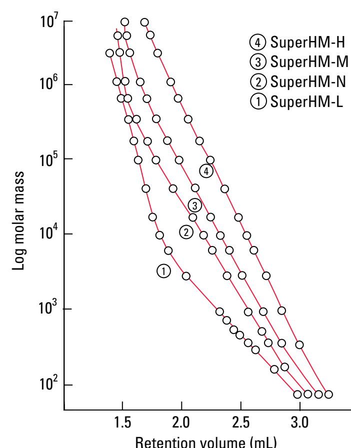
TSKgel column	Particle size (mean)	Pore size (mean)	Exclusion limit
SuperH1000	3 µm	1.5 nm	1,000 Da
SuperH2000	3 µm	2 nm	1.0×10^4 Da
SuperH2500	3 µm	3 nm	2.0×10^4 Da
SuperH3000	3 µm	7.5 nm	6.0×10^4 Da
SuperH4000	3 µm	20 nm	4.0×10^5 Da
SuperH5000	3 µm	65 nm	4.0×10^6 Da
SuperH6000	5 µm	>65 nm	4.0×10^7 Da
SuperH7000	5 µm	>65 nm	4.0×10^8 Da
SuperHM-H	3 µm	mixed pore sizes	4.0×10^8 Da
SuperHM-L	3 µm	mixed pore sizes	4.0×10^6 Da
SuperHM-M	3 µm	mixed pore sizes	4.0×10^6 Da
SuperHM-N	3 µm	mixed pore sizes	4.0×10^5 Da

Figure 102: Calibration curves for TSKgel SuperH columns



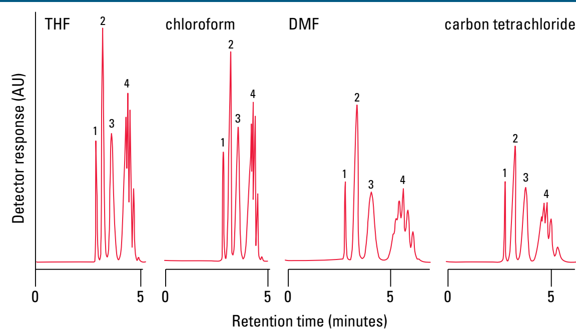
Column:	**TSKgel SuperH columns, 6.0 mm ID × 15 cm**
Mobile phase:	THF
Flow rate:	0.6 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Sample:	polystyrene standards

Figure 103: Calibration curves for TSKgel SuperH mixed bed columns



Column:	**TSKgel SuperH columns, 6.0 mm ID × 15 cm**
Mobile phase:	THF
Flow rate:	0.6 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Sample:	polystyrene standards

Figure 104: Separation of standard polystyrenes using a TSKgel SuperH2500 column



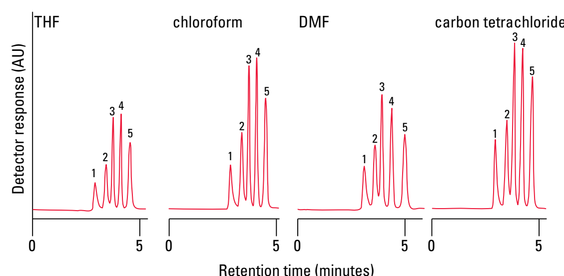
Column:	**TSKgel SuperH2500, 3 µm, 6 mm ID × 15 cm**
Mobile phase:	THF, chloroform, DMF, carbon tetrachloride
Flow rate:	0.6 mL/min
Temperature:	25 °C
Detection:	UV/VIS @ 254 nm or 270 nm
Samples:	1) polystyrene (1.9×10^5 Da)
	2) polystyrene (9.1×10^4 Da)
	3) polystyrene (2,800 Da)
	4) polystyrene A-500

Polystyrene Mixtures

Figure 104 compares chromatograms of standard polystyrene mixtures separated using a TSKgel SuperH2500 column with various organic solvents (THF, $CHCl_3$, DMF, and CCl_4) and Figure 105 compares chromatograms of standard polystyrene mixtures separated using a TSKgel SuperHM-H column with various organic solvents.

Due to the interaction between the packing material and standard polystyrene when using DMF as the mobile phase, the elution volume of standard polystyrenes is greater than it is with “good” solvents such as THF and $CHCl_3$. This effect is particularly noticeable with TSKgel SuperH2500, a column for the analysis of low molar mass samples. Under these circumstances, polyethylene oxide (PEO) is recommended as the standard sample, as this reacts very little with the packing material.

Figure 105: Separation of standard polystyrenes using a TSKgel SuperHM-H column



Column:	**TSKgel SuperHM-H, 3 µm, 6 mm ID × 15 cm**
Mobile phase:	THF, chloroform, DMF, carbon tetrachloride
Flow rate:	0.6 mL/min
Temperature:	25 °C
Detection:	UV/VIS @ 254 nm
Sample:	1. polystyrene (2.89×10^6 Da)
	2. polystyrene (4.22×10^5 Da)
	3. polystyrene (1.07×10^5 Da)
	4. polystyrene (1.67×10^4 Da)
	5. polystyrene (2,800 Da)

Band Broadening in the Detector

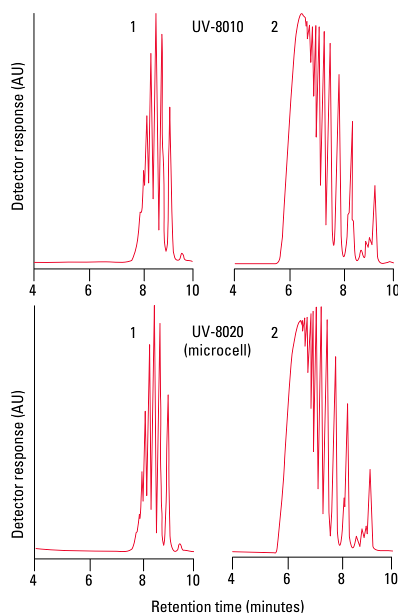
Table 27 compares the number of theoretical plates for a low molar mass sample (DCHP) using a TSKgel SuperH2500 column with various types of UV detectors and different flow cell volumes. Figure 106 compares the separation performance of each of these using standard polystyrene A-500 and epoxy resin samples. Based on these results, it is clear that the number of theoretical plates and the separation performance of the TSKgel SuperH column are significantly affected by band broadening in the detector, including the size of the flow cell. In analyses performed with a TSKgel SuperH column, a UV-8020 microcell (or an equivalent device) with reduced dead volume must be used in the detector.

Table 27: Comparison of number of theoretical plates with various detectors

Number of theoretical plates (TP/15 cm)		
UV-8020*[1]	UV-8010*[2]	UV-8010*[3]
28,100	23,860	17,890
Column:	TSKgel SuperH2500, 3 µm, 6 mm ID × 15 cm	
Mobile phase:	THF	
Flow rate:	0.6 mL/min	
Temperature:	25 °C	
Detection:	UV @ 254 nm	
Sample:	DCHP 0.1%, 2 µL	

*[1] flow cell volume: 2 µL microcell
*[2] flow cell volume: 10 µL low dead volume type
*[3] flow cell volume: 10 µL

Figure 106: Dependence of separation performance on band spreading in detector



Column: **TSKgel SuperH2500, 3 µm, 6 mm ID × 15 cm × 2**
Mobile phase: THF
Flow rate: 0.6 mL/min
Detection: UV/VIS @ 254 nm
Temperature: 25 °C
Samples: 1. standard polystyrene A-500 (0.1%), 10 µL
2. Epikote 1004 (0.1%), 10 µL

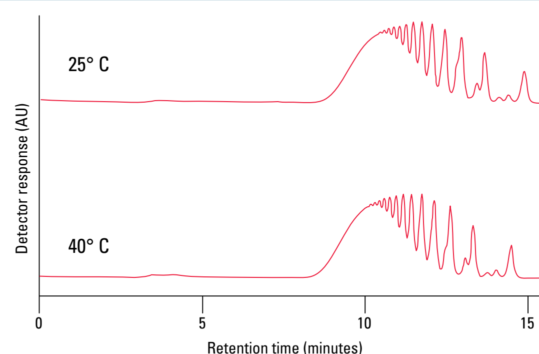
Column Temperature

The following advantages are gained by conducting analysis at high temperature:

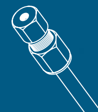
- Peaks become sharper as separation performance is increased. This is especially noticeable at higher flow rates.
- Viscosity of the mobile phase is lowered and operating pressure is decreased. This is an especially effective method with high-viscosity solvents such as DMSO, DMF, HFIP, etc.

Figure 107 demonstrates the temperature dependence of the separation of epoxy resin and a standard polystyrene mixture in TSKgel SuperH3000 and SuperH2500 columns.

Figure 107: Temperature dependence of separation on epoxy resin



Columns: **TSKgel SuperH3000, 3 µm, 6 mm ID × 15 cm × 2 + TSKgel SuperH2500, 3 µm, 6 mm ID × 15 cm × 3**
Mobile phase: THF
Flow rate: 0.6 mL/min
Detection: UV @ 254 nm
Sample: Epikote 1004 (0.1%), 10 µL



About: TSKgel SuperHZ Size Exclusion Columns

The TSKgel SuperHZ column line consists of five columns of 4.6 mm ID and 6.0 mm ID × 15 cm containing spherical particles composed of PS-DVB, TSKgel Super HZ1000 – 4000. Each column consists of a different pore size packing material. Subsequently, a unique separation range for each column exists, allowing researchers to choose a column that is designed for the sample type being analyzed.

The TSKgel SuperHZ column line also contains three linear, or mixed bed columns in which particles with different pore sizes are blended to provide an extended linear calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel SuperHZM-M to SuperHZM-N to SuperHZM-H. The mixed bed columns are also available in 4.6 mm ID and 6.0 mm ID × 15 cm.

The following eight columns are available within the TSKgel SuperHZ column line:

- TSKgel SuperHZ1000
- TSKgel SuperHZ2000
- TSKgel SuperHZ2500
- TSKgel SuperHZ3000
- TSKgel SuperHZ4000
- TSKgel SuperHZM-H mixed bed
- TSKgel SuperHZM-M mixed bed
- TSKgel SuperHZM-N mixed bed

TSKgel SuperHZ column dimensions are 6 mm ID × 15 cm and 4.6 mm ID × 15 cm versus 7.8 mm ID × 30 cm for conventional GPC columns. The smaller column dimensions translate to a reduction of peak volume by a factor of 3.4 (6 mm ID) and a factor of 5.8 (4.6 mm ID) versus the same component eluting from a corresponding TSKgel H_{XL} column. Thus, your HPLC system may require optimization of components that can give rise to extra-column band broadening, such as connecting tubing, injector, injection volume, detector cell volume, and detector time constant.

Attributes and Applications:

TSKgel SuperHZ columns have been developed for high throughput, high efficiency GPC applications such as those encountered in combinatorial chemistry experiments. These columns feature ultra-low sample adsorption, i.e., the columns show true size exclusion behavior for most polymers.

TSKgel SuperHZ1000 – 4000 columns are capable of measuring monomers, polymer additives, oligomers and polymers up to a molar mass of several hundred thousand with proper selection of pore size. Ultra-fine particles (3 µm) have been developed to provide high resolution over the entire molar mass range. This is especially important for the separation of low molar mass compounds.

Additionally, the mixed bed columns (TSKgel SuperHZM-N, M-M, and M-H) are capable of measuring oligomers and polymers with molar masses up to tens of millions with proper selection of the pore size. The various particle sizes of the mixed bed packing materials have been optimized to ensure resolution in the low molar mass range while avoiding shear degradation of polymers in the high molar mass region.

The columns are shipped in THF, which can be exchanged for a limited number of organic solvents as shown in the table within the TSKgel H series column overview.

Table 28 shows the product attributes of TSKgel SuperHZ columns, while Table 29 lists the features of the TSKgel SuperHZ column line and the corresponding benefits. The calibration curves for the TSKgel SuperHZ columns are shown in Figures 106 and 109.

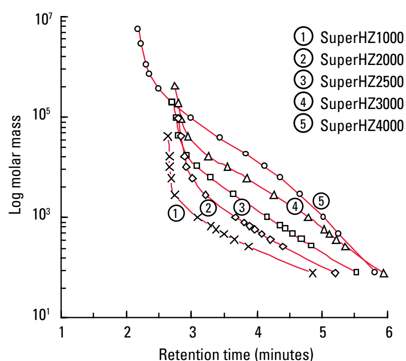
Table 28: Product attributes

TSKgel column	Particle size	Pore size	Exclusion limit	Max. temp.
SuperHZ1000	3 µm	1.5 nm	1,000 Da	60 °C
SuperHZ2000	3 µm	2 nm	1.0×10^4 Da	60 °C
SuperHZ2500	3 µm	3 nm	2.0×10^4 Da	60 °C
SuperHZ3000	3 µm	7.5 nm	6.0×10^4 Da	60 °C
SuperHZ4000	3 µm	20 nm	4.0×10^5 Da	80 °C
SuperHZM-N	3 µm	mixed pore sizes	7.0×10^5 Da	80 °C
SuperHZM-M	3 µm	mixed pore sizes	4.0×10^6 Da	80 °C
SuperHZM-H	10 µm	mixed pore sizes	4.0×10^8 Da	80 °C

Table 29: Features and benefits of TSKgel SuperHZ columns

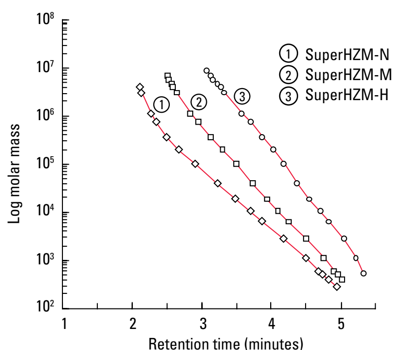
Feature	Benefit
Ultra-fine particles used in packing material	• Short measurement time is achieved. • Resolution equivalent to conventional columns (30 cm) can be obtained in ½ measurement time • Resolution does not deteriorate even under a high flow rate.
Semi-micro columns (4.6 mm ID and 6.0 mm ID)	• Reduction in solvent consumption (running costs, effluent processing costs) 1/6 to 1/3 solvent consumption compared to conventional columns
Optimization of particle size in the packing materials	• Shear degradation in polymers with high molar mass can be prevented
Adoption of low-adsorption packing materials	• Applicable to wide range of samples

Figure 108: Calibration curves for TSKgel SuperHZ columns



Column:	**TSKgel SuperHZ columns, 4.6 mm ID × 15 cm**
Mobile phase:	THF
Flow rate:	0.35 mL/min
Temperature:	25 °C
Injection vol.:	2 µL
Samples:	polystyrene standards

Figure 109: Calibration curves for TSKgel SuperHZ mixed bed columns

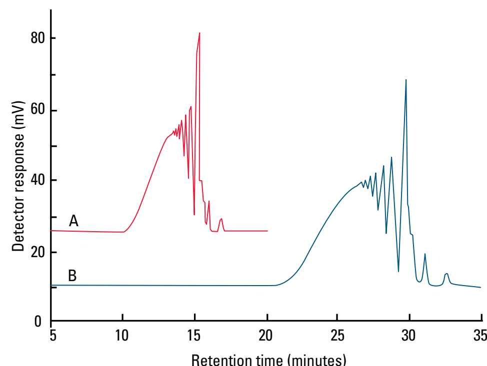


Column:	**TSKgel SuperHZ columns, 4.6 mm ID × 15 cm**
Mobile phase:	THF
Flow rate:	0.35 mL/min
Temperature:	25 °C
Injection vol.:	2 µL
Samples:	polystyrene standards

Faster Analysis

TSKgel SuperHZ1000-SuperHZ4000 columns are packed with 3 µm particles. The ultra-fine particles allow for high efficiency separations of low molar mass substances such as oligomers. These columns have theoretical plate values (per unit length) which are twice those of the conventional 5 µm columns. As a result, equal resolution can be obtained within half the analysis time. An example showing the analysis of phenolic resin is demonstrated in Figure 110.

Figure 110: Comparison of analysis on TSKgel SuperHZ and TSKgel HXL columns

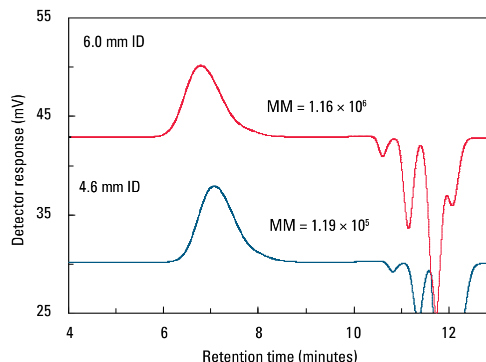


Columns:	**A. TSKgel SuperHZ columns (4000, 3000, 2500), 4.6 mm ID × 15 cm × 3** **B. TSKgel HXL columns (4000, 3000, 2500), 7.8 mm ID × 30 cm × 3**
Mobile phase:	THF
Flow rate:	A. 0.35 mL/min B. 1.0 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol.:	A. 5 µL B. 30 µL
Sample:	phenolic resin

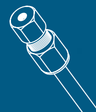
Polyisobutylene

The chromatogram in Figure 111 shows the analysis of polyisobutylene using two TSKgel SuperHZM-M columns in series

Figure 111: Analysis of polyisobutylene



Column:	**TSKgel SuperHZM-M, 3 µm × 2**
Mobile phase:	THF
Flow rate:	0.35 mL/min (4.6 mm ID), 0.6 mL/min (6.0 mm ID)
Detection:	RI
Temperature:	40 °C
Injection vol.:	10 µL (4.6 mm ID), 17 µL (6.0 mm ID)
Sample:	polyisobutylene (0.5 g/L)



About: TSKgel SuperMultiporeHZ Size Exclusion Columns

TSKgel SuperMultiporeHZ columns represent a new strategy for the separation of polymers with a wide range of molar masses. These columns are packed with particles of a uniform size, with each particle having a very broad pore size distribution. This innovative multi-pore approach, pioneered by Tosoh Bioscience, essentially creates a linear calibration curve within each particle. The spherical monodisperse, 3, 4 or 6 µm particles consist of cross-linked polystyrene/divinylbenzene copolymer. This base material, coupled with the semi-micro column dimensions (4.6 mm ID × 15 cm), offers users high speed and low solvent consumption analyses with precise results. Three columns are available within the TSKgel SuperMultiporeHZ series, each with a different particle size and separation range.

The TSKgel SuperMultiporeHZ columns offered include:

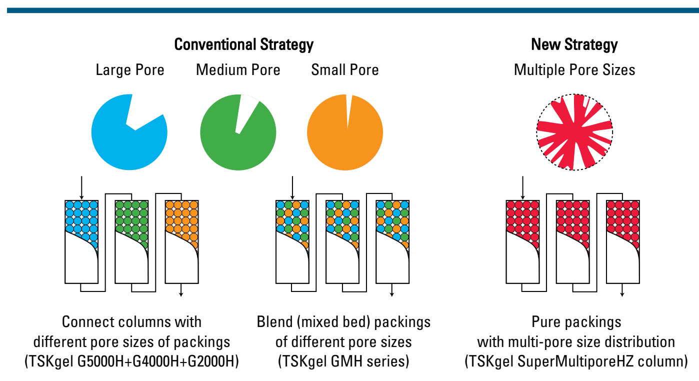
- TSKgel SuperMultiporeHZ-N
- TSKgel SuperMultiporeHZ-M
- TSKgel SuperMultiporeHZ-H

About: Multi-pore Technology

Prior to the introduction of TSKgel SuperMultiporeHZ columns, scientists separating polymers with a wide range of molar masses were left with two options. One option was to use multiple columns of different pore sizes linked together in series. A second was to use a column packed with a mixed bed resin of different pore sizes at an optimized mix ratio. However, problems can occur with both of these methods, which include distortion of the chromatogram or deviations between the actual calibration curve and the calibration curve approximated from data obtained from the molar mass standards.

As is shown in Figure 112, a novel approach to solve this problem was developed by Tosoh scientists and is incorporated in TSKgel SuperMultiporeHZ columns. Small particles of uniform size are synthesized with a broad distribution of pore sizes. This novel approach creates a linear calibration curve within each particle. Therefore, columns with an extended linear calibration curve can now be prepared without mixing particles of different pore sizes.

Figure 112: Graphical representations illustrate the multi-pore particle synthesis technology



Attributes and Applications:

Product attributes for the TSKgel SuperMultiporeHZ columns are listed in Table 30. Table 31 lists features and benefits of these columns. TSKgel SuperMultiporeHZ columns can be utilized for the analysis of polymers with a wide MM distribution range. The columns are shipped in THF, which cannot be replaced for any other organic solvent. Figure 113 shows the calibration curves for the TSKgel SuperMultiporeHZ columns.

Table 30: Product attributes

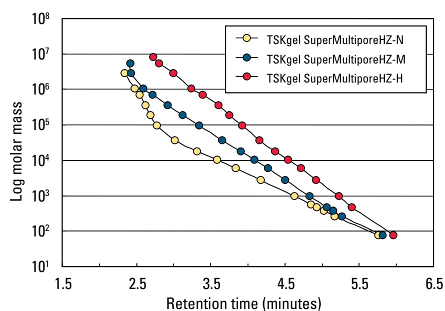
TSKgel column	SuperMultipore HZ-N	SuperMultipore HZ-M	SuperMultipore HZ-H
Base material	PS-DVB	PS-DVB	PS-DVB
Particle size	3 µm*	4 µm*	6 µm*
Pore size	8 nm	14 nm	>14 nm
Exclusion limit (PST/THF)	1.2×10^5 Da	2.0×10^6 Da	4.0×10^7 Da
Separation range	300 ~ 5.0×10^4 Da	500 ~ 1.0×10^6 Da	1,000 ~ 1.0×10^7 Da
Theoretical plates/15 cm column	20,000	16,000	11,000

* Particle size distribution is monodisperse.

Table 31: Features and benefits

Feature	Benefit
Multi-pore packing material (wide range of pores contained in single particle)	• Calibration curves with superior linearity • No observable distortion of chromatograms • Improved accuracy and repeatability of molar mass data • Capable of rapid analysis with high separation performance
Smaller particle size (monodisperse particles)	• Capable of achieving the same separation performance as conventional columns (30 cm) in half the analysis time • No reduction in separation performance even for analysis at high flow rates • Improved robustness of column performance
Semi-micro column	• Reduced solvent consumption • 1/6th the consumption of conventional (30 cm) columns
Low adsorption packing material	• Can be used for a wide variety of samples

Figure 113: Calibration curves for TSKgel SuperMultiporeHZ columns

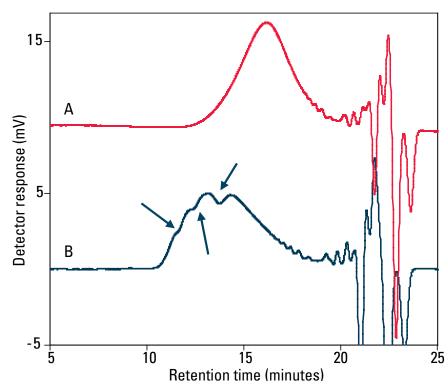


Columns:	**TSKgel SuperMultiporeHZ-N, 3 µm, 4.6 mm ID ×15 cm**
	TSKgel SuperMultiporeHZ-M, 4 µm, 4.6 mm ID × 15 cm
	TSKgel SuperMultiporeHZ-H, 6 µm, 4.6 mm ID × 15 cm
Mobile phase:	THF
Flow rate:	0.35 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	PStQuick polystyrene standards

Acrylic Resin

Figure 114 demonstrates that inflection points are no longer observed with columns packed from particles prepared by multi-pore technology.

Figure 114: Comparison for separation of acrylic resin

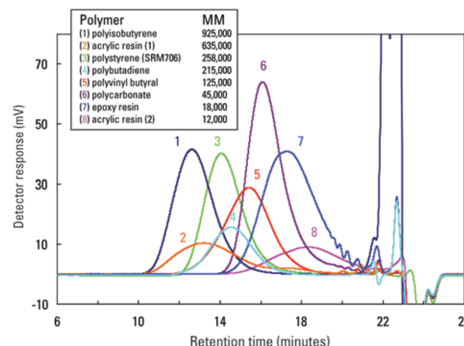


Columns:	**A. TSKgel SuperMultiporeHZ-M, 4.6 mm ID × 15 cm × 4**
	B. TSKgel SuperHZ4000+3000+2500+2000, 4.6 mm ID × 15cm × 1
Mobile phase:	THF
Detection:	RI
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	acrylic resin

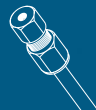
Various Polymers

Various polymers were analyzed on four TSKgel SuperMultiporeHZ-M columns in series. The superimposed chromatograms in Figure 115 clearly demonstrate that these new GPC columns can be utilized for the analysis of polymers with a wide molar mass distribution range.

Figure 115: Separation of various polymers



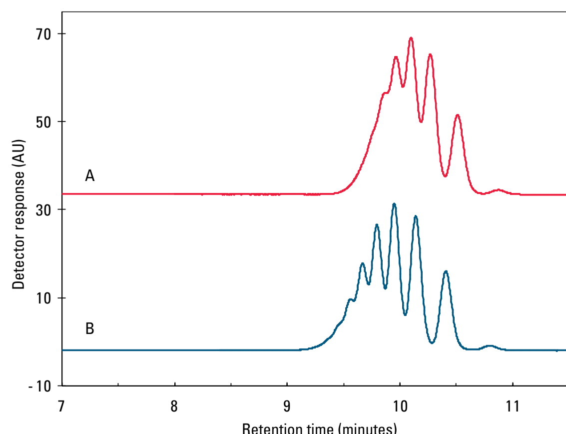
Columns:	**SuperMultiporeHZ-M, 4 µm, 4.6 mm ID × 15 cm × 4**
Mobile phase:	THF
Flow rate:	0.35 mL/min
Detection:	RI
Temperature:	25 °C
Injection vol.:	10 µL
Sample conc.:	0.3%



Standard Polystyrene

Figure 116 compares separation on the TSKgel SuperMultiporeHZ-N column versus the TSKgel SuperMultiporeHZ-M column in the low molar mass region (standard polystyrene A-500). The calibration curve for the TSKgel SuperMultiporeHZ-N column is not as steep and better separation is provided in the low molar mass region due to the smaller particle size (higher number of theoretical plates) of the TSKgel SuperMultiporeHZ-N column.

Figure 116: Analysis of standard polystyrene

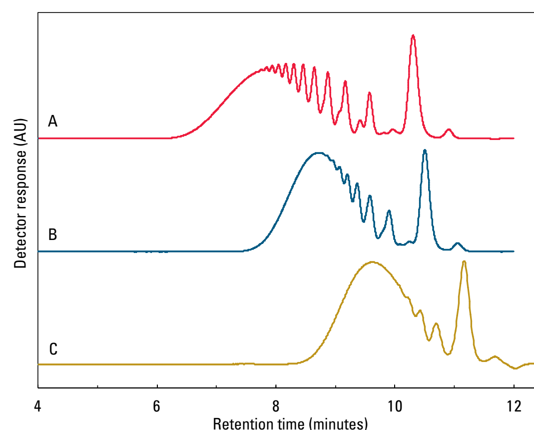


Columns:	**A. TSKgel SuperMultiporeHZ-M, 4 µm, 4.6 mm ID × 15 cm × 2**
	B. TSKgel SuperMultiporeHZ-N, 3 µm, 4.6 mm ID × 15 cm × 2
Mobile phase:	THF
Flow rate:	0.35 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Injection vol.:	5 µL
Sample:	standard polystyrene oligomer (TSKgel standard polystyrene A-500) (5 g/L)

Epoxy Resin

Figure 117 is a chromatogram of an epoxy resin (approximately 6,000 Da) created using the TSKgel SuperMultiporeHZ columns. The best separation performance is shown by the TSKgel SuperMultiporeHZ-N column, the particle size used for low molar mass samples, and it is clear that the TSKgel SuperMultiporeHZ-H column does not provide adequate separation performance.

Figure 117: Analysis of epoxy resin

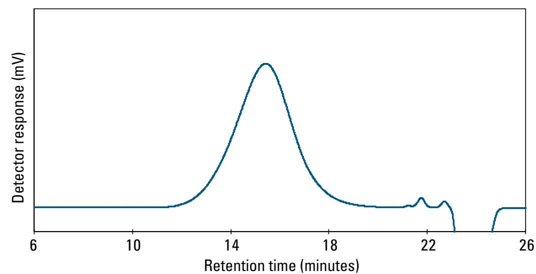


Columns:	**A. TSKgel SuperMultiporeHZ-N, 3 µm, 4.6 mm ID × 15 cm × 2**
	B. TSKgel SuperMultiporeHZ-M, 4 µm, 4.6 mm ID × 15 cm × 2
	C. TSKgel SuperMultiporeHZ-H, 6 µm, 4.6 mm ID × 15 cm × 2
Mobile phase:	THF
Flow rate:	0.35 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	epoxy resin (3 g/L)

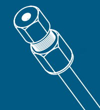
Polyvinylbutyral

The analysis of polyvinylbutyral using a TSKgel SuperMultiporeHZ-H column is show in Figure 118. A smooth chromatogram without any distortion is obtained.

Figure 118: Analysis of polyvinylbutyral



Column: **TSKgel SuperMultiporeHZ-H, 6 µm, 4.6 mm ID × 15 cm × 4**
Mobile phase: THF
Flow rate: 0.35 mL/min
Detection: RI
Temperature: 40 °C
Injection vol.: 10 µL
Sample: polyvinylbutyral (3 g/L)



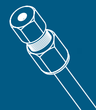
Ordering Information - TSKgel SW columns

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
23449	TSKgel UP-SW3000, 2 µm, 25 nm	silica	Stainless Steel	4.6	15
23448	TSKgel UP-SW3000, 2 µm, 25 nm	silica	Stainless Steel	4.6	30
23450	TSKgel Guard Column for TSKgel UP-SW3000, column, 2 µm	silica	Stainless Steel	4.6	2
23451	TSKgel Guard Column DC* for TSKgel UP-SW3000, column, 2 µm	silica	Stainless Steel	4.6	2
	* The guard column can be directly connected to the analytical column without tubing between the two columns. A male-type outlet endfitting on the guard column enables the direct connection to the screw-type endfitting of the analytical column.				
22854	TSKgel SuperSW mAb HR, 4 µm, 25 nm	silica	Stainless Steel	7.8	30
22855	TSKgel SuperSW mAb HTP, 4 µm, 25 nm	silica	Stainless Steel	4.6	15
22856	TSKgel UltraSW Aggregate, 3 µm, 30 nm	silica	Stainless Steel	7.8	30
22857	TSKgel Guard Column for TSKgel SuperSW mAb HR column, 4 µm	silica	Stainless Steel	6	4
22858	TSKgel Guard Column for TSKgel SuperSW mAb HTP column, 4 µm	silica	Stainless Steel	3	2
22859	TSKgel Guard Column for TSKgel UltraSW Aggregate column, 3 µm	silica	Stainless Steel	6	4
16215	QC-PAK GFC 200, 5 µm, 12.5 nm	silica	Stainless Steel	7.8	15
16049	QC-PAK GFC 300, 5 µm, 25 nm	silica	Stainless Steel	7.8	15
08543	TSKgel Guard Column for 7.8 mm ID TSKgel QC-PAK GFC columns, 7 µm	silica	Stainless Steel	6	4
08800	TSKgel G3000SW Glass, 10 µm, 25 nm	silica	Glass	8	30
08801	TSKgel G4000SW Glass, 13 µm, 45 nm	silica	Glass	8	30
05788	TSKgel G2000SW, 10 µm, 12.5 nm	silica	Stainless Steel	7.5	30
05102	TSKgel G2000SW, 10 µm, 12.5 nm	silica	Stainless Steel	7.5	60
05789	TSKgel G3000SW, 10 µm, 25 nm	silica	Stainless Steel	7.5	30
05103	TSKgel G3000SW, 10 µm, 25 nm	silica	Stainless Steel	7.5	60
05790	TSKgel G4000SW, 13 µm, 45 nm	silica	Stainless Steel	7.5	30
05104	TSKgel G4000SW, 13 µm, 45 nm	silica	Stainless Steel	7.5	60
06727	TSKgel G2000SW, 13 µm, 12.5 nm	silica	Stainless Steel	21.5	30
05146	TSKgel G2000SW, 13 µm, 12.5 nm	silica	Stainless Steel	21.5	60
06728	TSKgel G3000SW, 13 µm, 25 nm	silica	Stainless Steel	21.5	30
05147	TSKgel G3000SW, 13 µm, 25 nm	silica	Stainless Steel	21.5	60
06729	TSKgel G4000SW, 17 µm, 45 nm	silica	Stainless Steel	21.5	30
05148	TSKgel G4000SW, 17 µm, 45 nm	silica	Stainless Steel	21.5	60
08805	TSKgel Guard Column for 8 mm ID TSKgel G3000SW-G4000SW glass columns, 10 µm	silica	Glass	8	4
05371	TSKgel Guard Column for 7.5 mm ID TSKgel G2000SW-G4000SW columns, 10 µm	silica	Stainless Steel	7.5	7.5
05758	TSKgel Guard Column for 21.5 mm ID TSKgel G2000SW-G4000SW columns, 13 µm	silica	Stainless Steel	21.5	7.5
21463	TSKgel BioAssist DS, 15 µm for Desalting Applications	silica	PEEK	4.6	15
21464	TSKgel BioAssist DS, 15 µm for Desalting Applications	silica	PEEK	10	15
20027	TSKgel BioAssist G2SWXL, 5 µm, 12.5 nm	silica	PEEK	7.8	30

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
20026	TSKgel BioAssist G3SWXL, 5 µm, 25 nm	silica	PEEK	7.8	30
20025	TSKgel BioAssist G4SWXL, 8 µm, 45 nm	silica	PEEK	7.8	30
08540	TSKgel G2000SWXL, 5 µm, 12.5 nm	silica	Stainless Steel	7.8	30
08541	TSKgel G3000SWXL, 5 µm, 25 nm	silica	Stainless Steel	7.8	30
08542	TSKgel G4000SWXL, 8 µm, 45 nm	silica	Stainless Steel	7.8	30
18008	TSKgel Guard Column for 7.8 mm ID TSKgel G2SWXL-G4SWXL BioAssist columns, 7 µm	silica	PEEK	6	4
08543	TSKgel Guard Column for 7.8 mm ID TSKgel G2000SWXL-G4000SWXL columns, 7 µm	silica	Stainless Steel	6	4
21845	TSKgel SuperSW3000, 4 µm, 25 nm	silica	Stainless Steel	1	30
21485	TSKgel SuperSW3000, 4 µm, 25 nm	silica	Stainless Steel	2	30
18674	TSKgel SuperSW2000, 4 µm, 12.5 nm	silica	Stainless Steel	4.6	30
18675	TSKgel SuperSW3000, 4 µm, 25 nm	silica	Stainless Steel	4.6	30
18762	TSKgel Guard Column for 4.6 mm ID TSKgel SuperSW2000 & SuperSW3000 columns, 4 µm	silica	Stainless Steel	4.6	3.5
08544	TSKgel SWXL Top-Off, 5 µm, for TSKgel SWXL and QC-PAK columns, 1 g	silica			
06819	TSKgel SW Top-Off, 10 µm, for 7.5 mm ID TSKgel SW columns, 1 g	silica			

Ordering Information - TSKgel PW columns

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
20024	TSKgel BioAssist G6PW, 17 µm, >100 nm	polymer	PEEK	7.8	30
05761	TSKgel G2000PW, 12 µm, 12.5 nm	polymer	Stainless Steel	7.5	30
05105	TSKgel G2000PW, 12 µm, 12.5nm	polymer	Stainless Steel	7.5	60
08028	TSKgel G2500PW, 12 µm, 12.5 nm	polymer	Stainless Steel	7.5	30
08029	TSKgel G2500PW, 12 µm, 12.5 nm	polymer	Stainless Steel	7.5	60
05762	TSKgel G3000PW, 12 µm, 20 nm	polymer	Stainless Steel	7.5	30
05106	TSKgel G3000PW, 12 µm, 20 nm	polymer	Stainless Steel	7.5	60
05763	TSKgel G4000PW, 17 µm, 50 nm	polymer	Stainless Steel	7.5	30
05107	TSKgel G4000PW, 17 µm, 50 nm	polymer	Stainless Steel	7.5	60
05764	TSKgel G5000PW, 17 µm, 100 nm	polymer	Stainless Steel	7.5	30
05108	TSKgel G5000PW, 17 µm, 100 nm	polymer	Stainless Steel	7.5	60
05765	TSKgel G6000PW, 17 µm, >100 nm	polymer	Stainless Steel	7.5	30
05109	TSKgel G6000PW, 17 µm, >100 nm	polymer	Stainless Steel	7.5	60
08026	TSKgel GMPW, 17 µm, mixed bed	polymer	Stainless Steel	7.5	30
08027	TSKgel GMPW, 17 µm, mixed bed	polymer	Stainless Steel	7.5	60
16248	TSKgel G2500PW, 17 µm, 12.5 nm	polymer	Stainless Steel	21.5	30
16249	TSKgel G3000PW, 17 µm, 20 nm	polymer	Stainless Steel	21.5	30
08030	TSKgel G2500PW, 17 µm, 12.5 nm	polymer	Stainless Steel	21.5	60
06763	TSKgel Guard Column for 7.5 mm ID TSKgel G2000PW columns, 13 µm	polymer	Stainless Steel	7.5	7.5
06762	TSKgel Guard Column for 7.5 mm ID TSKgel G2500PW-GMPW columns, 12 µm	polymer	Stainless Steel	7.5	7.5
06758	TSKgel Guard Column for 21.5 mm ID TSKgel G2500-G3000PW columns, 17 µm	polymer	Stainless Steel	21.5	7.5



Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
08020	TSKgel G2500PWXL, 7 µm, 12.5 nm	polymer	Stainless Steel	7.8	30
08021	TSKgel G3000PWXL, 7 µm, 20 nm	polymer	Stainless Steel	7.8	30
08022	TSKgel G4000PWXL, 10 µm, 50 nm	polymer	Stainless Steel	7.8	30
08023	TSKgel G5000PWXL, 10 µm, 100 nm	polymer	Stainless Steel	7.8	30
08024	TSKgel G6000PWXL, 13 µm, >100 nm	polymer	Stainless Steel	7.8	30
08025	TSKgel GMPWXL, 13 µm, mixed bed	polymer	Stainless Steel	7.8	30
08032	TSKgel G-DNA-PW, 10 µm, >100 nm	polymer	Stainless Steel	7.8	30
08031	TSKgel G-Oligo-PW, 7 µm, 12.5 nm	polymer	Stainless Steel	7.8	30
22792	TSKgel SuperOligoPW, 3 µm, 12.5 nm	polymer	Stainless Steel	6	15
08033	TSKgel Guard Column for 7.8 mm ID TSKgel G2500PWXL-GMPWXL columns, 12 µm	polymer	Stainless Steel	6	4
08033	TSKgel Guard Column for 7.8 mm ID TSKgel G-DNA-PW column, 12 µm	polymer	Stainless Steel	6	4
08034	TSKgel Guard Column for 7.8 mm ID TSKgel G-Oligo-PW column, 13 µm	polymer	Stainless Steel	6	4
22796	TSKgel Guard colμmn for 6 mm ID TSKgel SuperOligoPW colμmn, 4 µm	polymer	Stainless Steel	4.6	3.5
21873	TSKgel G3000PWXL-CP, 7 µm, 20 nm	polymer	Stainless Steel	7.8	30
21874	TSKgel G5000PWXL-CP, 10 µm, 100 nm	polymer	Stainless Steel	7.8	30
21875	TSKgel G6000PWXL-CP, 13 µm, >100 nm	polymer	Stainless Steel	7.8	30
21876	TSKgel Guard Column for 7.8 mm ID TSKgel G3000-G6000PWXL-CP columns, 13 µm	polymer	Stainless Steel	6	4
22789	TSKgel SuperMultiporePW-N, 4 µm, 20 nm	polymer	Stainless Steel	6	15
22790	TSKgel SuperMultiporePW-M, 5 µm, 100 nm	polymer	Stainless Steel	6	15
22791	TSKgel SuperMultiporePW-H, 8 µm, >100 nm	polymer	Stainless Steel	6	15
22794	TSKgel SuperMP(PW)-M Guard, 8 µm	polymer	Stainless Steel	4.6	3.5
22793	TSKgel SuperMP(PW)-N Guard, 5 µm	polymer	Stainless Steel	4.6	3.5
22795	TSKgel SuperMP(PW)-H Guard, 12 µm	polymer	Stainless Steel	4.6	3.5
08035	TSKgel Top-Off for PWXL and G-DNA-PW, 10 µm, 1 g	polymer			

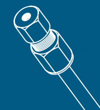
Ordering Information - TSKgel SuperAW and Alpha columns

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
19315	TSKgel SuperAW2500, 4 µm, 2.5 nm	polymer	Stainless Steel	6	15
19316	TSKgel SuperAW3000, 4 µm, 15 nm	polymer	Stainless Steel	6	15
19317	TSKgel SuperAW4000, 6 µm, 45 nm	polymer	Stainless Steel	6	15
19318	TSKgel SuperAW5000, 7 µm, 100 nm	polymer	Stainless Steel	6	15
19319	TSKgel SuperAW6000, 9 µm, >100 nm	polymer	Stainless Steel	6	15
19320	TSKgel SuperAWM-H, 9 µm, mixed bed	polymer	Stainless Steel	6	15
19321	TSKgel Guard Column for 6.0 mm ID TSKgel SuperAW2500-4000 columns, 7 µm	polymer	Stainless Steel	4.6	3.5
19322	TSKgel Guard Column for 6.0 mm ID TSKgel SuperAW5000-AWM-H columns, 13 µm	polymer	Stainless Steel	4.6	3.5

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
18339	TSKgel Alpha-2500, 7 µm, 12.5 nm	polymer	Stainless Steel	7.8	30
18340	TSKgel Alpha-3000, 7 µm, 15 nm	polymer	Stainless Steel	7.8	30
18341	TSKgel Alpha-4000, 10 µm, 45 nm	polymer	Stainless Steel	7.8	30
18342	TSKgel Alpha-5000, 10 µm, 100 nm	polymer	Stainless Steel	7.8	30
18343	TSKgel Alpha-6000, 13 µm, >100 nm	polymer	Stainless Steel	7.8	30
18344	TSKgel Alpha-M, 13 µm, mixed bed	polymer	Stainless Steel	7.8	30
18345	TSKgel Guard Column for 7.8 mm ID TSKgel Alpha-2500-Alpha-M columns, 13 µm	polymer	Stainless Steel	6	4

Ordering Information - TSKgel H columns

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
16131	TSKgel G1000HXL, 5 µm, 1.5 nm	polymer	Stainless Steel	7.8	30
16134	TSKgel G2000HXL, 5 µm, 2 nm	polymer	Stainless Steel	7.8	30
16135	TSKgel G2500HXL, 5 µm, 3 nm	polymer	Stainless Steel	7.8	30
16136	TSKgel G3000HXL, 6 µm, 7.5 nm	polymer	Stainless Steel	7.8	30
16137	TSKgel G4000HXL, 5 µm, 20 nm	polymer	Stainless Steel	7.8	30
16138	TSKgel G5000HXL, 9 µm, 65 nm	polymer	Stainless Steel	7.8	30
16139	TSKgel G6000HXL, 9 µm, > 65 nm	polymer	Stainless Steel	7.8	30
16140	TSKgel G7000HXL, 9 µm, > 65 nm	polymer	Stainless Steel	7.8	30
16141	TSKgel GMHXL, 9 µm, mixed bed	polymer	Stainless Steel	7.8	30
16652	TSKgel GMHXL-L, 5 µm, mixed bed	polymer	Stainless Steel	7.8	30
18403	TSKgel MultiporeHXL-M, 5 µm	polymer	Stainless Steel	7.8	30
07113	TSKgel Guard Column for 7.8 mm ID TSKgel G1000HXL-G4000HXL columns, 8 µm	polymer	Stainless Steel	6	4
13727	TSKgel Guard Column for 7.8 mm ID TSKgel G5000HXL-GMHXL & GMHXL-L columns, 13 µm	polymer	Stainless Steel	6	4
18404	TSKgel Guard Column for TSKgel MultiporeHXL-M column, 5 µm	polymer	Stainless Steel	6	4
17352	TSKgel G1000HHR, 5 µm, 1.5 nm	polymer	Stainless Steel	7.8	30
17353	TSKgel G2000HHR, 5 µm, 2 nm	polymer	Stainless Steel	7.8	30
17354	TSKgel G2500HHR, 5 µm, 3 nm	polymer	Stainless Steel	7.8	30
17355	TSKgel G3000HHR, 5 µm, 7.5 nm	polymer	Stainless Steel	7.8	30
17356	TSKgel G4000HHR, 5 µm, 20 nm	polymer	Stainless Steel	7.8	30
17357	TSKgel G5000HHR, 5 µm, 65 nm	polymer	Stainless Steel	7.8	30
17358	TSKgel G6000HHR, 5 µm, > 65 nm	polymer	Stainless Steel	7.8	30
17359	TSKgel G7000HHR, 5 µm, > 65 nm	polymer	Stainless Steel	7.8	30
17362	TSKgel GMHHR-L, 5 µm, mixed bed	polymer	Stainless Steel	7.8	30
17392	TSKgel GMHHR-M, 5 µm, mixed bed	polymer	Stainless Steel	7.8	30
18055	TSKgel GMHHR-N, 5 µm, mixed bed	polymer	Stainless Steel	7.8	30
17360	TSKgel GMHHR-H, 5 µm, mixed bed	polymer	Stainless Steel	7.8	30
17361	TSKgel GMHHR-H (S), 13 µm, mixed bed	polymer	Stainless Steel	7.8	30
17393	TSKgel GMHHR-M (S), 13 µm, mixed bed	polymer	Stainless Steel	7.8	30
18399	TSKgel GMHHR-H (20), 20 µm, mixed bed	polymer	Stainless Steel	7.8	30
18398	TSKgel GMHHR-H (30), 30 µm, mixed bed	polymer	Stainless Steel	7.8	30
18393	TSKgel GMHHR-H (S) HT, 13 µm, mixed bed	polymer	Stainless Steel	7.8	30



Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
18395	TSKgel G2000HHR (20) HT, 20 µm, 2 nm	polymer	Stainless Steel	7.8	30
18392	TSKgel GMHHR-H (20) HT, 20 µm, mixed bed	polymer	Stainless Steel	7.8	30
18391	TSKgel GMHHR-H (30) HT, 30 µm, mixed bed	polymer	Stainless Steel	7.8	30
18420	TSKgel GMHHR-H HT, 5 µm, mixed bed	polymer	Stainless Steel	7.8	30
22890	TSKgel G2000HHR (20) HT2, 20 µm, 2 nm	polymer	Stainless Steel	7.8	30
22888	TSKgel GMHHR-H (20) HT2, 20 µm, mixed bed	polymer	Stainless Steel	7.8	30
22887	TSKgel GMHHR-H (30) HT2, 30 µm, mixed bed	polymer	Stainless Steel	7.8	30
22889	TSKgel GMHHR-H (S) HT2, 13 µm, mixed bed	polymer	Stainless Steel	7.8	30
17368	TSKgel Guard Column for 7.8 mm ID TSKgel G1000HHR-G4000HHR & GMHHR-L columns, 5 µm	polymer	Stainless Steel	6	4
17369	TSKgel Guard Column for 7.8 mm ID TSKgel G5000HHR-G7000HHR & GMHHR-M;-N;-H columns, 7 µm	polymer	Stainless Steel	6	4
17367	TSKgel Guard Column for TSKgel GMHHR-H (S), -M (S) columns, 13 µm	polymer	Stainless Steel	7.5	7.5
18402	TSKgel Guard Column for TSKgel GMHHR-H (20), -H (30) columns, 30 µm	polymer	Stainless Steel	7.5	7.5
18397	TSKgel Guard Column for 7.8 mm ID TSKgel GMHHR-H (S) HT column, 13 µm	polymer	Stainless Steel	7.5	7.5
18396	TSKgel Guard Column for 7.8 mm ID TSKgel GMHHR-H (20) HT and GMHHR-H (30) HT columns, 30 µm	polymer	Stainless Steel	7.5	7.5
22891	TSKgel Guard Column for 7.8 mm ID TSKgel GMHHR-H (20) HT2 and GMHHR-H (30) HT2 columns, 30 µm	polymer	Stainless Steel	7.5	7.5
22892	TSKgel Guard Column for 7.8 mm ID TSKgel GMHHR-H (S) HT2 column, 13 µm	polymer	Stainless Steel	7.5	7.5
17990	TSKgel SuperH1000, 3 µm, 1.5 nm	polymer	Stainless Steel	6	15
17991	TSKgel SuperH2000, 3 µm, 2 nm	polymer	Stainless Steel	6	15
17992	TSKgel SuperH2500, 3 µm, 3 nm	polymer	Stainless Steel	6	15
17993	TSKgel SuperH3000, 3 µm, 7.5 nm	polymer	Stainless Steel	6	15
17994	TSKgel SuperH4000, 3 µm, 20 nm	polymer	Stainless Steel	6	15
17995	TSKgel SuperH5000, 3 µm, 65 nm	polymer	Stainless Steel	6	15
17996	TSKgel SuperH6000, 5 µm, > 65 nm	polymer	Stainless Steel	6	15
17997	TSKgel SuperH7000, 5 µm, > 65 nm	polymer	Stainless Steel	6	15
17998	TSKgel SuperHM-L, 3 µm, mixed bed	polymer	Stainless Steel	6	15
17999	TSKgel SuperHM-N, 3 µm, mixed bed	polymer	Stainless Steel	6	15
18000	TSKgel SuperHM-M, 3 µm, mixed bed	polymer	Stainless Steel	6	15
18001	TSKgel SuperHM-H, 3 µm, mixed bed	polymer	Stainless Steel	6	15
18002	TSKgel Guard Column for 6 mm ID TSKgel SuperH1000-SuperH4000 columns, 3 µm	polymer	Stainless Steel	4.6	3.5
18003	TSKgel Guard Column for 6 mm ID TSKgel SuperH5000-7000;HM-L;-N;-M;-H columns, 3 µm	polymer	Stainless Steel	4.6	3.5
19309	TSKgel SuperHZ1000, 3 µm, 1.5 nm	polymer	Stainless Steel	4.6	15
19310	TSKgel SuperHZ2000, 3 µm, 2 nm	polymer	Stainless Steel	4.6	15
19311	TSKgel SuperHZ2500, 3 µm, 3 nm	polymer	Stainless Steel	4.6	15
19312	TSKgel SuperHZ3000, 3 µm, 7.5 nm	polymer	Stainless Steel	4.6	15
19313	TSKgel SuperHZ4000, 3 µm, 20 nm	polymer	Stainless Steel	4.6	15
19660	TSKgel SuperHZM-N, 3 µm, mixed bed	polymer	Stainless Steel	4.6	15
19662	TSKgel SuperHZM-M, 3 µm, mixed bed	polymer	Stainless Steel	4.6	15

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
19664	TSKgel SuperHZM-H, 10 µm, mixed bed	polymer	Stainless Steel	4.6	15
19302	TSKgel SuperHZ1000, 3 µm, 1.5 nm	polymer	Stainless Steel	6	15
19303	TSKgel SuperHZ2000, 3 µm, 2 nm	polymer	Stainless Steel	6	15
19304	TSKgel SuperHZ2500, 3 µm, 3 nm	polymer	Stainless Steel	6	15
19305	TSKgel SuperHZ3000, 3 µm, 7.5 nm	polymer	Stainless Steel	6	15
19306	TSKgel SuperHZ4000, 3 µm, 20 nm	polymer	Stainless Steel	6	15
19661	TSKgel SuperHZM-N, 3 µm, mixed bed	polymer	Stainless Steel	6	15
19663	TSKgel SuperHZM-M, 3 µm, mixed bed	polymer	Stainless Steel	6	15
19665	TSKgel SuperHZM-H, 10 µm, mixed bed	polymer	Stainless Steel	6	15
19314	TSKgel Guard Column for 4.6 mm ID TSKgel SuperHZ1000-4000 and HZM-N & -M columns, 3 µm	polymer	Stainless Steel	4.6	2
19668	TSKgel Guard Column for 4.6 mm ID TSKgel SuperHZM-H column, 10 µm	polymer	Stainless Steel	4.6	2
19666	TSKgel Guard Column for 6 mm ID TSKgel SuperHZ1000-4000 and HZM-N & -M columns, 3 µm	polymer	Stainless Steel	4.6	3.5
19667	TSKgel Guard Column for 6 mm ID TSKgel SuperHZM-H column, 10 µm	polymer	Stainless Steel	4.6	3.5
21815	TSKgel SuperMultiporeHZ-N, 3 µm, 8 nm	polymer	Stainless Steel	4.6	15
21885	TSKgel SuperMultiporeHZ-H, 6 µm, > 14 nm	polymer	Stainless Steel	4.6	15
21488	TSKgel SuperMultiporeHZ-M, 4 µm, 14 nm	polymer	Stainless Steel	4.6	15
21816	TSKgel SuperMPHZ-N Guard, 3 µm	polymer	Stainless Steel	4.6	2
21886	TSKgel SuperMPHZ-H Guard, 6 µm	polymer	Stainless Steel	4.6	2
21489	TSKgel SuperMPHZ-M Guard, 4 µm	polymer	Stainless Steel	4.6	2

Anion Exchange columns

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

Cation Exchange columns

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

Ion Exchange Tips:

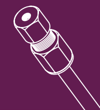
- TSKgel ion exchange columns are offered in glass, PEEK, and stainless steel hardware. Stainless steel (SS) or Pyrex frits are embedded in the body of the column end-fittings of metal and glass columns, respectively. The nominal frit size for SS columns is engraved in the end-fittings; Pyrex frits in the glass columns have a 10 µm nominal pore size.

- Halide salts corrode stainless steel tubing, fitting, and frits. Do not store SS columns in a mobile phase containing NaCl and, where possible, use another salt in the operating buffer. Chlorotrifluorethylene and tetrafluorethylene are the materials in the glass column fittings that come into contact with the mobile phase and sample.

- Good laboratory procedures demand that the analytical column be protected by a guard column. TSKgel guardgel kits, containing column hardware and gel packing, are available to pack your own guard column. In addition, guard cartridges and packed guard columns are available for use with TSKgel ion exchange columns.

- TSKgel ion exchange columns are supplied with an Inspection Data Sheet, which includes a QC chromatogram and test data, and an OCS Sheet summarizing the recommended operating conditions for optimum column performance.

- A separate TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems, can be downloaded from the Tosoh Bioscience LLC website (www.tosohbioscience.com).



About Ion Exchange Chromatography

Ion Exchange Chromatography (IEC) is a technique based on the difference in the strength of the interaction between a sample ion and an oppositely charged functional group on the support. The sample ion competes for the functional group with a counter ion that has been added to the mobile phase as a salt. Elution is most often accomplished by increasing the salt concentration over time.

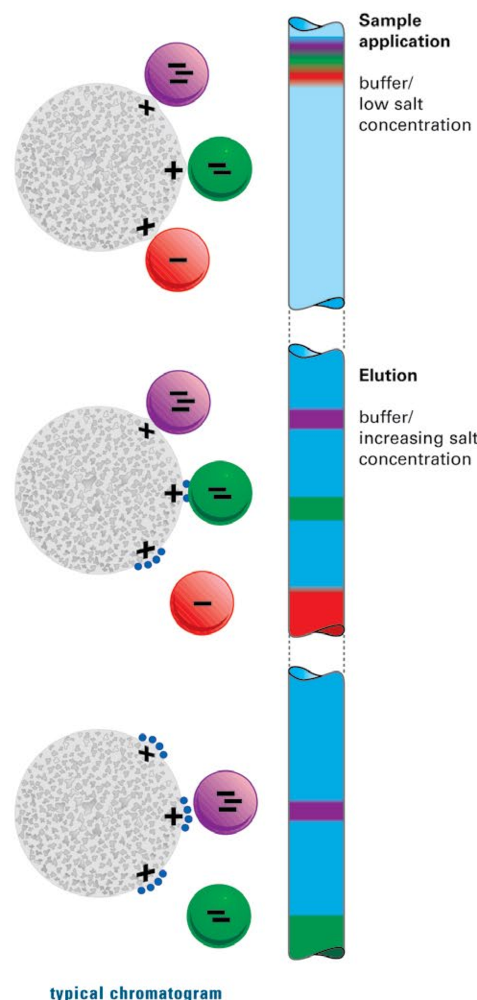
Ion exchange chromatography is the most common separation mode for protein purification schemes. Biomolecules generally have charged groups on their surfaces, which change with the pH of the solution.

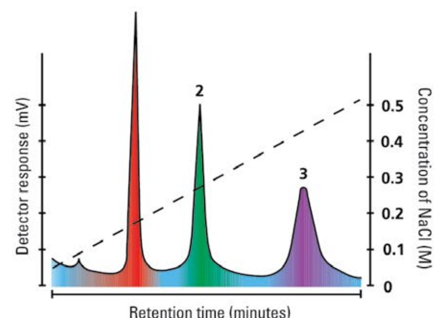
Anion Exchange Chromatography is performed with either a strong anion exchange column, containing a quaternary ammonium ion, or with a weak anion exchanger, having either a tertiary or secondary amine functional group, such as DEAE (diethylaminoethyl). A counter ion, often Cl^-, maintains electroneutrality.

Cation Exchange Chromatography is performed with either a strong cation exchanger, containing a bonded sulfonic acid group, such as sulfopropyl (SP), or with a weak cation exchanger, containing a weak acid such as carboxymethyl (CM). A counter ion, often Na^+, maintains electroneutrality. The advantage of strong vs. weak ion exchangers is that the first are charged over a wider pH range. Weak ion exchangers often provide slightly different selectivity from strong exchangers.

In ion exchange chromatography, the pH of the mobile phase buffer must be between the pI or pKa of the charged molecule and the pKa of the charged groups on the solid support. For example, a molecule with a pI of 8.2 is run in a mobile phase buffer at pH 6.0 with the solid support pKa at 1.2 in cation exchange chromatography. In anion exchange chromatography a molecule with a pI of 6.8 is run in a mobile phase buffer at pH 8.0 with the solid support pKa at 10.3.

Figure 1: Ion Exchange Chromatography





TSKgel Anion and Cation Exchange Chromatography Columns

Tosoh Bioscience offers a broad line of high efficiency columns for analysis and isolation of biomolecules by ion exchange chromatography. Methacrylate, silica, hydrophilic polymer, and polystyrene are used as matrices for the TSKgel line of anion and cation exchange columns. Tables 1 and 2 list the available columns according to matrix and summarize the features and benefits of TSKgel ion exchange columns.

- TSKgel STAT ion exchange columns:
  These are nonporous polymer columns with high surface density of functional groups: quaternary ammonium for anion exchange (Q- and DNA-STAT), carboxymethyl (CM-STAT) and sulfopropyl (SP-STAT) for cation exchange. Particle sizes and dimensions of the TSKgel STAT columns are optimized either for highest throughput or for highest efficiency. Applications for the TSKgel STAT columns include the separation of proteins, protein aggregates, charge isomers of monoclonal antibodies, PEGylated proteins, DNA fragments, nucleic acids, oligo DNA, and siRNA.

- TSKgel DEAE-5PW, SP-5PW, CM-5PW, SuperQ-5PW ion exchange columns:
  The polymethacrylate-based resin, TSKgel 5PW, is a spherical 10 µm particle with approximately 100 nm pores. It is derivatized with diethylaminoethyl (DEAE), sulfopropyl (SP) or carboxymethyl (CM) functionalities to provide a weak anion, a strong cation, and a weak cation exchanger, respectively. The polyamine chemistry employed in TSKgel SuperQ-5PW results in a high capacity strong anion exchanger with a smaller effective pore size than TSKgel DEAE-5PW. Proteins, peptides, DNA- and RNA-derived oligonucleotides, and other nucleic acid fragments are typical samples that are analyzed or isolated on the methacrylate-based TSKgel ion exchange columns.

- TSKgel BioAssist ion exchange columns:
  These columns are also based on methacrylate particle design technology. Particles in TSKgel BioAssist Q columns contain very large pores (~400 nm) that are functionalized with polyamine groups to form a network structure. The capacity of the TSKgel BioAssist Q columns is high over a wide molecular weight range (up to 1.0×10^6 Da). TSKgel BioAssist S columns are packed with particles possessing 130 nm pores functionalized with sulfopropyl groups. TSKgel BioAssist columns are available exclusively in PEEK housing.

- TSKgel DEAE-NPR, DNA-NPR and SP-NPR ion exchange columns:
  Methacrylate is the backbone of these nonporous resin columns, which 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. Due to the absence of large pores, protein recovery is generally very high on TSKgel NPR columns.

- TSKgel DEAE-2SW, DEAE-3SW, QAE-2SW, SP-2SW, CM-2SW, CM-3SW ion exchange columns:
  Silica-based TSKgel anion and cation exchange columns with diethylaminoethyl (DEAE), sulfopropyl (SP), trimethylamino (QAE), and carboxymethyl (CM) functional groups are available for analyzing smaller molar mass samples such as nucleotides, drug candidates, catecholamines, and small peptides or proteins. Binding capacity for small to medium size proteins on these columns is approximately double that of the TSKgel 5PW packings due to the smaller pore size and larger surface area.

- Specialty TSKgel polystyrene-based ion exchange columns:
  These columns are available for the analysis of mono- and disaccharides, organic acids and sugar alcohols.

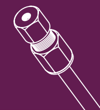


Table 1: Features and benefits of TSKgel cation exchange columns

TSKgel Column Type	Type/Matrix	Benefit
CM-STAT, SP-STAT	strong(SP-STAT), weak (CM-STAT/polymer	Nonporous with high surface density of carboxymethyl (CM) and sulfopropyl (SP) groups
CM-5PW, SP-5PW	strong (SP-5PW), weak (CM-5PW)/polymethacrylate	Polymethacrylate resin derivatized with carboxymethyl (CM) and sulfopropyl (SP) ligands
BioAssist S	strong/polymethacrylate	Contain very large pores (130 nm), resulting in high binding capacity and improved recovery of activity; available exclusively in PEEK housing
SP-NPR	strong/polymethacrylate	Nonporous with 2.5 µm particles; fast analysis; high protein recovery
CM-2SW, CM-3SW, SP-2SW	strong (SP-2SW), weak (CM-2SW, CM-3SW)/silica	Silica-based with carboxymethyl (CM) and sulfopropyl (SP) functional groups
SCX, OApak-A	strong (SCX), weak (OApak-A)/ polymethacrylate	Specialty columns for the analysis of organic acids, saccharides and alcohols

Table 2: Features and benefits of TSKgel anion exchange columns

TSKgel Column Type	Type/Matrix	Benefit
Q-STAT, DNA-STAT	strong (Q-STAT), weak (DNA-STAT)/polymer	Nonporous with high surface density of quaternary ammonium groups
DEAE-5PW, SuperQ-5PW	strong (SuperQ-5PW), weak (DEAE-5PW)/polymethacrylate	Polymethacrylate resin derivatized with diethylaminoethyl (DEAE) and trimethylamino (SuperQ) ligands
BioAssist Q	strong/polymethacrylate	Contain very large pores (400 nm), resulting in high binding capacity and improved recovery of activity; available exclusively in PEEK housing
DEAE-NPR, DNA-NPR	weak/polymethacrylate	Nonporous with 2.5 µm particles; fast analysis; high protein recovery
DEAE-2SW, DEAE-3SW, QAE-2SW	strong (QAE-2SW), weak (DEAE-2SW, DEAE-3SW)/silica	Silica-based with diethylaminoethyl (DEAE), and trimethylamino (QAE) functional groups
Sugar AXG, Suger AXI, SAX	strong/polystyrene	Specialty columns for the analysis of mono and disaccharides, as well as organic acids and sugar alcohols

About: TSKgel BioAssist Q Anion Exchange Columns

Especially designed for the separation of large biomolecules, such as antibodies, the large pores of the TSKgel BioAssist Q anion exchange column offer superior capacity and resolution at a modest column back pressure. The anion exchange functionality of BioAssist Q columns is introduced via a special graft polymerization technique that results in a high density of ionic exchange groups in the large particle pores that normally could only be achieved by using particles containing a much smaller pore size.

TSKgel BioAssist Q anion exchange columns are offered in a 4.6 mm ID × 5 cm format and a 10 mm ID × 10 cm semi-preparative column for scale-up. The hardware for both columns is made of PEEK to reduce protein adsorption.

Attributes and Applications:

Table 3 lists the product attributes of TSKgel BioAssist Q columns. The capacity of these columns is high over a wide molecular weight range (up to 1.0 × 10^6 Da) and they are an excellent choice for high throughput applications.

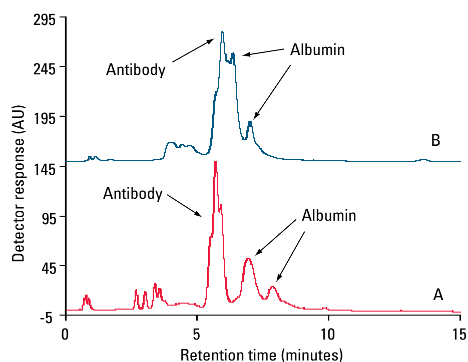
Table 3: Product attributes

Attribute	Value
Matrix	polymethacrylate
Particle size (mean)	10 µm and 13 µm
Pore size (mean)	400 nm
Functional group	polyamine
Counter ion	Cl^-
pH stability	2.0-12.0
Capacity (mg BSA/mL)	70
Small ion capacity	0.1 eq/L
pKa	9.4

Mouse Ascites

Figure 2 compares chromatograms of mouse ascites fluid. Excellent separation between the antibody and albumin has been obtained using a TSKgel BioAssist Q column versus a competitive Q-type product.

Figure 2: Analysis of mouse ascites fluid



Columns:	**A: TSKgel BioAssist Q, 10 µm, 4.6 mm ID × 5 cm**
	B: Commercial Q-type product A, 5.0 mm ID × 5 cm
Mobile phase:	15 min linear gradient of NaCl from 0 to 1.0 mol/L in 20 mmol/L Tris-HCl buffer, pH 8.0
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	5 µL
Sample:	mouse ascites fluid (3-fold dilution with initial eluent)

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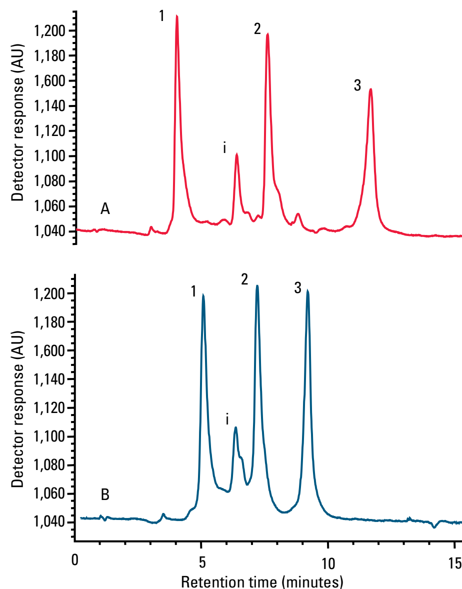
Performance Enhancement on FPLC System

Figure 3 demonstrates the performance enhancement of a TSKgel BioAssist Q column over a competitive product when operated side-by-side on an FPLC system.

Figure 3: Performance enhancement



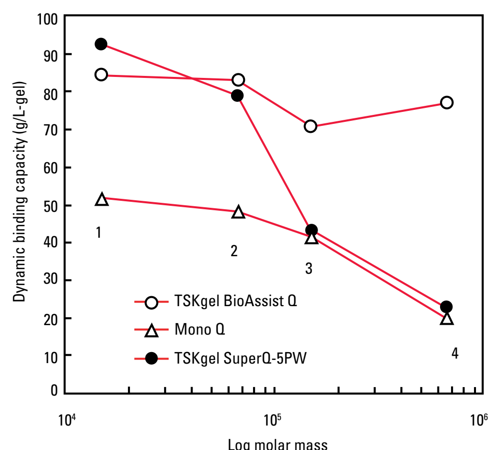
Columns:	**A: TSKgel BioAssist Q, 10 µm, 4.6 mm × 5 cm**
	B: Competitor Q, 5.0 mm ID × 5 cm
Mobile phase:	30 min linear gradient from 0 to 1 mol/L NaCl in 20 mmol/L sodium phosphate, pH 8.0
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Samples:	1. conalbumin
	i. ovalbumin impurity
	2. ovalbumin
	3. trypsin inhibitor

Dynamic Binding Capacity

The dynamic binding capacity for a TSKgel BioAssist Q column and two commercially available columns is shown in Figure 4 as a function of protein molar mass. Dynamic capacity is plotted against the molar mass of 4 proteins varying in molar mass from 2.0×10^4 Da to 6.7×10^5 Da and is determined by continuously loading the column with the protein solution and calculating the amount of protein adsorbed at 10% height of the breakthrough curve.

The binding capacity on TSKgel BioAssist Q is uniformly high for all proteins, while that of Mono Q (80 nm pores) and TSKgel SuperQ-5PW (100 nm pores) is distinctly lower for the larger proteins. It is evident that neither material is optimized for the analysis of monoclonal antibodies, which have a molar mass of 1.5×10^5 Da. Antibodies are blood components and as such are most stable at pH 7.35; they become more labile at acidic pH. Their excess positive charge makes anion exchange chromatography the method of choice for their chromatographic analysis.

Figure 4: Dynamic binding capacity as function of protein molar mass



Columns:	**TSKgel BioAssist Q, 10 µm, 4.6 mm ID × 1 cm**
	Conventional Q-type product A, 5.0 mm ID × 1 cm
	TSKgel SuperQ-5PW, 4.6 mm ID × 1 cm
Mobile phase:	20 mmol/L Tris-HCl buffer, pH 8.0
Flow rate:	0.38 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Samples:	1. trypsin inhibitor, 10 g/L
	2. human serum albumin, 10 g/L
	3. IgG_1, 2.3 g/L
	4. thyroglobulin, 5 g/L

About: TSKgel DEAE-2SW, DEAE-3SW and QAE-2SW Anion Exchange Columns

TSKgel DEAE-2SW, DEAE-3SW, and QAE-2SW columns are packed with porous spherical silica beads which are chemically modified with a weak anion exchange group. These columns are for analyzing smaller molar mass samples such as nucleotides, drug candidates, catecholamines, and small peptides or proteins.

Attributes and Applications:

Table 4 lists the product attributes of the TSKgel DEAE-2SW, DEAE-3SW, and QAE-2SW columns. These columns are packed with particles composed of silica with 12.5 nm and 25 nm pores and are stable in a pH range from 2.0 – 7.5.

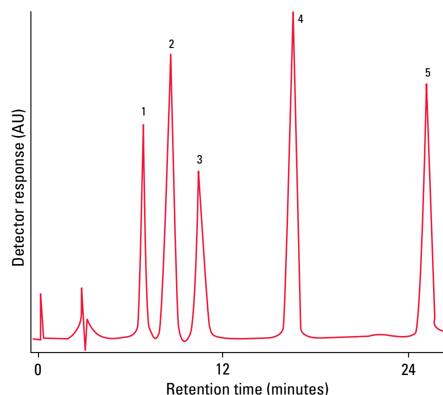
Table 4: Product attributes

TSKgel column	DEAE-2SW	DEAE-3SW	QAE-2SW
Matrix	Silica		
Particle size (mean)	5 µm	10 µm	5 µm
Pore size (mean)	12.5 nm	25 nm	12.5 nm
Functional group	$CH_2CH_2N^+(C_2H_5)_3$	$CH_2CH_2N^+(C_2H_5)_3$	trimethylamino
Counter ion	$H_2PO_4^-$	Cl^-	$H_2PO_4^-$
pH stability	2.0-7.5		
Small ion capacity	>0.3 eq/L		
pKa	11.2		

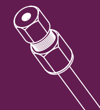
Nucleotides

High performance analyses of small anionic species are best performed on small pore silica-based anion exchange columns, such as TSKgel DEAE-2SW. This is demonstrated in Figure 5.

Figure 5: Separation of nucleotides



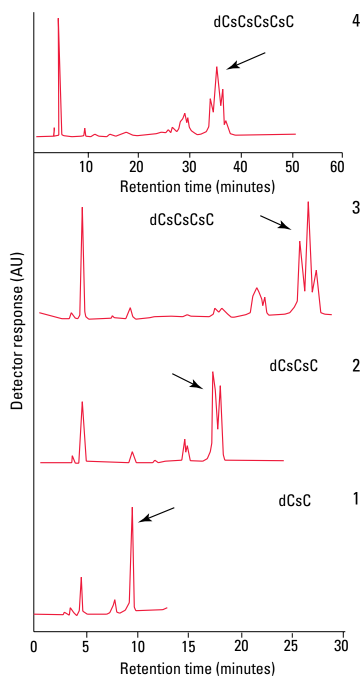
Column: **TSKgel DEAE-2SW, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase: A: CH_3CN in 0.1 mol/L phosphate, pH 3.0, 20/80
B: CH_3CN in 0.5 mol/L phosphate, pH 3.0, 20/80
Gradient: 30 min linear gradient from buffer A to B
Flow rate: 1.0 mL/min
Detection: UV @ 260 nm
Samples: 1. AMP 2. IMP 3. GMP 4. ADP 5. ATP



Oligonucleotides

Backbone-modified oligonucleotides are increasingly used for antisense therapy. These novel oligos have the benefit of longer half-lives due to resistance to endogenous nucleases. One common type of backbone-modified oligonucleotides is phosphorothioates where one of the two nonbridged oxygen atoms in the phosphate linkage has been replaced by a sulfur atom. The separation of several phosphorothioates on a TSKgel DEAE-2SW column is shown in Figure 6.

Figure 6: Separation of phosphorothioates

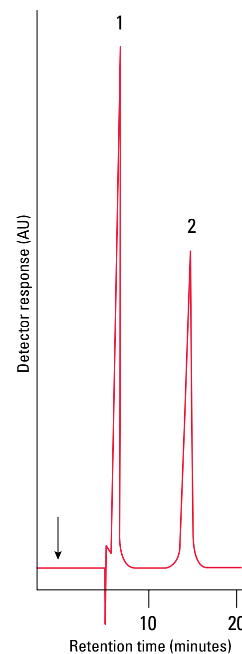


Column:	**TSKgel DEAE-2SW, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase:	A: 50 mmol/L ammonium acetate B: 1.5 mol/L ammonium acetate
Gradient:	linear, 0-100% B in 60 minutes
Flow rate:	1 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. 2 base phosphorothioate oligonucleotide 2. 3 base phosphorothioate oligonucleotide 3. 4 base phosphorothioate oligonucleotide 4. 5 base phosphorothioate oligonucleotide

Deoxyribonucleic Acids

Figure 7 demonstrates the successful separation of adenosine triphosphate (ATP) and deoxyribonucleic acid (DNA) using a TSKgel DEAE-3SW column.

Figure 7: Separation of ATP and DNA



Column:	**TSKgel DEAE-3SW, 10 µm, 6 mm ID × 15 cm**
Mobile phase:	ACN/0.6 mol/L ammonium formate buffer, pH 7.0 = 20/80
Flow rate:	0.7 mL/min
Detection:	UV @ 260 nm
Temperature:	23 °C
Samples:	1. adenosine triphosphate (ATP) 2. deoxyribonucleic acid (d-TCGAGCATAATA), DNA

About: TSKgel DEAE-5PW and SuperQ-5PW Anion Exchange Columns

The polymethacrylate-based resin, TSKgel 5PW, is a spherical 10 µm particle with approximately 100 nm pores. It is derivatized with a diethylaminoethyl (DEAE) functionality to provide the weak anion exchange column, TSKgel DEAE-5PW, and with a polyamine functionality to provide the strong anion exchange column, TSKgel SuperQ-5PW. The polyamine network chemistry employed in TSKgel SuperQ-5PW columns results in a much higher capacity, but also a smaller effective pore size than TSKgel DEAE-5PW columns.

The TSKgel SuperQ-5PW columns are used for the separation and analysis of proteins, oligonucleotides, and other biomolecules. These columns are offered in a stainless steel housing in dimensions of 7.5 mm ID × 7.5 cm and 21.5 mm ID × 15 cm and in an 8 mm ID × 7.5 cm glass format.

TSKgel DEAE-5PW columns are also used for the separation and analysis of proteins, along with nucleotides, nucleosides, and other biomolecules. These columns are available in internal diameters varying from 2 mm to 21.5 mm and in column housings of either glass or stainless steel.

Attributes and Applications:

Table 5 lists the product attributes of TSKgel SuperQ-5PW and DEAE-5PW columns. These columns are an excellent choice for biologically active molecules. TSKgel SuperQ-5PW and DEAE-5PW columns are stable over a pH range from 2.0 – 12.0 and have a mean pore size of 100 nm.

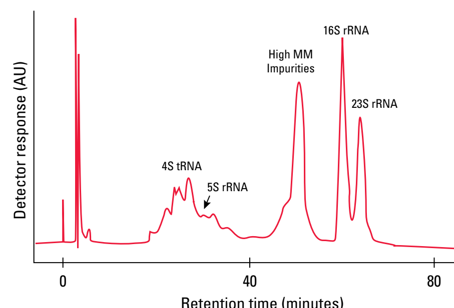
Table 5: Product attributes

TSKgel column	SuperQ-5PW	DEAE-5PW
Matrix	polymethacrylate	
Particle size (mean)	10 µm and 13 µm	10 µm, 13 µm, and 20 µm
Pore size (mean)	100 nm	
Functional group	trimethylamino	$CH_2CH_2N^+(C_2H_5)_3$
Counter ion	Cl^-	
pH stability	2.0-12.0	
Capacity (mg BSA/mL)	100	30
Small ion capacity	>0.13 eq/L	0.1 eq/L
pKa	12.2	11.5

E. coli RNA

Figure 8 shows the fractionation of high molar mass *E. coli* RNA on TSKgel DEAE-5PW, effectively utilizing the large 100 nm pores of the TSKgel 5PW resin.

Figure 8: Analysis of high MM RNA

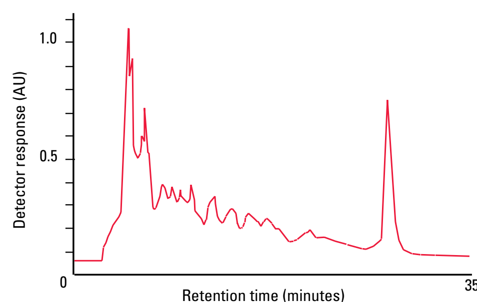


Column: **TSKgel DEAE-5PW, 10 µm, 6 mm ID × 15 cm (custom)**
Mobile phase: 300 min linear gradient from 0.3 mol/L to 1.0 mol/L NaCl in 0.1 mol/L Tris-HCl, pH 7.6
Flow rate: 1.0 mL/min
Detection: UV @ 260 nm
Sample: total *E. coli* RNA

Plasmid

Figure 9 illustrates the separation of crude pBR322 plasmid on a TSKgel DEAE-5PW column. This chromatographic separation provides purified plasmid in one hour, as opposed to a conventional Cs-Cl density gradient ultracentrifugation, which can take up to three days.

Figure 9: Detection of HIV-1 PCR-Amplified 130 bp target

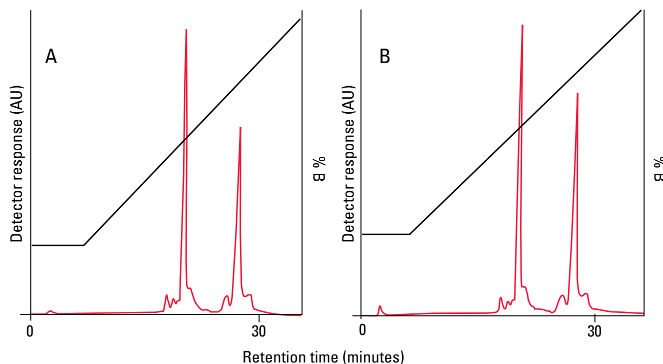


Column: **TSKgel DEAE-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase: A: 25 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0
B: 25 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0, with 1 mol/L NaCl
Gradient: 25-60% B in 30 minutes
Flow rate: 1 mL/min
Detection: UV @ 260 nm
Temperature: 25 °C
Sample: pBR322 plasmid
Sample load: 2.5 mg in 1 mL

Column Stability

Figures 10A & 10B demonstrate the stability of the TSKgel SuperQ-5PW columns. Ovalbumin and trypsin inhibitor were initially loaded onto a TSKgel SuperQ-5PW, 7.5 mm ID × 7.5 cm column (Figure 10A). The column was then cleaned in place (CIP) using a solution of 0.5 mol/L NaOH. This cleaning procedure was repeated once each day for a total of 15 days. The resolution after this cleaning protocol was equivalent to the resolution of the initial injection of the compounds on the column (Figure 10B).

Figures 10A & 10B: Stability of TSKgel SuperQ-5PW columns

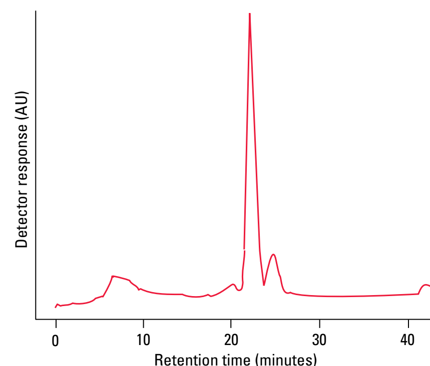


Column:	**TSKgel SuperQ-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	A: 50 mmol/L Tris-HCl, pH 8.6 B: 0.5 mmol/L sodium cloride in 50 mmol/L Tris-HCl, pH 8.6
Gradient:	A-B (60min)
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	100 µL
Sample load:	each of 1 mg
Samples:	1. ovalbumin 2. trypsin inhibitor
Note:	A: before CIP B: after 15 times (15 days)

Oligonucleotides

Figure 11 shows the analysis of a 16-mer morpholine oligonucleotide on a TSKgel SuperQ-5PW column using a NaCl gradient in a 10 mmol/L sodium hydroxide mobile phase.

Figure 11: Analysis of 16-mer oligonucleotide

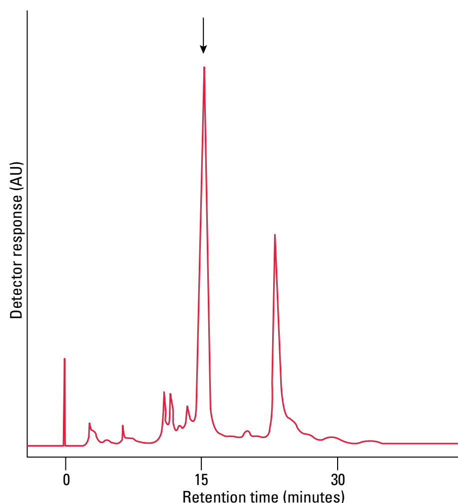


Column:	**TSKgel SuperQ-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	A: 10 mmol/L NaOH B: 10 mmol/L NaOH with 1 mol/L NaCl
Gradient	0 min (0% B) 40 min (50% B) 41 min (100%B) 46 min (100%B)
Flow Rate:	1 mL/min
Detection:	UV @ 254 nm
Sample:	16-mer morpholine oligonucleotide, AAG AAG AAG AGG GGA G
Sample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

Monoclonal Antibody

The separation of a monoclonal antibody (IgG_1) from mouse ascites fluid using a TSKgel DEAE-5PW column is shown in Figure 12. IgG_1 elutes in about 15 minutes, well separated from the impurities that elute before and after IgG_1, such as transferrin (about 11 minutes) and albumin (about 22 minutes).

Figure 12: Separation of monoclonal antibody

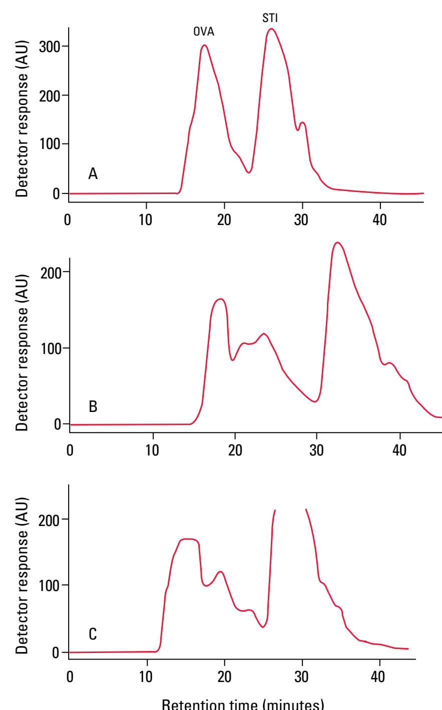


Column:	**TSKgel DEAE-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	A: 20 mmol/L Tris-HCl, pH 8.5 B: A + 0.5 mol/L NaCl A → B linear gradient (60 min)
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Sample:	anti-human albumin (IgG_1), diluted solution of mouse ascites (168 µg in 40 µL)

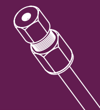
Performance Data

In Figure 13, a separation performed under high sample load conditions was compared on various anion exchange columns. When a 20 mg protein sample is loaded on a 1 mL column volume, only the TSKgel SuperQ-5PW column shows a chromatogram with "normal" looking peaks. Other anion exchange columns show multiple artifact peaks from sample overloading. A 5 mm ID × 5 cm TSKgel SuperQ-5PW column provides sufficient retention and resolution. Thus, isolation of proteins at semi-preparative scale is possible on TSKgel SuperQ-5PW when using an analytical column.

Figure 13: Comparison of various anion exchange columns under large sample load



Columns:	**A: TSKgel SuperQ-5PW, 10 µm, 5 mm ID × 5 cm** B: Company A, Q type, 5 mm ID × 5 cm C: Company A, perfusion Q type, 6.4 mm ID × 3 cm All column volumes were 1.0 mL
Mobile phase:	A: 50 mmol/L Tris-HCl buffer, pH 8.3 B: A + 0.5 mol/L NaCl A → B linear gradient (60 min)
Flow rate:	0.8 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	2 mL
Samples:	ovalbumin, 20 mg trypsin inhibitor, 20 mg



About: TSKgel DEAE-NPR and DNA-NPR Anion Exchange Columns

Methacrylate is the backbone of nonporous resin (NPR) columns such as TSKgel DEAE-NPR and DNA-NPR, which 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. Due to the absence of pores, protein recovery is generally very high on TSKgel DEAE-NPR and DNA-NPR columns.

TSKgel DNA-NPR columns are packed with hydrophilic polymer beads which are surface modified with a weak anion exchange group. Because TSKgel DNA-NPR columns contain nonporous particles, binding capacity is low compared to porous columns with the same ligand functionality. Column dimensions are optimized for the high efficiency separation of DNA fragments, PCR products, or plasmids.

The hydrophilic polymer beads used to pack the TSKgel DEAE-NPR columns are also surface modified with a weak anion exchange group. These columns are used for the high speed separation and analysis of proteins and poly- and oligonucleotides. TSKgel DEAE-NPR columns are particularly useful for high resolution separation of DNA digests or fragments.

Attributes and Applications:

Table 6 lists the product attributes of TSKgel DNA-NPR and DEAE-NPR columns. These columns are stable in a pH range from 2.0 – 12.0 and are packed with spherical 2.5 µm, nonporous particles.

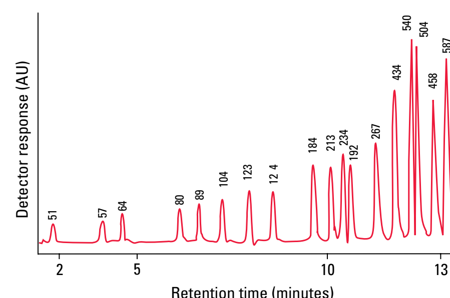
Table 6: Product attributes

TSKgel column	DEAE-NPR	DNA-NPR
Matrix	polymethacrylate	
Particle size (mean)	2.5 µm	
Pore size (mean)	nonporous	
Functional group	$CH_2CH_2N^+(C_2H_5)_3$	proprietary
Counter ion	Cl^-	ClO_4^-
pH stability	2.0-12.0	
Capacity (mg BSA/mL)	5	
Small ion capacity	>0.1 eq/L	
pKa	11.2	

DNA Digests

Because of their small (2.5 µm) particle size, TSKgel DEAE-NPR nonporous columns excel in rapid separations of large polynucleotides in DNA digests. A chromatogram of a standard Hae III digest of pBR322 plasmid DNA is shown in Figure 14.

Figure 14: Analysis of DNA digest

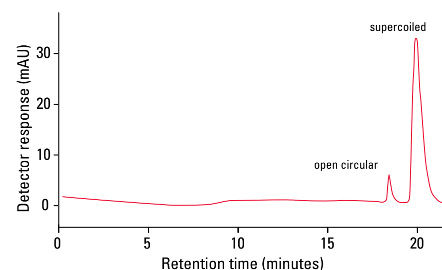


Column:	**TSKgel DEAE-NPR, 2.5 µm, 4. 6 mm ID × 3.5 cm, with guard column, 4.6 mm ID × 0.5 cm**
Mobile phase:	A: 0.02 mol/L Tris-HCl, pH 9.0 B: mobile phase A plus 1.0 mol/L NaCl
Gradient:	15 min linear gradient from 48% to 65% mobile phase B
Flow rate:	1.5 mL/min
Detection:	UV @ 260 nm
Pressure:	14 MPa
Temperature:	40 °C
Sample:	Hae III digest of pBR322 DNA, (base pair number for each peak is indicated)

Plasmid

One of the purity checks used for plasmids in gene therapy assays is the measure of the relative amount of open circular plasmid versus supercoiled plasmid. Figure 15 demonstrates the utility of the TSKgel DNA-NPR column for this type of analysis.

Figure 15: Plasmid analysis



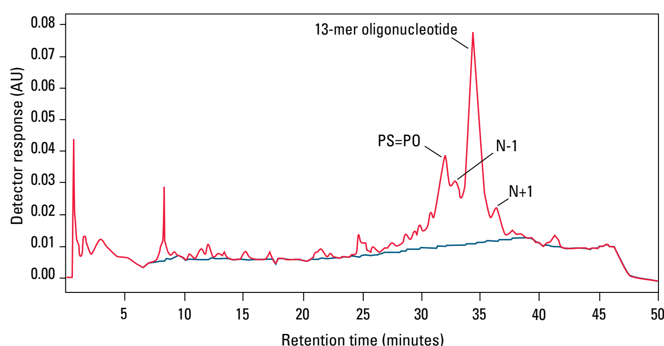
Column:	**TSKgel DNA-NPR, 2.5 µm, 4. 6 mm ID × 7.5 cm**
Mobile phase:	A. 20 mmol/L Tris, pH 9.0 B. 20 mmol/L Tris, pH 9.0 with 1 mol/L NaCl
Gradient:	linear gradient from 50% to 65%B in 10 column volumes
Flow rate:	1 mL/min
Detection:	UV @ 260 nm
Sample:	PUC19 plasmid

Oligonucleotides

Figure 16 contains the chromatographic trace of the crude deprotected 13-mer oligonucleotide using a TSKgel DNA-NPR column. The early eluting peaks from 0–5 minute exhibit a lambda max range of 220–230 nm, indicating the presence of protecting groups used in the synthesis. The N-1 peak as confirmed by mass spectrometry elutes just before the main substance peak. The PS=PO peak elutes before N-1. Structurally, the N-1 analog is completely thioated but is missing one nucleotide. As a result, the N-1 compound is more thioated and hydrophobic than the PS=PO analog. The backside peak is an N+1 impurity verified by mass spectrometry.

The method conditions are designed to optimize resolution of all impurity peaks and inhibit any aggregation, secondary structure formation, and PS=PO conversion. Specifically, sodium bromide acts as the eluting agent and diethylamine provides the buffering capacity while contributing mild chaotropic effects. The step gradient is designed to remove all the protecting groups from the column before elution of the impurity analogs.

Figure 16: Oligonucleotide analysis

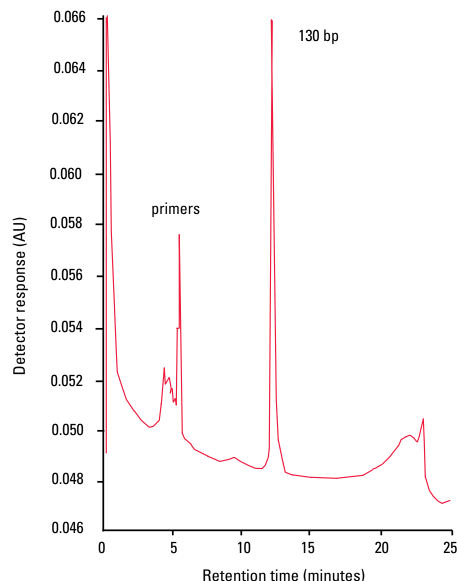


Column:	**TSKgel DNA-NPR, 2.5 µm, 4.6 mm ID × 7.5 cm**
Mobile phase:	A: 10 mmol/L sodium bromide, 20 mmol/L NaOH, pH 12, 1% diethylamine B: 1 mol/L sodium bromide, 20 mmol/L NaOH, pH 12, 1% diethylamine
Gradient	3.5 min (20%B) 12 min (20%B) 45 min (55%B)
Flow rate:	1.0 mL/min
Temperatures:	60 °C (column), 4 °C (sample chamber)
Sample:	crude deprotected 13-mer oligonucleotide

HIV-1 PCR-amplified 130 bp Target

Figure 17 shows the detection of a 130 bp target derived from HIV using a nonporous TSKgel DEAE-NPR column.

Figure 17: Detection of HIV-1 PCR-Amplified 130 bp target



Column:	**TSKgel DEAE-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase:	A: 20 mmol/L Tris-HCl with 0.25 mol/L NaCl, pH 7.7 B: 20 mmol/L Tris-HCl with 1 mol/L NaCl, pH 7.7
Flow rate:	1 mL/min
Detection:	UV @ 260 nm
Temperature:	ambient
Sample:	HIV-1 PCR-amplified 130 bp target
Sample load:	20 µL

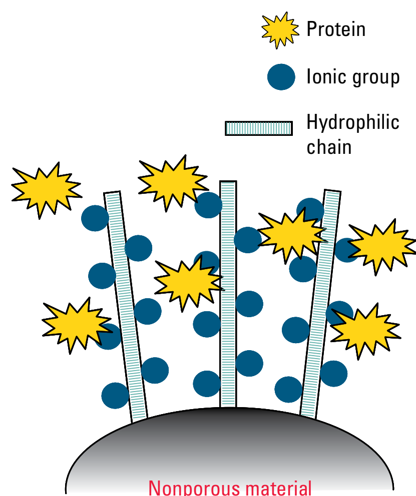
About: TSKgel Q-STAT and DNA-STAT Anion Exchange Columns

TSKgel Q-STAT and DNA-STAT columns are packed with hydrophilic nonporous resin particles of which the surface consists of an open access network of multi-layered anion exchange groups (see Figure 18). The innovative bonding chemistry, combined with a relatively large particle size of these nonporous columns, results in a respectable loading capacity and a low operating pressure.

TSKgel Q-STAT columns are packed with 7 or 10 µm nonporous particles. Applications for these columns include the separation of proteins, peptides, low molar mass nucleic acids, aggregates and charge isomers of monoclonal antibodies, PEGylated proteins, oligo DNA, and siRNA.

Applications for the 5 µm TSKgel DNA-STAT columns include the separation of DNA fragments, nucleic acids and nucleotides.

Figure 18: Schematic diagram of TSKgel STAT columns



Attributes and Applications:

Table 7 lists the product attributes of the TSKgel Q-STAT and DNA-STAT columns. These columns are an excellent choice for high resolution protein and DNA separations. TSKgel Q-STAT and DNA-STAT nonporous columns are supplied in stainless steel (SS) housing with SS fittings and PEEK frits and are stable in a pH range from 3.0 – 10.0.

Table 7: Product attributes

TSKgel column	Q-STAT	DNA-STAT
Matrix	hydrophilic polymer	
Particle size (mean)	7 µm and 10 µm	5 µm
Pore size (mean)	nonporous	
Functional group	quaternary ammonium	
Counter ion	Cl^-	
pH stability	3.0-10.0	
Static binding capacity (mg BSA/g dry gel)	ca. 25 (7 µm) ca. 20 (10 µm)	ca. 35 (5 µm)
Small ion capacity	270 µeq/g dry gel	
pKa	10.5	

Binding Capacities

Table 8 illustrates that despite the fact that surface area decreases with increasing particle size, the larger TSKgel Q-STAT and TSKgel DNA-STAT particles have higher binding capacities than the smaller particles used in TSKgel NPR columns. The novel bonding chemistry used in the preparation of the TSKgel STAT resin resulted in a dramatic increase in static binding capacity, more than compensating for the lower external surface area of the larger particles.

Table 8: Binding capacities of TSKgel STAT anion exchange columns

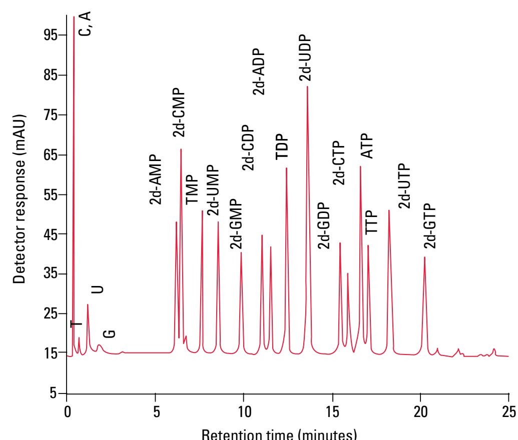
Property	TSKgel NPR column	TSKgel DNA-STAT	TSKgel Q-STAT	
Particle size	2.5 µm	5 µm	7 µm	10 µm
Capacity*	9.1	38.6	27.0	20.9

*Static binding capacity in mg BSA/mg dry gel.

DNA Fragments

Mono-, di-, and tri-nucleotides were separated with excellent peak shape on a TSKgel DNA-STAT column packed with 5 µm particles. The narrow, symmetrical peaks, as shown in Figure 19, demonstrate the absence of micro-pores on this new generation of nonporous resin columns. TSKgel DNA-STAT columns are, as the name implies, first choice for large nucleic acid fragments.

Figure 19: Separation of large DNA fragments

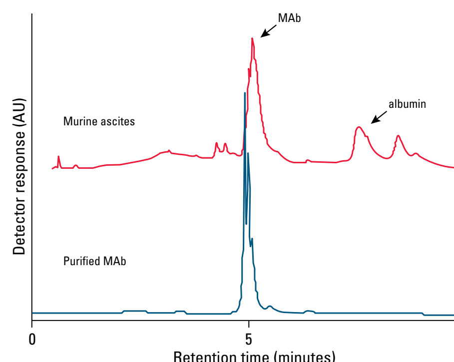


Column:	**TSKgel DNA-STAT, 5 µm, 4.6 mm ID × 10 cm**
Mobile phase:	A: 20 mmol/L Tris-HCl, pH 8.5 B: 0.75 mol/L NaCl in buffer A
Gradient:	0 min (50% B) 25 min (75% B)
Flow rate:	0.8 mL/min
Detection:	UV @ 260 nm

Mouse Ascites Fluid

Figure 20 shows the separation of mouse ascites fluid containing a monoclonal antibody (top) and a partially purified monoclonal antibody (bottom) on a TSKgel Q-STAT column. The top chromatogram clearly shows that the antibody and albumin components are well separated. The bottom chromatogram shows that multiple peaks are present in the partially purified monoclonal sample.

Figure 20: Separation of mouse ascites fluid containing monoclonal antibodies and purified monoclonal antibodies

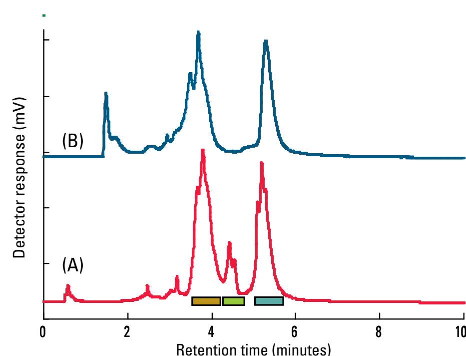


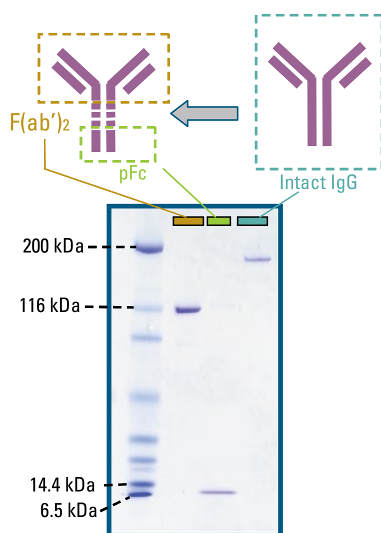
Column:	**TSKgel Q-STAT, 7 µm, 4.6 mm ID × 10 cm**
Mobile phase:	A:20 mmol/L Tris-HCl buffer, pH 8.5 B:0.5 mol/L NaCl in 20 mmol/L Tris-HCl buffer, pH 8.5
Gradient:	A → B linear gradient (10 min)
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	Top: 1/10 dilution of mouse ascites containing mAb Bottom: purified mouse mAb Sample diluted 10-fold with eluent A

Immunoglobulin G (IgG)

Immunoglobulin G (IgG) is a monomeric immunoglobulin, built of two heavy chains and two light chains. Each IgG has two antigen binding sites. It is the most abundant immunoglobulin and is approximately equally distributed in blood and in tissue liquids, constituting 75% of serum immunoglobulins in humans. IgG was digested using pepsin and separated on a TSKgel Q-STAT column and a competitive nonporous WAX-10 column. As shown in Figure 21, three peaks were isolated from the TSKgel Q-STAT column and assigned as F(ab')2, pFc, and intact IgG by SDS-PAGE. No correlation could be established between the peaks obtained on the WAX-10 column and SDS-PAGE results.

Figure 21: Analysis of IgG





Non-reduced SDS-PAGE

Columns:	**A: TSKgel Q-STAT, 7 µm, 4.6 mm ID × 10 cm**
	B: ProPac® WAX-10, 10 µm, 4 mm ID × 25 cm
Mobile phase:	A: 20 mmol/L Tris-HCl, pH 8.5
	B: 0.5 mol/L NaCl in buffer A
Gradient:	0 min (0% B) 10 min (100% B)
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Sample:	pepsin digested mAb

About: TSKgel Sugar AXG, Sugar AXI and SAX Anion Exchange Columns

TSKgel Sugar AXG and Sugar AXI columns are specialty columns for the analysis of mono- and disaccharides, as well as sugar alcohols. Both columns are packed with porous spherical polymer beads which are surface modified with a strong anion exchange group.

The TSKgel Sugar AXG column contains 10 µm particles for the gradient separation and analysis of monosaccharides, disaccharides, and sugar alcohols, whereas the TSKgel Sugar AXI column is packed with 8 µm particles for the isocratic separation of carbohydrates where lower and constant back pressures may be generated.

TSKgel SAX columns are packed with 5 µm porous spherical polymer beads which are surface modified with a strong anion exchange group. They are used for the separation of isomerized sugars, alcohols, and low molar mass organic acids.

Attributes and Applications:

Table 9 lists the product attributes of the TSKgel Sugar AXG, Sugar AXI, and SAX columns. These columns are packed with silica particles and are stable in a pH range from 1.0 – 14.0.

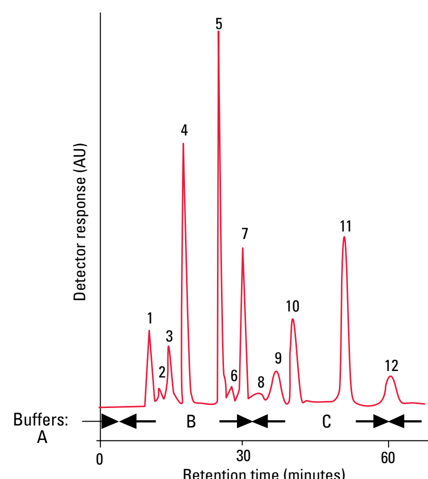
Table 9: Product attributes

TSKgel column	Sugar AXG	Sugar AXI	SAX
Matrix	PS-DVB polymer		
Particle size (mean)	10 µm	8 µm	5 µm
Pore size (mean)	6 nm		
Functional group	trimethylamino		
Counter ion	HBO_3^-	HBO_3^-	Cl^-
pH stability	1.0-14.0		
Small ion capacity	>1.2 eq/L	>1.2 eq/L	>1.0 eq/L
pKa	12.5		

Saccharide Mixture

Saccharides are retained on TSKgel Sugar AX columns following the formation of negatively charged complexes with boric acid at alkaline pH. Figure 22 shows the separation of twelve mono- and disaccharides using a TSKgel Sugar AXG column.

Figure 22: Separation of saccharide mixture

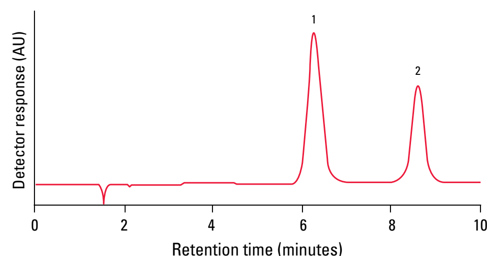


Column: **TSKgel Sugar AXG, 10 µm, 4. 6 mm ID × 15 cm**
Mobile phase: step gradient: 6 min buffer A, 0.6 mol/L boric acid, pH 7.7; then 27 min buffer B, 0.7 mol/L boric acid, pH 7.25; then 30 min buffer C, 0.7 mol/L boric acid, pH 8.7
Flow rate: 0.4 mL/min (column and post column reagent solution)
Detection: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

Sugar Alcohol

Palatinit is a sugar alcohol used as a low-calorie and anti-decay food additive. It can be obtained by reducing palatinose and is composed of two isomers, 6-O-alpha-D-Glucopyranosyl-D-glucitol and 1-O-alpha-D-glucopyranosyl-D-mannitol. As shown in Figure 23, a TSKgel Sugar AXG column can separate the isomers.

Figure 23: Analysis of palatinit

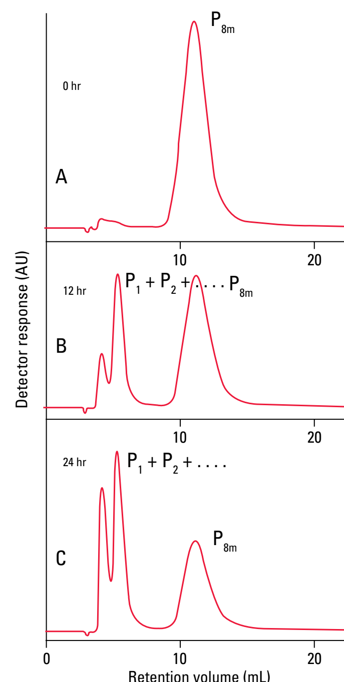


Column:	**TSKgel Sugar AXG, 10 µm, 4.6 mm ID × 15 cm**
Mobile phase:	0.7 mol/L borate buffer, pH 8.6
Flow rate:	0.8 mL/min
Detection:	RI
Temperature:	65 °C
Injection vol.:	10 µL
Samples:	1. alpha-D-glucopyranosyl-1,6-soribitol (GPS) 2. alpha-D-glucopyranosyl-1,6-mannitol (GPM)

Polyphosphates

The stability of the TSKgel SAX column allows a wide pH range for separations of polyphosphates. Figure 24 shows the monitoring of cyclooctaphosphate hydrolysis products over the course of 24 hours with a pH 10.2 mobile phase.

Figure 24: Hydrolysis products of cyclooctaphosphate



Column:	**TSKgel SAX, 5 µm, 4 mm ID × 25 cm**
Mobile phase:	0.4 mol/L KCl, 0.1% EDTA, pH 10.2
Sample:	cyclooctaphosphate hydrolysis products A. 0 hours B. 12 hours C. 24 hours

About: TSKgel BioAssist S Cation Exchange Columns

Specially designed for the separation of large biomolecules such as antibodies, the large pores of the TSKgel BioAssist S cation exchange column offer superior capacity and resolution at a low column pressure drop. Constructed via a polymerization technique that allows an equivalent density of ionic exchange groups to be incorporated into the particle without reducing pore size, the TSKgel BioAssist S column is unlike other ion exchange columns that use graft polymerization for polymer chain introduction. The TSKgel BioAssist S columns' large pores are very accessible even for high molar mass proteins. This leads to higher chromatographic efficiency and binding capacity for purification.

TSKgel BioAssist S cation exchange columns are offered in a 4.6 mm ID × 5 cm format and a 10 mm ID × 10 cm semi-preparative column for scale up. Both columns are made of PEEK to reduce protein adsorption.

Attributes and Applications:

Table 10 lists the product attributes of TSKgel BioAssist S columns. The pore structure and bonding chemistry of TSKgel BioAssist S columns provide high capacity for medium to large molar mass proteins.

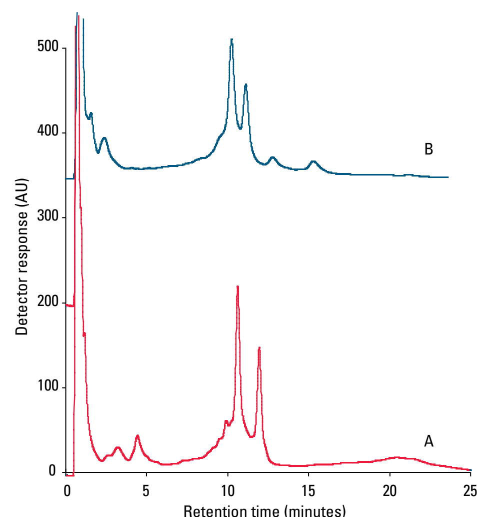
Table 10: Product attributes

Attribute	Value
Matrix	polymethacrylate
Particle size (mean)	7 µm and 13 µm
Pore size (mean)	130 nm
Functional group	sulfopropyl
Counter ion	Na^+
pH stability	2.0-12.0
Capacity (gamma globulin)	70
Small ion capacity	0.1 eq/L
pKa	2.4

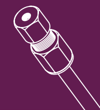
Bromelain

The application in Figure 25 shows the analysis of bromelain, a proteolytic enzyme that is used as a nutritional supplement. Bromelain is a basic glycoprotein with a molar mass of 33 kDa and pI of 9.55.

Figure 25: Analysis of bromelain



Columns:	**A: TSKgel BioAssist S, 7 µm, 4.6 mm ID × 5 cm** B: Competitor S, 5 mm ID × 5 cm
Mobile phase:	20 min (TSKgel) or 30 min (Competitor S) linear gradient of NaCl from 0 to 0.5 mol/L in 20 mmol/L sodium phosphate buffer, pH 7.0
Flow Rate:	0.8 mL/min for TSKgel; 1.0 mL/min for Competitor S
Detection:	UV @ 280 nm
Temperature:	25 °C
Sample:	crude bromelain (C4882, Sigma), 1 mg in 100 L

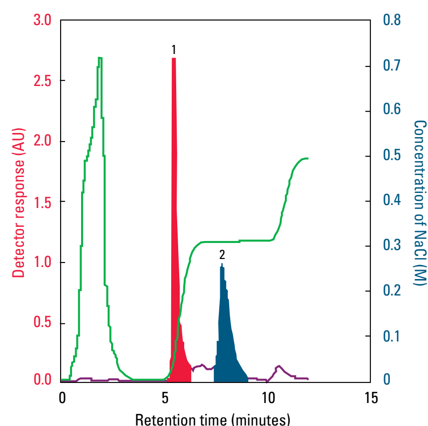


Immunoglobulin M (IgM)

IgM is known to possess unique and beneficial characteristics relative to other immunoglobulin classes; it is a large molecule comprised of five IgG subunits, resulting in a relatively unstable and difficult to purify protein. Unlike single chain antibodies, IgM cannot be purified by Protein A (an affinity material commonly used for its high binding capacity and excellent selectivity for antibodies) due to steric hindrance. Alternative affinity methods have been developed with thiophillic absorbents but these methods often result in low binding capacity.

An alternative purification method of IgM by ion exchange chromatography using a TSKgel BioAssist S column was developed. As shown in Figure 26, baseline separation of IgM from other contaminants is achieved using a 0.3 mol/L NaCl step gradient after elution of albumin.

Figure 26: Separation of IgM by cation exchange chromatography

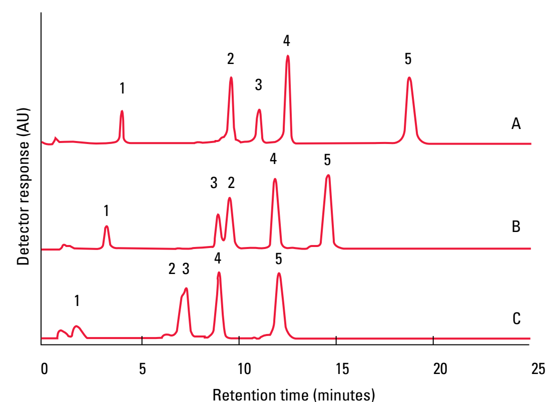


Column:	**TSKgel BioAssist S, 7 µm, 4.6 mm ID × 5 cm**
Mobile phase:	20 mmol/L sodium phosphate buffer, pH 6.0
Gradient	0 mol/L - 0.3 mol/L NaCl (5 min) 0.3 mol/L - 0.5 mol/L NaCl (10 min)
Flow Rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	500 µL of 9.5 mg/mL IgM in mouse ascites fluid; shaded peaks represent albumin and IgM respectively

Protein Standards

Figure 27 shows a comparison of a standard protein separation on a TSKgel BioAssist S column and conventional ion exchange columns. It is clear that the TSKgel BioAssist S column is more retentive and provides a higher resolution of the sample proteins compared to the conventional products.

Figure 27: Analysis of protein standards

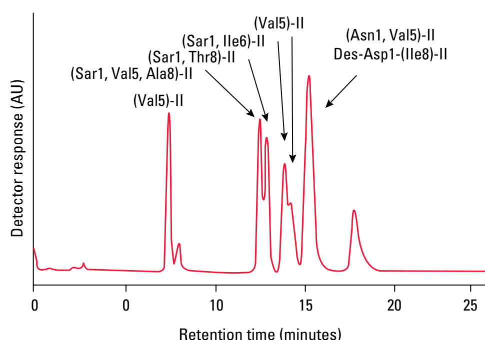


Columns:	**A: TSKgel BioAssist S, 7 µm, 4.6 mm ID × 5 cm** B: Conventional S type product C, 5.0 mm ID × 5 cm C: Conventional S type product D, 4.6 mm ID × 5 cm
Mobile phase:	A: 20 mmol/L sodium phosphate buffer, pH 6.5 B: 20 mmol/L sodium phosphate buffer containing 1.0 mol/L NaCl, pH 6.5
Gradient:	32 min (A-B)
Flow rate:	0.8 mL/min
Detection:	UV @ 280 nm
Temperature:	10 °C
Injection vol.:	20 µL
Samples:	1. myoglobin, 1 g/L 2. α-chymotrypsinogen A, 2 g/L 3. ribonuclease A, 4 g/L 4. cytochrome C, 2 g/L 5. lysozyme, 2 g/L

Peptides

Figure 28 shows chromatograms of peptides on a TSKgel BioAssist S column. It is generally known that an accurate quantification is difficult to obtain when peptides are analyzed on a column with a styrene-type base material, due to secondary interaction with the hydrophobic packing material. However, a TSKgel BioAssist S column is capable of analyzing such peptides as angiotensins without the need to add an organic solvent to the mobile phase since the acrylate packing material is hydrophilic.

Figure 28: Analysis of peptides



Column:	**TSKgel BioAssist S, 7 µm, 4.6 mm ID × 5 cm**
Mobile phase:	A: 20 mmol/L sodium acetate buffer, pH 5.0
	B: 20 mmol/L sodium acetate buffer containing 1.0 mol/L NaCl, pH 5.0
Gradient:	A → B linear gradient (20 min)
Detection:	UV @ 280 nm
Temperature:	25 °C

About: TSKgel SP-2SW, CM-2SW, and CM-3SW Cation Exchange Columns

The TSKgel SP-2SW, TSKgel CM-2SW, and TSKgel CM-3SW columns are silica-based columns derivatized with sulfopropyl (SP) and carboxymethyl (CM) ligands to provide a strong cation and weak cation exchange column, respectively. They are used for the separation and analysis of small proteins, peptides, and other biologically active molecules. TSKgel CM-2SW has a smaller pore size than TSKgel CM-3SW.

Attributes and Applications:

Table 11 shows the product attributes of the TSKgel SP-2SW, TSKgel CM-2SW, and TSKgel CM-3SW columns. These columns are typically used for analyzing smaller molar mass samples such as nucleotides, drug candidates, catecholamines, and small peptides or proteins.

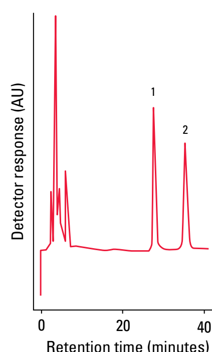
Table 11: Product attributes

TSKgel column	SP-2SW	CM-2SW	CM-3SW
Matrix		silica	
Particle size (mean)	5 µm	5 µm	10 µm
Pore size (mean)	12.5 nm	12.5 nm	25 nm
Functional group	sulfopropyl	$-CH_2COO^-$	$-CH_2COO^-$
Counter ion		Na^+	
pH stability		2.0-7.5	
Capacity (mg Hb/mL)	ND	110	ND
Small ion capacity	0.3 eq/L	>0.3 eq/L	>0.3 eq/L
pKa	2.2	4.2	4.2

Herbicides

Figure 29 shows the rapid analysis of the herbicides paraquat and diquat in urine on the TSKgel SP-2SW column.

Figure 29: Rapid analysis for the herbicides paraquat and diquat

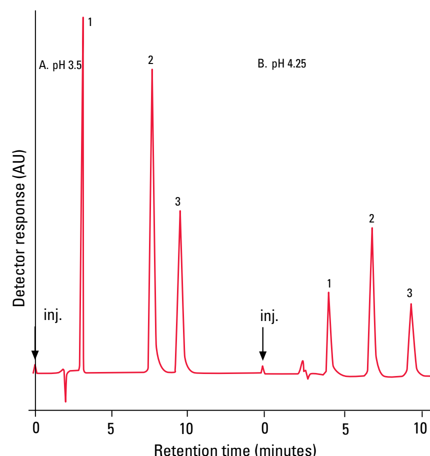


Column:	**TSKgel SP-2SW, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase:	20% CH_3CN in 0.2 mol/L phosphate, pH 3.0
Flow rate:	1.0 mL/min
Detection:	UV @ 290 nm
Samples:	1. paraquat, 5 g/mL 2. diquat, 5 g/mL

Nucleosides

Silica-based cation exchange columns are typically used in the separation of low molar mass compounds, such as pharmaceuticals, nucleotides, and small peptides. For example, Figure 30 shows the separation of nucleosides on the TSKgel SP-2SW column.

Figure 30: Separation of nucleosides



Column:	**TSKgel SP-2SW, 5 µm, 4.6 mm ID × 25 cm**
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
Detection:	UV @ 260 nm
Temperature:	23 °C
Samples:	nucleoside standards: 1. guanosine 2. cytidine 3. adenosine

About: TSKgel SP-5PW and CM-5PW Cation Exchange Columns

The polymethacrylate-based resin, TSKgel 5PW, is a spherical 10 µm particle with approximately 100 nm pores. It is derivatized with sulfopropyl (SP) ligands to provide the strong cation exchange column, TSKgel SP-5PW, and with carboxymethyl (CM) ligands to provide the weak cation exchange column, TSKgel CM-5PW.

TSKgel CM-5PW columns are used for the separation and analysis of proteins, peptides, and other biologically active molecules. These columns are offered in dimensions of 7.5 mm ID × 7.5 cm in stainless steel housing.

TSKgel SP-5PW columns are also used for the separation and analysis of proteins, peptides, and other biologically active molecules. These columns are available in internal diameters varying from 2 mm to 21.5 mm and in column housings of either glass or stainless steel.

Attributes and Applications:

Table 12 lists the product attributes of TSKgel SP-5PW and CM-5PW columns. These columns are an excellent choice for analyzing biologically active molecules. TSKgel SP-5PW and CM-5PW columns are stable over the pH range of 2.0 – 12.0 and the porous particles have a mean pore size of 100 nm.

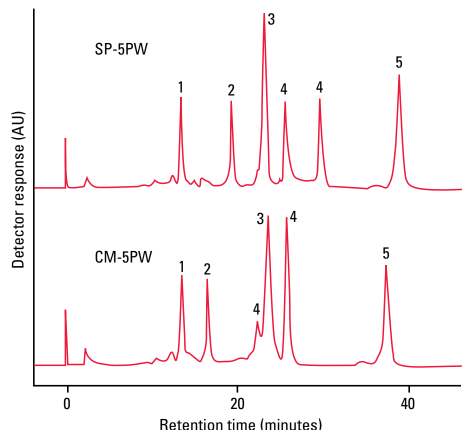
Table 12: Product attributes

TSKgel column	SP-5PW	CM-5PW
Matrix	polymethacrylate	
Particle size (mean)	10 µm, 13 µm, and 20 µm	10 µm and 13 µm
Pore size (mean)	100 nm	
Functional group	$-(CH_2)_3SO_3^-$	$-CH_2COO^-$
Counter ion	Na^+	
pH stability	2.0-12.0	
Capacity (mg Hb/mL):	40	45
Small ion capacity	>0.1 eq/L	
pKa	2.3	4.2

Differences in Selectivity

Differences in selectivity between strong (TSKgel SP-5PW) and weak (TSKgel CM-5PW) cation exchange columns are demonstrated in Figure 31, which is a separation of globular proteins.

Figure 31: Selectivity of strong and weak TSKgel cation exchange columns

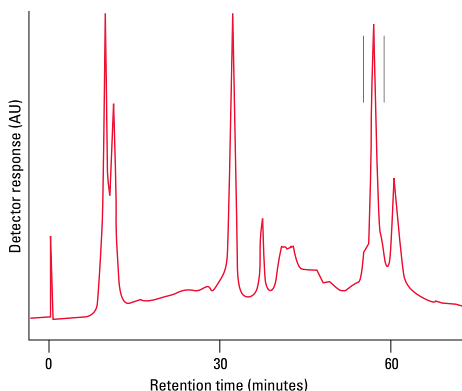


Columns: **TSKgel SP-5PW and TSKgel CM-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase: 60 min linear gradient from 0 mol/L to 0.5 mol/L NaCl in 0.02 mol/L phosphate, pH 7.0
Flow rate: 1.0 mL/min
Detection: UV @ 280 nm
Samples: 1. trypsinogen
2. ribonuclease A
3. α-chymotrypsinogen
4. cytochrome C
5. lysozyme

Crude Lipoxidase

The purification of 200 mg of crude lipoxidase on a 21.5 mm ID TSKgel SP-5PW column is illustrated in Figure 32. 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 32: Semi-preparative purification of lipoxidase

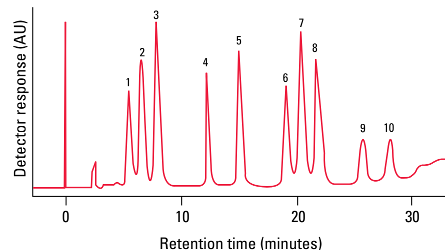


Column:	**TSKgel SP-5PW, 13 µm, 21.5 mm ID × 15 cm**
Mobile phase:	120 min linear gradient from 0 mol/L to 0.5 mol/L Na_2SO_4 in 0.02 mol/L acetate, pH 4.5
Flow rate:	4.0 mL/min
Detection:	UV @ 280 nm
Recovery:	lipoxidase activity collected between the two vertical lines was 84%
Sample:	crude lipoxidase, 200 mg

Peptides

One of the common HPLC modes for analysis and separation of peptides is cation exchange. Figure 33 shows that separations of peptides can be efficiently separated on the strong cation exchange column TSKgel SP-5PW.

Figure 33: Separation of peptide mixture



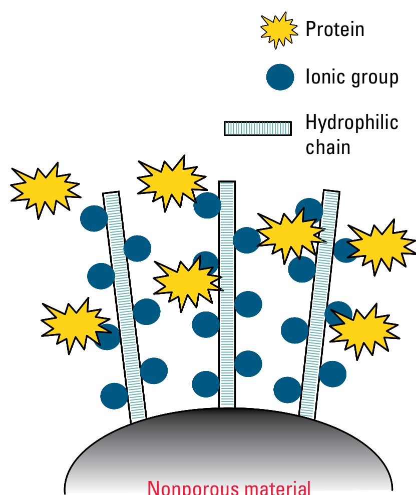
Column:	**TSKgel SP-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	30 min linear gradient from 0.02 mol/L to 0.5 mol/L phosphate, pH 3.0, in 30% acetonitrile
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Injection vol.:	50 µL
Samples:	2 µg each of: 1. oxytocin 2. met-enkephalin 3. TRH 4. α-MSH 5. LH-RH (1 µg) 6. neurotensin 7. α-MSH 8. angiotensin II 9. substance P 10. β-endorphin

About: TSKgel SP-STAT and CM-STAT Cation Exchange Columns

TSKgel CM-STAT and SP-STAT columns are packed with 7 or 10 µm hydrophilic nonporous resin particles of which the surface consists of an open access network of multi-layered weak cation exchange groups (see Figure 34). The innovative bonding chemistry, combined with a relatively large particle size, results in a respectable loading capacity, low operating pressure, and rapid analysis.

Applications for the TSKgel CM-STAT and SP-STAT columns include the separation of proteins, protein aggregates, charge variants of monoclonal antibodies, PEGylated proteins, and peptide digests.

Figure 34: Schematic diagram of TSKgel STAT columns

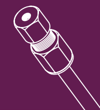


Attributes and Applications:

Table 13 lists the product attributes of TSKgel CM-STAT and SP-STAT columns. These columns are an excellent choice for high throughput protein separations. Nonporous TSKgel CM-STAT and SP-STAT columns are supplied in stainless steel (SS) housing with SS fittings and PEEK frits and are stable in a pH range from 3.0 – 10.0.

Table 13: Product attributes

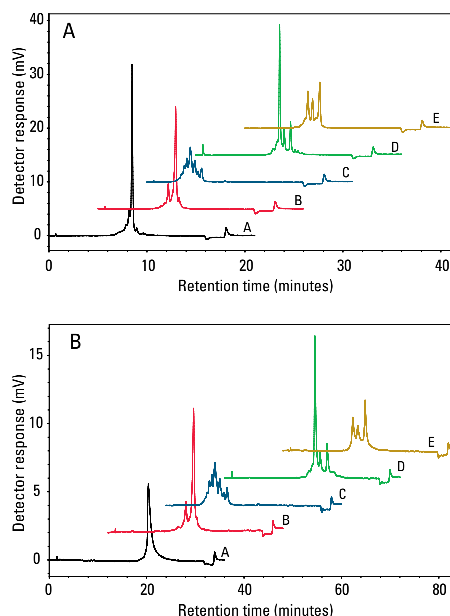
TSKgel column	TSKgel CM-STAT	TSKgel SP-STAT
Particle size (mean)	7 µm and 10 µm	
Pore size (mean)	nonporous	
Functional group	carboxymethyl	sulfopropyl
Counter ion	Na^+	
pH stability	3.0-10.0	
Static binding capacity (mg lysozyme/g dry gel)	ca. 20 (7 µm) ca. 15 (10 µm)	ca. 15 (7 µm) ca. 10 (10 µm)
Small ion capacity	100 µeq/g dry gel	23 µeq/g dry gel
pKa	4.9	2.6



Antibody Analysis

The analysis profiles for five antibodies separated on a TSKgel CM-STAT column (Figure 35A) were compared with the profiles obtained on a competitive WCX-10 column (Figure 35B). Similar or higher resolution profiles were obtained on the TSKgel CM-STAT column in approximately half the time.

Figures 35A & 35B: Antibody analysis

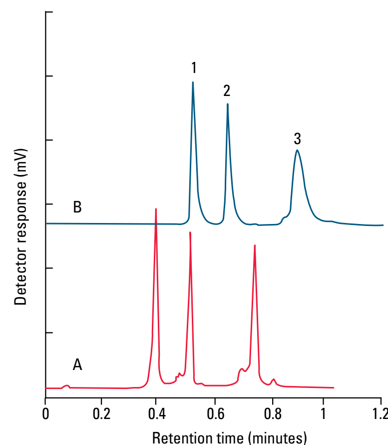


Columns:	**A: TSKgel CM-STAT, 7 µm, 4.6 mm ID × 10 cm**
	B: ProPac WCX-10, 10 µm, 4 mm ID × 25 cm
Mobile phase:	A: 20 mmol/L MES, pH 6.0
	B: 20 mmol/L MES + 0.5 mol/L NaCl, pH 6.0
Gradient:	A: 0 min (10% B) 15 min (30% B) 15 min (100% B) 17 min (0% B) 17 min (10% B) 21 min (10% B)
	B: 0 min (10% B) 30 min (30% B) 30 min (100% B) 32 min (100% B) 32 min (10% B) 36 min (10% B)
Flow rate:	A: 1.0 mL/min B: 2.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	20 µL
Samples:	monoclonal antibodies (mAb A through E)

Protein Standards

The fast separation of protein standards was investigated using short cation exchange columns (see Figure 36). A TSKgel SP-STAT column shows superior resolution, better peak shape, and a shorter analysis time (<60 seconds) compared to a competitive monolithic SP-type column.

Figure 36: Fast separation of protein standards

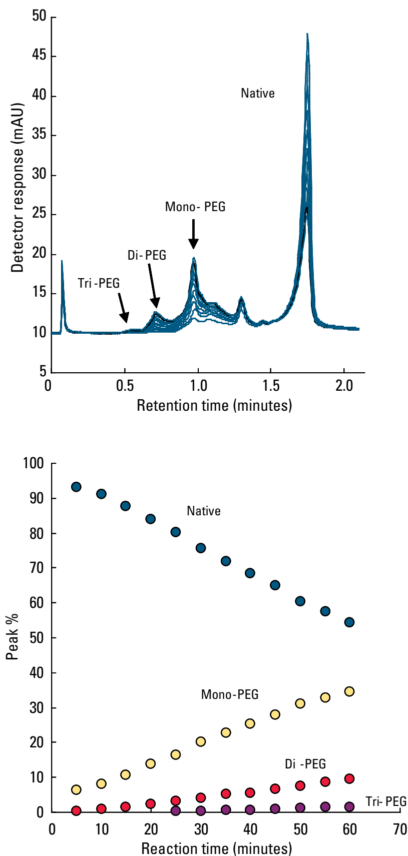


Columns:	**A: TSKgel SP-STAT, 10 µm, 3.0 mm ID × 3.5 cm**
	B: ProSwift® SCX-1S Monolith, 4.6 mm ID × 5 cm
Mobile phase:	A: 20 mmol/L sodium acetate, pH 5.0
	B: 1.0 mol/L NaCl in mobile phase A, pH 5.0 for column A
	1.5 mol/L NaCl in mobile phase A, pH 5.0 for column B
Gradient:	0 min (0% B) 1 min (100% B)
Flow rate:	A: 2.0 mL/min
	B: 4.73 mL/min
Detection:	UV @ 280 nm
Samples:	1. α-chymotrypsinogen A
	2. cytochrome C
	3. lysozyme

Reaction Monitoring

A sample of β-lactoglobulin (5 mg/mL) was reacted with polyethylene glycol (5 kDa) in a pH 6.5 phosphate buffer. The formation of pegylated protein reaction products was monitored in 5 minute intervals on a 3.5 cm TSKgel SP-STAT column. As demonstrated in Figure 37, peak areas of mono-, di-, and tri-pegylated β-lactoglobulin increased with reaction time, while the area of unreacted β-lactoglobulin declined.

Figure 37: Monitoring of reaction products

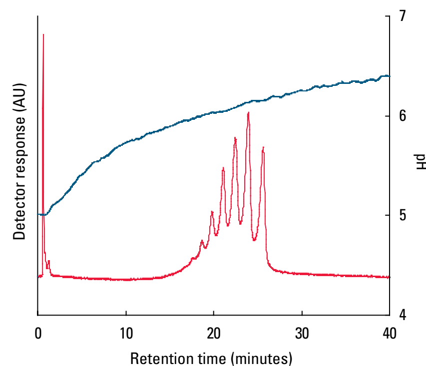


Column:	**TSKgel SP-STAT, 10 µm, 3 mm ID × 3.5 cm**
Mobile phase:	A: 20 mmol/L sodium acetate buffer, pH 5.0 B: 1.0 mol/L NaCl in mobile phase A, pH 5.0
Gradient:	0 min (0%B) 2 min (100%B)
Flow rate:	2.0 mL/min
Detection:	UV @ 280 nm
Sample:	pegylated β-lactoglobulin

Charge Isomers

As shown in Figure 38, the TSKgel CM-STAT column can also be used to separate charge isomers of a purified monoclonal antibody by pH gradient.

Figure 38: Separation of charge isomers



Column:	**TSKgel CM-STAT, 7 µm, 4.6 mm ID × 10 cm**
Mobile phase:	A: 50 mmol/L sodium acetate buffer, pH 5.0 B: 30 mmol/L sodium acetate buffer (pH not adjusted) Column equilibrated with mobile phase A, the sample is injected, then eluted stepwise to 100% mobile phase B
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	purified mAb
Sample concentration:	1 g/L

About: TSKgel SCX and OApak-A Cation Exchange Columns

The TSKgel SCX column is packed with porous polystyrene divinylbenzene polymer beads of which the surface has been modified with strong cation exchange groups that are surrounded by Na^+ counterions. This column is optimized for the separation and analysis of organic acids, saccharides, and alcohols. The TSKgel SCX column is also available in the H^+ form for the separation of isomerized sugars, alcohols, and lower organic acids.

A TSKgel OApak-A column is packed with porous hydrophilic polymer beads which have been chemically modified with a weak cation exchange group. This column is optimized for the separation and analysis of organic acids by an ion exclusion mechanism. Applications include: organic acids in fruit juices, wine, beer, coffee, and salt solutions.

The TSKgel OApak-A column is to be used in conjunction with the TSKgel OApak-P guard column which has a strong cation exchange group for the removal of dissociated strong acids under the isocratic mobile phase conditions of 0.75 mmol/L H_2SO_4.

Attributes and Applications:

Table 14 shows the product attributes of the TSKgel SCX column and the TSKgel OApak-A column. Both of these columns are composed of 5 µm particles and are stable in the pH range of 2.0 – 12.0.

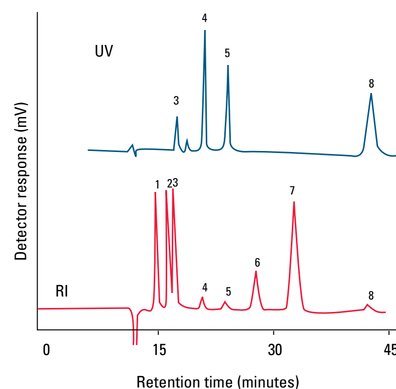
Table 14: Product attributes

TSKgel column	SCX	OApak-A
Matrix	PS-DVB	hydrophilic polymer
Particle size (mean)	5 µm	
Pore size (mean)	6 nm	ND
Functional group	sulfonic acid	proprietary
Counter ion	H^+ and Na^+	H^+
pH stability	2.0-12.0	
Small ion capacity	>1.5 eq/L	

Saccharide, Organic Acid, and Alcohol Mixture

Ion exclusion chromatography can be used as an effective method for separating alcohols. An example of saccharide, organic acid, and alcohol separation is shown in Figure 39 on two TSKgel SCX (H^+) columns in series.

Figure 39: Separation of saccharide, organic acid, and alcohol mixture

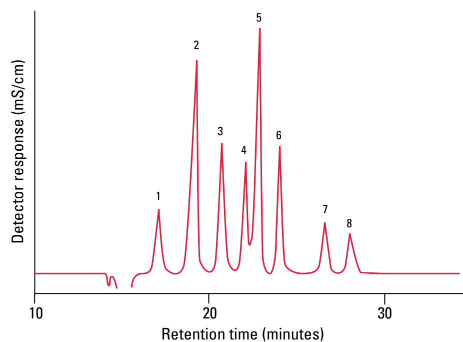


Column: **TSKgel SCX (H^+), 5 µm, 7.8 mm ID × 30 cm × 2**
Mobile phase: 0.05 mol/L $HClO_4$
Flow rate: 0.8 mL/min
Detection: UV @ 210 nm, RI
Samples:
1. maltose
2. glucose
3. fructose
4. lactic acid
5. acetic acid
6. methanol
7. ethanol
8. butyric acid

Organic Acids in Wine and Beer

Figure 40 demonstrates the separation of organic acids commonly found in wines and beers on the TSKgel OApak-A column.

Figure 40: Separation of organic acids commonly found in beer and wine

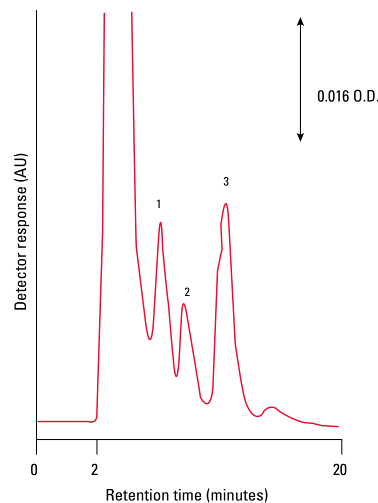


Column:	**TSKgel OApak-A, 5 µm, 7.8 mm ID × 30 cm**
	TSKgel OApak-P guard, 6 mm ID × 4 cm
Mobile phase:	0.75 mmol/L sulfuric acid
Flow rate:	0.8 mL/min
Detection:	conductivity
Temperature:	60 °C
Injection vol.:	20 µL
Samples:	1. pyruvic acid (50 ppm)
	2. tartaric acid (500 ppm)
	3. citric acid (500 ppm)
	4. malic acid (500 ppm)
	5. pyroglutamic acid (500 ppm)
	6. lactic acid (1,000 ppm)
	7. acetic acid (2,000 ppm)
	8. succinic acid (1,000 ppm)

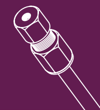
Column Stability

An example of the stability of the TSKgel SCX column is demonstrated in Figure 41 where 1 mol/L NaOH is used as the mobile phase for the separation of organic acids.

Figure 41: Separation of acids



Column:	**TSKgel SCX (Na+), 5 µm, 8 mm ID × 10 cm**
Mobile phase:	1 mol/L NaOH
Flow rate:	0.8 mL/min
Detection:	UV @ 210 nm
Samples:	1. formic acid (50 ppm)
	2. acetic acid (50 ppm)
	3. propionic acid (100 ppm)



About: TSKgel SP-NPR Cation Exchange Columns

The TSKgel SP-NPR column is packed with spherical, nonporous (NPR) hydrophilic polymer beads of which the surface has been modified with a strong cation exchange group. Nonporous resin columns provide fast separations due to their small (2.5 µm) particle size. High column efficiency coupled with low sample capacity restricts the application of these columns to fast analysis and micro-scale preparative isolation.

The TSKgel SP-NPR column is used for the separation and analysis of proteins and peptides. This column is particularly useful for adeno-associated viruses and other large biopolymers.

Attributes and Applications

Table 15 shows the product attributes of the TSKgel SP-NPR column. Due to the absence of all but very small pores, protein recovery is generally high on TSKgel NPR columns.

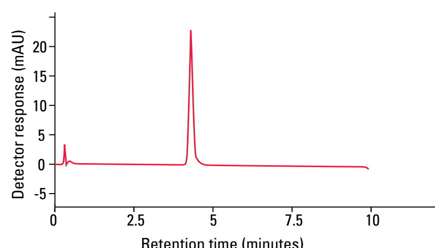
Table 15: Product attributes

TSKgel column	SP-NPR
Matrix	hydroxylated methacrylic polymer
Particle size (mean)	2.5 µm
Pore size (mean)	nonporous
Functional group	sulfopropyl
Counter ion	Na^+
pH stability	2.0-12.0
Capacity (mg Hb/mL):	5
Small ion capacity	>0.1 eq/L
pKa	2.3

Purified Adeno-Associated Virus

A purity check of adeno-associated virus (AAV), commonly used in gene therapy research, on a TSKgel SP-NPR column is shown in Figure 42. This 10 minute HPLC method replaces an existing assay that took two days to perform.

Figure 42: Analysis of purified AAV

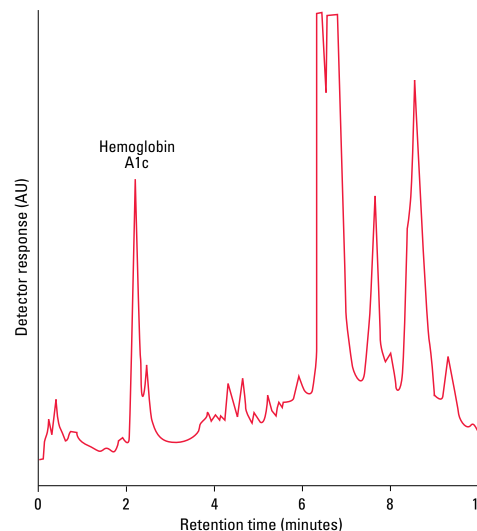


Column: **TSKgel SP-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase: A. 50 mmol/L HEPES, 1 mmol/L EDTA, 5 mmol/L MgCl, pH 7.5
B. 50 mmol/L HEPES, 1 mmol/L EDTA, 5 mmol/L MgCl, pH 7.5 with 0.5 mol/L NaCl; linear gradient from 20% to 100% B in 10 column volumes
Gradient: 0 min (0% B) 2 min (100% B)
Flow rate: 1 mL/min
Detection: UV @ 280 nm
Sample: purified adeno-associated virus

Hemoglobin A1c

The analysis of hemoglobin A1c levels in blood is used to monitor glucose levels in diabetic patients. Figure 43 shows that the HbA1c fraction can be separated from other human Hb variants on a TSKgel SP-NPR column by running a linear pH gradient in 10 minutes.

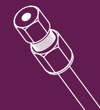
Figure 43: pH gradient analysis of hemoglobin A1c



Column: **TSKgel SP-NPR, 2.5 µm, 4. 6 mm ID × 3.5 cm**
Mobile phase: A: 0.02 mol/L MES, and 0.02 mol/L HEPES-NaOH, pH 6.0
B: 0.02 mol/L MES, and 0.02 mol/L HEPES- NaOH, pH 8.0
Gradient: 10 min linear gradient from 32% to 75% buffer B (pH 6.66 to pH 7.43)
Flow rate: 1.5 mL/min
Detection: VIS @ 415 nm
Sample: hemoglobin standard

Ordering Information - TSKgel Anion Exchange columns

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
19685	TSKgel BioAssist Q, 10 µm, 400 nm	polymer	PEEK	4.6	5
21410	TSKgel BioAssist Q, 13 µm, 400 nm	polymer	PEEK	10	10
18761	TSKgel DEAE-2SW, 5 µm 12.5 nm	silica	Stainless Steel	2	25
07168	TSKgel DEAE-2SW, 5 µm, 12.5 nm	silica	Stainless Steel	4.6	25
42154	TSKgel Guard Cartridge for 2 mm ID TSKgel DEAE-2SW column, 3 pk, 5 µm	silica	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
07648	TSKgel Guardgel Kit for 4.6 mm ID TSKgel DEAE-2SW column, 10 µm	silica	Stainless Steel		
07163	TSKgel DEAE-3SW, 10 µm, 25 nm	silica	Stainless Steel	7.5	7.5
07648	TSKgel Guardgel Kit for 7.5 mm ID TSKgel DEAE-3SW column, 10 µm	silica	Stainless Steel		
13061	TSKgel DEAE-5PW Glass, 10 µm, 100 nm	polymer	Glass	5	5
08802	TSKgel DEAE-5PW Glass, 10 µm, 100 nm	polymer	Glass	8	7.5
14016	TSKgel DEAE-5PW Glass, 13 µm, 100 nm	polymer	Glass	20	15
18757	TSKgel DEAE-5PW, 10 µm, 100 nm	polymer	Stainless Steel	2	7.5
07164	TSKgel DEAE-5PW, 10 µm, 100 nm	polymer	Stainless Steel	7.5	7.5
07574	TSKgel DEAE-5PW, 13 µm, 100 nm	polymer	Stainless Steel	21.5	15
08806	TSKgel Glass Guardgel Kit for 5 mm ID and 8 mm ID TSKgel DEAE-5PW columns, 20 µm	polymer	Glass		
14466	TSKgel Glass Guard Column for 20 mm ID TSKgel DEAE-5PW Glass column, 13 µm	polymer	Glass	20	2
42152	TSKgel Guard Cartridge for 2 mm ID TSKgel DEAE-5PW column, 3 pk, 10 µm	polymer	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
07210	TSKgel Guardgel Kit for 7.5 mm ID TSKgel DEAE-5PW column, 20 µm	polymer	Stainless Steel		
16092	TSKgel Guardgel Kit for 21.5 mm ID TSKgel DEAE-5PW column, 20 µm	polymer	Stainless Steel		
13075	TSKgel DEAE-NPR, 2.5 µm, nonporous	polymer	Stainless Steel	4.6	3.5
17088	TSKgel Guard Column for 4.6 mm ID TSKgel DEAE-NPR column, 5 µm	polymer	Stainless Steel	4.6	0.5
18249	TSKgel DNA-NPR, 2.5 µm, nonporous	polymer	Stainless Steel	4.6	7.5
18253	TSKgel Guard Column for 4.6 mm ID TSKgel DNA-NPR column, 5 µm	polymer	Stainless Steel	4.6	0.5
21962	TSKgel DNA-STAT, 5 µm, nonporous	polymer	Stainless Steel	4.6	10
07166	TSKgel QAE-2SW, 5 µm, 12.5 nm	silica	Stainless Steel	4.6	25
07646	TSKgel Guardgel Kit for 4.6 mm ID TSKgel QAE-2SW column, 5 µm	silica	Stainless Steel		

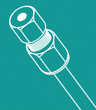


Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
21960	TSKgel Q-STAT, 10 µm, nonporous	polymer	Stainless Steel	3	3.5
21961	TSKgel Q-STAT, 7 µm, nonporous	polymer	Stainless Steel	4.6	10
07157	TSKgel SAX, 5 µm, 6 nm	polymer	Stainless Steel	6	15
08640	TSKgel Sugar AXG, 10 µm, 6 nm	polymer	Stainless Steel	4.6	15
08639	TSKgel Sugar AXI, 8 µm, 6 nm	polymer	Stainless Steel	4.6	15
18386	TSKgel SuperQ-5PW Glass, 10 µm, 100 nm	polymer	Glass	8	7.5
18257	TSKgel SuperQ-5PW, 10 µm, 100 nm	polymer	Stainless Steel	7.5	7.5
18387	TSKgel SuperQ-5PW, 13 µm, 100 nm	polymer	Stainless Steel	21.5	15
18388	TSKgel Guardgel Kit for 7.5 mm ID TSKgel SuperQ-5PW column, 20 µm	polymer	Stainless Steel		

Ordering Information - TSKgel Cation Exchange columns

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
19686	TSKgel BioAssist S, 7 µm, 130 nm	polymer	PEEK	4.6	5
21411	TSKgel BioAssist S, 13 µm, 130 nm	polymer	PEEK	10	10
07167	TSKgel CM-2SW, 5 µm, 12.5 nm	silica	Stainless Steel	4.6	25
07650	TSKgel Guardgel Kit for TSKgel CM-2SW column, 10 µm	silica	Stainless Steel		
07162	TSKgel CM-3SW, 10 µm, 25 nm	silica	Stainless Steel	7.5	7.5
07650	TSKgel Guardgel Kit for TSKgel CM-2SW & CM-3SW columns, 10 µm	silica	Stainless Steel		
13068	TSKgel CM-5PW, 10 µm, 100 nm	polymer	Stainless Steel	7.5	7.5
13069	TSKgel Guardgel Kit for 7.5 mm ID TSKgel CM-5PW column, 20 µm	polymer	Stainless Steel		
21965	TSKgel CM-STAT, 10 µm, nonporous	polymer	Stainless Steel	3	3.5
21966	TSKgel CM-STAT, 7 µm, nonporous	polymer	Stainless Steel	4.6	10
16653	TSKgel OApak-A, 5 µm, 100 nm	polymer	Stainless Steel	7.8	30
16654	TSKgel Guard Column for TSKgel OApak-A, 10 µm	polymer	Stainless Steel	6	4
07156	TSKgel SCX, Strong Cation Exchange, 5 µm (Na^+), 6 nm	polymer	Stainless Steel	6	15
07158	TSKgel SCX, Strong Cation Exchange, 5 µm (H^+), 6 nm	polymer	Stainless Steel	7.8	30
07165	TSKgel SP-2SW, 5 µm, 12.5 nm	silica	Stainless Steel	4.6	25
07644	TSKgel Guardgel Kit for 4.6 mm ID TSKgel SP-2SW column, 5 µm	silica	Stainless Steel		

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
13062	TSKgel SP-5PW Glass, 10 µm, 100 nm	polymer	Glass	5	5
08803	TSKgel SP-5PW Glass, 10 µm, 100 nm	polymer	Glass	8	7.5
14017	TSKgel SP-5PW Glass, 13 µm, 100 nm	polymer	Glass	20	15
18758	TSKgel SP-5PW, 10 µm, 100 nm	polymer	Stainless Steel	2	7.5
07161	TSKgel SP-5PW, 10 µm, 100 nm	polymer	Stainless Steel	7.5	7.5
07575	TSKgel SP-5PW, 13 µm, 100 nm	polymer	Stainless Steel	21.5	15
08807	TSKgel Glass Guardgel Kit for 5 mm ID & 8 mm ID TSKgel SP-5PW columns, 20 µm	polymer	Glass		
42153	TSKgel Guard Cartridge for 2 mm ID TSKgel SP-5PW column, 3 pk, 10 µm	polymer	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
07211	TSKgel Guardgel Kit for 7.5 mm ID TSKgel SP-5PW column, 20 µm	polymer	Stainless Steel		
16093	TSKgel Guardgel Kit for 21.5 mm ID TSKgel SP-5PW column, 20 µm	polymer	Stainless Steel		
13076	TSKgel SP-NPR, 2.5 µm, nonporous	polymer	Stainless Steel	4.6	3.5
21963	TSKgel SP-STAT, 10 µm, nonporous	polymer	Stainless Steel	3	3.5
21964	TSKgel SP-STAT, 7 µm, nonporous	polymer	Stainless Steel	4.6	10



TSKgel Butyl-NPR

TSKgel Ether-5PW

TSKgel Phenyl-5PW

Hydrophobic Interaction Tips:

- TSKgel Hydrophobic Interaction Chromatography (HIC) columns are offered in glass and stainless steel. Stainless steel (SS) or Pyrex frits are embedded in the body of the column end-fittings of metal and glass columns, respectively. The nominal frit size for SS columns is engraved in the end-fittings; Pyrex frits in the glass columns have a 10 μm nominal pore size.
- Halide salts corrode stainless steel tubing, fitting, and frits. Do not store SS columns in a mobile phase containing NaCl and, where possible, use another salt in the operating buffer. Chlorotrifluorethylene and tetrafluorethylene are the materials in the glass column fittings that come into contact with the mobile phase and sample.
- Good laboratory procedures demand that the analytical column be protected by a guard column. TSKgel guardgel kits, containing column hardware and gel packing, are available to pack your own guard column. Guard cartridges and packed guard columns are also available for use with TSKgel HIC columns.
- A guard column is not available for the TSKgel Butyl-NPR column. Be sure to use an in-line filter with 0.5 μm pores to avoid frequent plugging of the 1.0 μm pores in the NPR column frit. We also recommend a pre-injector membrane filter to prevent particles generated by pump seal wear from reaching the column.
- All TSKgel HIC columns can be routinely operated from pH 2.0-12.0.
- The TSKgel Ether-5PW and Phenyl-5PW HIC columns are physically and chemically stable in water-soluble organic solvents of concentration ratios under 50%, such as methanol, ethanol, and acetonitrile (DMF, DMSO, or chloroform <30%). Change the solvent gradually by reducing the flow rate (preferably with a gradient) because rapid change may cause degradation of column efficiency. Note: Reduce your salt concentration to prevent the precipitation of the salt on the column. Also chaotropic agents (urea, SDS, etc.) will reduce the adsorption of the biomolecule; therefore, use low levels of chaotropic agents (<2 mol/L).
- The TSKgel Butyl-NPR columns are compatible with water-soluble organic solvents of concentration range ≤ 50%.
- The addition of organic solvents or chaotropic agents in the final buffer can improve separation. Relative elution positions can change, however, so add chaotropic agent and organic solvent in small quantities.
- Periodic injections of one column volume of 0.2 mol/L NaOH remove strongly retained contaminants from the top of the column by hydrolysis or dissolution. Additionally, acetic acid (20-40%) can be used to regenerate the column. Note: Acid can precipitate protein.
- If the inlet frit of the column becomes plugged, rinse the column with water when operating the column in reverse flow direction. When all else fails, and at your own risk, remove the column end-fitting at the top of the column and sonicate it in 6 mol/L nitric acid. The end-fitting and frit are one piece. Be careful not to disturb the packing and rinse the fitting well after cleaning.
- The shipping solvent for all TSKgel HIC columns is distilled water.
- TSKgel HIC columns are supplied with an Inspection Data Sheet, which includes a QC chromatogram and test data, and an OCS Sheet summarizing the recommended operating conditions for optimum column performance.
- A separate TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems, can be downloaded from the Tosoh Bioscience LLC website (www.tosohbioscience.com).

About Hydrophobic Interaction Chromatography

Hydrophobic interaction chromatography (HIC) is based on non-polar interactions that are induced by high salt mobile phases. Stationary phases are similar to reversed phase chromatography (RPC) but the density of functional groups is lower. A weakly non-polar stationary phase is used with an aqueous mobile phase containing a high concentration of a chaotropic salt.

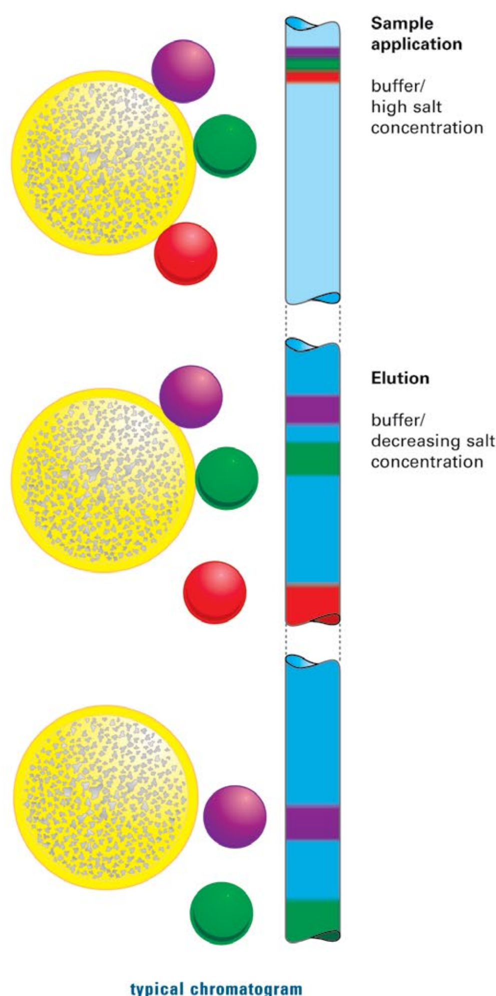
The technique is mainly applied to the separation of proteins, which are eluted by decreasing the salt concentration or by adding a low percentage of organic solvent. Although also based on hydrophobic interactions, selectivity in HIC separations is distinctly different from that in reversed phase chromatography. Despite the lower peak capacity in HIC compared to RPC, HIC has the advantage that the mobile phase conditions (primarily aqueous) do not usually disrupt higher-order protein structures.

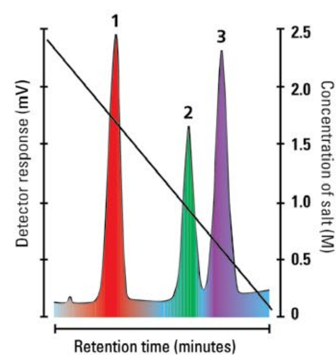
HIC is used in the biopharmaceutical industry for the analysis of antibody drug conjugates (ADCs) or to determine the aggregate content of monoclonal antibodies. The features and benefits of HIC are detailed in Table 1 below.

Table 1: Features and benefits of Hydrophobic Interaction Chromatography

Features	Benefits
Choice of three hydrophobic ligands (ether, phenyl, or butyl)	Added flexibility during method development
Rigid polymeric base resin	Wide pH range (2-12) of the base enabling robust cleaning options
Similar chemistry to TOYOPEARL resins	Seamless scalability from analytical to preparative scale
TSKgel Phenyl-5PW columns offered in PEEK hardware	Eliminates undesirable interactions with column hardware
TSKgel Ether-5PW and Phenyl-5PW columns available in 2 mm ID format	LC/MS applications

Figure 1: Hydrophobic Interaction Chromatography





TSKgel Hydrophobic Interaction Chromatography Columns

TSKgel HIC columns are polymethacrylate-based with a choice of three ligands (butyl, ether, and phenyl) with varied hydrophobicities from low to high, respectively (see Table 2). The HIC packing materials are based on the polymeric TSKgel G5000PW resin which is then derivatized with oligoethylene-glycol (Ether-5PW) or phenyl (Phenyl-5PW) groups. The base material used to prepare TSKgel Butyl-NPR colunms is of the same chemical composition as the TSKgel G5000PW base material used to prepare TSKgel Phenyl-5PW and Ether-5PW columns. The difference between the two packings is that the TSKgel 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.

The TSKgel HIC columns are compatible with water-soluble organic solvents at concentrations below 50% (20% for TSKgel Butyl-NPR). See Figure 2 for the structure of the TSKgel HIC resins. Table 3 lists well known applications for each type of TSKgel HIC column.

Figure 2: Structure of TSKgel HIC resins

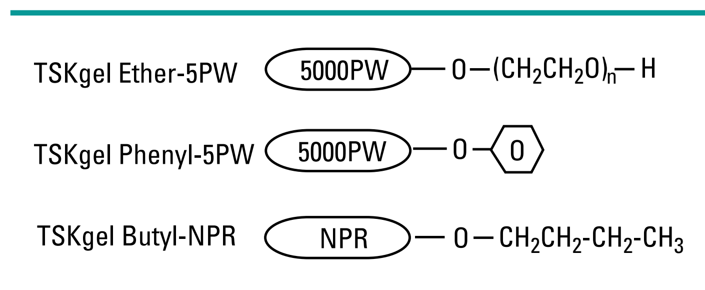
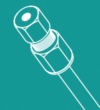


Table 2: Comparison of TSKgel HIC columns

TSKgel Column	Hydrophobicity	Benefits
Phenyl-5PW	Most hydrophobic	Requires modest salt concentration to retain proteins. Most popular column applicable for the widest range of hydrophobicities.
Ether-5PW	Less hydrophobic	Excellent choice for hydrophobic proteins such as membrane proteins or monoclonal antibodies.
Butyl-NPR	Least hydrophobic	Excellent choice for monoclonal antibody analysis and high speed applications; usually high recovery due to absence of pores.

Table 3: Column selection for TSKgel HIC columns

Sample	MM Range (Da)	TSKgel Column
Peptides	$<1.0 \times 10^4$	Butyl-NPR
Medium to large proteins	$>1.0 \times 10^4$	Phenyl-5PW Ether-5PW Butyl-NPR
DNA, RNA & PCR products	$>5.0 \times 10^5$	Phenyl-5PW Butyl-NPR
Oligonucleotides	$>1.0 \times 10^4$	Phenyl-5PW Butyl-NPR



About: TSKgel Butyl-NPR Hydrophobic Interaction Chromatography Columns

The 2.5 µm nonporous methacrylate packing material of the TSKgel Butyl-NPR columns is bonded with butyl groups. In terms of hydrophobicity, the TSKgel Butyl-NPR columns are the least hydrophobic of the HIC column offerings and require a higher salt concentration for binding. They are the best choice for high speed separations with excellent recovery, even for more hydrophobic samples. As in other modes of liquid chromatography, 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. Since the binding kinetics occur only on the bead's surface, nonporous resins are more efficient than porous particles of the same size. 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. Because the surface area of nonporous particles is much lower, analysts need to be aware that sample mass and volume need to be adjusted to maintain optimum column efficiency.

Attributes and Applications

Product attributes of the TSKgel Butyl-NPR columns are shown in Table 4. The ultra-efficient 2.5 µm nonporous resin makes TSKgel Butyl-NPR columns the preferred choice for time-critical QC analysis and sample limited applications.

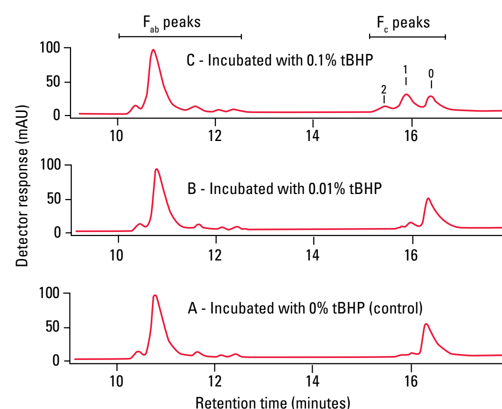
Table 4: Product attributes

Attribute	Value
Pore size (mean)	nonporous
Particle size (mean)	2.5 µm
pH stability	2.0-12.0
Functional group	butyl

Antibody Fragments

Figure 3 shows the separation of Fab and Fc fragments of an antibody on a TSKgel Butyl-NPR column. The appearance of additional Fc fragments is due to the oxidation of methionine residues by 0.10% t-butyl hydroperoxide (tBHP). The numbers above the Fc peaks correspond to the number of oxidized residues in each fragment.

Figure 3: Separation of Fab and Fc fragments



Column:	**TSKgel Butyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase:	A: 2 mol/L $(NH_4)_2SO_4$, 20 mmol/L Tris, pH 7.0 B: 20 mmol/L Tris, pH 7.0
Gradient:	0 min (10%B) 34 min (100%B)
Flow rate:	1 mL/min
Temperature:	30 °C

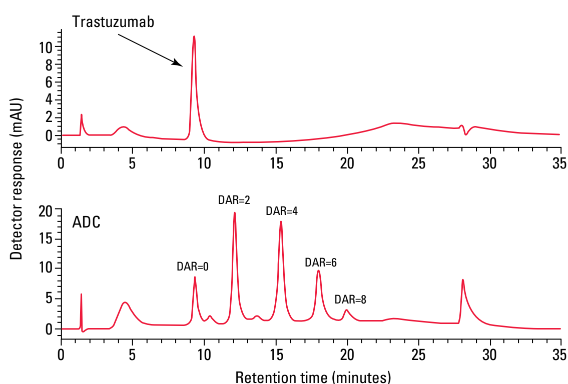
Drug to Antibody Ratio (DAR)

Both unconjugated monoclonal antibody (Trastuzumab) and drug conjugated Trastuzumab (Trastuzumab-vcMMAE) samples were independently injected onto a TSKgel Butyl-NPR, 2.5 μm, 4.6 mm ID × 10 cm column. After samples were injected onto the column, they were eluted with 2-propanol, an organic solvent, mixed in with a low concentration of sodium phosphate buffer as mentioned in the experimental conditions.

The unconjugated Trastuzumab sample was eluted as a major single peak at approximately 9.5 minutes (Figure 4, upper panel). This single peak indicated that the unconjugated Trastuzumab consisted of mostly homogeneous molecules. The profile of the drug conjugated Trastuzumab (an ADC) exhibited well resolved peaks with different retention times than that of the unconjugated drug and with baseline separation (Figure 4, lower panel). These well resolved peaks were suggested to have different drug-to-antibody ratio (DAR). These peaks ranged in DAR from 0 to 8, estimated based on the mobility of the peaks. The heterogeneity of this sample was due to the addition of different drug loads. Consequently, this caused a decrease in mobility which resulted in differing elution times; the lower drug-loaded peaks eluted first and the higher drug-loaded peaks eluted later.

The ADC peak with a retention time of 9.5 minutes displayed the same retention time as the unconjugated Trastuzumab peak (compare Figure 4 lower panel to upper panel). The retention time similarity of the two peaks indicated that it contained a group of non-conjugated Trastuzumab; therefore, it had the same mobility (retention time) to that of the non-conjugated Trastuzumab. This peak was called DAR = 0. When Trastuzumab was conjugated with 2 cell-killing drugs, it eluted later than the non-conjugated Trastuzumab and was called DAR = 2. DARs = 4, 6 and 8 peaks were labeled due their retention times, respectively.

Figure 4: Analysis of unconjugated Trastuzumab (upper panel) and drug conjugated Trastuzumab (lower panel) using TSKgel Butyl-NPR column

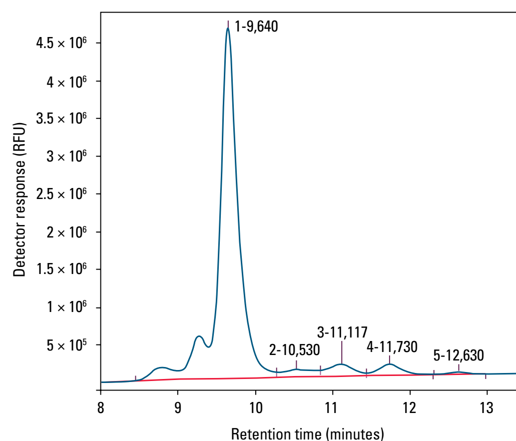


Column:	**TSKgel Butyl-NPR, 2.5 μm, 4.6 mm ID × 10 cm**
Mobile phase:	A: 25 mmol/L phosphate buffer, pH 7.0, + 1.5 mol/L ammonium sulfate B: 25 mmol/L phosphate buffer, pH 7.0, + 2-propanol - (80:20)
Gradient:	0 - 100 % B (20 minutes)
Flow rate:	0.5 mL/min
Detection:	UV @ 280 nm
Injection vol.:	10 μL
Samples:	Trastuzumab, 0.24 g/L ADC(Trastuzumab-vcMMAE), 2.2 g/L

Proteins

The use of a TSKgel Butyl-NPR column as an alternative to the size exclusion separation of a monoclonal antibody and its high molar mass aggregates is shown in Figure 5 below. Because of the high efficiency of the nonporous particles in the TSKgel Butyl-NPR column, only low sample amounts are needed for aggregate analysis.

Figure 5: Analysis of monoclonal antibody and aggregates using a TSKgel Butyl-NPR column

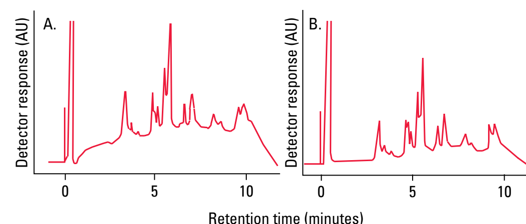


Column:	**TSKgel Butyl-NPR, 2.5 μm, 4.6 mm ID × 3.5 cm**
Mobile phase:	A: 3 mol/L NaCl B: H_2O
Gradient:	0-100% B in 10 min
Flow rate:	1.0 mL/min
Detection:	flourescence; Ex: 280 nm, Em: 348 nm
Injection vol.:	5 μg
Sample:	IgG_1

Phosphoglucose Isomerase

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 using a TSKgel Butyl-NPR column as shown in Figure 6.

Figure 6: Effect of sample load on the separation of phosphoglucose isomerase



Column:	**TSKgel Butyl-NPR, 2.5 μm, 4.6 mm ID × 3.5 cm**
Mobile phase:	10 min linear gradient of $(NH_4)_2SO_4$ from 1.8 mol/L to 0 mol/L in 0.1 mol/L phosphate buffer, pH 7.0
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Samples:	crude sample of phosphoglucose isomerase
Sample loads:	A: 25 μg B: 100 μg

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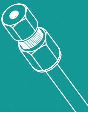


About: TSKgel Ether-5PW Hydrophobic Interaction Chromatography Columns

Of the three TSKgel HIC columns, the TSKgel Ether-5PW columns have intermediate hydrophobicity. TSKgel Ether-5PW columns are stable in either acid or caustic cleaning regimens and provide excellent access to larger molecules with low diffusion coefficients.

Attributes and Applications

Table 5 lists the product attributes of the TSKgel Ether-5PW columns. The TSKgel Ether-5PW columns are an excellent choice for separating hydrophobic molecules, including membrane proteins or monoclonal antibodies such as IgG or IgM.

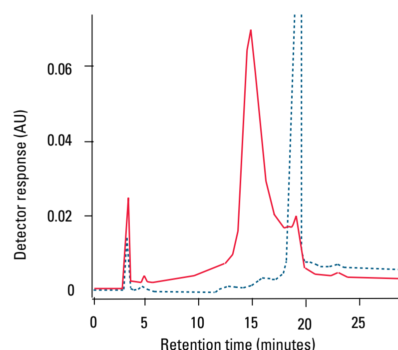
Table 5: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Particle size (mean)	10 µm
pH stability	2.0-12.0
Functional group	ether

Antibiotics

A 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 7. 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.

Figure 7: Purification of anti-tumor antibiotic

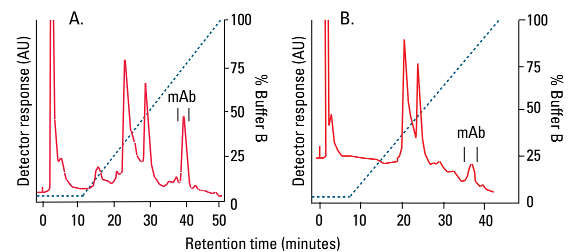


Column: **TSKgel Ether-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase: linear gradient from 1.5 mol/L to 0 mol/L $(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer, pH 7.0
Flow rate: 0.8 mL/min
Detection: UV @ 220 nm
Injection vol.: 20 µL
Sample: C-1027 ——— C-1027-AG - - - -
concentration: 1 g/L

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 8 shows typical results from the screening of two mAbs using a TSKgel Ether-5PW column.

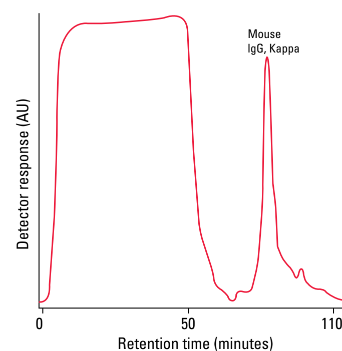
Figure 8: Screening of mouse monoclonal antibodies



Column: **TSKgel Ether-5PW, 10 µm, 8.0 mm ID × 7.5 cm, glass**
Mobile phase: linear gradient from A to B as shown
A: 0.05 mol/L sodium phosphate, pH 7.0, 2.0 mol/L ammonium sulfate, 1.0 mol/L glycine
B: 0.05 mol/L sodium phosphate, pH 7.0, 1.0 mol/L glycine
Flow rate: 10 mL/min
Detection: UV @ 280 nm
Samples: A: 20 µL unequilibrated mouse IgG_{2b} κ ascites
B: 20 µL unequilibrated mouse IgM κ ascites

TSKgel Ether-5PW columns have been used successfully for purifying membrane-bind proteins such as immunoglobulins. Figure 9 demonstrates this for a 50 mL injection of mouse IgG_{1k} monoclonal antibody on a TSKgel Ether-5PW, 8 mm ID × 7.5 cm glass column.

Figure 9: Monoclonal antibody purification



Column: **TSKgel Ether-5PW, 10 µm, 8.0 mm ID × 7.5 cm, glass**
Mobile phase: 67.5 min isocratic load and wash with 1 mol/L $(NH_4)_2SO_4$ in 1 mol/L glycine, 0.5 mol/L phosphate buffer, pH 7.0, followed by a 37.5 min linear gradient from 1.0 mol/L to 0 mol/L $(NH_4)_2SO_4$ in 1.0 mol/L glycine, 0.05 mol/L phosphate, pH 7.0
Flow rate: 1.0 mL/min
Detection: UV @ 280 nm, 3.0 AUFS
Injection vol.: 50 mL
Sample: 25 mL raw cell culture supernatant
- 200 mg total protein
- 15 mg total antibody diluted to 50 mL with initial elution buffer

About: TSKgel Phenyl-5PW Hydrophobic Interaction Chromatography Columns

TSKgel Phenyl-5PW columns were the first commercially available, polymer-based columns for high performance HIC. These columns have been instrumental in the increase in popularity of this technique for analytical, preparative, and process scale separations of biopolymers. The high porosity of TSKgel Phenyl-5PW packings allows very large proteins to enter the internal pore structure, thereby maintaining high capacity for such compounds.

Attributes and Applications

Product attributes of the TSKgel Phenyl-5PW columns are shown in Table 6. The most hydrophobic among the three TSKgel HIC columns, TSKgel Phenyl-5PW columns are an excellent choice to screen for the selectivity, retention, and recovery of most biomolecules.

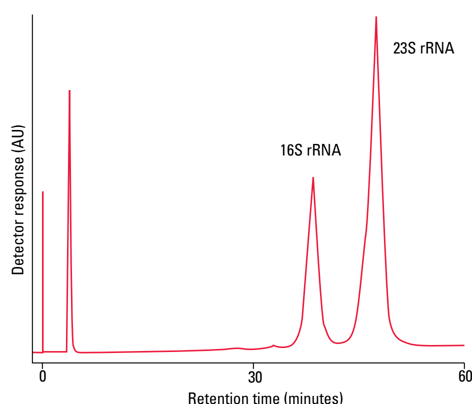
Table 6: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Particle size (mean)	10 µm and 13 µm
pH stability	2.0-12.0
Functional group	phenyl

RNA

Figure 10 illustrates the separation of 16S and 23S ribosomal RNA on a TSKgel Phenyl-5PW column. The approximate molar masses of these RNAs are 5.6×10^5 and 1.1×10^6 Da, respectively.

Figure 10: Analysis of ribosomal RNA

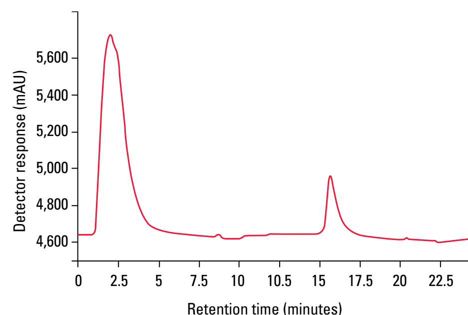


Column: **TSKgel Phenyl-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase: 60 min linear gradient from 2 mol/L to 0 mol/L $(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer, pH 7.0
Flow rate: 0.5 mL/min
Detection: UV @ 280 nm
Sample: 16S and 23S rRNA from *E. coli,* 0.05 mg in 0.1 mL

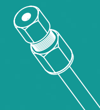
Calcium-Binding Proteins

Calcium-binding proteins are involved in signal transduction processes. One of these proteins, myristoylated frequenin, has a myristoyl group that protrudes in the presence of calcium. This characteristic can be exploited using HIC to purify the protein, as shown in Figure 11. A step gradient is employed on a TSKgel Phenyl-5PW glass column to purify myristoylated frequenin from crude *E. coli* lysate.

Figure 11: Purification of myristoylated frequenin



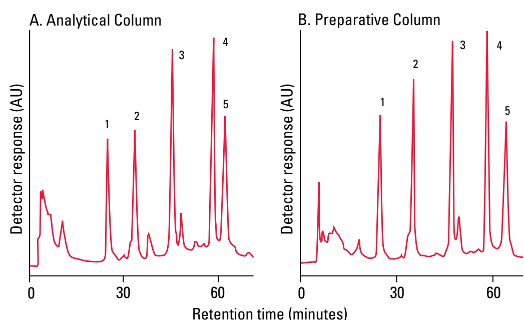
Column: **TSKgel Phenyl-5PW, 10 µm, 5 mm ID × 5 cm, glass**
Mobile phase: equilibration in 50 mmol/L HEPES, 100 mmol/L KCl, 1 mmol/L DTT, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, pH 7.5 step gradient at 12.5 min to 50 mmol/L HEPES, 1 mmol/L DTT, 1 mmol/L $MgCl_2$, 2 mmol/L EGTA, pH 7.5
Flow rate: 1 mL/min
Detection: UV @ 280 nm
Temperature: ambient
Sample: *E. coli* lysate containing myristoylated frequenin, 100 µL



Proteins: Scale up to Preparative Separations

Figure 12 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.

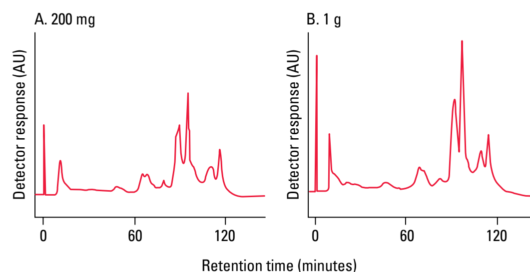
Figure 12: Scale up to preparative separations



Columns:	**A: TSKgel Phenyl-5PW, 10 µm, 7.5 mm ID × 7. 5 cm** **B: TSKgel Phenyl-5PW, 13 µm, 21.5 mm ID × 15 cm**
Mobile phase:	60 min linear gradient from 1.8 mol/L to 0 mol/L $(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer, pH 7.0
Flow rate:	0.5 mL/min (7.5 mm ID) or 4 mL/min (21.5 mm ID)
Detection:	UV @ 280 nm
Samples:	1. myoglobin 2. ribonuclease 3. lysozyme 4. α-chymotrypsinogen 5. α-chymotrypsin

Figure 13 compares resolution for a 200 mg injection of crude lipoxidase on a 21.5 mm ID × 15 cm TSKgel Phenyl-5PW column with that of a 1 g injection on a 55 mm ID × 20 cm column. As shown, the increase in particle size from 13 µm to 20 µm did not influence chromatographic resolution, keeping in mind that the sample load was only scaled up five-fold.

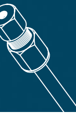
Figure 13: Purify grams of protein



Columns:	**A: TSKgel Phenyl-5PW, 13 µm, 21.5 mm ID × 15 cm** **B: TSKgel Phenyl-5PW, 20 µm, 55 mm ID × 20 cm**
Mobile phase:	120 min linear gradient from 1.5 mol/L to 0 mol/L$(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer, pH 7.0
Flow rate:	4 mL/min (21.5 mm ID) and 40 mL/min (55 mm ID)
Detection:	UV @ 280 nm
Sample:	crude lipoxidase, 200 mg (21.5 mm ID) and 1 g (55 mm ID)

Ordering Information

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
14947	TSKgel Butyl-NPR, 2.5 µm, nonporous	polymer	Stainless Steel	4.6	3.5
42168	TSKgel Butyl-NPR, 2.5 µm, nonporous	polymer	Stainless Steel	4.6	10
14013	TSKgel Ether-5PW Glass, 10 µm, 100 nm	polymer	Glass	5	5
14014	TSKgel Ether-5PW Glass, 10 µm, 100 nm	polymer	Glass	8	7.5
18760	TSKgel Ether-5PW, 10 µm, 100 nm	polymer	Stainless Steel	2	7.5
08641	TSKgel Ether-5PW, 10 µm, 100 nm	polymer	Stainless Steel	7.5	7.5
14025	TSKgel Glass Guardgel Kit for 5 mm ID and 8 mm ID TSKgel Ether-5PW glass columns, 20 µm	polymer	Glass		
42156	TSKgel Guard Cartridge for 2 mm ID TSKgel Ether-5PW column, 3 pk, 10 µm	polymer	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
08643	TSKgel Guardgel Kit for 7.5 mm ID TSKgel Ether-5PW column, 20 µm	polymer	Stainless Steel		
20023	TSKgel BioAssist Phenyl, 10 µm, 100 nm	polymer	PEEK	7.8	5
13063	TSKgel Phenyl-5PW Glass, 10 µm, 100 nm	polymer	Glass	5	5
08804	TSKgel Phenyl-5PW Glass, 10 µm, 100 nm	polymer	Glass	8	7.5
18759	TSKgel Phenyl-5PW, 10 µm, 100 nm	polymer	Stainless Steel	2	7.5
07573	TSKgel Phenyl-5PW, 10 µm, 100 nm	polymer	Stainless Steel	7.5	7.5
07656	TSKgel Phenyl-5PW, 13 µm, 100 nm	polymer	Stainless Steel	21.5	15
42155	TSKgel Guard Cartridge for 2 mm ID TSKgel Phenyl-5PW column, 3 pk, 10 µm	polymer	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
07652	TSKgel Guardgel Kit for 7.5 mm ID TSKgel Phenyl-5PW column, 20 µm	polymer	Stainless Steel		
16095	TSKgel Guardgel Kit for 21.5 mm ID TSKgel Phenyl-5PW column, 20 µm	polymer	Stainless Steel		

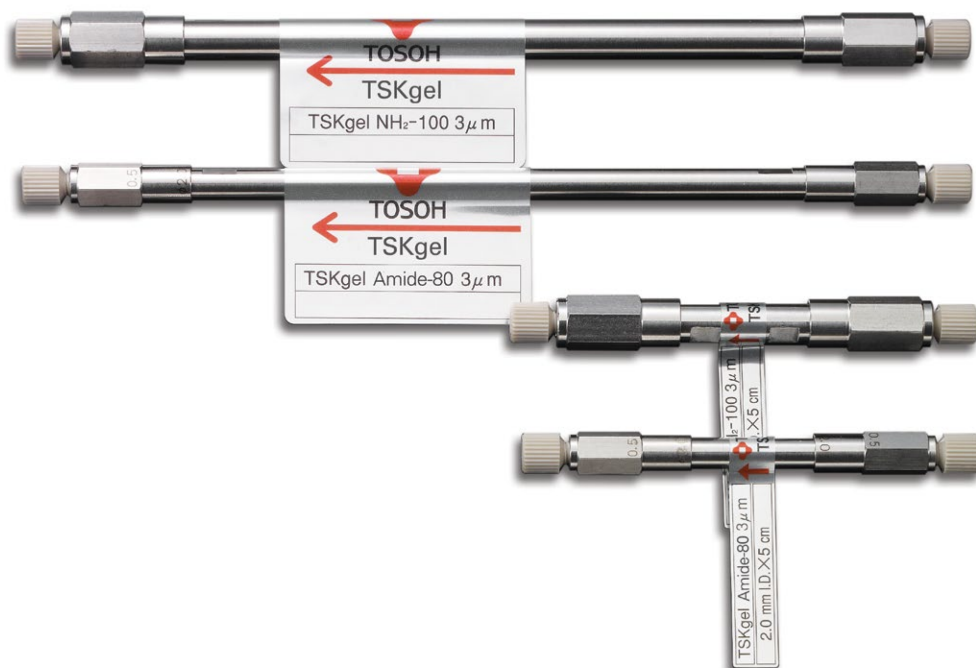


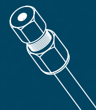
TSKgel Amide-80

TSKgel NH_2-100

HILIC Tips:

- TSKgel HILIC columns are offered in stainless steel. Stainless steel (SS) frits are embedded in the body of the column end-fittings of the metal columns. The nominal frit size for SS columns is engraved in the end-fittings.
- Halide salts corrode stainless steel tubing, fitting, and frits. Do not store SS columns in a mobile phase containing NaCl and, where possible, use another salt in the operating buffer.
- Good laboratory procedures demand that the analytical column be protected by a guard column. Guard cartridges and packed guard columns are available for use with TSKgel HILIC columns.
- Column shipping solvents are: 85% acetonitrile - 15% water for TSKgel Amide-80, 2 µm, 3 µm & HR columns and TSKgel NH_2-100 columns; 75% acetonitrile - 25% water for 5 µm & 10 µm TSKgel Amide-80 columns.
- TSKgel HILIC columns are supplied with an Inspection Data Sheet, which includes a QC chromatogram and test data, and an OCS Sheet summarizing the recommended operating conditions for optimum column performance.
- A separate TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems, can be downloaded from the Tosoh Bioscience LLC website (www.tosohbioscience.com).





About Hydrophilic Interaction Chromatography

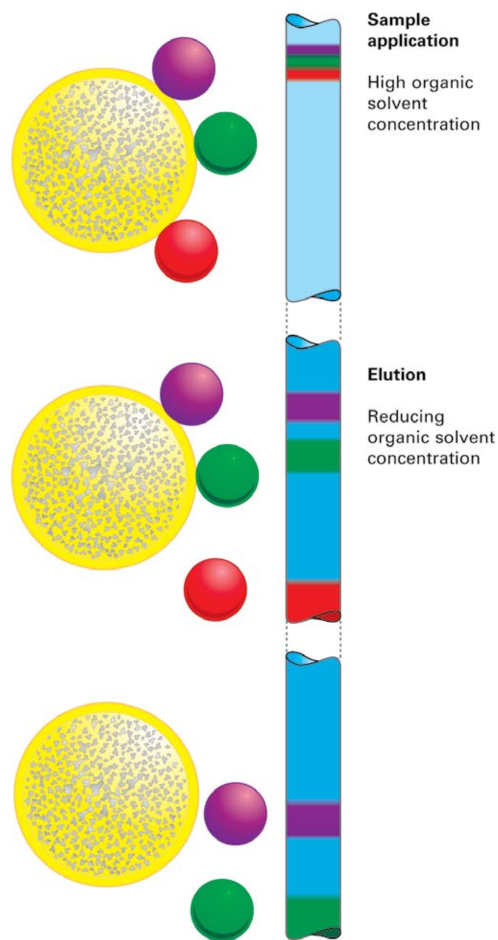
Normal phase and hydrophilic interaction liquid chromatography (HILIC) are primarily used to separate polar and hydrophilic compounds. In reversed phase mode very polar compounds are often not sufficiently retained in low percent organic, or even in 100% aqueous mobile phase. The order of elution in normal phase is opposite that found in reversed phase for the same mixture of compounds. Although non-polar organic mobile phases and a silica stationary phase were used traditionally in normal phase LC, today most separations are performed with aqueous-organic mobile phases and a more polar-bonded stationary phase. This mode of HPLC is now commonly referred to as HILIC, hydrophilic interaction liquid chromatography.

By using an amide or amino bonded phase column, polar compounds can be retained by a normal phase or hydrophilic interaction chromatography retention mechanism. Typical mobile phases in HILIC are aqueous buffers with organic modifiers – primarily acetonitrile. In contrast to the retention behavior in reversed phase, in HILIC, solutes will be retained longer when increasing the percent acetonitrile.

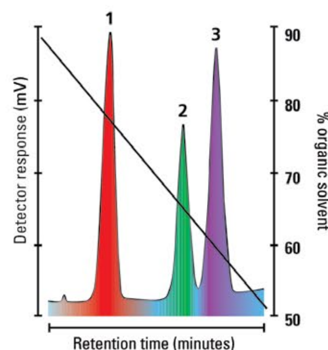
Typical applications for HILIC are:

- Analysis of polyols, carbohydrates, or vitamins
- Characterization of protein glycosylation by fluorescence or mass spectrometric detection
- Separation of polar peptides, e.g. after enzymatic digestion of proteins (peptide mapping)
- Analysis of polar drugs and separation of drug metabolites
- LC/MS analysis of polar compounds

Figure 1: Hydrophilic Interaction Chromatography



typical chromatogram



TSKgel HILIC Chromatography Columns

The TSKgel HILIC chromatography column line consists of TSKgel Amide-80 and TSKgel NH_2-100. TSKgel Amide-80 columns are covalently bonded with carbamoyl groups. Offering a different selectivity from the TSKgel Amide-80 columns, the TSKgel NH_2-100 amino-bonded phase columns offer superior chemical stability. The structures of the TSKgel Amide-80 and NH_2-100 resins are shown in Figure 2. See Table 1 for details on these two TSKgel HILIC columns.

Figure 2: Structures of TSKgel HILIC resins

R = Spacer

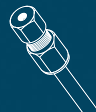
TSKgel NH_2-100

*The spacer (R) contains secondary as well as tertiary amino groups.

TSKgel Amide-80

Table 1: Features of TSKgel HILIC columns

	TSKgel Amide-80	TSKgel NH_2-100
Bonded Phase	Does not react with reducing sugars. Anomer formation can be prevented by raising mobile phase temperature up to 50 °C for 2 & 3 µm columns and up to 80 °C for 5 & 10 µm columns.	More stable than conventional amino phases due to a special endcapping prior to introduction of aminoalkyl groups. Amino-bonded phase can react with a reducing sugar to form a Schiff base
Detectors	Can be used with evaporative light scattering (ELS) and mass spec (MS) detectors	
Mobile Phase	Stable in 100% organic eluents	
Solutes	Retains very polar drug candidates and drug metabolites	
Applications	Saccharides	
	Polyols (polyalcohols)	
	Drug metabolites	Methotrexate polyglutamate derivatives
	Peptides	Pyridylaminated oligosaccharides
	Melamine and cyanuric acid	Water-soluble vitamins
	Oligonucleotides	Nucleic acid fragments
	Polar compounds in fermentation broth	
	Cytosine	



About: TSKgel Amide-80 HILIC Chromatography Columns

TSKgel Amide-80 columns are packed with 2, 3, 5, or 10 µm spherical silica particles that are covalently bonded with carbamoyl groups. The amide stationary phase provides a unique selectivity under regular normal phase conditions or in the hydrophilic interaction mode of chromatography. TSKgel Amide-80 columns possess superior stability in aqueous/organic solvent systems, an advantage over other hydrophilic stationary phases such as traditional amino-bonded phase columns. 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 2 µm TSKgel Amide-80 columns offer higher resolution and faster analysis with equivalent retention and selectivity as TSKgel Amide-80, 3 µm columns. This allows for smooth method transfer from a 3 µm to a 2 µm column. In addition, TSKgel Amide-80, 2 µm columns more highly retain hydrophilic compounds than competitive amide columns on the market.

Also available within this line is a TSKgel Amide-80 HR column in 5 µm, 4.6 mm ID × 25 cm size. These columns are prepared from the same 5 µm packing material and thus offer the same selectivity as existing 5 µm TSKgel Amide-80 columns. In contrast to traditional 4.6 mm ID × 25 cm TSKgel Amide-80 columns, the new TSKgel Amide-80 HR columns offer higher specifications for plate count (N = >18,000 vs. 8,000). Because HR columns are prepared from the same bonded phase lots as standard 5 µm TSKgel Amide-80 columns, the only change you will notice is more efficient chromatography!

Attributes and Applications

Table 2 lists the attributes of TSKgel Amide-80 columns. Target applications for the TSKgel Amide-80 columns include the analysis of saccharides, glycans, oligosaccharides, peptides, and polar compounds from natural products. Unique advantages of the TSKgel Amide-80 phase for saccharide analysis include a novel hydrogen bonding retention mechanism between hydroxyl groups of the sample and the carbamoyl group in the stationary phase. The stationary phase does not react with reducing sugars and can be used at elevated temperatures (4 to 80 °C) to prevent peak splitting of carbohydrates that can occur at lower temperatures.

Table 2: Product attributes

Attribute	Value
Pore size (silica)*	8 nm
Particle size	2 µm, 3 µm, 5 µm, or 10 µm
pH stability	2.0-7.5
Functional group	carbamoyl
Max. temperature	50 °C (2 & 3 µm), 80 °C (5 & 10 µm)
Surface area (m^2/g)	450

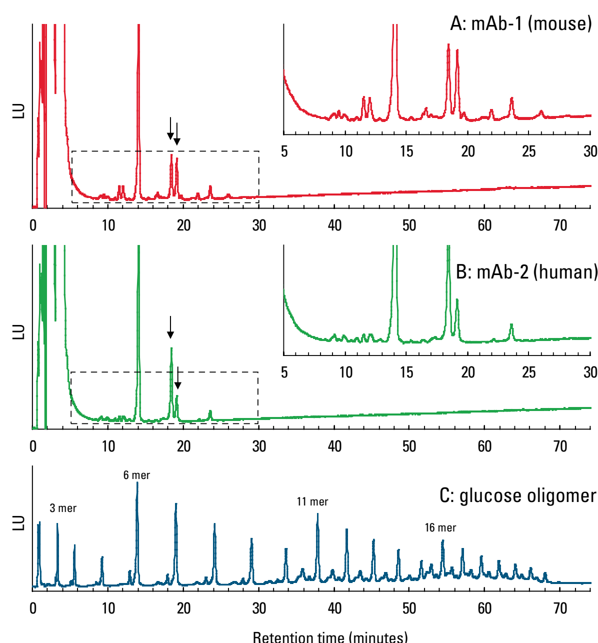
*The pore size of the bonded phase is indicated by the number in the product description, in this case TSKgel Amide-80 has 8 nm nominal pore size. The nominal pore size of the starting base silica is 10 nm.

Glycans

Pyridylamination is a fluorescence-tagging method for oligosaccharides that enables measurement and structural analyses of glycans. Glycans do not contain fluorophores and thus must be labeled with fluorescent tags prior to analysis. 2-aminobenzamide (2-AB) is one of the most common labels used.

Several peaks of glycans were separated from both mouse IgG and human IgG (Figure 3). These peaks were similar in elution time to 6-8 mer glucose. When comparing chromatograms A and B, different ratios of glycan attached to each mAb is implied.

Figure 3: Comparison of Glycan Peak Profile of Monoclonal Antibodies



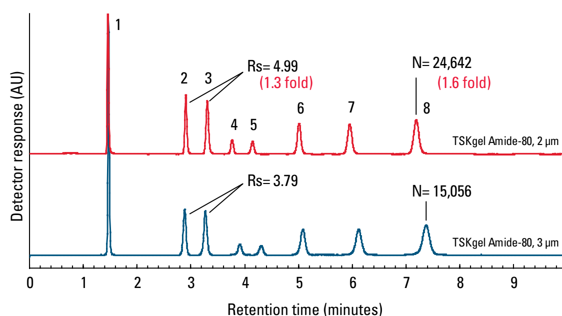
Column:	**TSKgel Amide-80, 2 µm, 2.0 mm ID × 15 cm**
Mobile phase:	A: 200 mmol/L acetic acid + triethylamine, pH 7.3 B: ACN
Gradient:	75% B (0 - 5 min) 75 - 50% B (5 - 80 min, linear)
Flow rate:	0.5 mL/min
Detection:	fluorescence (λ_{ex}: 315 nm, λ_{em}: 380 nm)
Temperature:	40 °C
Injection vol.:	50 µL
Samples:	A: pyridylaminated oligosaccharides released from mAb-1 (mouse) B: pyridylaminated oligosaccharides released from mAb-2 (human) C: PA-glucose oligomer (3 - 22 mer) (TaKaRa Bio)

Hydrophilic Molecules

A set of hydrophilic molecules, such as nucleosides, sugars, hydrotropes etc., were analyzed using TSKgel Amide-80, 3.0 mm ID × 15 cm columns of 2 and 3 µm particle size. As seen in Figure 4, similar chromatographic profiles were obtained with similar selectivity. The smaller particle size of the TSKgel Amide-80, 2 µm column yielded a 1.6-fold increase in theoretical plates and a 1.3-fold higher resolution.

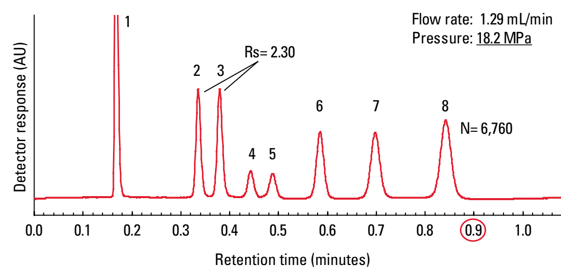
A TSKgel Amide-80, 2 µm column showed impressive results for an ultra-high speed analysis of these same samples (Figure 5). A less than one minute separation was obtained using a TSKgel Amide-80, 2 µm column at a flow rate of 1.29 mL/min. In addition, the 2 µm column showed a lower pressure drop than the maximum pressure of a conventional HPLC system. Therefore, it is not necessary to use a UHPLC system for this type of ultra-high fast separation.

Figure 4: Higher Resolution with TSKgel Amide-80, 2 µm Column

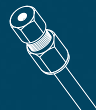


Columns:	**TSKgel Amide-80, 2 µm, 2.0 mm ID × 15 cm**
	TSKgel Amide-80, 3 µm, 3.0 mm ID × 15 cm
Mobile phase:	20 mmol/L NH_4OAc, pH 4.7 / acetonitrile = 10/90
Flow rate:	0.43 mL/min
Detection:	UV @ 254 nm
Temperature:	40 °C
Injection vol.:	2 µL
Samples:	1. toluene (1 g/L)
	2. theophylline (0.1 g/L)
	3. theobromine (0.1 g/L)
	4. NPβGlu (0.1 g/L)
	5. NPαGlu (0.1 g/L)
	6. 2'-deoxyuridine (0.1 g/L)
	7. 5-methyluridine (0.1 g/L)
	8. uridine (0.1 g/L)

Figure 5: Ultra-high Fast Separation with TSKgel Amide-80, 2 µm Column



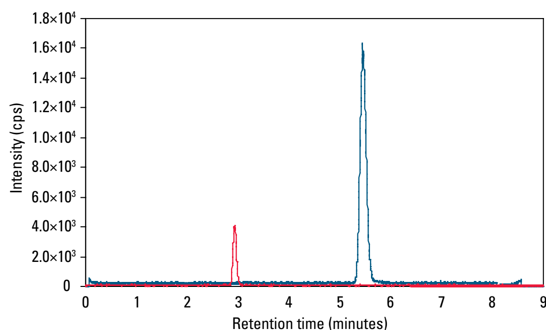
Column:	**TSKgel Amide-80, 2 µm, 2.0 mm ID × 5 cm**
Mobile phase:	20 mmol/L NH_4OAc, pH 4.7 / acetonitrile = 10/90
Flow rate:	1.29 mL/min
Detection:	UV @ 254 nm
Temperature:	40 °C
Injection vol.:	2 µL
Samples:	1. toluene (1 g/L)
	2. theophylline (0.1 g/L)
	3. theobromine (0.1 g/L)
	4. NPβGlu (0.1 g/L)
	5. NPαGlu (0.1 g/L)
	6. 2'-deoxyuridine (0.1 g/L)
	7. 5-methyluridine (0.1 g/L)
	8. uridine (0.1 g/L)



Melamine and Cyanuric Acid in Milk

To aid chemists charged with the determination of melamine and related products in milk, Tosoh scientists developed a method for the simultaneous determination of melamine and cyanuric acid by HILIC/MS/MS using a 3 µm TSKgel Amide-80 column. Milk was spiked with melamine and cyanuric acid standards to serve as a model sample. High recovery and excellent resolution was obtained for both compounds, as shown in Figure 6.

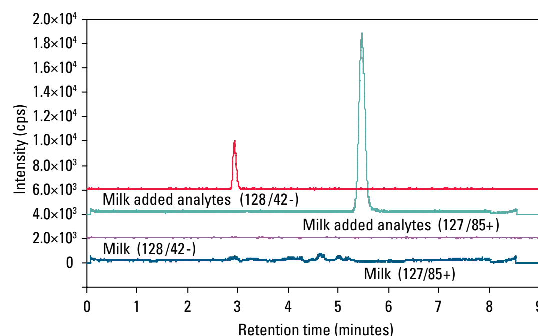
Figure 6: Separation of melamine and cyanuric acid in milk



Column:	**TSKgel Amide-80, 3 µm, 2.0 mm ID × 15 cm**
Mobile phase:	A: 0.05% formic acid in H_2O
	B: 0.05% formic acid in ACN
	A/B = 25/75
Flow rate:	0.2 mL/min
Temperature:	40 °C
Injection vol.:	5 µL
Instrument:	Q TRAP® (AB Sciex)
Ion source:	ESI
	127/85+ (melamine)
	128/42- (cyanuric acid)

Multiple Reaction Monitoring is a mode of MS/MS that yields maximum sensitivity and selectivity for known target analytes. Figure 7 shows the results of this type of mass analysis on unspiked and spiked milk samples. The figure demonstrates that the original milk sample did not contain any amount of either melamine or cyanuric acid. After adding the compounds to the milk sample, melamine and cyanuric acid were independently detected, with more than sufficient resolution between the compounds.

Figure 7: Multiple Reaction Monitoring (MRM) chromatograms of milk and spiked milk samples - 10 ppb each



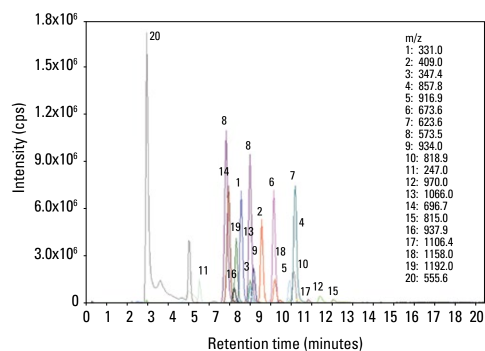
Column:	**TSKgel Amide-80, 3 µm, 2.0 mm ID × 15 cm**
Mobile phase:	A: 0.05% formic acid in H_2O
	B: 0.05% formic acid in ACN
	A/B = 25/75
Flow rate:	0.2 mL/min
Temperature:	40 °C
Injection vol.:	5 µL
Instrument:	Q TRAP (AB Sciex)
Ion source:	ESI
	127/85+ (melamine)
	128/42- (cyanuric acid)

Tryptic Digest

Scientists have begun to employ the hydrophilic stationary phase in separations of natural product extracts, peptide digests, and other polar compound mixtures. Often, these complex mixtures are analyzed using the power of mass spectrometry in conjunction with liquid chromatography. TSKgel Amide-80 columns are advantageous for these applications because unwanted secondary ionic interactions from residual silanols can be eliminated by adding trifluoroacetic acid (TFA) to the mobile phase. The use of "mass-spec friendly" TFA eliminates extra steps involved with removing salts or non-volatile acids required by amino-bonded columns to eliminate ionic interactions.

Figure 8 details the use of a 5 µm TSKgel Amide-80 column for the separation of a β-lactoglobulin tryptic digest, with separation achieved within 12 minutes.

Figure 8: Separation of β-lactoglobulin tryptic digest

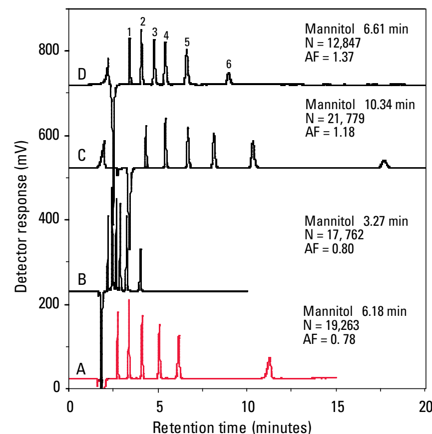


Column:	**TSKgel Amide-80, 5 µm, 2.0 mm ID × 15 cm**
Mobile phase:	A: 0.1% TFA in H_2O B: 0.1% TFA in ACN
Gradient:	0 min (95%B) 15 min (50%B) 17 min (50%B)
Flow rate:	0.2 mL/min
Injection vol.:	1 µL
Sample:	β-lactoglobulin tryptic digest
Instrument:	Q TRAP (AB Sciex)
Ion Source:	ESI+

Sugar Alcohols

Figure 9 shows comparative chromatograms of the separation of sugar alcohols using a TSKgel Amide-80 column and competitive HILIC columns. The TSKgel Amide-80, 3 µm column showed high column efficiency for mannitol, while the selectivity for saccharides was as good or better for the TSKgel Amide-80 column compared to the competitive HILIC columns.

Figure 9: Comparing the retention of sugar alcohols



Columns:	**A: TSKgel Amide-80, 3 µm, 4.6 mm ID × 15 cm** B: Luna® NH_2, 4.6 mm ID × 15 cm C: Polyamine®, 4.6 mm ID × 25 cm D: NH_2P-50 4E®, 4.6 mm ID × 25 cm
Mobile phase:	H_2O/ACN (25/75)
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. ethylene glycol 2. glycerin 3. erythritol 4. xylitol 5. mannitol 6. inositol

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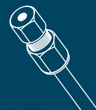
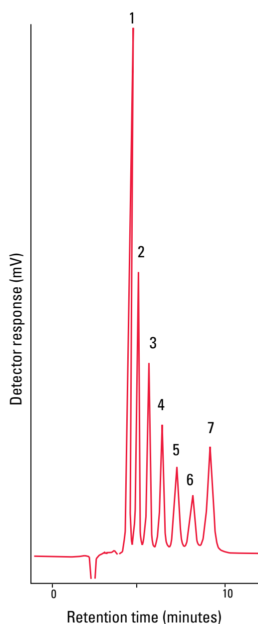
technical service: 800-366-4875



Carbohydrates

A TSKgel Amide-80 column can separate oligosaccharides very rapidly and efficiently. Figure 10 shows a separation of a β-cyclodextrin hydrolysate in less than 10 minutes. The peak numbers indicate the degree of polymerization of the repeating base sugar in the oligosaccharide with 1 representing a single glucose unit, 2 a dimer, etc.

Figure 10: Separation of oligosaccharides

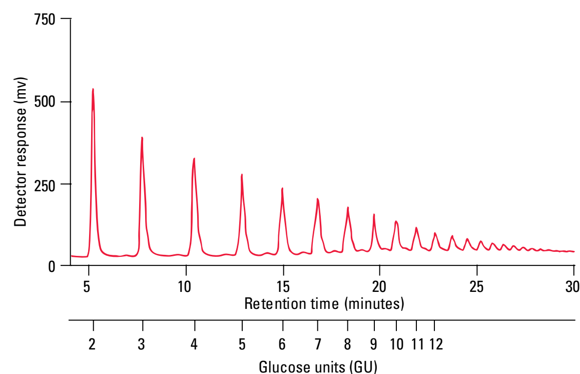


Column:	**TSKgel Amide-80, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase:	ACN/H_2O (55/45)
Flow Rate:	1.0 mL/min
Detection:	RI
Temperature:	25 °C
Sample:	2 µL, β-cyclodextrin hydrolysate, 1-7 indicate degree of polymerization (4.6 g/L)

Carbohydrates

TSKgel Amide-80 chemistry is ideally suited for the separation of carbohydrate structures. Figure 11 shows the high resolution separation of a 2-aminobenzamide (2AB) labeled dextran ladder within 30 minutes on a TSKgel Amide-80, 3 µm column. This ladder can be used as a calibration standard for HPLC and MS analysis of glycans. The ladder contains glucose homopolymer species from degree of polymerization (dp) 1 to dp 22 (i.e. the glucose monomer GU1-2AB to GU22-2AB).

Figure 11: 2-AB Labeled Glucose Homopolymer (GHP) Ladder



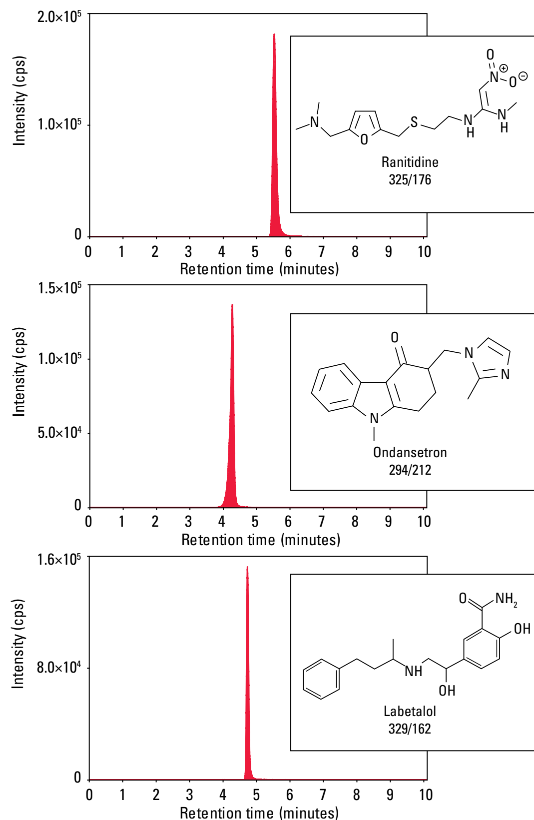
Column:	**TSKgel Amide-80, 3 µm, 2.0 mm ID × 15 cm**
Mobile phase:	A: 50 mmol/L ammonium formate, pH 4.3 B: ACN
Gradient:	0-35 min - 75-35% B
Flow rate:	0.22 mL/min
Detection:	fluorescence Ex: 360 nm, Em: 425 nm
Temperature:	50 °C
Injection vol.:	3 µL
Sample:	CAB-GHP dextran ladder (Ludger: ~ 300 fmol for GU2)

*Courtesy of K. Darsow & H. Lange, Institute of Bioprocessing, University of Nurnberg/ Erlangen

Polar Drugs

TSKgel Amide-80 columns are also a valuable tool for the analysis of small molar mass polar drugs that are not sufficiently retained on reversed phase columns. Figure 12 shows the separation of polar drug standards in HILIC mode using a 3 µm TSKgel Amide-80 column coupled with electrospray ionization mass spectroscopy (ESI/MS). Due to the high organic content of the eluent, HILIC analysis provides increased detection sensitivity.

Figure 12: Separation of polar drug standards

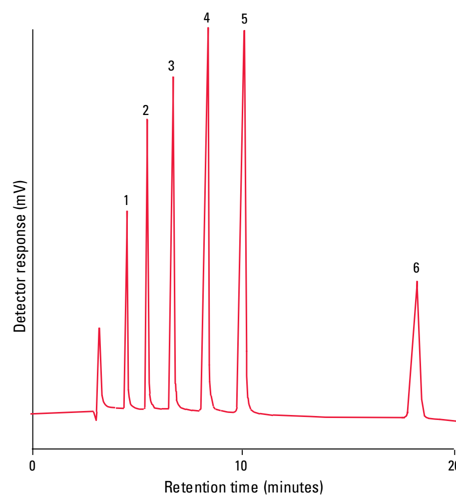


Column:	**TSKgel Amide-80, 3 µm, 2.0 mm ID × 15 cm**
Mobile phase :	A: 10 mol/L ammonium formate, pH 3.75 B: ACN
Gradient :	0 min (90%B) 10 min (40%B) 13 min (40%B)
Flow rate :	0.2 mL/min
Injection vol.:	5 µL (50 µg/L)
Samples:	ranitidine ondansetron labetalol
Instrument :	Q TRAP (AB Sciex) LC/MS/MS
Ion Source:	ESI+

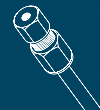
Polyalcohols

Polyalcohols are typically separated using a TSKgel Amide-80 column with a mobile phase of organic solvent and water as shown in Figure 13.

Figure 13: Separation of polyalcohols



Column:	**TSKgel Amide-80, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase:	ACN/H_2O (75/25)
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	25 °C
Samples:	1. ethylene glycol, 10 mmol/L, 20 µL 2. glycerin, 10 mmol/L, 20 µL 3. erythritol, 10 mmol/L, 20 µL 4. xylitol, 10 mmol/L, 20 µL 5. mannitol, 10 mmol/L, 20 µL 6. inositol, 4 mmol/L, 20 µL



About: TSKgel NH_2-100 HILIC Chromatography Columns

TSKgel NH_2-100 amino columns expand the range of TSKgel columns for hydrophilic interaction chromatography (HILIC). Offering a different selectivity from the well known TSKgel Amide-80 columns, these novel amino-bonded phase columns stand out by providing much improved chemical stability, a prerequisite for achieving reproducible and reliable results.

TSKgel NH_2-100 columns are packed with 3 µm silica particles containing 10 nm pores. A novel bonding strategy was adopted to improve chemical stability of the bonded phase. First, the silica is reacted with a trimethylsilane endcapping reagent at a low stoichiometric ratio before reacting residual and accessible silanol groups with trifunctional alkylaminosilane reagent. The resulting bonded phase provides a better safeguard against hydrolysis of the underlying silica.

TSKgel NH_2-100 columns are unique in that the bonded phase ligand not only has a terminal primary amino group as expected, but that the spacer also incorporates secondary as well as tertiary amino groups. Anionic compounds are retained on the column by ionic interaction. This allows for the use of gradients in salt concentration in addition to gradient elutions with acetonitrile. Since the TSKgel NH_2-100 columns have cationic sites, the columns can be used as mixed mode columns under some conditions.

Also available within this line is a TSKgel NH_2-100 DC column that connects directly to TSKgel reversed phase columns. The DC in TSKgel NH_2-100 DC emphasizes this Direct Connect aspect. This column has the same packing material as the TSKgel NH_2-100 columns, so therefore shows high retention for hydrophilic compounds/ions. A male-type outlet end-fitting enables the direct connection to the screw-type end-fitting of a TSKgel reversed phase column. This allows for the simultaneous separation using a linear gradient of an active pharmaceutical ingredient (API) and its counterion without the loss of column efficiency normally experienced when connecting two columns with capillary tubing.

Attributes and Applications

Product attributes of the TSKgel NH_2-100 columns are listed in Table 3. TSKgel NH_2-100 columns are well suited for the analysis of all types of hydrophilic compounds, including carbohydrates and peptides. Due to a high ligand density and large surface area, these columns show stronger retention of polar compounds than TSKgel Amide-80 columns.

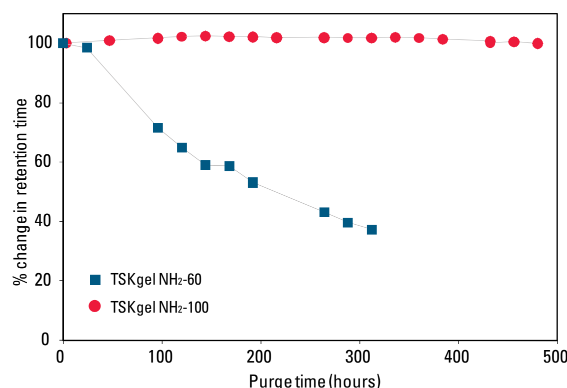
Table 3: TSKgel NH_2-100 product attributes

Attribute	Value
Pore size (silica)	10 nm
Particle size (mean)	3 µm
pH stability	2.0-7.5
Functional group	alkylamine
Temperature range (°C)	10-50
Surface area (m^2/g)	450

Performance Data

Figure 14 shows the result of a long term chemical stability comparison between the more recently developed TSKgel NH_2-100 column and the older TSKgel NH_2-60 column. Both columns were purged for 300 hours in 25% H_2O/75% ACN and you can see that the retention time of inositol on the TSKgel NH_2-60 column decreased more than 60% from its initial retention time. In the case of the endcapped TSKgel NH_2-100 HILIC column, the retention time of inositol decreased less than 20%.

Figure 14: Chemical stability study

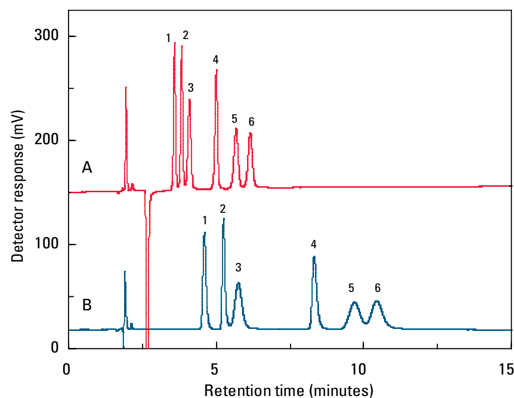


Columns:	**TSKgel NH_2-100, 3 µm, 4.6 mm ID × 15 cm** **TSKgel NH_2-60, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase:	H_2O/ACN (25/75)
Flow Rate:	1.0 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol.:	10 µL
Sample:	inositol

Sugars

A TSKgel NH2-100 column was used to analyze sugars - a typical analysis done in the food industry. Two different eluents were used to compare retention times and peak shapes. As shown in Figure 15, a mixture of water and acetone provided superior resolution within 6 minutes; whereas an eluent mixture of triethylaluminium-hydroxymethylfuranal and acetone did not produce as sharp of peaks and the retention time was over 4 minutes longer.

Figure 15: Analysis of common sugars

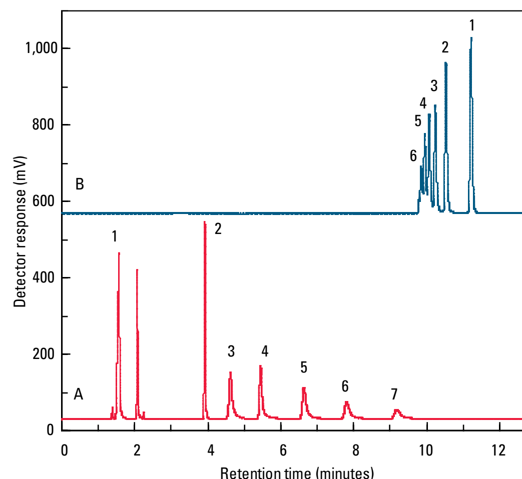


Column:	**TSKgel NH2-100, 3 µm, 4.6 mm ID × 15 cm**
Mobile phase:	A: H_2O/acetone (25/75)
	B: 100 mmol/L TEA-FA, pH 10.0/acetone (25/75)
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	50 °C
Injection vol.:	10 µL
Samples:	1. fructose
	2. sorbitol
	3. glucose
	4. sucrose
	5. maltose
	6. lactose

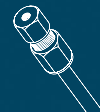
Methotrexate and Derivatives

Figure 16 shows the results of methotrexate and its derivatives ($MTXPG_{2\text{~}7}$) analyzed on TSKgel NH2-100, 3 µm HILIC and TSKgel ODS-100V, 3 µm reversed phase narrow bore columns. The MTX and polyglutamate derivatives were eluted in the order of the number of glutamate groups in their molecules on the TSKgel NH2-100 HILIC column, but eluted in reverse order on the TSKgel ODS-100V column. Despite the early elution of MTX and $MTXPG_2$ on the TSKgel NH2-100 HILIC column, the overall separation is better than what can be accomplished on the C18 column.

Figure 16: Separation of methotrexate and derivatives



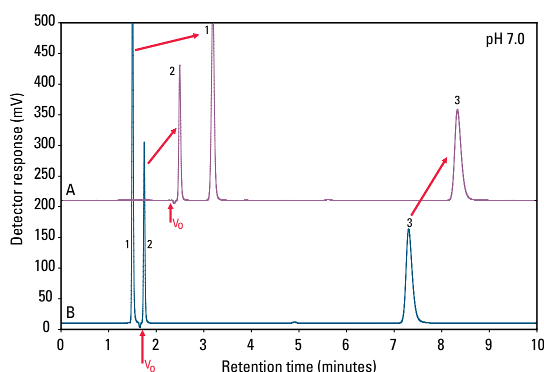
Columns:	**A. TSKgel NH2-100, 3 µm, 2.0 mm ID × 15 cm**
	B. TSKgel ODS-100V, 3 µm, 2.0 mm ID × 15 cm
Mobile phase:	A: A) H_2O/ACN (10/90) + 0.1% TFA
	B) H_2O + 0.1% TFA
	B: A) H_2O/ACN (10/90) + 0.1% TFA
	B) ACN + 0.1% TFA
Gradient:	0 min (0%B) 15 min (40%B) 17 min (0%B)
Flow rate:	0.20 mL/min
Detection:	UV @ 313 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. MTX (MTXPG) 2. $MTXPG_2$
	3. $MTXPG_3$ 4. $MTXPG_4$
	5. $MTXPG_5$ 6. $MTXPG_6$
	7. $MTXPG_7$



Drug and Counter Ions at pH 7.0

Maleic acid and p-toluene sulfonic acid are commonly used as counter ions in pharmaceutical preparations. Both of these organic acids are hydrophilic and are not retained on a TSKgel ODS-100V reversed phase column at pH 7.0 in 70% methanol eluent (Figure 17). With the connection of a TSKgel NH_2-100 DC column prior to the TSKgel ODS-100V column, the simultaneous determination of maleic acid and the API desipramine becomes possible. Maleic acid is slightly retained on the TSKgel NH_2-100 DC column by an anion exchange interaction. Desipramine, on the other hand, does not interact with the protonated amino groups as it is positively charged.

Figure 17: Simultaneous determination of maleic acid and the API desipramine at pH 7.0

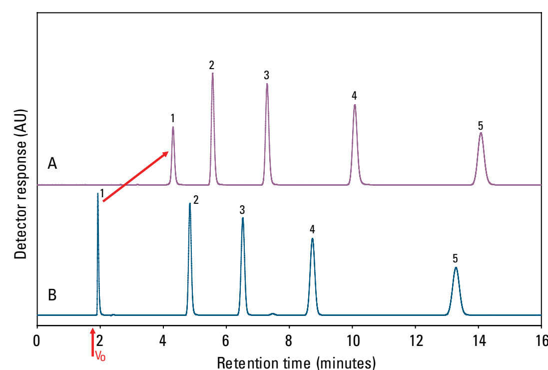


Columns:	**A: TSKgel NH_2-100 DC, 3 µm, 4.6 mm ID × 5 cm**
	+ TSKgel ODS-100V, 3 µm, 4.6 mm ID × 15 cm
	B: TSKgel ODS-100V, 3 µm, 4.6 mm ID × 15 cm
Mobile phase:	50 mmol/L phosphate buffer, pH 7.0/MeOH = 30/70
Flow rate:	1.0 mL/min
Detection:	UV @ 210 nm
Temperature:	40 °C
Inj. volume:	5 µL
Samples:	1. maleic acid (50 mg/L)
	2. *p*-toluene sulfonic acid (50 mg/L)
	3. desipramine (50 mg/L)

Cold Medicine Ingredients

Guaiacol sulfonic acid, a hydrophilic counter ion, is an expectorant used in pharmaceutical cold preparations that are sold over the counter (OTC) in many countries, but not in the US. Guaiacol sulfonic acid elutes in the solvent front on a C18 column, but is retained on a TSKgel NH_2-100 DC, 3 µm column. Direct Connection (DC) of the TSKgel NH_2-100 DC, 3 µm column to a TSKgel ODS-100V, 3 µm column allows for the simultaneous determination of APIs and guaiacol sulfonic acid in a single run as shown in Figure 18.

Figure 18: Separation of cold medicine ingredients



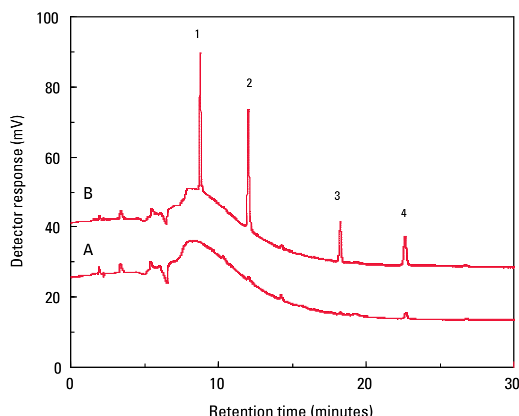
Columns:	**A) TSKgel NH_2-100 DC, 3 µm, 4.6 mm ID × 5 cm**
	+ TSKgel ODS-100V, 3 µm, 4.6 mm ID × 15 cm
	B) TSKgel ODS-100V, 3 µm, 4.6 mm ID × 15 cm
Mobile phase:	50 mmol/L NaH_2PO_4, pH 2.5/MeOH = 65/35
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	40 °C
Injection vol.:	5 µL
Samples:	1. guaiacol sulfonic acid (50 mg/L)
	2. anhydrous caffeine (25 mg/L)
	3. salicylamide (125 mg/L)
	4. aspirin (250 mg/L)
	5. ethenzamide (125 mg/L)

Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) by HPLC is required for several drug classes, as the effective dosage range, varying between insufficient activity and toxic levels, is patient dependent. Although a reversed phase column is typically adopted for separating drugs in blood samples, hydrophilic compounds show poor retention times on an ODS column. A TSKgel NH_2-100, 3 µm HILIC column was investigated for the separation of hydrophilic drugs and its metabolites in blood.

Theophylline is a medication that is used for treating airway spasms in people with asthma or chronic obstructive pulmonary disease (COPD). Serum levels of this drug correlate well with both therapeutic and toxic effects. As demonstrated in Figure 19, spiked samples of theophylline and its metabolites could be separated successfully with a TSKgel NH_2-100 column using an off-line deproteinization procedure.

Figure 19: Separation of theophylline and metabolites



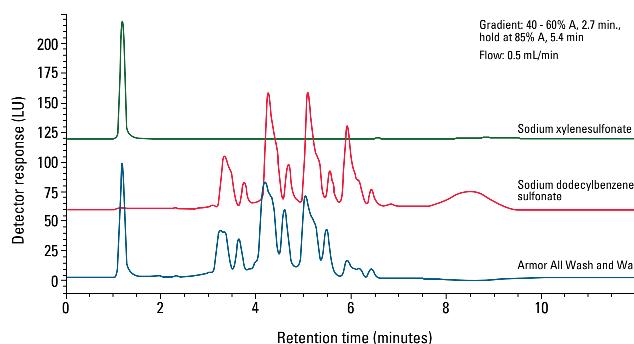
Column:	**TSKgel NH_2-100, 3 µm, 2.0 mm ID × 15 cm**
	A: Supernatant of serum deproteinated with 10-fold ACN B: Supernatant of spiked serum deproteinated with 10-fold ACN
Mobile phase:	A: 0.1 mol/L triethylamine-formic acid, pH 10.0/ACN (5/95) B: 0.1 mol/L triethylamine-formic acid, pH 10.0/ACN (50/50)
Gradient:	0 min-2 min (0% B) 2-30 min (80% B) 30-32 min (0% B)
Flow rate:	0.25 mL/min
Detection:	UV @ 254 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. theophylline 2. 3-methylxanthine 3. 1,3-dimethyluric acid 4. 1-methyluric acid (concentration: 10 mg/L for each)

2D-LC Separation of Cationic, Anionic, and Hydrotropic Surfactants

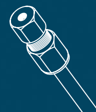
Surfactants are frequently found in pharmaceutical and biopharmaceutical drug applications as well as in common household products. Because they can be polar, non-polar, or amphoteric, the structural diversity of the surfactants and complexity of the sample matrix can make their separation and identification by HPLC challenging.

Figure 20 illustrates the characterization of the surfactant profile of Armor All™ Wash and Wax using a TSKgel ODS-140HTP and TSKgel NH_2-100 columns in series. As shown, the use of these columns yielded excellent separation and retention of the anionic surfactant sodium dodecylbenzene sulfonate and the hydrotropic surfactant sodium xylene sulfonate present in the Armor All formulation. Additionally, the use of fluorescence detection (λex: 225 nm, λem: 300-400 nm) allowed for increased sensitivity of the low level surfactants found in the product.

Figure 20: Characterization of surfactant profile in Armor All Wash and Wax using 2D-LC with the TSKgel ODS-140HTP and TSKgel NH_2-100 columns



Columns:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 5 cm** **TSKgel NH_2-100, 3 µm, 2 mm ID × 15 cm**
Mobile phase:	A: CH_3CN B: 100 mmol/L ammonium acetate, pH 5.4
Gradient:	40-60% A, 2.7 minutes, hold at 85% A, 5.4 minutes
Flow rate:	0.5 mL/min
Detection:	UV @ 280 nm, 254 nm, and 210 nm FLD λex 280 nm, λem 350 nm
Temperature:	30 °C
Injection vol.:	1 µL
Samples:	Triton™ X Triton N sodium xylenesulfonate sodium dodecylbenzene sulfonate



Ordering Information

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
21999	TSKgel NH_2-100 DC, 3 µm, 10 nm	silica	Stainless Steel	4.6	5
21967	TSKgel NH_2-100, 3 µm, 10 nm	silica	Stainless Steel	2	5
21968	TSKgel NH_2-100, 3 µm, 10 nm	silica	Stainless Steel	2	15
21969	TSKgel NH_2-100, 3 µm, 10 nm	silica	Stainless Steel	4.6	5
21970	TSKgel NH_2-100, 3 µm, 10 nm	silica	Stainless Steel	4.6	15
21971	TSKgel Guard Cartridge for 2 mm ID TSKgel NH_2-100 columns, 3 pk, 3 µm	silica	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
21972	TSKgel Guard Cartridge for 4.6 mm ID TSKgel NH_2-100 columns, 3 pk, 3 µm	silica	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID cartridges		Stainless Steel	3.2	1.5
23454	TSKgel Amide-80, 2 µm, 10 nm	silica	Stainless Steel	2	5
23455	TSKgel Amide-80, 2 µm, 10 nm	silica	Stainless Steel	2	10
23456	TSKgel Amide-80, 2 µm, 10 nm	silica	Stainless Steel	2	15
23457	TSKgel Amide-80, 2 µm, 10 nm	silica	Stainless Steel	3	5
23458	TSKgel Amide-80, 2 µm, 10 nm	silica	Stainless Steel	3	10
23459	TSKgel Amide-80, 2 µm, 10 nm	silica	Stainless Steel	3	15
23460	TSKgel Guard Column for 10 µm TSKgel Amide-80 columns	silica	Stainless Steel	2	1
21864	TSKgel Amide-80, 3 µm, 10 nm	silica	Stainless Steel	2	5
21865	TSKgel Amide-80, 3 µm, 10 nm	silica	Stainless Steel	2	15
22850	TSKgel Amide-80, 3 µm, 10 nm	silica	Stainless Steel	3	5
22851	TSKgel Amide-80, 3 µm, 10 nm	silica	Stainless Steel	3	10
22852	TSKgel Amide-80, 3 µm, 10 nm	silica	Stainless Steel	3	15
21866	TSKgel Amide-80, 3 µm, 10 nm	silica	Stainless Steel	4.6	5
22849	TSKgel Amide-80, 3 µm, 10 nm	silica	Stainless Steel	4.6	10
21867	TSKgel Amide-80, 3 µm, 10 nm	silica	Stainless Steel	4.6	15
21982	TSKgel Amide-80 HR, 5 µm, 10 nm	silica	Stainless Steel	4.6	25
20009	TSKgel Amide-80, 5 µm, 10 nm	silica	Stainless Steel	1	5
20010	TSKgel Amide-80, 5 µm, 10 nm	silica	Stainless Steel	1	10
21486	TSKgel Amide-80, 5 µm, 10 nm	silica	Stainless Steel	1	15
21487	TSKgel Amide-80, 5 µm, 10 nm	silica	Stainless Steel	1	25
19694	TSKgel Amide-80, 5 µm, 10 nm	silica	Stainless Steel	2	5
19695	TSKgel Amide-80, 5 µm, 10 nm	silica	Stainless Steel	2	10
19696	TSKgel Amide-80, 5 µm, 10 nm	silica	Stainless Steel	2	15
19697	TSKgel Amide-80, 5 µm, 10 nm	silica	Stainless Steel	2	25
19532	TSKgel Amide-80, 5 µm, 10 nm	silica	Stainless Steel	4.6	5
19533	TSKgel Amide-80, 5 µm, 10 nm	silica	Stainless Steel	4.6	10
13071	TSKgel Amide-80, 5 µm, 10 nm	silica	Stainless Steel	4.6	25
14459	TSKgel Amide-80, 10 µm, 10 nm	silica	Stainless Steel	7.8	30
14460	TSKgel Amide-80, 10 µm, 10 nm	silica	Stainless Steel	21.5	30

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
21862	TSKgel Guard Cartridge for 2 mm ID TSKgel Amide-80 columns, 3 pk, 3 µm	silica	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
21863	TSKgel Guard Cartridge for 4.6 mm ID TSKgel Amide-80 columns, 3 pk, 3 µm	silica	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID cartridges		Stainless Steel	3.2	1.5
21941	TSKgel Guard Cartridge for 2 mm ID TSKgel Amide-80 columns, 3 pk, 5 µm	silica	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
19021	TSKgel Guard Column for 4.6 mm ID TSKgel Amide-80 columns, 5 µm	silica	Stainless Steel	4.6	1
19010	TSKgel Guard Cartridge for 4.6 mm ID TSKgel Amide-80 columns, 3 pk, 5 µm	silica	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID cartridges		Stainless Steel	3.2	1.5
14461	TSKgel Guard Column for 21.5 mm ID TSKgel Amide-80 column, 10 µm	silica	Stainless Steel	21.5	7.5

Silica-based:

TSKgel Protein C4-300
TSKgel ODS-140HTP
TSKgel ODS-100V
TSKgel ODS-100Z
TSKgel Super-ODS
TSKgel Super-Octyl
TSKgel Super-Phenyl
TSKgel CN-80Ts
TSKgel Octyl-80Ts
TSKgel ODS-80TM
TSKgel ODS-80Ts
TSKgel ODS-80Ts QA
TSKgel ODS-120A
TSKgel ODS-120T
TSKgel OligoDNA-RP
TSKgel TMS-250

Polymer-based:

TSKgel Octadecyl-2PW
TSKgel Octadecyl-4PW
TSKgel Octadecyl-NPR
TSKgel Phenyl-5PW RP

Reversed Phase Tips:

- TSKgel reversed phase columns are offered in stainless steel hardware. Stainless steel (SS) frits are embedded in the body of the column endfittings. The nominal frit size for SS columns is engraved in the endfittings.

- Halide salts corrode stainless steel tubing, fitting, and frits. Do not store SS columns in a mobile phase containing NaCl and, where possible, use another salt in the operating buffer.

- Good laboratory procedures demand that the analytical column be protected by a guard column. TSKgel guardgel kits, containing column hardware and gel packing, are available to pack your own guard column. In addition, guard cartridges, guardfilters, and packed guard columns are available for use with TSKgel reversed phase columns.

- Caution: The silica particles in TSKgel Super series columns have a relatively small pore volume, which results in shorter retention times than obtained on most other reversed phase columns. For instance, to achieve similar retention times as obtained on TSKgel ODS-100V, the percentage organic solvent in the mobile phase has to be reduced by about 5-10% on a TSKgel Super-ODS column.

- Optimizing results with the TSKgel Super series columns: TSKgel 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.

- TSKgel reversed phase columns are supplied with an Inspection Data Sheet, which includes a QC chromatogram and test data, and an OCS Sheet summarizing the recommended operating conditions for optimum column performance.

- A separate TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems, can be downloaded from the Tosoh Bioscience LLC website (www.tosohbioscience.com).

About Reversed Phase Chromatography

Reversed Phase Chromatography (RPLC or RPC) is the most efficient of all biomolecule separation techniques. It has been the technique of choice for the analysis of small molar mass compounds in both the pharmaceutical and chemical industries, as well as in biomedical research, since the late 1970s. More recently, RPC has become the accepted tool for the separation of peptides, proteins and other biopolymers, making it largely responsible for the widespread popularity of HPLC as a chromatographic technique.

The opposite of normal phase chromatography, RPC requires a non-polar stationary phase and a mobile phase that consists of a mixture of water and polar-solvent mobile phase. The so-called "hydrophobic effect" is the major driving force for retention in RPC. The hydrophobic effect is related to the non-polar surface area of the solute molecule, which varies as a function of mobile phase composition, while the strength of the hydrophobic bond is proportional to the decrease in molecular surface area when the solute associates with the carbon-based stationary phase. Mobile phase additives, such as trifluoroacetic acid, increase protein hydrophobicity by forming ion pairs that strongly adsorb to the stationary phase. Typically, the mobile phase consists of a mixture of water (buffer) and acetonitrile, methanol or, less common, THF, or 2-propanol. The biological molecules are eluted from the chromatographic support by a change in the polarity of the mobile phase.

Silica particles are most commonly used as the support, which then is derivatized with octadecylsilane (ODS). Polymer-based supports have been introduced as an alternative to silica-based reversed phase columns, particularly for analyzing basic compounds in their neutral state at high pH.

RPC columns can be applied to the analysis of a wide variety of compounds, ranging from neutral polar and non-polar solutes to acidic, basic, and amphoteric compounds. RPC is also an efficient technique for the analysis of derivatized amino acids, peptides and proteins, although protein structure is not always maintained due to the high concentration of organic solvent required for their elution.

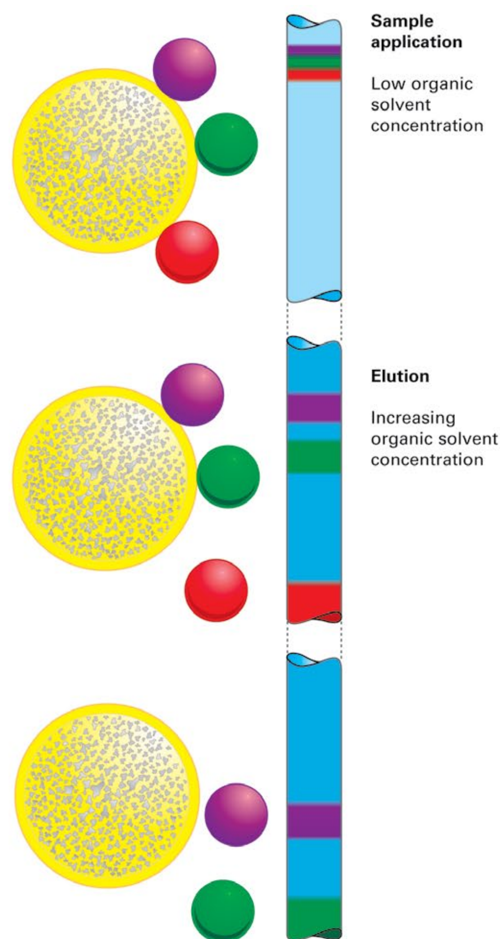
TSKgel Reversed Phase Chromatography Columns

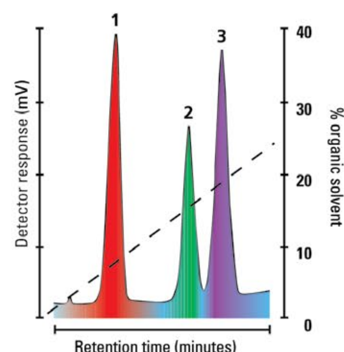
Tosoh Bioscience offers 18 distinct Reversed Phase column types which are based on either silica or methacrylate particles, as discussed in Table 1.

Table 1: Overview of TSKgel RPC columns

Silica-based columns	Polymer-based columns
High purity Type B silica High efficiencies Excellent recoveries Low bleed for MS	Hydrophilic backbone to improve recovery and reduce secondary interactions. pH stable from 1 to 12. Compatibility with organic solvents eliminates swelling.
An excellent choice for analysis of small molecules and peptides. Grouped into 6 product families.	An excellent choice for large MW biomolecules (>1.0 × 10^4 Da) and for analyzing small MM compounds at high pH. Offered in 4 different chemistries.
• Protein C4-300 • ODS-140HTP • ODS-100V and 100Z (10 nm) • Monomeric bonded silica (8 nm) • High efficiency (14 nm) • Specialty silica columns	• Octadecyl-2PW (12.5 nm) • Octadecyl-4PW (50 nm) • Phenyl-5PW RP (100 nm) • Octadecyl-NPR (nonporous)

Figure 1: Reversed Phase Chromatography





The silica-based TSKgel reversed phase product line consists of ten stationary phases designed for the analysis of low molar mass compounds, including pharmaceutical drugs, forensic compounds, derivatized amino acids, carbohydrates, steroids, lipids, and fatty acids, as well as two stationary phases with larger pore size designed for protein analysis.

TSKgel silica packings consist of spherical particles with uniform pore sizes of 8, 10, 12, 14, 25, or 30 nm bonded with a monomeric or polymeric layer of octadecyl, octyl, cyano, trimethylsilyl, or phenyl groups. Several of the stationary phases are subsequently derivatized with trimethylsilyl groups by a proprietary method that deactivates residual but accessible silanol groups.

Polymethacrylate-based reversed phase columns are available in a range of pore and particle sizes. Although often not as efficient as and less robust than silica-based RPC columns, key advantages of polymer-based columns are the fact that they are chemically stable from pH 2 to 12, which allows many basic compounds to be analyzed in their uncharged form, thus reducing secondary adsorption and improving peak shape and improving recovery for peptides and proteins due to reduced secondary interactions.

Tables 2 and 3 feature the properties and applications of each of the TSKgel silica-based and polymer-based reversed phase columns.

Table 2: Properties of TSKgel silica-based RPC columns

Properties of Silica-Based TSKgel RPC Columns						
Column	**Functional group**	**End-capped**	**% Carbon**	**Particle size (μm)**	**Pore size (nm)**	**Application/Features**
Protein C_4-300	C4 alkyl, polymeric	Yes	3	3	30	For recovery and resolution of large biomolecules, such as proteins
ODS-140HTP	C18 alkyl, polymeric	Yes	6	2.3	14	UHPLC applicable; high throughput separations; high resolution and short analysis time at moderate pressures
ODS-100V	C18 alkyl, monomeric	Yes	15	3, 5	10	Initial choice; general purpose column
ODS-100Z	C18 alkyl, monomeric	Yes	20	3, 5	10	Initial choice; general purpose column
ODS-120T	C18 alkyl, polymeric	Yes	22	5, 10	15	Specialty column for analysis of peptides, small proteins, and small molecular weight compounds
ODS-120A	C18 alkyl, polymeric	No	22	5, 10	15	Specialty column for analysis of polyaromatic hydrocarbons. Best choice for steric selectivity
ODS-80Ts	C18 alkyl, monomeric	Yes	15	5, 10	8	Low MW pharmaceuticals, bases, nucleosides and nucleotides. Ideal for strongly basic or charged compounds
ODS-80Ts QA	C18 alkyl, monomeric	Yes	15	5	8	Tighter specs than standard ODS-80Ts
ODS-80Tm	C18 alkyl, monomeric	Yes	15	5, 10	8	General purpose column for low MW pharmaceuticals, bases, nucleosides and nucleotides
Oligo-DNA RP	C18 alkyl, monomeric	No	10	5	25	For analysis and purification of oligonucleotides, RNA and DNA-fragments
Octyl-80Ts	C8 alkyl, monomeric	Yes	10	5	8	Ideal choice for highly hydrophobic small molecules; Reduced tailing when analyzing basic compounds

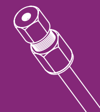


Table 2 Continued: Properties of TSKgel silica-based RPC columns

Properties of Silica-Based TSKgel RPC Columns						
Column	**Functional group**	**Endcapped**	**% Carbon**	**Particle size (µm)**	**Pore size (nm)**	**Application/Features**
Super-ODS	C18 alkyl, polymeric	Yes	6	2.3	14	UHPLC-like resolution and speed with conventional HPLC systems; improved sensitivity; savings in time and solvent; less hydrophobic than C18; allows for rapid, high resolution separations of small proteins, pharmaceuticals, and aromatic compounds
Super-Octyl	C8 alkyl, polymeric	Yes	5	2.3	14	
Super-Phenyl	Phenyl alkyl, polymeric	Yes	3	2.3	14	
CN-80Ts	CN, monomeric	Yes	9	5	8	Polar peptides, amino acids, and other pharmaceutical and food & beverage products
TMS-250	C1 alkyl, monomeric	Yes	5	10	25	For recovery and resolution of large biomolecules, such as proteins

Table 3: Properties of TSKgel polymer-based RPC columns

Properties of Polymer-Based TSKgel RPC Columns						
Column	**Functional group**	**Endcapped**	**% Carbon**	**Particle size (µm)**	**Pore size (nm)**	**Application/Features**
Octadecyl-2PW	C18 alkyl, monomeric	-	-	5	12.5	Peptides up to 8,000 Da and small proteins
Octadecyl-4PW	C18 alkyl, monomeric	-	-	7, 13	50	Great for high pH separations of small molecules and proteins; Available in analytical and semi-preparative scale
Phenyl-5PW RP	Phenyl, monomeric	-	-	10, 13	100	Ideal for large, globular protein samples up to 1.0×10^6 Da; highly stable in low and high pH environments
Octadecyl-NPR	C18 alkyl, monomeric	-	-	2.5	nonporous	High efficiency separations and fast analysis of peptides and proteins with excellent pH stability

About: TSKgel Protein C4-300 Reversed Phase Chromatography Columns

TSKgel Protein C4-300 columns are designed for the optimal recovery and resolution of proteins such as recombinant proteins, antibody fragments or PEGylated proteins.

The 30 nm pore size of the TSKgel Protein C4-300 columns are ideal for the separation of proteins. A particle size of 3 µm and optimized ligand density and alkyl length result in better protein and peptide resolution compared to other leading RP-C4 HPLC phases.

The C4 short alkyl chain ligand and its controlled bonding density provide moderate hydrophobicity to the stationary phase, which results in protein separations with high recovery and less peak tailing. The large pore size, allowing macromolecules to enter the interior of the pore, provides higher peak capacities than reversed phase columns with 10 nm pore size.

Attributes and Applications

The silica-based, wide pore TSKgel Protein C4-300 HPLC columns are suitable for highly efficient, reversed phase separations of large biomolecules such as proteins. Table 4 lists the attributes of TSKgel Protein C4-300.

Table 4. Product attributes

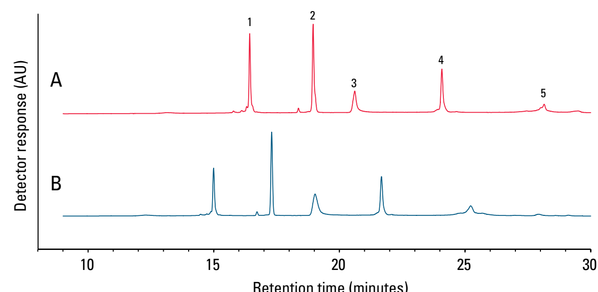
Attribute	Value
Pore size	30 nm
Endcapped	Yes (Trimethylsilyl)
Particle size	3 µm
pH stability	1.5-7.5
Ligand	C4 (Butyl)
Surface area (m^2/g):	100
% Carbon	3%

Standard Proteins

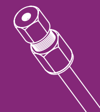
Figure 2 shows the separation of a mixture of standard proteins on the TSKgel Protein C4-300 column compared to a competitor column with 3.5 µm particle size. The resolution between cytochrome c and lysozymes reaches 24.8 on the TSKgel Protein C4-300 column compared to 18.6 on the competitor C4 column.

Furthermore, the TSKgel column shows higher theoretical plates and less peak tailing, especially for BSA (Peak 3), and also a better resolution of minor peaks.

Figure 2. Comparison of standard protein separation



Columns:	**A. TSKgel Protein C4-300, 3 µm, 4.6 mm ID × 15 cm**
	B. Competitor A, 3.5 µm, 4.6 mm ID × 15 cm
Mobile phase:	A: $H_2O/CH_3CN/TFA$ = 90/10/0.05 (v/v/v)
	B: $H_2O/CH_3CN/TFA$ = 20/80/0.05 (v/v/v)
Gradient:	0 min (0% B) 45 min (100% B)
Flow rate:	1.0 mL/min
Detection:	UV @ 210 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. cytochrome C 2. lysozyme
	3. BSA 4. α-chymotrypsinogen A
	5. ovalbumin (each 2 µg/10 µL)

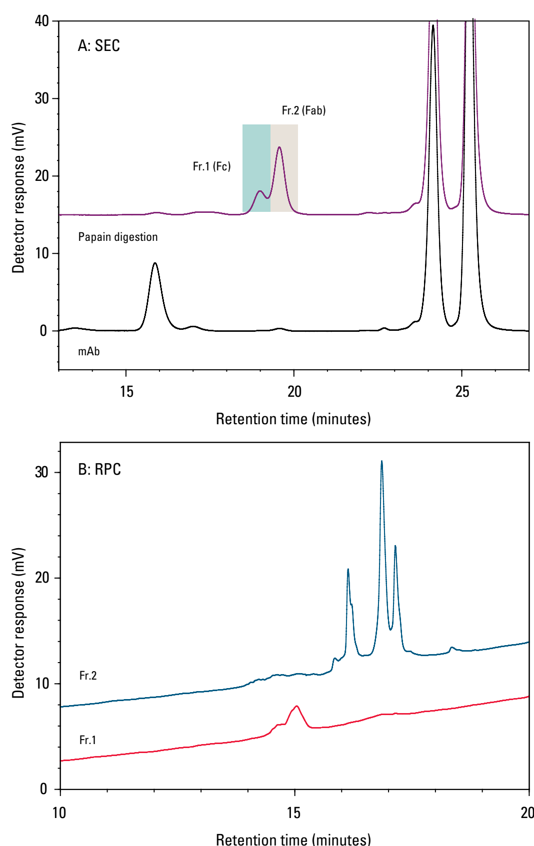


Antibody Fragments

Figures 3A & 3B show the analysis of antibody fragments. The monoclonal antibody human IgG_1 was first papain digested and separated using a TSKgel G3000SWXL SEC column (Figure 3A). The intact form of the antibody, partially digested fragments, and completely digested fragments were separated on the basis of molecular size.

Two fractions were obtained from the SEC analysis and each fraction was analyzed with the TSKgel Protein C4-300 reversed phase column, as shown in Figure 3B. Several peaks were observed in each chromatogram of the analysis of Fc (fragment 1) and Fab (fragment 2), indicating that the antibody used in this study was heterogeneous in hydrophobicity.

Figure 3. Analysis of antibody fragments



Conditions for SEC

Column:	**TSKgel G3000SWXL, 3 µm, 7.8 mm ID × 30 cm × 2**
Mobile phase:	20 mmol/L phosphate buffer, pH 7.0 + 0.3 mol/L NaCl
Flow rate:	1.0 mL/min
Temperature:	25 °C
Sample:	monoclonal antibody (human IgG_1)

Conditions for RPC

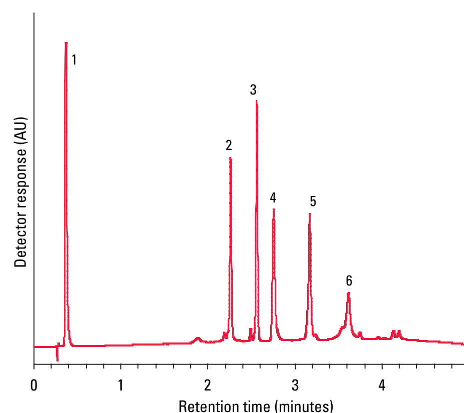
Column:	**TSKgel Protein C4-300, 3 µm, 4.6 mm ID × 30 cm**
Mobile phase:	A: 0.05% TFA in H_2O B: 0.05% TFA in ACN
Gradient:	0 min (5%B) 20 min (50%B)
Flow rate:	1.0 mL/min
Temperature:	70 °C
Sample:	monoclonal antibody (human IgG_1)

Reduced Analysis Time in Protein Separation

For high speed separations, the analysis time can be reduced by more than eighty percent when using the short 5 cm TSKgel Protein C4-300 column and increasing the flow rate to 3 mL/min (see Figure 4). The backpressure remains below 15 MPa, allowing the use of standard HPLC systems. The long term stability of the new C4 phase in acidic solution was tested by flushing the column with 30% acetonitrile, 0.2% TFA (4 times the standard TFA concentration) at 40 °C.

There was no change in theoretical plates even after 1,000 hours of run time under this chromatographic condition. Also retention times of standard proteins didn't have significant loss when compared to the initial values.

Figure 4. High speed separation of proteins



Column:	**TSKgel Protein C4-300, 3 µm, 4.6 mm ID × 5 cm**
Mobile phase A:	$H_2O/CH_3CN/TFA$ = 90/10/0.05 (v/v/v)
Mobile phase B:	$H_2O/CH_3CN/TFA$ = 20/80/0.05 (v/v/v)
Gradient:	0 min (0%B) 5 min (100%B)
Flow rate:	3.0 mL/min
Detection:	UV @ 210 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. phenylalanine 2. cytochrome C 3. lysozyme 4. BSA 5. α-chymotrypsinogen A 6. ovalbumin (each 0.2 g/µL)

About: TSKgel ODS-140HTP Reversed Phase Chromatography Columns

TSKgel ODS-140HTP columns provide high resolution and short analyses times at moderate pressures, enabling high throughput separations. The polylayer bonding chemistry of the 2.3 µm particle size of these columns results in highly efficient and durable columns. The lower pressure drop reduces the burden on the hardware, allowing the TSKgel ODS-140HTP columns to be used with either UPLC® (up to 62 MPa) or conventional HPLC systems.

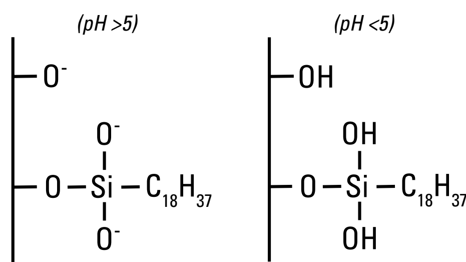
Attributes and Applications

Table 5 lists the attributes of TSKgel ODS-140HTP columns, while Figure 5 displays the structure. For use in high throughput applications, including drug discovery, pharmacokinetics and peptide digest separations, TSKgel ODS-140HTP columns offer excellent peak shape for basic compounds.

Table 5: Product attributes

Attribute	Value
Pore size (mean)	14 nm
Endcapped	Yes
Particle size	2.3 µm
pH stability	2.0-7.5
Functional group	C18 (polymeric bonding chemistry)
% Carbon	6

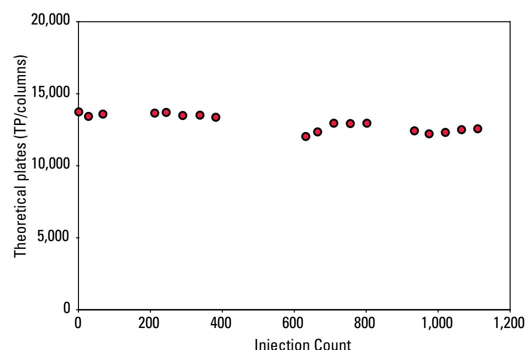
Figure 5: TSKgel ODS-140HTP structure



Column Stability

Figures 6 and 7 demonstrate that TSKgel ODS-140HTP columns are stable at high flow rates under demanding step gradient conditions. Figure 6 shows that consistent theoretical plate values were obtained on the TSKgel ODS-140HTP column during 1,110 gradient cycles consisting of five minute step gradients from 10% to 50% and from 50% to 100% methanol at 0.6 mL/min. During each cycle, pressure fluctuated between 30 and 60 MPa.

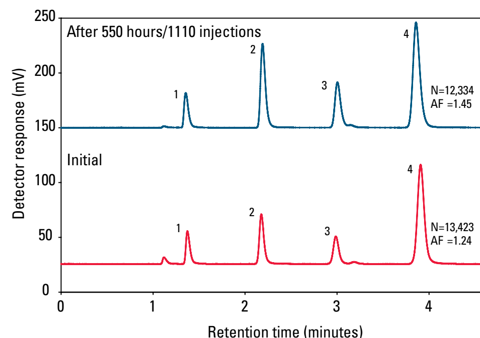
Figure 6: Stability of TSKgel ODS-140HTP columns



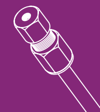
Column: **TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 10 cm**
Mobile phase: A: H_2O/MeOH = 90/10
B: H_2O/MeOH = 50/50
C: MeOH
Gradient: A→B→C (5 min., Step gradient)
Flow rate: 0.6 mL/min
Temperature: 25 °C
Pressure: A: 45 MPa B: 59 MPa C: 32 MPa
Sample: napthalene

Figure 7 shows injections of test solutes after the first step gradient cycle and after 1,110 cycles. The results clearly demonstrate the durability of the TSKgel ODS-140HTP columns when operated at high flow rate and high pressure.

Figure 7: Durability of TSKgel ODS-140HTP columns



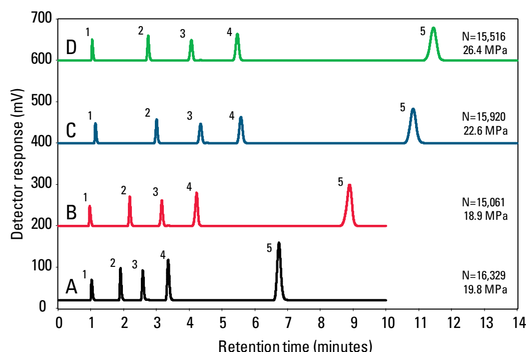
Column: **TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 10 cm**
Mobile phase: H_2O/MeOH = 30/70
Flow Rate: 0.2 mL/min
Detection: UV @ 254 nm
Temperature: 25 °C
Injection vol.: 2 µL
Samples: 1. phenol 2. benzene 3. toluene 4. napthalene



Performance Data

Column efficiency of a TSKgel ODS-140HTP column compares favorably with other sub-3 µm ODS columns (see Figure 8). Higher efficiency and a shorter retention time make the TSKgel ODS-140HTP column more suitable for high throughput separations.

Figure 8: Comparison of 2.3 µm and sub-3 µm columns

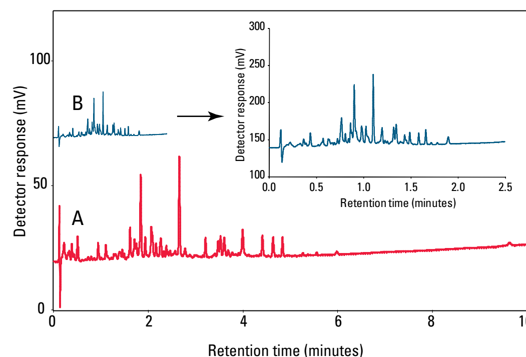


Columns:	**A. TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 10 cm**
	B. Ascentis® Express C18, 2.7 µm, 2.1 mm ID × 10 cm
	C. Luna C18(2)-HST, 2.5 µm, 2 mm ID × 10 cm
	D. YMC UltraHT® Pro C18, 2 µm, 2 mm ID × 10 cm
Mobile phase:	H_2O/MeOH = 30/70
Flow Rate:	0.2 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Injection vol.:	2 µL
Samples:	1. uracil
	2. benzene
	3. toluene
	4. napthalene
	5. fluorene

Tryptic Digest

Excellent resolution at high speed was achieved on a TSKgel ODS-140HTP column for the separation of a β-lactoglobulin tryptic digest (see Figure 9). As expected, peak capacity improved when using a longer gradient time.

Figure 9: Separation of β-lactoglobulin tryptic digest

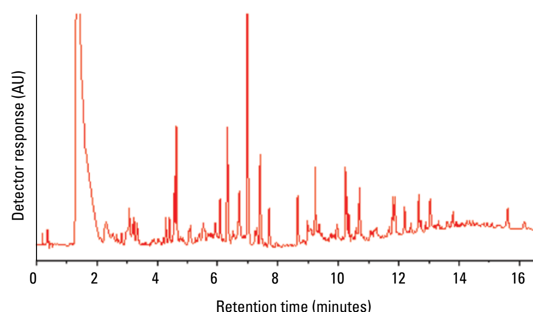


Column:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 5 cm**
Mobile phase:	A: H_2O/ACN (95/5) + 0.1% TFA
	B: H_2O/ACN (50/50) + 0.1% TFA
Gradient:	0-100%B (linear gradient)
Gradient time:	A: 10 min
	B: 2.5 min
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Temperature:	40 °C
Injection vol.:	10 µL
Sample:	β-lactoglobulin tryptic digest

Herbal Extract

In Chinese traditional medicine, an extract of *Crinum latifolium L.* is used to invigorate blood circulation. It 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 of components, the column needs to provide high peak capacity. As shown in Figure 10, a TSKgel ODS-140HTP column is an excellent choice for plant extract separations.

Figure 10: Separation of Crinum latifolium L.

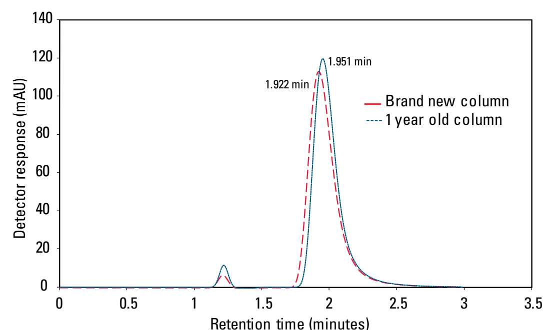


Column:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 10 cm**
Mobile phase:	A: H_2O B: ACN
Gradient:	0 min (5%B) 0.08 min (5%B) 7.47 min (40%B) 13.66 min (100%B) 16.13 min (100%B) 16.14 min (5%B)
Flow rate:	0.523 mL/min
Detection:	UV @ 220 nm
Temperature:	35 °C
Injection vol.:	2 µL
Sampling rate:	80 Hz
Sample:	50 g/L extract of *Crinum latifolium L* by 95% ethanol
Instrument:	Acquity UPLC® System with TUV detector

Caffeine Analysis

HPLC methods are commonly used for the analysis of caffeine in beverages. A caffeine USP standard eluted from a TSKgel ODS-140HTP, 2.3 µm column within two minutes under isocratic chromatographic conditions using a conventional HPLC system. The durability of the column was tested under these isocratic conditions using a fresh TSKgel ODS-140HTP column and one run frequently for over a year (more than 1,000 injections). No significant change in elution profile was noted. Caffeine eluted at 1.922 minutes from the new column while the used column yielded a retention time of 1.951 minutes (Figure 11).

Figure 11: Isocratic elution of caffeine USP and test of column stability

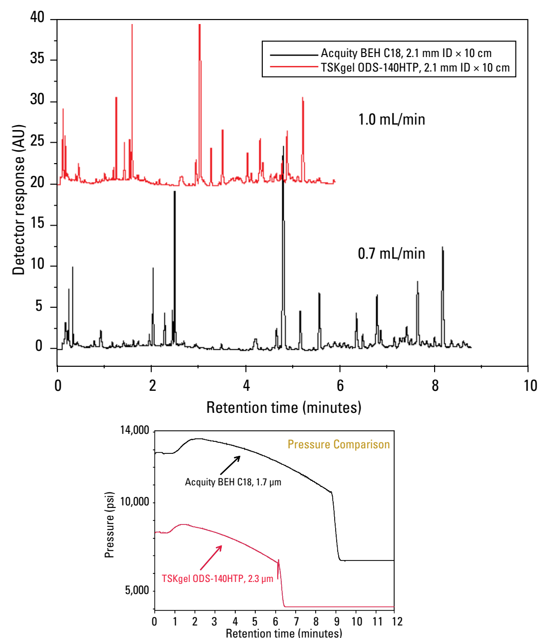


Column:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 5 cm**
Mobile phase (Isocratic):	10% ACN in H_2O containing 0.15% TFA
Flow rate:	0.2 mL/min
Detection:	UV @ 275 nm
Temperature:	40 °C
Injection vol.:	10 µL
Sample:	caffeine USP (1.427 mg/mL)

Root Extract

Figure 12 details the separation using a TSKgel ODS-140HTP column of a root tuber extract of *cynanchum auriculatum* Royle ex Wight. This weed, also known as climbing milkweed, is used in traditional Chinese medicine for its anti-tumor and anti-gastric lesion activity. The TSKgel ODS-140HTP column delivers a faster analysis at a higher flow rate under a lower pressure compared to a competitive sub-2 µm column when run on an Acquity UPLC system.

Figure 12: Comparative separation of C. auriculatum Royle ex Wight



Columns:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 10 cm** Acquity BEH C18, 1.7 µm, 2.1 mm ID × 10 cm
Mobile phase:	A: H_2O B: ACN
Flow rate:	1.0 mL/min (TSKgel ODS-140HTP) 0.7 mL/min (Acquity BEH C18)
Detection:	UV @ 220 nm
Temperature:	40 °C
Injection vol.:	1 µL
Sampling rate:	80 Hz
Sample:	10 g/L extract of *C. auriculatum* Royle ex Wight by 95% ethanol
Instrument:	Acquity UPLC System with TUV detector

Optimum gradient for Acquity BEH C18: 0 min (5%B) 0.68 min (5%B) 2.28 min (30%B) 8.57 min (68%B) 8.70 min (100%B) 20 min (100%B)

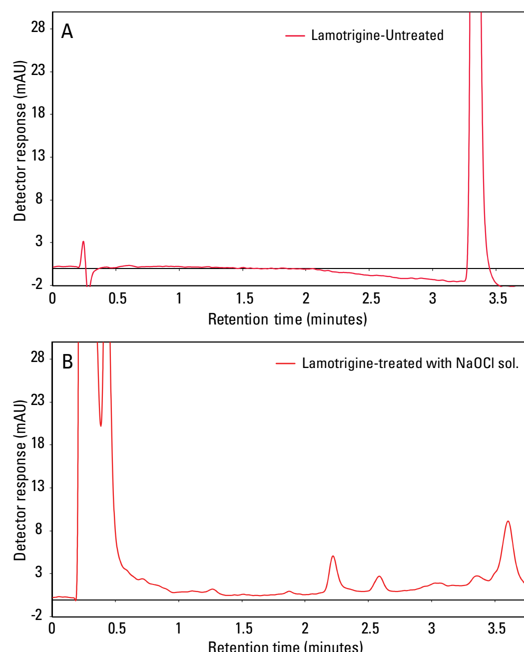
Optimum gradient for TSKgel ODS-140HTP: 0 min (5%B) 0.48 min (5%B) 1.6 min (30%B) 6.0 min (68%B) 6.1 min (100%B) 20 min (100%B)

Forced Degradation of Off-Patent Drug

In 2007, more than two thirds of all prescriptions in the United States were filled with generic drugs (http://www.nytimes.com/2009/01/06/us/06healthcare.html?r=1) Like the manufacturers of brand name drugs, generic manufacturers need to develop validated methods to meet regulatory compliance. Forced degradation studies are designed to determine the degradation products formed during accelerated pharmaceutical studies and long-term stability studies.

A TSKgel ODS-140HTP column was used to study the degradation of lamotrigine, an anti-epileptic drug that lost patent protection in 2009. Figure 13A shows the analysis of untreated lamotrigine. Lamotrigine is known to form two different N-chloro products when in contact with a 6% NaOCl solution. Upon treatment with NaOCl, the lamotrigine peak disappeared, leaving only evidence of degradation products (as demonstrated in Figure 13B).

Figure 13A & 13B: Forced degradation study of lamotrigine

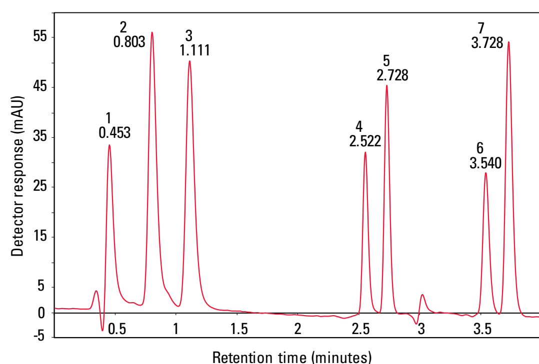


Column:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 5 cm**
Mobile phase:	A: H_2O + 0.15% TFA B: 100% ACN with 0.15% TFA
Gradient:	0 min (4%A) 15 min (100%B)
Flow rate:	0.8 mL/min
Detection:	UV @ 215 nm
Temperature:	40 °C
Injection vol.:	10 µL
Sample:	lamotrigine (25 mg/L, 750 µL) in mobile phase A treated with 750 µL of 6% NaOCl solution for 1 minute. Final concentration of lamotrigine: 12.5 mg/L

OTC Cold, Sinus and Allergy Medications

Six cold and sinus drug standards (Figure 14) were separated as sharp peaks with good resolution within 3.8 minutes using a TSKgel ODS-140HTP column. The peak labeled (1) was identified as maleate originating from the drug standard chlorpheniramine maleate (5). Diphenhydramine is considerably shorter retained than that reported using an ACQUITY UPLC HSS T3, 1.8 µm, 2.1 mm ID × 10 cm column (Mazzeo JR, LCGC Asia Pacific, Volume 10, Issue 1, May1, 2007). The two drug substances diphenhydramine and dextromethorphan have very similar and strong hydrophobic properties with a tendency to co-elute or elute with considerable overlap. These substances were separated with a resolution of 1.9.

Figure 14: Analyses of six cold and sinus drug standards



Column:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 5 cm**
Mobile phase:	A: H_2O with 0.15% TFA
	B: 100% ACN with 0.15% TFA
Gradient:	

Time (min)	Solvent B (%)	Flow (mL/min)
1.4	2.0	0.6
1.5	24.0	1.4
2.1		1.4
2.2		0.8
4.0	5.0	0.8
4.1	1.0	0.6

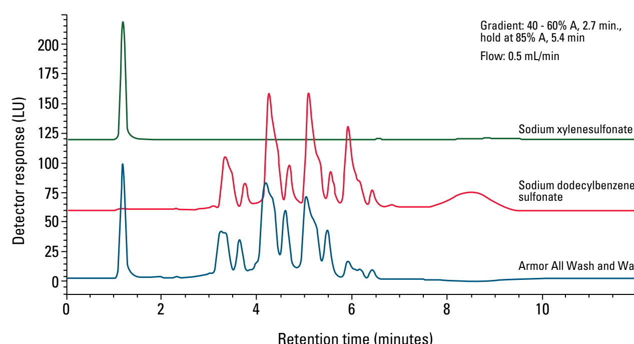
Detection:	UV @ 215 nm
Temperature:	50 °C
Injection vol.:	10 µL
Samples:	1. maleate peak
	2. phenylephrine
	3. acetaminophen
	4. doxylamine succinate
	5. chlorpheniramine maleate
	6. dextromethorphan HBr
	7. diphenhydramine HCl

2D-LC Separation of Cationic, Anionic, and Hydrotropic Surfactants

Surfactants are frequently found in pharmaceutical and biopharmaceutical drug applications as well as in common household products. Because they can be polar, non-polar, or amphoteric, the structural diversity of the surfactants and complexity of the sample matrix can make their separation and identification by HPLC challenging.

Figure 15 illustrates the characterization of the surfactant profile of Armor All™ Wash and Wax using a TSKgel ODS-140HTP and TSKgel NH2-100 columns in series. As shown, the use of these columns yielded excellent separation and retention of the anionic surfactant sodium dodecylbenzene sulfonate and the hydrotropic surfactant sodium xylene sulfonate present in the Armor All formulation. Additionally, the use of fluorescence detection (λex: 225 nm, λem: 300-400 nm) allowed for increased sensitivity of the low level surfactants found in the product.

Figure 15: Characterization of surfactant profile in Armor All Wash and Wax using 2D-LC with the TSKgel ODS-140HTP and TSKgel NH2-100 columns



Columns:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 5 cm**
	TSKgel NH2-100, 3 µm, 2 mm ID × 15 cm
Mobile phase:	A: CH_3CN
	B: 100 mmol/L ammonium acetate, pH 5.4
Gradient:	40-60% A, 2.7 minutes, hold at 85% A, 5.4 minutes
Flow rate:	0.5 mL/min
Detection:	UV @ 280 nm, 254 nm, and 210 nm
	FLD λex 280 nm, λem 350 nm
Temperature:	30 °C
Injection vol.:	1 µL
Samples:	Triton™ X
	Triton N
	sodium xylenesulfonate
	sodium dodecylbenzene sulfonate

About: TSKgel ODS-100V Reversed Phase Chromatography Columns

TSKgel ODS-100V reversed phase columns are general purpose columns suitable for the most demanding separations in quality control as well as in research and development. Containing a unique surface property utilizing highly efficient bonding and endcapping procedures, secondary interactions of basic, acidic, and chelating compounds are limited.

TSKgel ODS-100V columns provide strong retention for polar compounds as these types of compounds are retained by hydrophobic association, plus by enhanced interaction of their polar groups with the more polar surface of the TSKgel ODS-100V column. In addition to the strong retention, these columns also provide higher selectivity for polar compounds. Monomeric bonded phase chemistry of the TSKgel ODS-100V packing material provides complete wetting and retention stability in 100% aqueous mobile phases (see Figure 16).

TSKgel ODS-100V columns are available in 3 µm particle size in addition to the traditional 5 µm size. The 3 µm columns are well suited for high throughput LC/MS applications, providing fast and efficient separations.

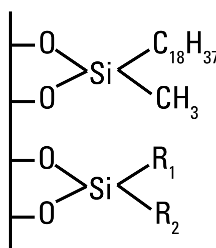
Attributes and Applications:

Product attributes of TSKgel ODS-100V columns are listed in Table 6. The structure is displayed in Figure 16. TSKgel ODS-100V columns are the best choice for challenging compounds, including acidic, basic, zwitterionic, and chelating compounds.

Table 6: Product attributes

Attribute	Value
Pore size (mean)	10 nm
Molar mass limit	1.0×10^4 Da
Endcapped	Yes
Particle size	3 µm and 5 µm
pH stability	2.0-7.5
Functional group	octadecylmethylsilane
% Carbon	15
Surface area (m^2/g)	450

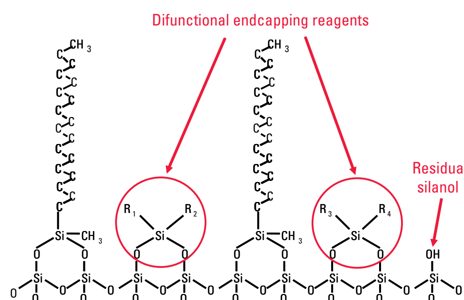
Figure 16: TSKgel ODS-100V structure



Novel Bonding Chemistry

The novel bonding chemistry employed in the preparation of TSKgel ODS-100V is depicted in Figure 17. The TSKgel ODS-100V bonded phase is prepared by an incomplete first reaction with a difunctional octadecylsilane reagent, which is followed by endcapping with a mixture of two difunctional dialkylsilane reagents. This material is made under conditions that promote the formation of a monomeric bonded phase layer.

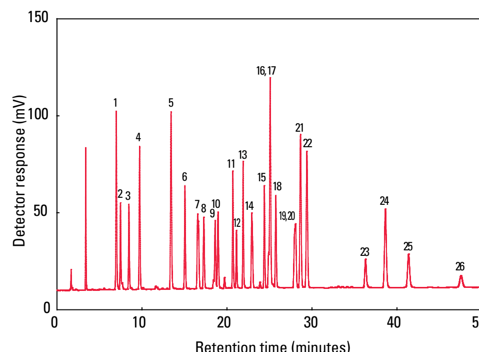
Figure 17: Bonded phase structure of TSKgel ODS-100V



Antioxidants and UV Absorbants

Small quantities of antioxidants and UV stabilizers are often added to commercial plastics to prevent or reduce degradation. It is of vital importance in the manufacturing process to accurately control these additives. The chromatogram in Figure 18 shows the separation of 26 commercially available antioxidants and UV absorbants in about 50 minutes using a TSKgel ODS-100V column.

Figure 18: Separation of antioxidants and UV absorbants

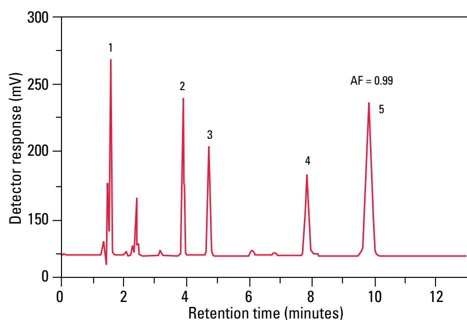


Column: **TSKgel ODS-100V, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase: A: H_2O
B: CH_3CN
Gradient: 0 min (60%B) 20 min (100%B)
Flow rate: 1.0 mL/min
Temperature: 50 °C
Detection: UV @ 225 nm
Injection Vol.: 10 µL
Concentration: 10 mg/L each
Samples: 1. Cyasorb® UV-24 2. BHA 3. Ionox 100 4. Seesorb 101 5. 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

Bonded Phase Characterization

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 19, symmetrical peaks are obtained on a TSKgel ODS-100V column for all compounds in this test mixture, clearly demonstrating the superior performance of this column for the analysis of basic and chelating compounds as well as for less challenging compounds.

Figure 19: Separation of SRM 870

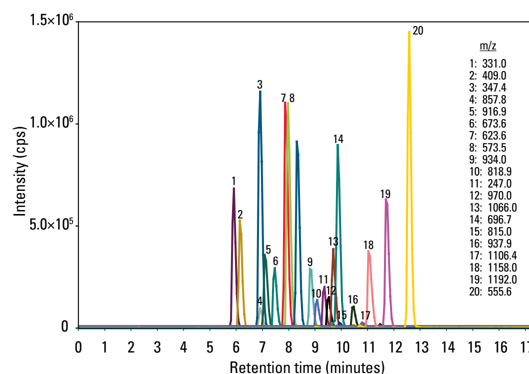


Column:	**TSKgel ODS-100V, 3 µm, 4.6 mm ID × 15 cm**
Mobile phase:	20 mmol/L phosphate buffer, pH 7.0/MeOH (20/80)
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. uracil
	2. toluene
	3. ethyl benzene
	4. quinizarin
	5. amitriptyline

Tryptic Digest

The rapid identification of 20 peptides using a TSKgel ODS-100V column is detailed in Figure 20. The high speed analysis and symmetrical peaks of basic compounds in low concentration ammonium formate buffer make this column an excellent choice for LC/MS work.

Figure 20: Rapid identification of 20 peptide fragments



Column:	**TSKgel ODS-100V, 3 µm, 2.0 mm ID × 15 cm**
Mobile phase:	A: 0.1% TFA in H_2O
	B: 0.1% TFA in ACN
Gradient:	0 min (10%B) 15 min (70%B) 17 min (70%B)
Flow rate:	0.2 mL/min
Injection vol.:	2 µL
Sample:	β-lactoglobulin tryptic digest
Instrument:	Q TRAP, ESI+

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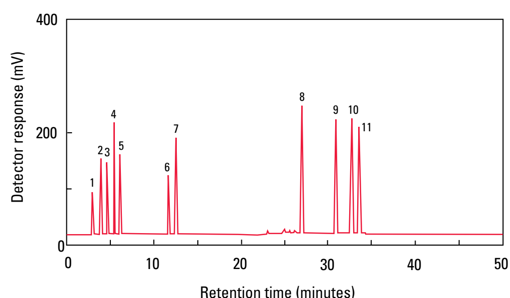
technical service: 800-366-4875

Vitamins

Water and lipid-soluble vitamins were separated in a single run on a TSKgel ODS-100V column as demonstrated in Figure 21. The sample is a mixture of vitamins ranging from the very polar water-soluble vitamin ascorbic acid to the very hydrophobic tocopherol derivatives. The polar vitamins elute in the beginning of the chromatogram under aqueous or low organic mobile phase conditions. A steep gradient from 40% ACN to 100% ACN is initiated from 20 to 22 minutes to elute retinol and the tocopherols. Clearly the TSKgel ODS-100V column provides high resolution for the polar compounds in the mixture, while at the same time delivers a short analysis time for the late eluting non-polar compounds.

Figure 21: Separation of water and lipid-soluble vitamins

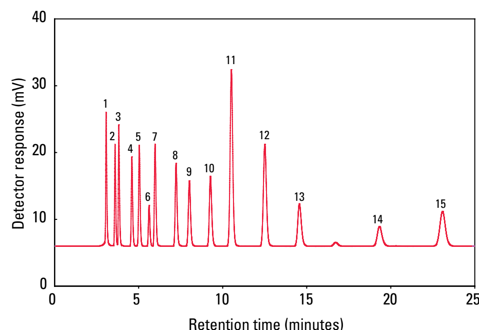


Column:	**TSKgel ODS-100V, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	A: 0.1% TFA in H_2O B: 0.1% TFA in ACN
Gradient:	0 min (0%B) 20 min (40%B) 22 min (100%B) 50 min (100%B)
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	40 °C
Injection vol.:	5 µL
Samples:	1. L-ascorbic acid 2. nicotinic acid 3. thiamine 4. pyridoxal 5. pyridoxine 6. caffeine 7. riboflavine 8. retinol 9. δ-tocopherol 10. α-tocopherol 11. α-tocopherol acetate

Organic Acids

Organic acids play an important role in many metabolic processes, fermentation and food products. Figure 22 shows a baseline separation of 15 organic acids in less than 25 minutes using a simple 0.1% phosphoric acid mobile phase with a TSKgel ODS-100V column.

Figure 22: Separation of organic acids

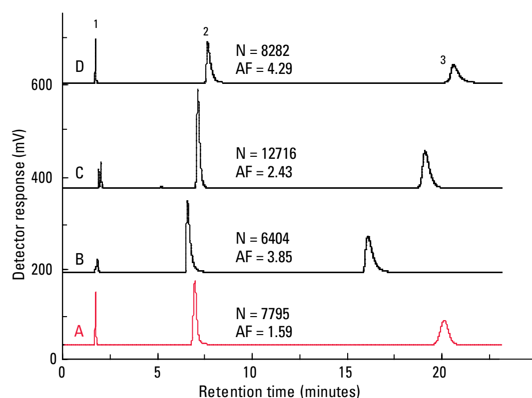


Column:	**TSKgel ODS-100V, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase:	0.1% H_3PO_4
Flow rate:	1.0 mL/min
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. oxalic acid (0.1 g/L) 2. l-Tartaric acid (0.5 g/L) 3. formic acid (1.0 g/L) 4. l-Malic acid (1.0 g/L) 5. l-Ascorbic acid (0.1 g/L) 6. lactic acid (1.0 g/L) 7. acetic acid (1.0 g/L) 8. maleic acid (0.01 g/L) 9. citric acid (1.0 g/L) 10. succinic acid (1.0 g/L) 11. fumaric acid (0.025 g/L) 12. acrylic acid (0.1 g/L) 13. propionic acid (2.0 g/L) 14. glutaric acid (1.0 g/L) 15. itaconic acid (0.025 g/L)

Performance Data

To demonstrate the absence of accessible silanol groups, Figure 23 compares retention and peak shape for two tricyclic antidepressant drugs on four water-wettable columns including TSKgel ODS-100V and three competitive C18 reversed phase columns. The ability to provide symmetrical peak shapes for basic compounds makes TSKgel ODS-100V the column of choice for method development and quantitative analysis of small molar mass compounds using from 100% aqueous to 100% organic mobile phase conditions.

Figure 23: Comparison of C18 columns



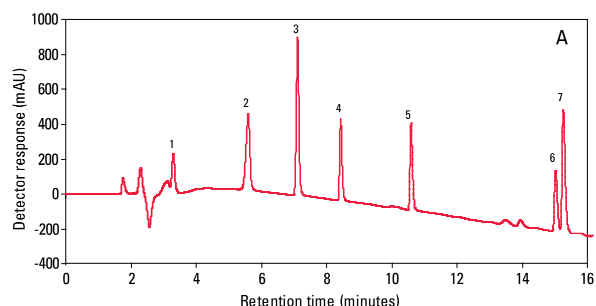
Columns:	**A. TSKgel ODS-100V, 5 µm, 4.6 mm ID × 15 cm**
	B. CAPCELL PAK C18AQ®, 5 µm, 4.6 mm ID × 15 cm
	C. Hydrosphere® C18, 5 µm, 4.6 mm ID × 15 cm
	D. Atlantis® dC18, 5 µm, 4.6 mm ID × 15 cm
Mobile phase:	50 mmol/L phosphate buffer, pH 7.0/MeOH (30/70)
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. uracil
	2. desipramine
	3. imipramine

Cold, Sinus and Analgesic Medications

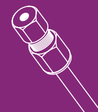
Because of FDA-mandated changes to the regulation of drugs containing the popular decongestant pseudoephedrine, many pharmaceutical companies reformulated their products using phenylephrine as a substitute. To support the need to revalidate test methods, we used a TSKgel ODS-100V column to separate phenylephrine from some of the most common combinations of cold and sinus medications on the market today.

Figure 24A shows the separation of a cold mixture containing six common ingredients using a TSKgel ODS-100V, 3 µm column. The TSKgel ODS-100V column produced a single sharp peak for the analysis of phenylephrine and also a single peak for doxylamine. All compounds were resolved by this column in less than 17 minutes.

Figure 24A: Analysis of cold mixture on TSKgel ODS-100V column

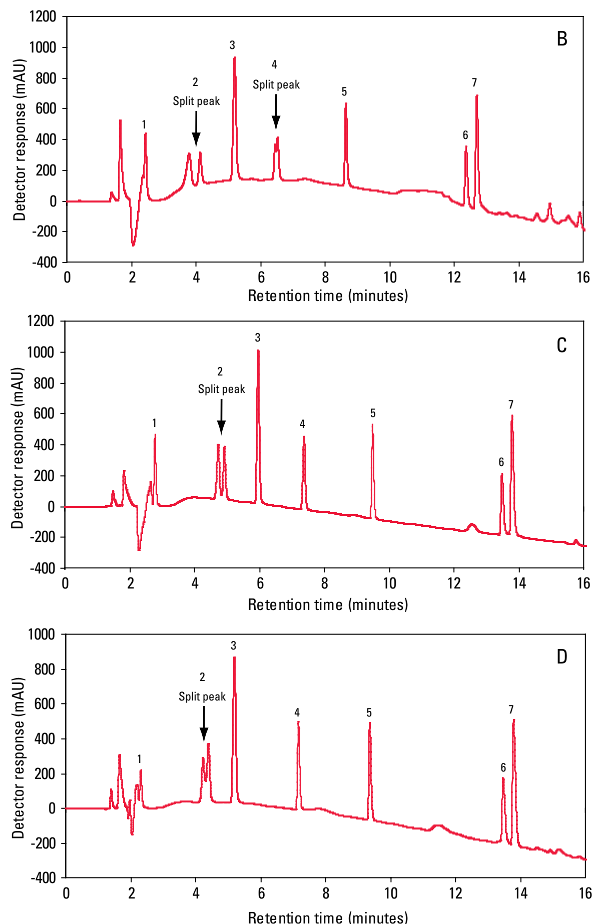


Column:	**A. TSKgel ODS-100V, 3 µm, 4.6 mm ID × 15 cm**
Mobile phase	A: 0.15% TFA in H_2O
	B: 0.02% TFA in ACN/MeOH (75/25)
Gradient:	0 min (96%A, 4%B)
	15 min (40%A, 60%B)
	17 min (40%A, 60%B)
Flow rate:	1.0 mL/min
Detection:	UV @ 210 nm
Temperature:	40 °C
Injection vol.:	20 µL
Samples:	1. maleate
	2. phenylephrine HCl
	3. acetaminophen
	4. doxylamine succinate
	5. chlorpheniramine
	6. dextromethorphan HBr
	7. diphenhydramine HCl



Figures 24B-D shows the same cold mixture run on three competitive ODS columns under the same chromatographic conditions. On all three columns, phenylephrine eluted as two distinct peaks with each peak having approximately half the area as the single peak produced on the TSKgel ODS-100V column. Also, one of the competitive columns exhibited peak-splitting on the doxylamine peak.

Figure 24B-D: Analysis of cold mixture on competitive ODS columns



Columns:	B. Symmetry® C18, 3.5 µm, 4.6 mm ID × 15 cm C. Luna C18(2), 3 µm, 4.7 mm ID × 15 cm D. Zorbax® Eclipse Plus C18, 3.5 µm, 4.7 mm ID × 15 cm
Mobile phase:	A: 0.15% TFA in H_2O B: 0.02% TFA in ACN/MeOH (75/25)
Gradient:	0 min (96%A, 4%B) 15 min (40%A, 60%B) 17 min (40%A, 60%B)
Flow rate:	1.0 mL/min
Detection:	UV @ 210 nm
Temperature:	40 °C
Injection vol.:	20 µL
Samples:	1. maleate 2. phenylephrine HCl 3. acetaminophen 4. doxylamine succinate 5. chlorpheniramine 6. dextromethorphan HBr 7. diphenhydramine HCl

About: TSKgel ODS-100Z Reversed Phase Chromatography Columns

TSKgel ODS-100Z reversed phase columns are a great choice when a change of selectivity from the TSKgel ODS-100V columns is needed to resolve one or more overlapping pairs. The TSKgel ODS-100Z columns contain a high density monomeric C18 bonded phase (Figure 26) for maximum retention and selectivity of small molar mass compounds. Exhaustive endcapping prevents secondary interaction with residual silanol groups. Available in 3 and 5 µm particle size, TSKgel ODS-100Z columns stand out for lot-to-lot reproducibility (see Figure 27).

Containing a high carbon content of 20%, TSKgel ODS-100Z columns exhibit a high stability at both low and high pH. This stability at low pH is important when running peptides and proteins. At low pH conditions, silanol groups get removed first by acid hydrolysis before hydrolysis of the alkyl chains takes place. Because of their high bonded phase surface coverage, the TSKgel ODS-100Z columns can be expected to last longer before showing appreciable changes in retention due to increased silanol interaction.

TSKgel ODS-100Z columns provide longer retention for non-polar compounds and a slightly higher selectivity for non-polar compounds, for example when you need to separate homologues series, than the TSKgel ODS-100V columns. Steric selectivity is also higher for TSKgel ODS-100Z columns. This plays a role with complex 3-D molecules, such as aromatic hydrocarbons, steroids, etc.

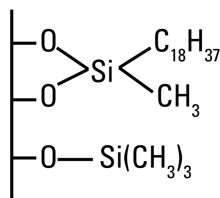
Attributes and Applications:

Table 7 lists the attributes of TSKgel ODS-100Z columns, while Figure 25 displays the structure. This general purpose column is the workhorse for analysis of small molar mass compounds in life science applications.

Table 7: Product attributes

Attribute	Value
Pore size (mean)	10 nm
Molar mass limit	1.0×10^4 Da
Endcapped	Exhaustive
Particle size	3 µm and 5 µm
pH stability	2.0-7.5
Functional group	octadecylmethylsilane
% Carbon	20
Surface area (m^2/g)	450

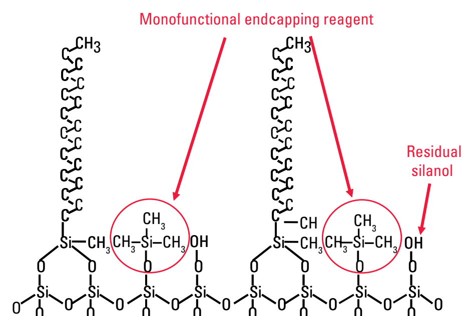
Figure 25: TSKgel ODS-100Z structure



Novel Bonding Chemistry

The novel bonding chemistry employed in the preparation of TSKgel ODS-100Z is depicted in Figure 26. TSKgel ODS-100V is prepared by reacting the surface with a difunctional octadecylsilane reagent, followed by repeated endcapping with monofunctional trimethylsilane reagent. The TSKgel ODS-100Z is prepared under conditions that promote the formation of a monomeric bonded phase layer.

Figure 26: Bonded phase structure of TSKgel ODS-100V

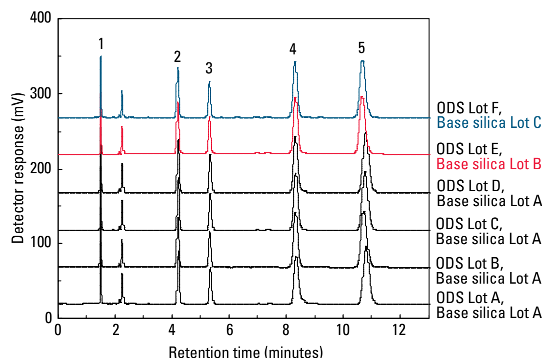


Lot-to-Lot Reproducibility

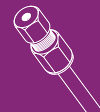
Figure 27 shows the chromatograms for SRM870 test mixture using 6 bonding lots of TSKgel ODS-100Z columns prepared from 3 different base silica lots. The results show no marked differences among the chromatograms, confirming that minimal lot-to-lot variability and high consistency of the manufactured packing material.

Note the good peak shape for the metal-chelating compound quinizarine (peak 4), and the symmetrical peak shape for the organic base amitriptyline (peak 5). These results indicate the low activity towards chelating compounds and the very low activity towards organic bases, respectively, of TSKgel ODS-100Z columns.

Figure 27: TSKgel ODS-100Z lot-to-lot variability



Column: **TSKgel ODS-100Z, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase: 20 mmol/L phosphate buffer, pH 7.0/MeOH = 20/80
Flow rate: 1.0 mL/min
Detection: UV @ 254 nm
Temperature: 40 °C
Injection vol.: 10 µL
Samples: 1. uracil 2. toluene 3. ethyl benzene 4. quinizarin 5. amitriptyline

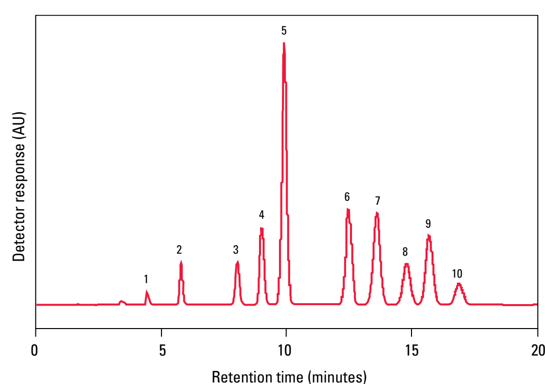


Indoor Air Pollutants

In the last several years, a growing body of scientific evidence has indicated that the air within homes and other buildings can be more seriously polluted than the outdoor air in even the largest and most industrialized cities. Other research indicates that people spend approximately 90 percent of their time indoors. Thus, for many people, the risks to health may be greater due to exposure to air pollution indoors than outdoors. This is the reason for the increased emphasis on the monitoring of indoor air pollutants.

Ten common indoor air pollutants were sharply resolved on a TSKgel ODS-100Z column (see Figure 28).

Figure 28: Analysis of indoor air pollutants

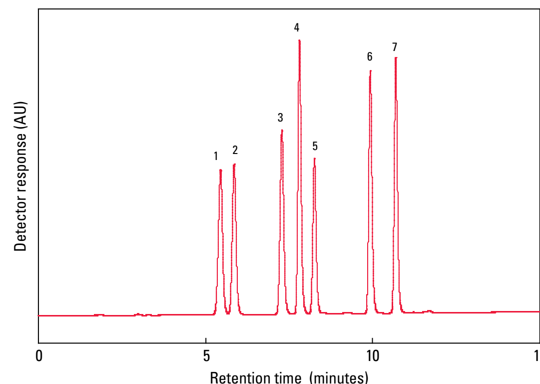


Column:	**TSKgel ODS-100Z, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	H_2O/CH_3OH = 32/68
Flow rate:	1.0 mL/min
Detection:	UV @ 210 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. chloroform (1.0 g/L) 2. benzene (0.1 g/L) 3. trichloroethylene (0.05 g/L) 4. toluene (0.05 g/L) 5. styrene (0.05 g/L) 6. o-dichlorobenzene (0.05 g/L) 7. ethylbenzene (0.05 g/L) 8. p-xylene (0.05 g/L) 9. m-dichlorobenzene (0.05 g/L) 10. tetrachloroethylene (0.05 g/L)

Polyphenols

Catechins, which are found in large quantities in tea, are polyphenols. Catechins have been extensively studied for their antioxidant properties. Figure 29 demonstrates the baseline separation of six catechins in the presence of caffeine on a 15 cm TSKgel ODS-100Z column.

Figure 29: Separation of catechins

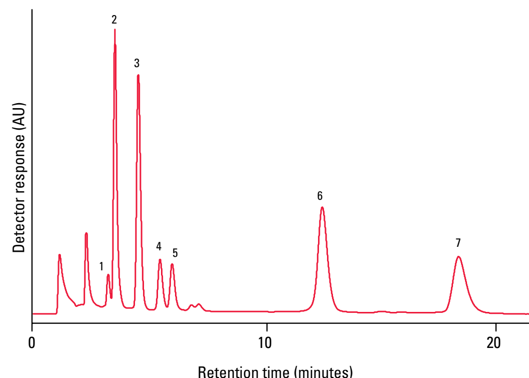


Column:	**TSKgel ODS-100Z, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	A: 10 mmol/L KH_2PO_4, pH 2.5 B: CH_3OH
Gradient:	0 min (18%B) 15 min (60%B)
Flow rate:	1.0 mL/min
Detection:	UV @ 270 nm
Temperature:	40 °C
Injection vol.:	5 µL
Samples:	1. (–)-epigallocatechin (175 mg/L) 2. (–)-catechin (87 mg/L) 3. (–)-epigallocatechin gallate (43 mg/L) 4. caffeine (217 mg/L) 5. (+)-epicatechin (87 mg/L) 6. (–)-epicatechin gallate (43 mg/L) 7. (–)-catechin gallate (43 mg/L)

Tetracycline Antibiotics

A 15 cm TSKgel ODS-100Z column was evaluated for its selectivity for a mixture of tetracycline-like chemical structures. Tetracycline is an impurity in oxytetracycline formulations. The two compounds have very similar structures and separation is difficult. As demonstrated in Figure 30, a TSKgel ODS-100Z column provides superior resolution for oxytetracycline (peak 2) and tetracycline (peak 3) within the mixture.

Figure 30: Separation of tetracycline antibiotics

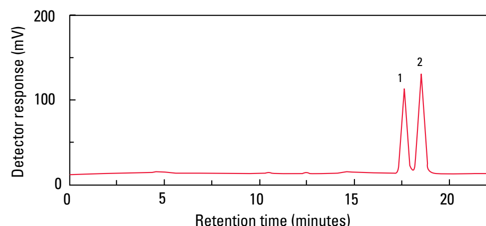


Column:	**TSKgel ODS-100Z, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	10 mmol/L formic acid/ACN = 82.5/17.5
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	10 °C
Injection vol.:	20 µL
Samples:	1. tetracycline derivative 2. oxytetracycline (20 mg/L) 3. tetracycline (20 mg/L) 4. doxycycline derivative 5. chlortetracycline derivative 6. chlortetracycline (30 mg/L) 7. doxycycline (30 mg/L)

Fat-Soluble Vitamins

Analysis of fat soluble vitamins D2 (ergocalciferol) and D3 (cholecalciferol) are critical because they differ only in one methyl group and one double bond. These compounds are very hydrophobic. As shown in Figure 31, separation was achieved using a TSKgel ODS-100Z column under isocratic conditions, demonstrating the ability of these columns to operate under non-aqueous reversed phase (NARP) conditions, in this case 100% acetonitrile.

Figure 31: Analysis of fat-soluble vitamins



Column:	**TSKgel ODS-100Z, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	ACN
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	5 µL
Samples:	1. ergocalciferol 2. cholecalciferol

About: TSKgel Super-ODS Reversed Phase Chromatography Columns

TSKgel Super-ODS columns are packed with monodispersed 2 µm* spherical silica particles covalently bonded with octadecyl groups. The small particle size makes the Super series the highest efficiency reversed phase columns in the TSKgel product line. The monodispersed packing generates operational back pressures more typical of larger particles allowing the use of higher flow rates than other 2 µm packings

*nominal particle size; mean particle size is 2.3 µm.

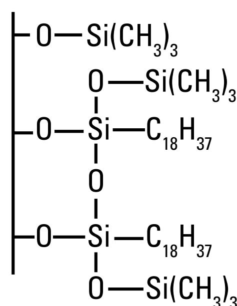
Attributes and Applications:

Table 8 lists the attributes of TSKgel Super-ODS columns, while Figure 32 displays the structure. TSKgel Super-ODS is an excellent choice for small peptides, amino acids, tryptic digests, nucleotides, pharmaceutical molecules, and food/beverage samples.

Table 8: Product attributes

Attribute	Value
Pore size	14 nm
Exclusion limit	2.0×10^4 Da
Endcapped	Yes
Particle size	2.3 µm
pH stability	2.0-7.5
Functional group	C18 (polymeric bonding chemistry)
% Carbon	6

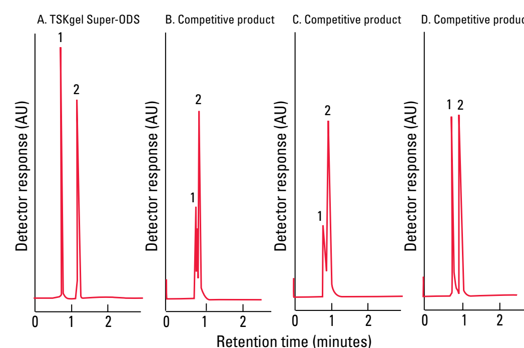
Figure 32: TSKgel Super-ODS structure



Superior Resolution

Figure 33 demonstrates the superior resolution of the TSKgel Super-ODS columns when compared with competitive 3 µm packings.

Figure 33: Comparison of resolution

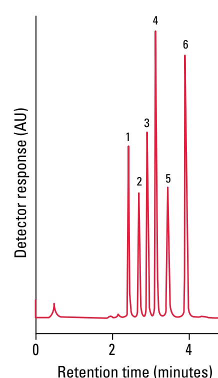


Columns: **A: TSKgel Super-ODS, 2.3 µm, 4.6 mm ID × 5 cm**
B, C & D: silica C18, 3 µm, 4.6 mm ID × 5 cm
Mobile phase: A: 30% CH_3CN B, C, D: 50% CH_3CN
Flow rate: 1.0 mL/min
Detection: UV @ 254 nm (2 mL cell)
Temperature: ambient
Samples: 1. pyradine 2. phenol

Peptide Separation

The chromatogram in Figure 34 shows the analysis of hydrophilic peptides using a TSKgel Super-ODS column. Since TSKgel Super-ODS has a large surface area, it shows favorable separation of peptides with high hydrophilicity.

Figure 34: Analysis of hydrophilic peptides



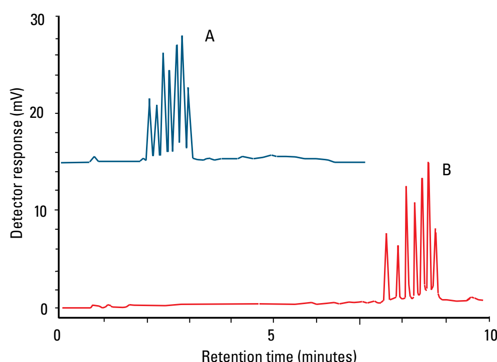
Column: **TSKgel Super-ODS, 2.3 µm, 4.6 mm ID × 5 cm**
Mobile phase: 13 mmol/L $HClO_4$/ACN
Linear gradient from 10% to 50% ACN over 10 minutes
Flow rate: 2 mL/min
Detection: UV @ 220 nm, micro-flow cell
Temperature: 25 °C
Samples: 1. oxytocin 2. a-endorphin
3. bombesin 4. Leu-enkephalin
5. gamma-endorphin 6. somatostatin
All peptides were injected at 0.1 to 0.2 µg each.

Oligonucleotides

Most synthesis protocols for oligonucleotides incorporate the use of a protective group on the 5′ terminal. Typically this protective group is dimethoxytrityl (DMT), which is a hydrophobic compound. One strategy for separating DMT on final products from DMT failures is the use of reversed phase chromatography.

The effect of gradient conditions on the separation of 12-18-mer polyadenylic oligonucleotides is shown in Figure 35. With the TSKgel Super-ODS column, this separation can be performed in less than five minutes under the conditions listed in Figure 35.

Figure 35: Separation of oligonucleotides

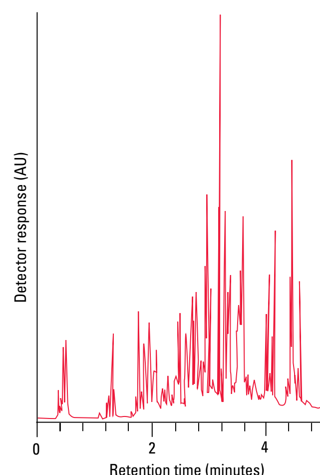


Column:	**TSKgel Super-ODS, 2.3 µm, 4.6 mm ID × 10 cm**
Mobile phase:	20 mmol/L phosphate buffer + 5 mmol/L t-butyl ammonium phospate, pH 6.0/CH_3CN
Gradient:	A: linear, 32-49% ACN in 5 minutes B: linear, 20-40% ACN in 10 minutes
Flow rate:	1.5 mL/min
Detection:	UV @ 260 nm
Temperature:	40 °C
Sample:	12-18-mer polyadenylic oligonucleotides

Trypsin Digest

A tryptic digest of α-chymotrypsinogen is separated on a TSKgel Super-ODS column as shown in Figure 36. The entire digest is separated in under five minutes.

Figure 36: Trypsin digest of α-chymotrypsinogen

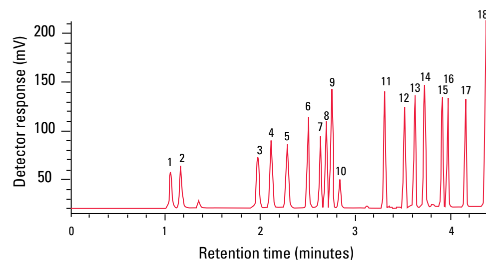


Column:	**TSKgel Super-ODS, 2.3 µm, 4.6 mm ID × 5 cm**
Mobile phase:	13 mmol/L $HClO_4$/CH_3CN; linear gradient of CH_3CN
Flow rate:	1.5 mL/min
Detection:	UV @ 220 nm
Temperature:	25 °C
Sample:	2 µL portion of trypsin digest of α-chymotrypsinogen

Amino Acids

The baseline separation of 18 PTC-derivatized amino acids in five minutes is demonstrated in Figure 37 using a TSKgel Super-ODS column.

Figure 37: PTC amino acids



Column:	**TSKgel Super-ODS, 2.3 µm, 4.6 mm ID × 10 cm**
Mobile phase:	A: ACN/50 mmol/L acetate buffer, pH 6.0 = 3/97 B: ACN/H_2O = 60/40
Flow rate:	1.5 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Injection vol.:	5 mL (250 pmol)
Samples:	1. Asp 2. Glu 3. Ser 4. Gly 5. His 6. Arg 7. Thr 8. Ala 9. Pro 10. PTC-NH_2 11. Try 12. Val 13. Met 14. Cys 15. Ile 16. Leu 17. Phe 18. Lys

About: TSKgel Super-Octyl Reversed Phase Chromatography Columns

TSKgel Super-Octyl columns are packed with monodispersed 2 µm* spherical silica particles covalently bonded with octyl groups. The small particle size makes the Super series the highest efficiency reversed phase columns in the TSKgel reversed phase column product line. The monodispersed packing generates operational back pressures more typical of larger particles allowing the use of higher flow rates than other 2 µm packings and offers less hydrophobicity than TSKgel Super-ODS.

* nominal particle size; mean particle size is 2.3 µm.

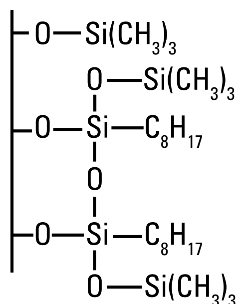
Attributes and Applications

Table 9 lists the attributes of TSKgel Super-Octyl columns, while Figure 38 displays the structure. TSKgel Super-Octyl columns are an excellent choice for peptides, proteins, amino acids, tryptic digests, nucleotides, pharmaceutical molecules, and food/beverage samples.

Table 9: Product attributes

Attribute	Value
Pore size	14 nm
Exclusion limit	2.0×10^4 Da
Endcapped	Yes
Particle size	2.3 µm
pH stability	2.0-7.5
Functional group	C8 (polymeric bonding chemistry)
% Carbon	5

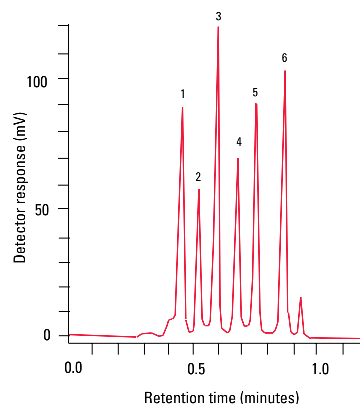
Figure 38: TSKgel Super-Octyl structure



Protein Mixture

The rapid analysis of a protein mixture using the TSKgel Super-Octyl column is shown in Figure 39. The separation was completed in one minute.

Figure 39: Rapid separation of protein mixture



Column: **TSKgel Super-Octyl, 2.3 µm, 4.6 mm ID × 5 cm**
Mobile phase: A: 13 mmol/L $HClO_4$,
B: 13 mmol/L $HClO_4/CH_3CN = 20/80$
40%B to 100%B in a 1.5 min linear gradient
Flow rate: 2.0 mL/min
Detection: UV @ 220 nm
Samples: 1. ribonuclease A
2. insulin
3. cytochrome C
4. lysozyme
5. α-lactalbumin
6. myoglobin

About: TSKgel Super-Phenyl Reversed Phase Chromatography Columns

TSKgel Super-Phenyl columns are packed with monodispersed 2 µm* spherical silica particles covalently bonded with phenyl groups. The small particle size makes the Super series the highest efficiency reversed phase columns in the TSKgel product line. The monodispersed packing generates operational back pressures more typical of larger particles allowing the use of higher flow rates than other 2 µm packings and offers less hydrophobicity than TSKgel Super-Octyl and TSKgel Super-ODS columns.

*nominal particle size; mean particle size is 2.3 µm.

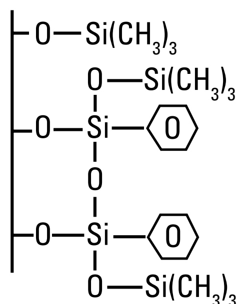
Attributes and Applications:

Table 10 lists the attributes of TSKgel Super-Phenyl columns; Figure 40 shows the structure. TSKgel Super-Phenyl is an excellent choice for peptides, proteins, amino acids, tryptic digests, nucleotides, pharmaceutical molecules, and food/beverage samples.

Table 10: Product attributes

Attribute	Value
Pore size	14 nm
Exclusion limit	2.0×10^4 Da
Endcapped	Yes
Particle size	2.3 µm
pH stability	2.0-7.5
Functional group	phenyl (polymeric bonding chemistry)
% Carbon	3

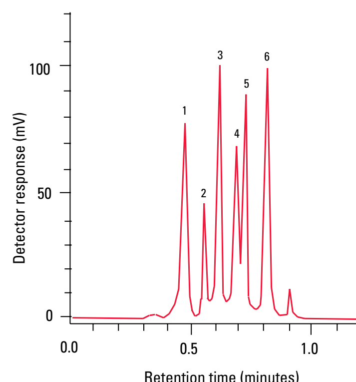
Figure 40: TSKgel Super-Phenyl structure



Protein Mixture

The chromatogram in Figure 41 shows retention and selectivity of TSKgel Super-Phenyl columns for proteins. The separation was achieved within one minute.

Figure 41: Rapid separation of protein mixture



Column: **TSKgel Super-Phenyl, 2.3 µm, 4.6 mm ID × 5 cm**
Mobile phase: A: 13 mmol/L $HClO_4$
B: 13 mmol/L $HClO_4/CH_3CN$ = 20/80
40%B to 100%B in a 1.5 min linear gradient
Flow rate: 2.0 mL/min
Detection: UV @ 220 nm
Samples: 1. ribonuclease A
2. insulin
3. cytochrome C
4. lysozyme
5. α-lactalbumin
6. myoglobin

About: TSKgel CN-80Ts Reversed Phase Chromatography Columns

TSKgel CN-80Ts is an alternative to C18 (ODS) and C8 (Octyl) phases. The resin is based on a high-purity, metal-free 80Ts silica bonded to a C_3CN group. The cyano group is the least hydrophobic of the 10 nm phases available and in some cases is used under normal phase conditions.

The nomenclature for TSKgel reversed phase columns is based on the characteristics of the individual packing. In the case of TSKgel CN-80Ts, the "T" indicates endcapping with TMS groups while the subscript "S" denotes that endcapping is complete. Bonded phase pore size is indicated by the number in the product description, in this case TSKgel CN-80Ts has 8 nm nominal pore size. The pore size of the base silica is 10 nm.

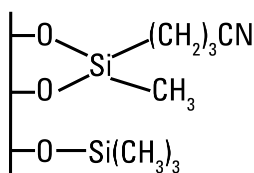
Attributes and Applications

Table 11 lists the attributes of TSKgel CN-80Ts columns, while Figure 42 displays the structure. TSKgel CN-80Ts is useful for the analysis of polar peptides, amino acids, and other pharmaceutical and food & beverage products. As with other 80Ts products, TSKgel CN-80Ts provides reproducible separations of molecules below 6,000 Da.

Table 11: Product attributes

Attribute	Value
Pore size	8 nm
Molar mass limit	6,000 Da
Endcapped	Yes - complete
Particle size	5 µm
pH stability	2.0-7.5
Functional group	cyano (monomeric bonding chemistry)
% Carbon	9

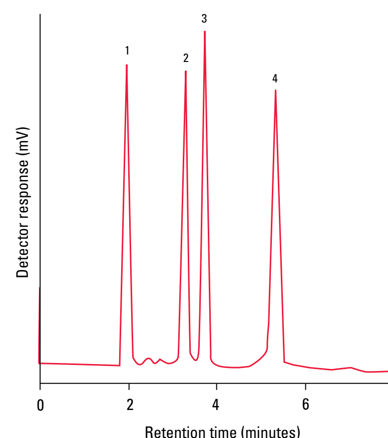
Figure 42: TSKgel CN-80Ts structure



Aromatic Compounds

The chromatogram in Figure 43 shows the symmetrical peaks obtained with the rapid separation of 3 aromatic compounds using a TSKgel CN-80Ts column.

Figure 43. Aromatic compounds on TSKgel CN-80Ts



Column: **TSKgel CN-80Ts, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase: 50% MeOH
Flow rate: 1.0 mL/min
Temperature: 25 °C
Samples: 1. uracil
2. benzene
3. toulene
4. napthalene

About: TSKgel Octyl-80Ts Reversed Phase Chromatography Columns

The high-purity, metal-free silica particles in TSKgel Octyl-80Ts columns contain 8 nm pores and are bonded with octylmethyl silyl groups. Featuring a proprietary technique for complete endcapping of residual silanol groups, TSKgel Octyl-80Ts columns reduce tailing when analyzing basic compounds. TSKgel Octyl-80Ts columns have a lower carbon load and hydrophobicity than the corresponding ODS products. The C8 alkyl ligand provides a unique selectivity for the analysis of low molar mass pharmaceuticals, bases, nucleosides, and nucleotides.

The nomenclature for TSKgel reversed phase columns is based on the characteristics of the individual packing. In the case of TSKgel Octyl-80Ts, the "T" indicates endcapping with TMS groups while the subscript "S" denotes that endcapping is complete. The pore size of the bonded phase particles is indicated by the number in the product description; in this case TSKgel Octyl-80Ts has 8 nm nominal pore size. The pore size of the starting or base silica is 10 nm.

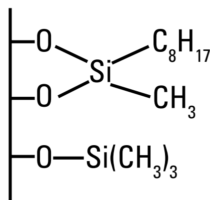
Attributes and Applications

Table 12 lists the attributes of TSKgel Octyl-80Ts columns. The stucture of the bonded phase is displayed in Figure 44. TSKgel Octyl-80Ts columns are recommended for molecules under 6,000 Da, such as amino acids, pharmaceuticals, nucleotides, and food and beverage components. Common applications include purity checks and peptide mapping.

Table 12: Product attributes

Attribute	Value
Pore size	8 nm
Molar mass limit	6,000 Da
Endcapped	Yes
Particle size	5 µm
pH stability	2.0-7.5
Functional group	C8 (monomeric bonding chemistry)
% Carbon	10

Figure 44: TSKgel Octyl-80Ts structure

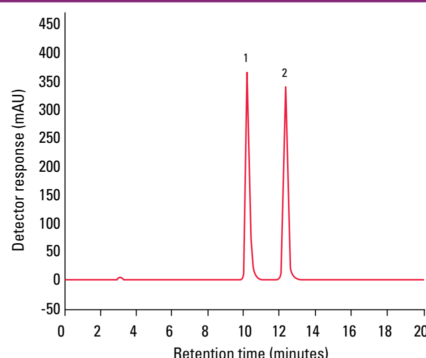


Asthma Medication

Pranlukast hydrate dry syrup is a medicine used in Japan that inhibits contraction of the airway and vascular permeability by binding with leukotriene receptors and blocking their action. It helps to prevent symptoms of bronchial asthma such as coughing, wheezing, and difficulty in breathing. Its action is similar to Merck & Co.'s Singulair (montelukast).

The Japanese Pharmaceutical Drug Standards recommends an octyl column for the analysis of pranlukast and the internal standard isoamyl p-oxybenzoate. Figure 45 shows the high resolution separation of pranlukast hydrate and isoamyl p-oxybenzoate using a TSKgel Octyl-80Ts column.

Figure 45: Analysis of pranlukast hydrate dry syrup



Column: **TSKgel Octyl-80Ts, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase: 20 mmol/L KH_2PO_4/ACN/ MeOH = 5/5/1(v/v/v)
Flow rate: 0.6 mL/min
Detection: UV/VIS @ 260 nm
Temperature: 25 °C
Injection vol.: 4 µL
Samples: 1. pranlukast hydrate, 0.2 mg/L
2. isoamyl p-oxybenzoate (4-hydroxybenzoic acid isoamyl ester), 0.2 mg/L

Sample preparation:
Pranlukast solution: To 400 mg of pranlukast hydrate dry syrup, 10 mL of acetonitrile/dimethyl sulfoxide = 3/1(v/v) was added and shaken vigorously. Solution was centrifuged at 3000 rpm for 5 min. To 1 mL of supernatant, 9 mL of acetonitrile/dimethyl sulfoxide = 3/1(v/v) was added.

Isoamyl p-oxybenzoate solution (IS): To 4.03 mg of isoamyl p-oxybenzoate, 10 mL of acetonitrile/dimethyl sulfoxide = 3/1(v/v) was added and dissolved. 5 mL of both solutions were mixed and applied.
Sample: pranlukast hydrate dry syrup

Call customer service: 866-527-3587,
technical service: 800-366-4875

About: TSKgel ODS-80TM Reversed Phase Chromatography Columns

TSKgel ODS-80TM is a packing with a C18 (ODS) group bonded to a 8 nm pore size, high-purity, metal-free silica. High endcapping of the TSKgel ODS-80TM bonded phase shields the silica surface from participating in solute retention through ionic interaction.

The nomenclature for TSKgel reversed phase columns is based on the characteristics of the individual packing. In the case of TSKgel ODS-80TM, the "T" indicates endcapping with TMS groups while the subscript "M" denotes a monolayer coverage of C18 groups. Bonded phase pore size is indicated by the number in the product description, in this case TSKgel ODS-80TM has 8 nm nominal pore size. The pore size of the base silica is 10 nm.

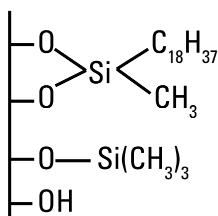
Attributes and Applications

The product attributes of TSKgel ODS-80TM columns are listed in Table 13; the structure is displayed in Figure 46. The TSKgel ODS-80TM column is a general purpose column for the analysis of low molar mass pharmaceuticals, basic compounds, nucleosides, nucleotides, purines, and pyrimidines. Common applications include purity checks and peptide mapping.

Table 13: Product attributes

Attribute	Value
Pore size	8 nm
Molar mass limit	6,000 Da
Endcapped	Yes
Particle size	5 µm and 10 µm
pH stability	2.0-7.5
Functional group	C18 (monomeric bonding chemistry)
% Carbon	15

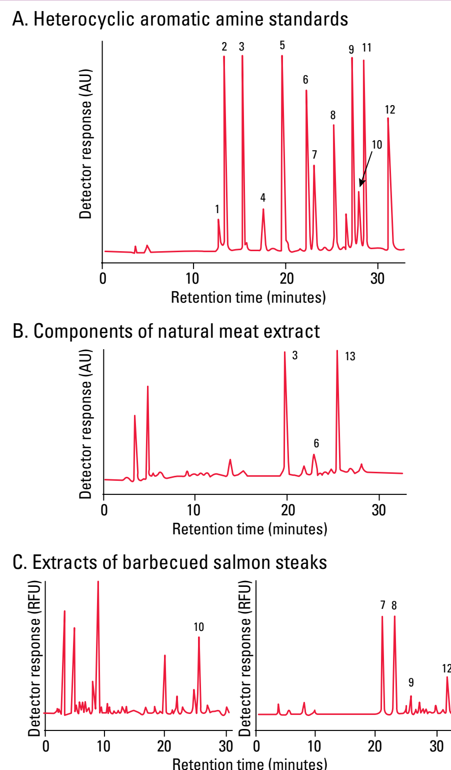
Figure 46: TSKgel ODS-80TM structure



Food Products

TSKgel ODS-80TM provides high efficiency and symmetrical peaks for basic, heterocyclic aromatic amines in food products, as shown in Figure 47. In this study, TSKgel ODS-80TM columns provided the best resolution of nanogram levels of the amines in barbecued food, known to be potential carcinogens.

Figure 47: Determination of carcinogens in food



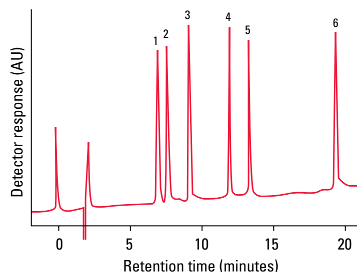
Column:	**TSKgel ODS-80TM, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	15 min linear gradient from 5% to 15% CH_3CN in 0.01 mol/L triethyl ammonium phosphate (TAP), pH 3.2; then switch to TAP buffer at pH 3.6 and conduct a 4 min linear gradient to 25% CH_3CN, followed by a 15 min linear gradient to 55% CH_3CN
Flow rate:	1.0 mL/min
Detection:	A: UV @ 263 nm B: UV @ 360 nm C: fluoresence: Ex: 360 nm, Em: 450 nm
Samples:	1. Glu-P-2 (18 ng) 2. IQ (12 ng) 3. MeIQ (14 ng) 4. Glu-P-1 (18 ng) 5. MeIQx (12 ng) 6. 4,8-DiMeIQx (15 ng) 7. norharman (10 ng) 8. harman (15 ng) 9. Trp-P-2 (12 ng) 10. PhIP (15 ng) 11. Trp-P-1 (8 ng) 12. A-alpha-C (17 ng) 13. 4,7,8-TriMeIQx
Legend:	see footnote below for explanation of abbreviations

Amino-imidazo-quinolines (IQ and MeIQ)
Amino-imidazo-quinoxalines (MeIQx and DIMEIQx)
Amino-pyrido-indoles (Trp-P-1 and Trp-P-2)
Amino-pyrido-imidazoles (Glu-P-1 and Glu-P-2)
Amino-alpha-carbolines (A-alpha-C and MeA-alpha-C)

Peptides

Figure 48 demonstrates the applicability of the TSKgel ODS-80TM column for the analysis of peptides. Very high resolution was achieved for each compound.

Figure 48: Peptide analysis

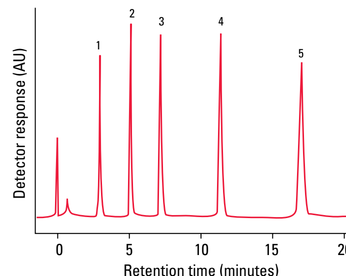


Column:	**TSKgel ODS-80TM, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	90 min linear gradient from 23.5% to 100% CH_3CN in 0.1% TFA
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Samples:	1. bradykinin (2 µg) 2. α-endorphin (2 µg) 3. angiotensin II (1.5 µg) 4. angiotensin I (1.5 µg) 5. substance P (2 µg) 6. β-endorphin (3 µg)

Pharmaceuticals

The TSKgel ODS-80TM column was used successfully for the baseline separation of 5 common pharmaceuticals, as shown in Figure 49.

Figure 49: Common pharmaceuticals



Column:	**TSKgel ODS-80TM, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	35% CH_3OH in 0.05 mol/L phosphoric acid, pH 2.5
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Samples:	1. p-aminoacetophenon (0.05 µg) 2. caffeine (0.25 µg) 3. salicylamide (0.6 µg) 4. aspirin I (1.56 µg) 5. phenacetin (0.16 µg)

About: TSKgel ODS-80Ts Reversed Phase Chromatography Columns

TSKgel ODS-80Ts columns contain packing that has C18 groups bonded to 8 nm pore size, high-purity, metal-free silica. The silica used in the OSD-80Ts is highly endcapped, which reduces cationic interactions. In addition, the silica does not contain metal ions or ammonium moieties that can broaden peaks of acidic compounds and chelating reagents.

The nomenclature for TSKgel reversed phase columns is based on the characteristics of the individual packing. In the case of TSKgel ODS-80Ts, the "T" indicates endcapping with TMS groups while the subscript "S" denotes that endcapping is complete. Bonded phase pore size is indicated by the number in the product description, in this case TSKgel ODS-80Ts has 8 nm nominal pore size. The pore size of the base silica is 10 nm.

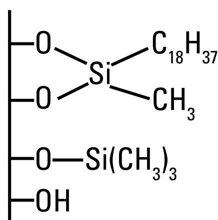
Attributes and Applications

Table 14 lists the attributes of TSKgel ODS-80Ts columns, while Figure 50 displays the structure. The TSKgel ODS-80Ts columns are useful for molecules in the 100-6,000 Da range, so small peptides and pharmaceuticals can be successfully separated on this column.

Table 14: Product attributes

Attribute	Value
Pore size	8 nm
Molar mass limit	6,000 Da
Endcapped	Yes
Particle size	5 µm and 10 µm
pH stability	2.0-7.5
Functional group	C18 (monomeric bonding chemistry)
% Carbon	15

Figure 50: TSKgel ODS-80Ts structure

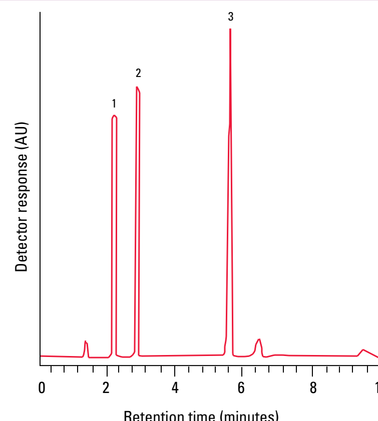


Food Products

Because of the stability of silica-based packings at acidic and neutral pH, most reversed phase separations are conducted in the pH range from 2.0 to 7.0. Under these pH conditions, however, organic bases have a charge and careful control of the eluent pH with buffers, and/or ion-pair liquid chromatography is employed to isolate them. An ion-pair reagent added to the buffer forms a complex with the stationary phase. For basic compounds, alkylsulfonic acids are most often used, while allyl amines are typical ion-pair reagents for strongly acidic analytes.

Since the ODS binding and trimethylsilyl endcapping techniques leave few residual silanol groups to cause tailing, the endcapped silica reduces cationic interactions, metal ion interactions, or ammonium moiety interactions that can broaden peaks of basic compounds, acidic compounds, and chelating reagents as shown in Figure 51 using a TSKgel ODS-80Ts column.

Figure 51: Test of column efficiency

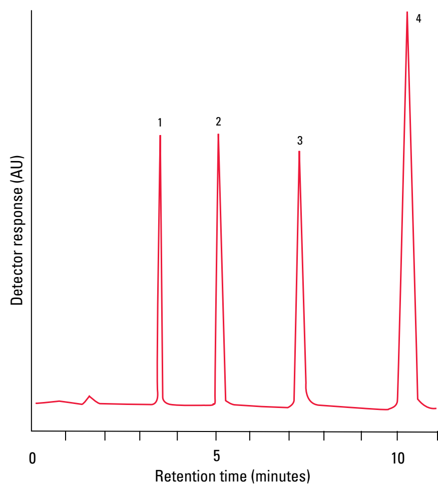


Column: **TSKgel ODS-80Ts, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase: 50% MeOH
Flow rate: 1.0 mL/min
Detection: UV @ 254 nm
Temperature: 25 °C
Samples: 1. pyridine
2. phenol
3. methyl benzoate

Pharmaceuticals

Figure 52 shows simple pharmaceuticals analyzed using a 2 mm ID TSKgel ODS-80Ts column.

Figure 52: Analysis of pharmaceuticals

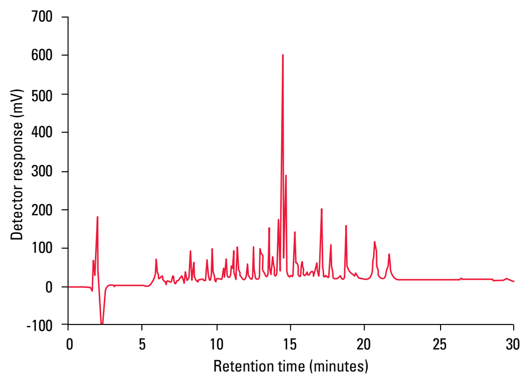


Column:	**TSKgel ODS-80Ts, 5 µm, 2 mm ID × 15 cm**
Mobile phase:	50 mmol/L phosphate buffer, pH 2.5/ MeOH = 60/40
Flow rate:	0.2 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. caffeine (12 ng) 2. salicylamide 3. aspirin (120 ng) 4. phenacetin (18 ng)

Trypsin Digest

Figure 53 shows the analysis of a trypsin digest of β-lactoglobulin on a TSKgel ODS-80Ts semi-micro column.

Figure 53: Trypsin digest



Column:	**TSKgel ODS-80Ts, 5 µm, 2.0 mm ID × 15 cm**
Mobile phase:	A: 0.1% TFA solution B: ACN + 0.1% TFA A (100%A) → A (30%) linear gradient (30 min)
Flow rate:	0.20 mL/min
Detection:	UV @ 215 nm, micro-cell
Temperature:	25 °C
Sample:	trypsin digest of β-lactoglobulin (10 µL)

About: TSKgel ODS-80Ts QA Reversed Phase Chromatography Columns

TSKgel ODS-80Ts QA columns were developed specifically for use by QA/QC departments that require highly reproducible separations. These columns are prepared from the same endcapped C18 packing material as TSKgel ODS-80Ts columns, but with narrower manufacturing specifications to meet the demand for high reproducibility.

The variation between different lots of TSKgel ODS-80Ts QA packing material is minimized by selecting batches of TSKgel ODS-80Ts that fall within a very narrow range of specifications, as demonstrated in Table 15 below. In addition, each column must pass demanding specifications for efficiency (N) and peak asymmetry, as are spelled out in the Operating Conditions and Specifications sheet. The end result is TSKgel ODS-80Ts QA columns that exhibit an unparalleled level of reproducibility for retention, selectivity (k´), efficiency (N), and peak symmetry.

Table 15: Product specifications

Attribute	Specification Range	Lot-to-Lot Reproducibility (CV%)
Particle Size: -Distribution (dp_{90}/dp_{10})	4.95-5.35 1.55 - 1.70	0.6 1.8
Surface area (m^2/g)	410 - 440	0.5
Pore size (nm) silica	9 - 10	0.5
Pore volume (mL/g silica)	0.96 - 1.04	0.7
Carbon content (wt%)	14.0 - 15.0	N/A
C18 coverage (μmol/m^2)	1.71 - 1.99	1.3
Metal ion content (ppm) -Na -Al -Fe -Ti	 <10 <10 <10 <10	 N/A N/A N/A N/A

Column classification:

TSKgel ODS-80Ts QA columns were submitted to several characterization tests to determine the level of hydrophobic retention, steric selectivity, and retention of basic compounds. The results of the characterization tests were used to establish specifications listed in Table 16.

Table 16: Characterization test results

Parameter	Specification	CV (%)	Test Conditions
k′ naphthalene (hydrophobicity)	1.53 - 1.63	1.3	1
α triphenylene/*o*-terphenyl (steric selectivity)	1.21 - 1.25	0.4	1
k′ procainamide (basic compounds)	1.35 - 1.55	2.6	2
k′ phenol	9.25 - 9.85	0.9	2
k′ oxine copper (inertness to chelating compounds)	1.13 - 1.35	3.5	3

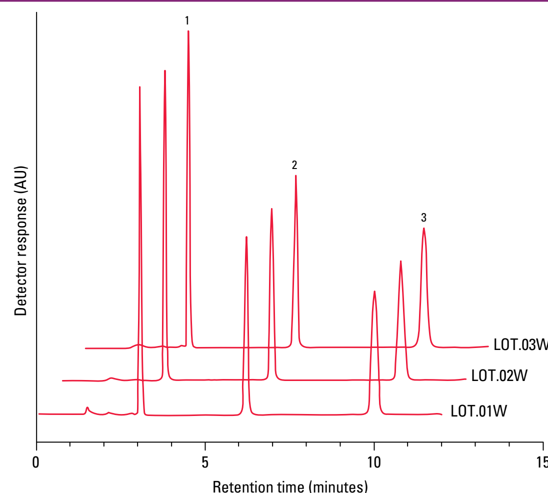
1. 80% CH_3OH/20% H_2O, T = 40 °C
2. 10% ACN/90% 20 mmol/L Na_2HPO_4 (pH 6.6, adjusted with 20 mmol/L NaH_2PO_4, T = 40 °C)
3. 7% ACN/93% 20 mmol/L H_3PO_4, T = 40 °C

Over-the-Counter Analgesic Pain Reliever

To demonstrate the lot-to-lot reproducibility that can be expected when using TSKgel ODS-80Ts QA columns, the contents of an over-the-counter analgesic pain reliever was analyzed on columns from three different lots of packing material. The results are shown in Figure 54 and Table 17 below. Excellent reproducibility of retention, peak height, peak area, efficiency, and peak shape is evident for all three ingredients.

Highly reproducible results can be achieved using the TSKgel ODS-80Ts QA columns as shown in this lot-to-lot reproducibility test.

Figure 54: Analysis of an over-the-counter analgesic pain reliever



Column: **TSKgel ODS-80Ts QA, 5 μm, 4.6 mm ID × 15 cm**
Mobile phase: 50 mmol/L phosphate buffer, pH 2.5/ACN = 80/20
Flow rate: 1.0 mL/min
Detection: UV @ 254 nm
Temperature: 40 °C
Injection vol.: 5 μL
Samples: 1. caffeine
2. salicylamide
3. acetylsalicylic acid

Table 17: Results demonstrating lot-to-lot reproducibility

Compound	Lot#	RT (min)	Peak Area (mv × sec)	Peak Height	N	AF
caffeine	01W 02W 03W	3.047 3.080 3.080	4.51575×10^2 4.50235×10^2 4.44175×10^2	99.26 94.04 95.63	10,396 9,597 10,143	1.10 1.13 1.13
salicylamide	01W 02W 03W	6.190 6.253 6.250	3.96172×10^2 3.94822×10^2 3.90617×10^2	54.06 52.23 52.40	16,441 15,797 16,333	1.03 1.07 1.06
acetylsalicylic acid	01W 02W 03W	9.983 10.080 10.063	4.34473×10^2 4.33835×10^2 4.27633×10^2	37.35 36.48 36.28	16,742 16,297 16,649	1.03 1.05 1.05

About: TSKgel ODS-120A Reversed Phase Chromatography Columns

TSKgel ODS-120A columns use a 15 nm pore size silica base support. The bonding method results in a polymeric coverage of C18 groups on the silica surface.

The "A" signifies that the material is not endcapped. For charged samples, the endcapped TSKgel ODS-120T column is a more suitable alternative.

Bonded phase pore size is indicated by the number in the product description, in this case TSKgel ODS-120A has 12 nm nominal pore size.

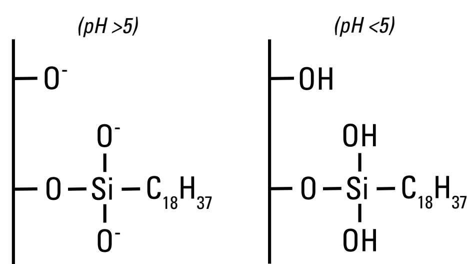
Attributes and Applications

Table 18 lists the attributes of TSKgel ODS-120A columns, while Figure 55 displays the structure. The silica base support's exclusion limit of 1.0×10^4 Da makes them a good choice for the reversed phase chromatography of peptides, small proteins, and environmental samples such as poly-aromatic hydrocarbons.

Table 18: Product attributes

Attribute	Value
Pore size (mean)	15 nm
Exclusion limit	1.0×10^4 Da
Endcapped	No
Particle size (mean)	5 µm and 10 µm
pH stability	2.0-7.5
Functional group	C18 (polymeric bonding chemistry)
% Carbon	22

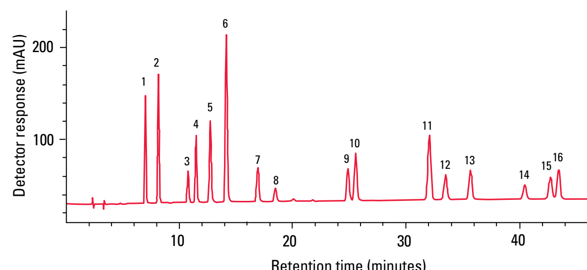
Figure 55: TSKgel ODS-120A structure



Polynuclear Aromatic Hydrocarbons

The polymeric stationary phase of the TSKgel ODS-120A column exhibits improved shape selectivity for the separation of complex geometric isomers, such as polynuclear aromatic hydrocarbons (PAH) as shown in Figure 56.

Figure 56: Separation of 16 poly-aromatic hydrocarbons



Column: **TSKgel ODS-120A, 5 µm, 4.6 mm ID × 25 cm**
Gradient: 40 min linear from 75% MeOH/25% H_2O to 95% MeOH/5% H_2O, 5 min hold
Flow rate: 1.2 mL/min
Detection: UV @ 254 nm
Temperature: 40 °C
Samples: 5 mL mixture of:
1. naphthalene
2. acenaphthylene
3. acenaphthene
4. fluorene
5. phenanthrene
6. anthracene
7. fluoranthene
8. pyrene
9. benzo(a)anthracene
10. chrysene
11. benzo(b)fluoranthene
12. benzo(k)fluoranthen
13. benzo(a)pyrene
14. dibenzo(a,h)anthracene
15. benzo(g,h,i)perylene
16. indeno(1,2,3-cd)pyrene

About: TSKgel ODS-120T Reversed Phase Chromatography Columns

TSKgel ODS-120T columns use a 15 nm pore size silica base support. The columns are endcapped with trimethyl silane groups to improve the peak shape of negatively charged analytes.

Bonded phase pore size is indicated by the number in the product description, in this case TSKgel ODS-120T has 12 nm nominal pore size.

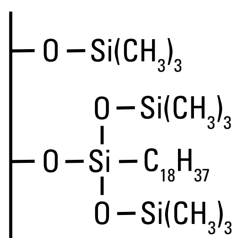
Attributes and Applications

Table 19 lists the attributes of TSKgel ODS-120T columns, while Figure 57 displays the structure. With an exclusion limit of 1.0×10^4 Da, the TSKgel ODS-120T are a good choice for the reversed phase chromatography of peptides, small proteins, and small molar mass compounds in organic and environmental samples.

Table 19: Product attributes

Attribute	Value
Pore size (mean)	15 nm
Exclusion limit	1.0×10^4 Da
Endcapped	Yes
Particle size	5 µm and 10 µm
pH stability	2.0-7.5
Functional group	C18 (polymeric bonding chemistry)
% Carbon	22

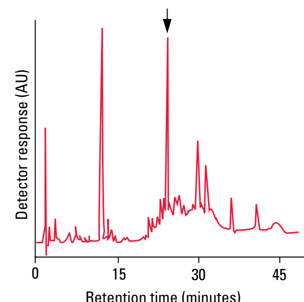
Figure 57: TSKgel ODS-120T structure



Peptides

Endcapped TSKgel ODS-120T is an alternative to TSKgel ODS-80TM for peptide and protein separation. Figure 58 demonstrates the applicability of the TSKgel ODS-120T column for the analysis of synthetic peptides.

Figure 58: Purification and rapid analysis of synthetic peptides

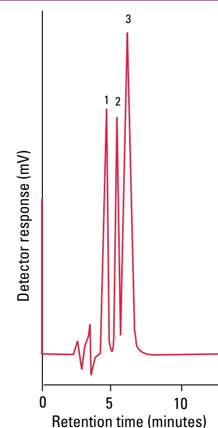


Column: **TSKgel ODS-120T, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase: 48 min linear gradient from 14% to 50% CH_3CN in 0.1% TFA
Flow rate: 1.0 mL/min
Detection: UV @ 215 nm
Sample: triacontadipeptide (EAEDLQVGQVELGGGPGAGSLQPLALEGSLQC) indicated by arrow; 50 µg in 50 µL

Bradykinins

The good peak shape of closely related bradykinins on TSKgel ODS-120T in the non-buffered eluent is due to reduced interaction with residual silanol groups as a result of endcapping (Figure 59).

Figure 59: Separation of bradykinins

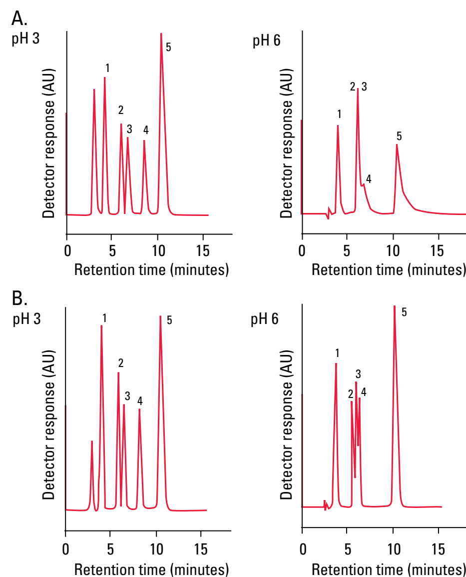


Column: **TSKgel ODS-120T, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase: 20% CH_3CN in 0.05% TFA
Flow rate: 1.0 mL/min
Detection: UV @ 220 nm
Temperature: 25 °C
Samples: 1. Lys-bradykinin
2. Met-Lys-bradykinin
3. bradykinin

Catecholamines

In Figure 60, the effect of pH on endcapped and non-endcapped packings is shown for the same columns in the separation of catecholamines. When the pH of the eluent is above the pKa of the non-endcapped silanol groups, the TSKgel ODS-120A packing is negatively charged, and the catecholamine peaks tail. However, notice the similar resolution on TSKgel ODS-120A and TSKgel ODS-120T columns when the eluent is buffered at an acidic pH, where the silanol groups will be protonated.

Figure 60: Separation of catecholamines



Columns:	**A. TSKgel ODS-120A, 5 µm, 4.6 mm ID × 25 cm**
	B. TSKgel ODS-120T, 5 µm, 4.6 mm ID × 25 cm (endcapped)
Mobile phase:	0.1 mol/L phosphate buffer, pH 3.0 or 6.0
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Samples:	1. norepinephrine
	2. epinephrine
	3. 3,4-dihydroxybenzylamine
	4. D,L-DOPA
	5. dopamine-HCl

About: TSKgel OligoDNA-RP Reversed Phase Chromatography Columns

Specifically designed for the purification of oligonucleotides, and RNA and DNA fragments (up to 500-mer), TSKgel OligoDNA-RP columns can provide excellent separations of samples with very similar sequences. The packing is prepared by monomeric binding of octadecyl silyl groups to 5 µm spherical silica gel with 25 nm pores. This packing is not endcapped and it has a relatively low carbon content of 10%.

The 25 nm pore size of the TSKgel OligoDNA-RP column provides excellent kinetics for molecules with helix shape structures processing large radii of gyration. The 5 µm particle size provides a minimum of 7,000 plates per 15 centimeter column.

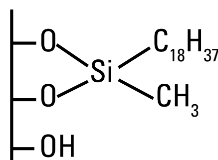
Attributes and Applications

Table 20 lists the attributes of TSKgel OligoDNA-RP columns, while Figure 61 displays the structure.

Table 20: Product attributes

Attribute	Value
Pore size (mean)	25 nm
Exclusion limit	500-mer
Endcapped	No
Particle size (mean)	5 µm
pH stability	2.0-7.5
Functional group	C18 (monomeric bonding chemistry)
% Carbon	10

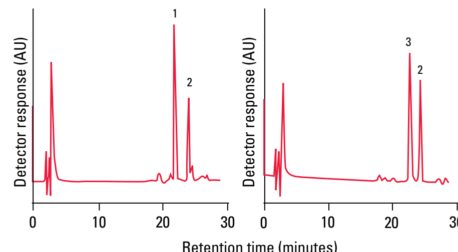
Figure 61: TSKgel OligoDNA-RP structure



Octamers

TSKgel OligoDNA-RP columns possess high-resolving power for octamers of similar sequence, as demonstrated in Figure 62.

Figure 62: Separation of octomers

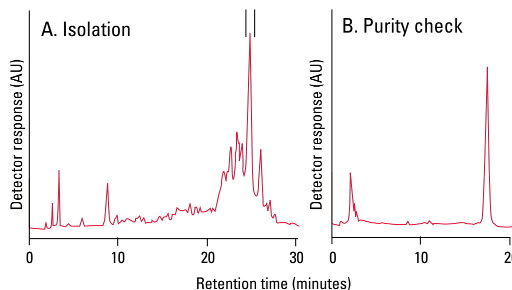


Column:	**TSKgel OligoDNA-RP, 5 µm, 4.6 mm ID × 5 cm**
Mobile phase:	120 min linear gradient from 5% to 25% CH_3CN in 0.1 mol/L ammonium acetate, pH 7.0
Flow rate:	1.0 mL/min
Detection:	UV @ 260 nm
Sample:	1. linker EcoR I, d(CGAATTCG) 2. *Hpa* I, d(CGTTAACG) 3. linker EcoR V, d(CGATATCG)

Oligonucleotides

The semi-preparative isolation of a 49-mer oligonucleotide from the crude synthetic reaction mixture using a 7.8 mm ID TSKgel OligoDNA-RP column is shown in Figure 63. The purity of the isolated oligonucleotide was subsequently verified on an analytical 4.6 mm ID TSKgel OligoDNA-RP column.

Figure 63: Purification of synthetic 49-mer oligonucleotide



Columns:	**A. TSKgel OligoDNA-RP, 5 µm, 7.8 mm ID × 15 cm** **B. TSKgel OligoDNA-RP, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	A. 120 min linear gradient from 6.25% to 25% CH_3CN (7.8 mm ID) column B. 90 min linear gradient from 7.5% to 25% CH_3CN (4.6 mm ID) column both in 0.1 mol/L ammonium acetate, pH 7.0
Flow rate:	A. 2.8 mL/min (7.8 mm ID) B. 1.0 mL/min (4.6 mm ID)
Detection:	UV @ 260 nm
Sample:	synthetic 49-mer oligonucleotide, d(AGCTTGGGCTGCAGGTCGTCTCTAGAGGATCCCC GGGCGAGCTCGAATT)

About: TSKgel TMS-250 Reversed Phase Chromatography Columns

TSKgel TMS-250 columns contain a unique C1 bonded phase. The packing is prepared by monomeric binding of trimethyl silyl groups to a 25 nm pore size spherical silica.

Due to the low hydrophobicity of the ligand, excellent recoveries are common even when used with large proteins. Proteins such as adolase (158 kDa) exhibit sharp peaks relative to wide pore C18 columns.

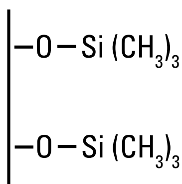
Attributes and Applications

Table 21 lists the attributes of TSKgel TMS-250 columns; Figure 64 displays the structure. TSKgel TMS-250 columns are an excellent choice for analysis of larger proteins by reversed phase HPLC.

Table 21: Product attributes

Attribute	Value
Pore size (mean)	25 nm
Exclusion limit	2.0×10^5 Da
Endcapped	Yes
Particle size	10 µm
pH stability	2.0-7.5
Functional group	C1 (monomeric bonding chemistry)
% Carbon	5

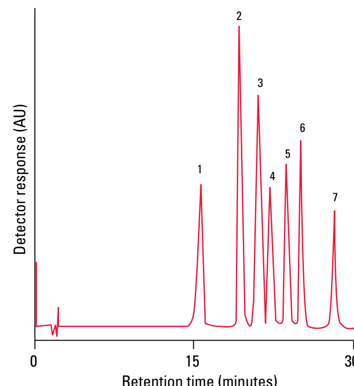
Figure 64: TSKgel TMS-250 structure



Protein Analysis

The resolution of proteins on TSKgel TMS-250 columns is shown in Figure 65. The wide pore packing of these columns can accommodate such large proteins as adolase.

Figure 65: High resolution protein separation

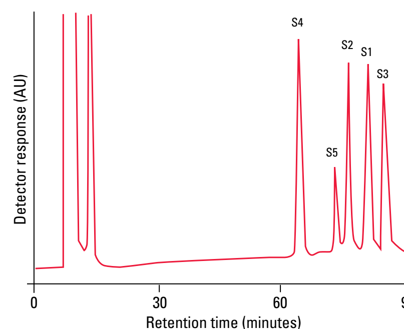


Column: **TSKgel TMS-250, 10 µm, 4.6 mm ID × 7.5 cm**
Mobile phase: 60 min linear gradient from 20% to 95% CH_3CN in 0.05% TFA, pH 2.2
Flow rate: 0.61 mL/min
Detection: UV @ 220 nm
Samples: 5 µg each of: 1 ribonuclease A 2. cytochrome C 3. lysozyme 4. bovine serum albumin 5. aldolase 6. carbonic anhydrase 7. ovalbumin

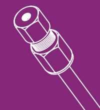
Protein Subunits

Figure 66 illustrates the high resolution and efficiency of TSKgel TMS-250 for the isolation of B. pertussis toxin (PT) subunit proteins. Five distinct PT subunits of molar mass ranging from 1.0×10^4 to 2.6×10^4 Da were resolved without significant cross-contamination using a specially packed 7.5 mm ID x 30 cm TSKgel TMS-250 column.

Figure 66: Separation of protein subunits



Column: **TSKgel TMS-250, 10 µm, 7.5 mm ID × 30 cm**
Mobile phase: Load and 12 min wash with 34% CH_3CN in 0.1% TFA, followed by a 100 min linear gradient from 34% to 47% CH_3CN in 0.1% TFA
Flow rate: 1.5 mL/min
Detection: UV @ 210 nm and 280 nm (not shown)
Injection vol.: 100 µg in 500 mL
Sample: purified *Bordetella pertussis* toxin in 0.1 mol/L phosphate buffer, pH 7.2, with 0.5 mol/L NaCl and 30% glycerol



About: TSKgel Octadecyl-2PW Reversed Phase Chromatography Columns

The highly cross-linked polymethacrylate base material of TSKgel Octadecyl-2PW columns provides excellent stability in high pH buffer systems and can withstand rigorous cleaning with either acid or base. The 12.5 nm pore size of TSKgel Octadecyl-2PW columns makes them ideally suited for peptides and small proteins. Large pores allow unhindered access to proteins and other large molar mass biopolymers. The TSKgel Octadecyl-2PW columns demonstrate faster analysis than other competitive reversed phase polymeric columns.

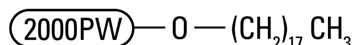
Attributes and Applications

Table 22 lists the attributes of TSKgel Octadecyl-2PW columns, while Figure 67 displays the structure. The 12.5 nm pores allow for analysis of peptides up to 8,000 Da.

Table 22: Product attributes

Attribute	Value
Pore size (mean)	12.5 nm
Exclusion limit	8,000 Da
Particle size (mean)	5 µm
pH stability	2.0-12.0
Functional group	C18 (monomeric bonding chemistry)

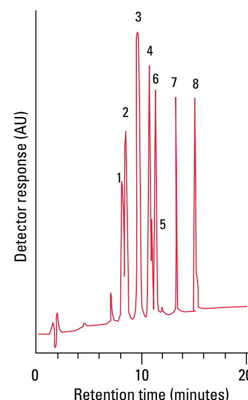
Figure 67: TSKgel Octadecyl-2PW structure



Neuropeptides

The rapid separation of a mixture of eight peptides using a TSKgel Octadecyl-2PW column is shown in Figure 68. The complexity of these peptides, found in neural tissue, requires an efficient column that is robust under low pH mobile phase conditions. A TSKgel Octadecyl-2PW column delivers symmetrical peaks and a sharp elution profile.

Figure 68: Separation of eight peptides

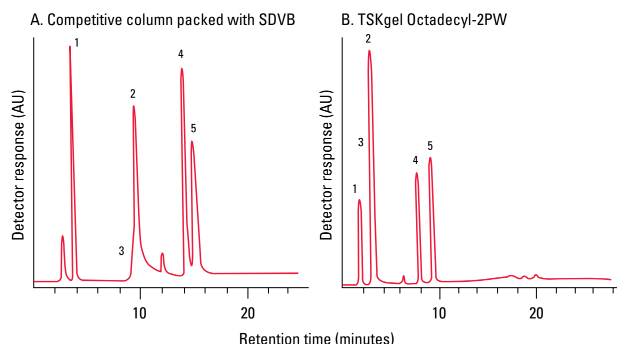


Column:	**TSKgel Octadecyl-2PW, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	30 min linear gradient from 0.1% TFA/CH_3CN from 90/10 to 30/70
Flow rate:	1.0 mL/min
Detection:	UV @ 215 nm
Temperature:	ambient
Samples:	1. met-enkephalin 2. bradykinin 3. leu-enkephalin 4. neurotensin 5. bombesin 6. angiotensin I 7. somatostatin 8. insulin (bovine)

Common Drugs

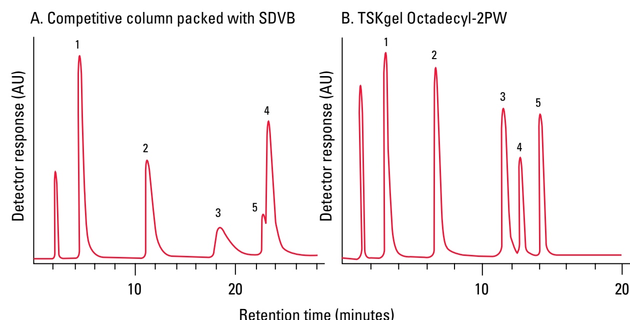
The polymeric backbone of TSKgel Octadecyl-2PW gives this column better pH stability than silica-based columns so the separations can be optimized over a wider pH range, as shown in Figure 69. A pH of 7.0 gives excellent resolution of a mixture of common drugs on the TSKgel Octadecyl-2PW column, while they tail or are unresolved on a competitive PSDVB column.

Figure 69: Comparison over a wide pH range



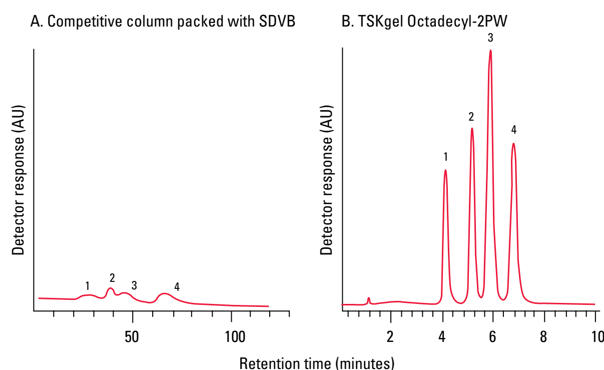
1. pH 2.5

Columns:	A. competitive column with styrene divinylbenzene (SDVB), 5 µm packing **B. TSKgel Octadecyl-2PW, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	20 mmol/L phosphate buffer, pH 2.5/ACN, 80/20 to 0/100, 30 min linear gradient
Flow rate:	A. 0.5 mL/min B. 1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. sulfide 2. disopyramide 3. chlorphenirmin 4. ciltrazem 5. hydroxyzine



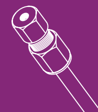
2. pH 7.0

Columns:	A. competitive column with styrene divinylbenzene (SDVB), 5 µm packing **B. TSKgel Octadecyl-2PW, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	20 mmol/L phosphate buffer, pH 7.0/ACN, 80/20 to 0/100, 30 min linear gradient
Flow rate:	A. 0.5 mL/min B. 1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. sulfide 2. disopyramide 3. chlorphenirmin 4. ciltrazem 5. hydroxyzine



3. pH 11.0

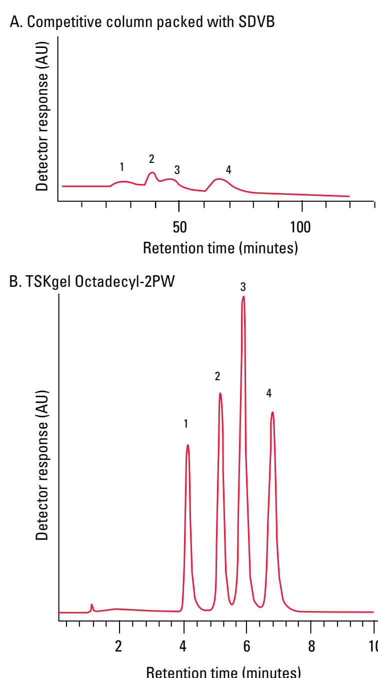
Columns:	A. competitive column with styrene divinylbenzene (SDVB), 5 µm packing **B. TSKgel Octadecyl-2PW, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	20 mmol/L phosphate buffer, pH 11.0/ACN, 40/60, 30 min linear gradient
Flow rate:	A. 0.5 mL/min B. 1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. desipramine 2. imiptamine 3. amitriptyline 4. trimipramine



Tricylic Antidepessant Drugs

Figure 70 shows a comparison of four tricyclic antidepressant drugs on a column packed with styrene-divinylbenzene and a TSKgel Octadecyl-2PW column, both operated at pH 11. Recovery of sample analytes is high with the TSKgel Octadecyl-2PW column due to the modest hydrophobic nature of the polymethacrylate base matrix in comparison to a competitive polystyrene-based column.

Figure 70: Comparison of common tricyclic antidepressant drugs



Columns:	A. competitive column with styrene divinylbenzene (SDVB), 5 µm packing **B. TSKgel Octadecyl-2PW, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	20 mmol/L phosphate buffer, pH 11.0/ ACN, 40/60
Flow rate:	A. 0.5 mL/min B. 1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. desipramine 2. imipramine 3. amitriptyline 4. trimipramine

About: TSKgel Octadecyl-4PW Reversed Phase Chromatography Columns

The highly cross-linked polymethacrylate base material of TSKgel Octadecyl-4PW provides excellent stability in high pH buffer systems and can withstand rigorous cleaning with either acid or base. The large pore size of TSKgel Octadecyl-4PW columns, 50 nm, allows unhindered access to proteins and other large molar mass biopolymers. The particle size offerings allow for analytical and semi-preparative scale separations.

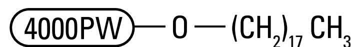
Attributes and Applications

Table 23 lists the attributes of TSKgel Octadecyl-4PW columns, while Figure 71 displays the structure. TSKgel Octadecyl-4PW columns are for the analysis of proteins up to 200 kDa.

Table 23: Product attributes

Attribute	Value
Pore size (mean)	50 nm
Exclusion limit	1,000 - 2.0 × 10^5 Da
Estimated ligand density	1 eq/L
Particle size (mean)	7 µm and 13 µm
pH stability	2.0-12.0
Functional group	C18 (monomeric bonding chemistry)

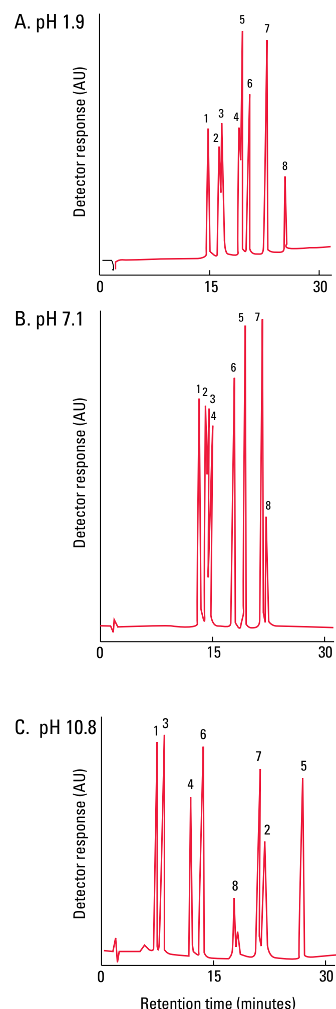
Figure 71: TSKgel Octadecyl-4PW structure



Peptides in Neural Tissue

The retention of eight peptides on a TSKgel Octadecyl-4PW column was compared under acidic, neutral, and basic pH conditions, as shown in Figure 72. This peptide mixture is well resolved only under high pH elution conditions that cannot be used with silica-based ODS columns. These high pH conditions also allow different selectivities of the eight peptides.

Figure 72: Comparison of pH conditions



Column:	**TSKgel Octadecyl-4PW, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	A. 0.2% TFA, pH 1.9 B. 0.05 mol/L phosphate buffer, pH 7.1 C. 0.2 mol/L NH_3, pH 10.8
Gradient:	50 min. linear gradient from 0% to 80% CH_3CN
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Samples:	1. met-enkephalin 2. bradykinin 3. leu-enkephalin 4. neurotensin 5. bombesin 6. angiotensin I 7. somatostatin 8. insulin

About: TSKgel Octadecyl-NPR Reversed Phase Chromatography Columns

The highly cross-linked polymethacrylate base material of TSKgel Octadecyl-NPR provides excellent stability in high pH buffer systems and can withstand rigorous cleaning with either acid or base.

NPR, nonporous resin, columns are prepared from nonporous methacrylate particles of uniform 2.5 µm size, which provides high efficiency separations and fast analyses of peptides and proteins. The nonporous particle structure limits product isolation to sub-microgram loads.

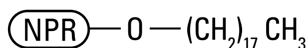
Attributes and Applications

Table 24 lists the attributes of TSKgel Octadecyl-NPR columns, while Figure 73 displays the structure. TSKgel Octadecyl-NPR columns are for the high efficiency purification of proteins and peptides at sub-microgram loads.

Table 24: Product attributes

Attribute	Value
Pore size (mean)	nonporous
Exclusion limit	>1.0 × 10^6 Da
Estimated ligand density	1 eq/L
Particle size (mean)	2.5 µm
pH stability	2.0-12.0
Functional group	C18 (monomeric bonding chemistry)

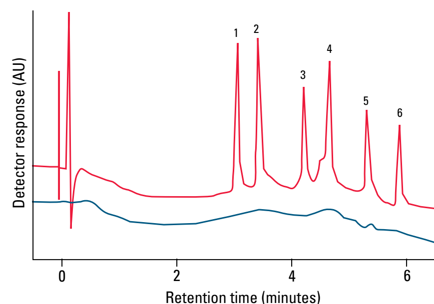
Figure 73: TSKgel Octadecyl-NPR structure



Nanogram Protein Samples

Protein mass and activity recovery is a principal objective in protein purifications. Non-specific protein binding is minimized on the hydrophilic backbone of both porous and nonporous TSKgel polymeric packings, thus making high mass recovery for proteins and peptides possible. Sub-microgram protein loads eluted quickly with high resolution and high sample recovery rates from a TSKgel Octadecyl-NPR column, shown in Figure 74. This example also shows the excellent baseline stability of perchloric acid at low wavelengths. When sensitive detection is needed, perchloric acid is preferred over trifluoroacetic acid.

Figure 74: Analysis and recovery of nanogram protein samples

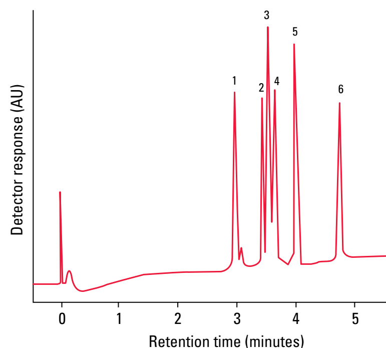


Column: **TSKgel Octadecyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase: 10 min linear gradient from 15% to 80% CH_3CN in 5 mmol/L $HClO_4$
Flow rate: 1.5 mL/min
Detection: UV @ 220 nm
Samples: 50 ng each of 1. ribonuclease A 2. insulin 3. cytochrome C 4. lysozyme 5. transferrin 6. myoglobin
Note: Blank gradient trace also shown

Natural Peptides

TSKgel Octadecyl-NPR columns are useful for the rapid analysis of natural peptides, as shown in Figure 75.

Figure 75: Rapid peptide separation

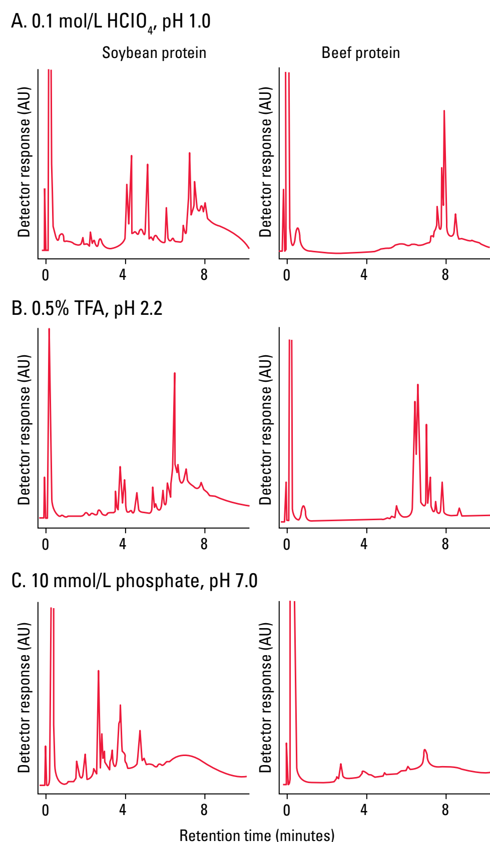


Column: **TSKgel Octadecyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase: 10 min linear gradient from 0% to 80% CH_3CN in 0.2% TFA
Flow rate: 1.5 mL/min
Detection: UV @ 220 nm
Samples: 1. α-endorphin 2. bombasin 3. γ-endorphin 4. angiotensin 5. somatosatin 6. calcitonin

Method Development

Method development is expedient with TSKgel Octadecyl-NPR columns. In Figure 76, two protein extracts were analyzed under three different elution conditions in a relatively short time.

Figure 76: Rapid method development

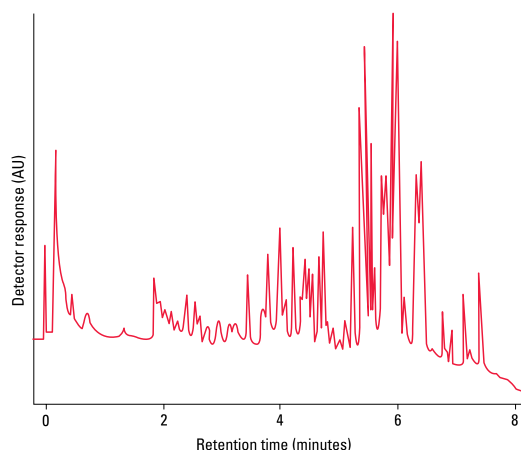


Column: **TSKgel Octadecyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase: A. 10 min linear gradient from 0% to 80% CH_3CN in 0.1 mol/L $HClO_4$
B. 10 min linear gradient from 0% to 80% CH_3CN in 0.05% TFA, pH 2.2
C. 10 min linear gradient from 0% to CH_3CN in 10 mmol/L phosphate buffer to 80% CH_3CN in 0.5 mmol/L phosphate buffer, pH 7.0
Flow rate: 1.5 mL/min
Detection: UV @ 220 nm
Samples: left column: water extract of soybean flour
right column: water extract of beef

Tryptic Digests

The 2.5 µm particle size of TSKgel Octadecyl-NPR columns also provides high resolution of tryptic digests, see Figure 77. The addition of a small quantity of surfactant to the mobile phase was necessary in this application to enhance retention of hydrophilic peptide fragments.

Figure 77: Fast, high resolution analysis



Column: **TSKgel Octadecyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase: 10 min linear gradient from 0% to 60% CH_3CN in 0.05 mol/L phosphate buffer, pH 2.8, containing 1 mmol/L sodium dodecyl sulfate
Flow rate: 1.5 mL/min
Detection: UV @ 210 nm
Sample: tryptic digest of reduced and S-carboxymethylated bovine serum albumin, 10 µg

About: TSKgel Phenyl-5PW RP Reversed Phase Chromatography Columns

TSKgel Phenyl-5PW RP columns are prepared by chemically bonding a high density of phenyl groups with an ether linkage to the base matrix of TSKgel G5000PW, a 10 µm high performance gel filtration packing. The TSKgel Phenyl-5PW RP column is structurally similar to the TSKgel Phenyl-5PW column used in hydrophobic interaction chromatography (HIC), but the RP column packing is prepared by bonding a higher density of phenyl groups. The greater level of hydrophobicity makes the packing more suitable for reversed phase chromatography.

The highly cross-linked polymethacrylate base material provides an advantage over silica when high pH buffer systems are needed. Additionally, TSKgel Phenyl-5PW RP can withstand rigorous cleaning protocols using either acid or base.

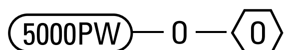
Attributes and Applications

Table 25 lists the attributes of TSKgel Phenyl-5PW RP columns, while Figure 78 displays the structure. The 100 nm pore size of the TSKgel Phenyl-5PW RP columns accommodates globular protein samples up to 1.0×10^6 Da.

Table 25: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Exclusion limit	1.0×10^6 Da
Estimated ligand density	1 eq/L
Particle size (mean)	10 µm and 13 µm
pH stability	2.0-12.0
Functional group	phenyl (monomeric bonding chemistry)

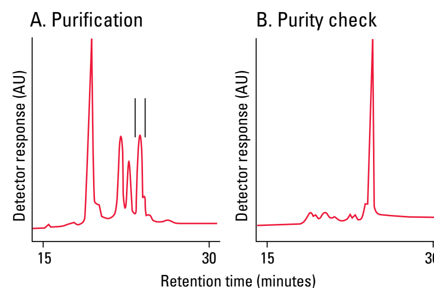
Figure 78: TSKgel Phenyl-5PW RP structure



Protein Analysis

Based on 100 nm pore size methacrylate resin, TSKgel Phenyl-5PW RP columns allow proteins unrestricted access to the available pore structure. Large proteins and biomolecules up to 1,000 kDa can be retained without being excluded from the pore structure, resulting in excellent peak symmetry and sharpness. For example, crude lactate dehydrogenase (approximately 120 kDa) eluted as a sharp peak during the purification and purity check performed on a TSKgel Phenyl-5PW RP column, as shown in Figure 79.

Figure 79: Purification and purity check

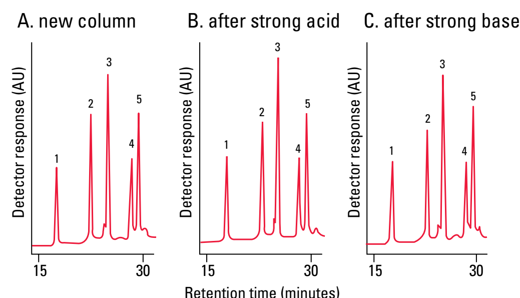


Column: **TSKgel Phenyl-5PW RP, 10 µm, 4.6 mm ID × 7.5 cm**
Mobile phase: 2 min linear gradient from 5% to 20% CH_3CN in 0.05% TFA, followed by (A - 48 min/B - 32 min) linear gradient to (80%A/60%B) CH_3CN in 0.05% TFA
Flow rate: 1.0 mL/min
Detection: UV @ 220 nm
Sample: lactate dehydrogenase
A. 40 µg in 100 µL
B. purity check of fraction collected in part A

Chemical Stability

The chromatograms in Figure 80 show the retention and selectivity of TSKgel Phenyl-5PW RP columns are stable under extended treatment with strong acid or base. Additionally, methods can be developed at pH extremes.

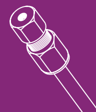
Figure 80: Chemical stability



Column: **TSKgel Phenyl-5PW RP, 10 µm, 4.6 mm ID × 7.5 cm**
Mobile phase: 60 min linear gradient from 5% to 80% CH_3CN in 0.05% TFA
Flow rate: 1.0 mL/min
Detection: UV @ 220 nm
Samples: 10 µg each of
1. ribonuclease A
2. cytochrome C
3. lysozyme
4. bovine serum albumin
5. myoglobin

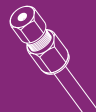
Ordering Information

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
22830	TSKgel Protein C_4-300, 3 µm, 30 nm	Silica	Stainless Steel	2	5
22831	TSKgel Protein C_4-300, 3 µm, 30 nm	Silica	Stainless Steel	2	10
22832	TSKgel Protein C_4-300, 3 µm, 30 nm	Silica	Stainless Steel	2	15
22827	TSKgel Protein C_4-300, 3 µm, 30 nm	Silica	Stainless Steel	4.6	5
22828	TSKgel Protein C_4-300, 3 µm, 30 nm	Silica	Stainless Steel	4.6	10
22829	TSKgel Protein C_4-300, 3 µm, 30 nm	Silica	Stainless Steel	4.6	15
22834	TSKgel Guard Cartridges for 2 mm ID TSKgel Protein C_4-300, 3 µm columns, 3 pk, 3 µm	Silica	Stainless Steel	2	1
22833	TSKgel Guard Cartridges for 4.6 mm ID TSKgel Protein C_4-300, 3 µm columns, 3 pk, 3 µm	Silica	Stainless Steel	3.2	1.5
21927	TSKgel ODS-140HTP, 2.3 µm, 14 nm	Silica	Stainless Steel	2.1	5
21928	TSKgel ODS-140HTP, 2.3 µm, 14 nm	Silica	Stainless Steel	2.1	10
21838	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	1	3.5
21839	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	1	5
21814	TSKgel ODS-100V, 3 µm, 10 nm, 3 pk	Silica	Stainless Steel	2	1
22700	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	2	2
21813	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	2	3.5
21812	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	2	5
21811	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	2	7.5
21938	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	2	10
21810	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	2	15
22701	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	2	25
22702	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	3	2
22703	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	3	3.5
22704	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	3	25
22705	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	4.6	2
22706	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	4.6	3.5
21831	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	4.6	5
21830	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	4.6	7.5
21940	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	4.6	10
21829	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	4.6	15
22707	TSKgel ODS-100V, 3 µm, 10 nm	Silica	Stainless Steel	4.6	25
22708	TSKgel ODS-100V, 5 µm, 10 nm, 3 pk	Silica	Stainless Steel	2	1
22709	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	2	2
22710	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	2	3.5
21457	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	2	5
22711	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	2	7.5
22712	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	2	10
21458	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	2	15
22713	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	2	25
22714	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	3	2
22715	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	3	3.5



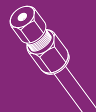
Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
22716	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	3	5
22717	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	3	7.5
22718	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	3	10
22719	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	3	15
22720	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	3	25
22721	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	4.6	2
22722	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	4.6	3.5
22723	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	4.6	5
22724	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	4.6	7.5
22725	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	4.6	10
21455	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	4.6	15
21456	TSKgel ODS-100V, 5 µm, 10 nm	Silica	Stainless Steel	4.6	25
21997	TSKgel Guard Cartridges for 2 mm ID TSKgel ODS-100V, 3 µm columns, 3 pk, 3 µm	Silica	Stainless Steel	2	1
21841	TSKgel Guard Cartridges for 2 mm ID TSKgel ODS-100V, 5 µm columns, 3 pk, 5 µm	Silica	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
21453	TSKgel Guard Cartridges for 4.6 mm ID TSKgel ODS-100V, 5 µm columns, 3 pk, 5 µm	Silica	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID cartridges		Stainless Steel	3.2	1.5
22726	TSKgel ODS-100Z, 3 µm, 10 nm, 3 pk	Silica	Stainless Steel	2	1
22727	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	2	2
22728	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	2	3.5
22729	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	2	5
22730	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	2	7.5
22731	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	2	10
22732	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	2	15
22733	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	2	25
22734	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	3	2
22735	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	3	3.5
22736	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	3	5
22737	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	3	7.5
22738	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	3	10
22739	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	3	15
22740	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	3	25
22741	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	4.6	2
22742	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	4.6	3.5
22743	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	4.6	5
22744	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	4.6	7.5
22745	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	4.6	10
22746	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	4.6	15
22747	TSKgel ODS-100Z, 3 µm, 10 nm	Silica	Stainless Steel	4.6	25
22748	TSKgel ODS-100Z, 5 µm, 10 nm, 3 pk	Silica	Stainless Steel	2	1
22749	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	2	2
22750	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	2	3.5

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
21460	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	2	5
22751	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	2	7.5
22752	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	2	10
21459	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	2	15
22753	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	2	25
22754	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	3	2
22755	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	3	3.5
22756	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	3	5
22757	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	3	7.5
22758	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	3	10
22759	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	3	15
22760	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	3	25
22761	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	4.6	2
22762	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	4.6	3.5
22763	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	4.6	5
22764	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	4.6	7.5
22765	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	4.6	10
21461	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	4.6	15
21462	TSKgel ODS-100Z, 5 µm, 10 nm	Silica	Stainless Steel	4.6	25
21996	TSKgel Guard Cartridges for 2 mm ID TSKgel ODS-100Z, 3 µm columns, 3 pk, 3 µm	Silica	Stainless Steel	2	1
21995	TSKgel Guard Cartridges for 2 mm ID TSKgel ODS-100Z, 5 µm columns, 3 pk, 5 µm	Silica	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
21454	TSKgel Guard Cartridges for 4.6 mm ID TSKgel ODS-100Z, 5 µm columns, 3 pk, 5 µm	Silica	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID cartridges		Stainless Steel	3.2	1.5
20015	TSKgel Super-ODS, 2 µm, 14 nm	Silica	Stainless Steel	1	5
19541	TSKgel Super-ODS, 2 µm, 14 nm	Silica	Stainless Steel	2	5
19542	TSKgel Super-ODS, 2 µm, 14 nm	Silica	Stainless Steel	2	10
18154	TSKgel Super-ODS, 2 µm, 14 nm	Silica	Stainless Steel	4.6	5
18197	TSKgel Super-ODS, 2 µm, 14 nm	Silica	Stainless Steel	4.6	10
19672	TSKgel Guard Cartridge for 2 mm ID TSKgel Super-ODS columns, 3 pk, 2 µm	Silica	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
18207	TSKgel Guardfilter for 4.6 mm ID TSKgel Super-ODS columns		Stainless Steel	4	0.4
18206	TSKgel Guardfilter Holder for 4 mm ID guardfilters		Stainless Steel		
20013	TSKgel Super-Octyl, 2 µm, 14 nm	Silica	Stainless Steel	2	5
20014	TSKgel Super-Octyl, 2 µm, 14 nm	Silica	Stainless Steel	2	10
18275	TSKgel Super-Octyl, 2 µm, 14 nm	Silica	Stainless Steel	4.6	5
18276	TSKgel Super-Octyl, 2 µm, 14 nm	Silica	Stainless Steel	4.6	10

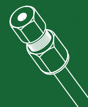


Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
42176	TSKgel Guard Cartridge for 2 mm ID TSKgel Super-Octyl columns, 3 pk, 2.3 µm	Silica	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
18207	TSKgel Guardfilter for 4.6 mm ID TSKgel Super-Octyl columns		Stainless Steel	4	0.4
18206	TSKgel Guardfilter Holder for 4 mm ID guardfilters		Stainless Steel		
20017	TSKgel Super-Phenyl, 2 µm, 14 nm	Silica	Stainless Steel	2	5
20018	TSKgel Super-Phenyl, 2 µm, 14 nm	Silica	Stainless Steel	2	10
18277	TSKgel Super-Phenyl, 2 µm, 14 nm	Silica	Stainless Steel	4.6	5
18278	TSKgel Super-Phenyl, 2 µm, 14 nm	Silica	Stainless Steel	4.6	10
42177	TSKgel Guard Cartridge for 2 mm ID TSKgel Super-Phenyl columns, 3 pk, 2.3 µm	Silica	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
18207	TSKgel Guardfilter for 4.6 mm ID TSKgel Super-Phenyl columns		Stainless Steel	4	0.4
18206	TSKgel Guardfilter Holder for 4 mm ID guardfilters		Stainless Steel		
18150	TSKgel ODS-80Ts, 5 µm, 8 nm	Silica	Stainless Steel	2	15
18151	TSKgel ODS-80Ts, 5 µm, 8 nm	Silica	Stainless Steel	2	25
17200	TSKgel ODS-80Ts, 5 µm, 8 nm	Silica	Stainless Steel	4.6	7.5
17201	TSKgel ODS-80Ts, 5 µm, 8 nm	Silica	Stainless Steel	4.6	15
17202	TSKgel ODS-80Ts, 5 µm, 8 nm	Silica	Stainless Steel	4.6	25
17380	TSKgel ODS-80Ts, 10 µm, 8 nm	Silica	Stainless Steel	21.5	30
18768	TSKgel ODS-80Ts QA, 5 µm, 8 nm	Silica	Stainless Steel	2	15
18769	TSKgel ODS-80Ts QA, 5 µm, 8 nm	Silica	Stainless Steel	2	25
18519	TSKgel ODS-80Ts QA, 5 µm, 8 nm	Silica	Stainless Steel	4.6	7.5
18520	TSKgel ODS-80Ts QA, 5 µm, 8 nm	Silica	Stainless Steel	4.6	15
18521	TSKgel ODS-80Ts QA, 5 µm, 8 nm	Silica	Stainless Steel	4.6	25
19325	TSKgel Guard Cartridge for 2 mm ID TSKgel ODS-80Ts & ODS-80Ts QA columns, 3 pk, 5 µm	Silica	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
19011	TSKgel Guard Cartridge for 4.6 mm ID TSKgel ODS-80Ts & ODS-80Ts QA columns, 3 pk, 5 µm	Silica	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID cartridges		Stainless Steel	3.2	1.5
17385	TSKgel Guard Column for 21.5 mm ID TSKgel ODS-80Ts column, 10 µm	Silica	Stainless Steel	21.5	7.5
17344	TSKgel Octyl(C8)-80Ts, 5 µm, 8 nm	Silica	Stainless Steel	4.6	15
17345	TSKgel Octyl(C8)-80Ts, 5 µm, 8 nm	Silica	Stainless Steel	4.6	25
19012	TSKgel Guard Cartridge for 4.6 mmID TSKgel Octyl-80Ts columns, 3 pk, 5 µm	Silica	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mmID cartridges		Stainless Steel	3.2	1.5
17348	TSKgel CN-80Ts, 5 µm, 8 nm	Silica	Stainless Steel	4.6	15
17349	TSKgel CN-80Ts, 5 µm, 8 nm	Silica	Stainless Steel	4.6	25

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
19013	TSKgel Guard Cartridge for 4.6 mm ID TSKgel CN-80TS columns, 3 pk, 5 µm	Silica	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID cartridges		Stainless Steel	3.2	1.5
16651	TSKgel ODS-80TM, 5 µm, 8 nm	Silica	Stainless Steel	4.6	7.5
08148	TSKgel ODS-80TM, 5 µm, 8 nm	Silica	Stainless Steel	4.6	15
08149	TSKgel ODS-80TM, 5 µm, 8 nm	Silica	Stainless Steel	4.6	25
14002	TSKgel ODS-80TM, 10 µm, 8 nm	Silica	Stainless Steel	21.5	30
19004	TSKgel Guard Cartridge for 4.6 mm ID TSKgel ODS-80TM columns, 3 pk, 5 µm	Silica	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID TSKgel ODS-80TM cartridges		Stainless Steel	3.2	1.5
14098	TSKgel Guard Column for 21.5 mm ID TSKgel ODS-80TM column, 10 µm	Silica	Stainless Steel	21.5	7.5
18152	TSKgel ODS-120T, 5 µm, 12 nm	Silica	Stainless Steel	2	15
18153	TSKgel ODS-120T, 5 µm, 12 nm	Silica	Stainless Steel	2	25
07637	TSKgel ODS-120T, 5 µm, 12 nm	Silica	Stainless Steel	4.6	15
07125	TSKgel ODS-120T, 5 µm, 12 nm	Silica	Stainless Steel	4.6	25
07130	TSKgel ODS-120T, 10 µm, 12 nm	Silica	Stainless Steel	7.8	30
07134	TSKgel ODS-120T, 10 µm, 12 nm	Silica	Stainless Steel	21.5	30
19325	TSKgel Guard Cartridge for 2 mm ID TSKgel ODS-120T columns, 3 pk, 5 µm	Silica	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges		Stainless Steel	2	1
19006	TSKgel Guard Cartridge for 4.6 mm ID TSKgel ODS-120T columns, 3 pk, 5 µm	Silica	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID cartridges		Stainless Steel	3.2	1.5
07636	TSKgel ODS-120A, 5 µm, 12 nm	Silica	Stainless Steel	4.6	15
07124	TSKgel ODS-120A, 5 µm, 12 nm	Silica	Stainless Steel	4.6	25
07129	TSKgel ODS-120A,10 µm, 12 nm	Silica	Stainless Steel	7.8	30
06172	TSKgel ODS-120A, 10 µm, 12 nm	Silica	Stainless Steel	21.5	30
19005	TSKgel Guard Cartridge for 4.6 mm ID TSKgel ODS-120A columns, 3 pk, 5 µm	Silica	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID cartridges		Stainless Steel	3.2	1.5
07190	TSKgel TMS-250, 10 µm, 25 nm	Silica	Stainless Steel	4.6	7.5
13352	TSKgel OligoDNA RP, 5 µm, 25 nm	Silica	Stainless Steel	4.6	15
13353	TSKgel OligoDNA RP, 5 µm, 25 nm	Silica	Stainless Steel	7.8	15
14007	TSKgel Phenyl-5PW RP Glass, 10 µm, 100 nm	Polymer	Stainless Steel	8	7.5
18756	TSKgel Phenyl-5PW RP, 10 µm, 100 nm	Polymer	Stainless Steel	2	7.5
08043	TSKgel Phenyl-5PW RP, 10 µm, 100 nm	Polymer	Stainless Steel	4.6	7.5
16260	TSKgel Phenyl-5PW RP, 13 µm, 100 nm	Polymer	Stainless Steel	21.5	15



Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
42159	TSKgel Guard Cartridge for 2 mm ID TSKgel Phenyl-5PW RP column, 3 pk, 10 µm	Polymer	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges	Polymer	Stainless Steel	2	1
19007	TSKgel Guard Cartridge for 4.6 mm ID TSKgel Phenyl-5PW RP column, 3 pk, 10 µm	Polymer	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID cartridges	Polymer	Stainless Steel	3.2	1.5
14005	TSKgel Octadecyl-NPR, C18, 2.5 µm, nonporous	Polymer	Stainless Steel	4.6	3.5
18754	TSKgel Octadecyl-2PW, 5 µm, 12.5 nm	Polymer	Stainless Steel	2	15
17500	TSKgel Octadecyl-2PW, 5 µm, 12.5 nm	Polymer	Stainless Steel	4.6	15
17501	TSKgel Octadecyl-2PW, 5 µm, 12.5 nm	Polymer	Stainless Steel	6	15
42161	TSKgel Guard Cartridge for 2 mm ID TSKgel Octadecyl-2PW column, 3 pk, 5 µm	Polymer	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges	Polymer	Stainless Steel	2	1
17502	TSKgel Guard Column for 4.6 mm ID TSKgel Octadecyl-2PW column, 5 µm	Polymer	Stainless Steel	4.6	1
17503	TSKgel Guard Column for 6 mm ID TSKgel Octadecyl-2PW column, 5 µm	Polymer	Stainless Steel	6	1
18755	TSKgel Octadecyl-4PW, 7 µm, 50 nm	Polymer	Stainless Steel	2	15
13351	TSKgel Octadecyl-4PW, 7 µm, 50 nm	Polymer	Stainless Steel	4.6	15
16257	TSKgel Octadecyl-4PW, 13 µm, 50 nm	Polymer	Stainless Steel	21.5	15
42160	TSKgel Guard Cartridge for 2 mm ID TSKgel Octadecyl-4PW column, 3 pk, 7 µm	Polymer	Stainless Steel	2	1
19308	TSKgel Guard Cartridge Holder for 2 mm ID cartridges	Polymer	Stainless Steel	2	1
19008	TSKgel Guard Cartridge for 4.6 mm ID TSKgel Octadecyl-4PW column, 3 pk, 7 µm	Polymer	Stainless Steel	3.2	1.5
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID cartridges	Polymer	Stainless Steel	3.2	1.5



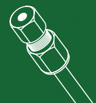
TSKgel Boronate-5PW

TSKgel Chelate-5PW

TSKgel Tresyl-5PW

Affinity Tips:

- TSKgel Affinity columns are offered in glass and stainless steel. Stainless steel (SS) or Pyrex frits are embedded in the body of the column end-fittings of metal and glass columns, respectively. The nominal frit size for SS columns is engraved in the end-fittings; Pyrex frits in the glass columns have a 10 µm nominal pore size.
- Halide salts corrode stainless steel tubing, fitting, and frits. Do not store SS columns in a mobile phase containing NaCl and, where possible, use another salt in the operating buffer. Chlorotrifluorethylene and tetrafluorethylene are the materials in the glass column fittings that come into contact with the mobile phase and sample.
- Good laboratory procedures demand that the analytical column be protected by a guard column. TSKgel guardgel kits, containing column hardware and gel packing, are available to pack your own guard column for use with TSKgel affinity columns.
- As with all columns used with gradient elution, affinity columns should be washed with final elution buffer prior to their re-equilibration with initial (binding) buffer. Always wash the column and the LC system with halide-free buffer at the end of the day.
- The recommended pH range for some TSKgel affinity columns is not as large as that for the base gel. Solvents outside of this pH range can be utilized for clean-up by injecting small volumes (100 µL) of the cleaning solution as part of a standard mobile phase. For the most appropriate clean-up procedure, consult the Operating Conditions and Specifications (OCS) Sheet that is shipped with each column.
- Column shipping solvents are: distilled water (TSKgel Boronate-5PW); 10 mmol/L acetate buffer, pH 4.5 (TSKgel Chelate-5PW); and acetone (TSKgel Tresyl-5PW).
- TSKgel affinity columns (except TSKgel Tresyl-5PW columns, since the ligand is activated) are supplied with an Inspection Data Sheet, which includes a QC chromatogram and test data, and an OCS Sheet summarizing the recommended operating conditions for optimum column performance.
- A separate TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems, can be downloaded from the Tosoh Bioscience LLC website (www.tosohbioscience.com).



About Affinity Chromatography

Affinity Chromatography (AFC) offers the greatest potential specificity and selectivity for the isolation or purification of biomolecules. Almost all biological molecules can be purified on the basis of a specific interaction between their chemical or biological structure and a suitable affinity ligand.

In AFC, the target molecule is specifically and reversibly adsorbed by a complementary ligand and immobilized on a matrix. Examples of a complementary ligand include an inhibitor, substrate analog or cofactor, or an antibody which specifically recognizes the target molecule. The selectivity is often based on spatial recognition, a 'lock-and-key' mechanism.

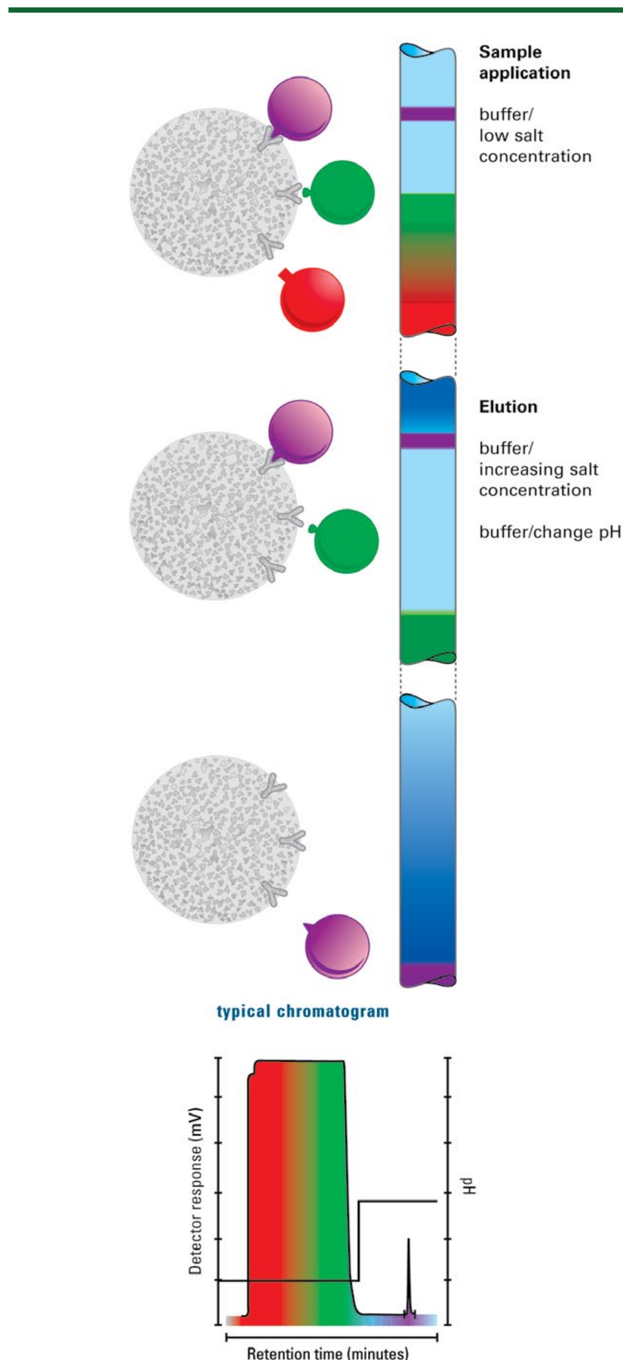
The adsorbed molecule is subsequently eluted either by competitive displacement or a conformation change through a shift in pH or ionic strength. Typical molecular pairs are antigens and antibodies, enzymes and coenzymes, and sugars with lectins.

Purification of several thousand-fold may be obtained due to the high selectivity of the affinity interactions. Although affinity chromatography is not specific, in that no enzyme interacts with only one substrate, it is the most selective method for separating proteins. The features and benefits of AFC are detailed in Table 1 below.

Table 1: Features and benefits of Affinity Chromatography

Features	Benefits
High size exclusion limit ($>5 \times 10^6$ 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 polymer 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 columns offered in PEEK hardware	Eliminates undesirable interactions with column hardware

Figure 1: Affinity Chromatography



TSKgel Affinity Chromatography Columns

The TSKgel affinity chromatography column line consists of two group-specific stationary phases: Boronate-5PW and Chelate-5PW, as well as one with a chemically-activated functionality, Tresyl-5PW. All analytical TSKgel AFC columns are based on the well-established 10 µm rigid TSKgel G5000PW resin. This resin features 100 nm pores that have an estimated exclusion limit of 1 million Dalton, along with excellent stability from pH 2 to 9.

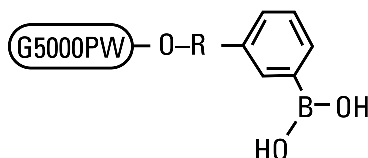
The structures of the available functional ligands are shown in Figure 2. The choice of a specific ligand is dictated by the expected interaction between the sample and the bonded phase. Table 2 lists well known applications for each type of TSKgel affinity column.

Table 2: Applications of TSKgel Affinity Chromatography columns

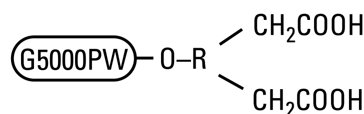
Group Specific Ligands	Application
TSKgel Boronate-5PW	carbohydrates, nucleic acids, nucleosides, nucleotides, catecholamines
TSKgel Chelate-5PW	immunoglobulins, transferrin lectins, milk proteins, membrane proteins, peptides
Activated Ligands	**Application**
TSKgel Tresyl-5PW	coupling of ligands to form a custom affinity resin

Figure 2: Structure of TSKgel Affinity ligands

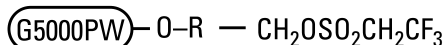
TSKgel Boronate-5PW



TSKgel Chelate-5PW



TSKgel Tresyl-5PW



About: TSKgel Boronate-5PW Affinity Chromatography Columns

The coupling of *m*-aminophenyl boronate to TSKgel G5000PW polymeric support results in the TSKgel Boronate-5PW column. This coupling makes 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 matrix 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.

Attributes and Applications

Table 3 lists the attributes of TSKgel Boronate-5PW columns. Applications for TSKgel Boronate-5PW columns include nucleic acids, nucleosides, nucleotides, catecholamines, and other biomolecules containing the 1,2 *cis*-diol functionality.

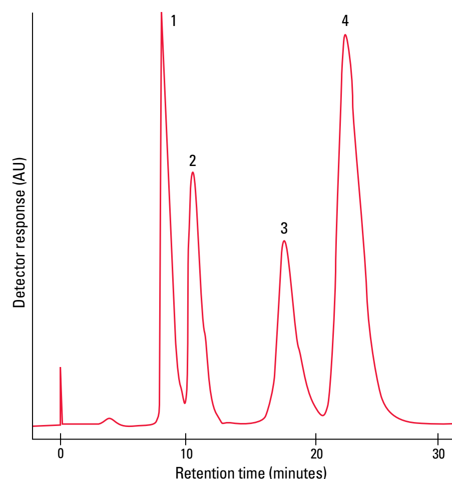
Table 3: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Exclusion limit (base resin, estimate)	<1.0 × 10^7 Da globular proteins
Adsorption capacity	40 µmol/L resin (sorbitol)
Particle size	10 µm
pH stability	2.0-9.0
Functional group	*m*-aminophenyl boronate

Nucleosides

Nucleosides are glycosylamines consisting of a nucleobase (often referred to as simply *base*) bound to a ribose or deoxyribose sugar via a beta-glycosidic linkage. Examples of nucleosides include cytidine, uridine, adenosine, guanosine, thymidine, and inosine. Figure 3 shows a TSKgel Boronate-5PW column for the selective separation of nucleosides using an isocratic mobile phase.

Figure 3: Isocratic separation of nucleosides

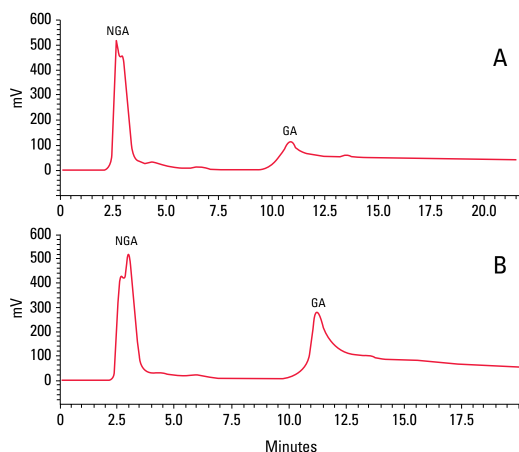


Column: **TSKgel Boronate-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase: 0.1 mol/L phosphate buffer, pH 8.0
Flow rate: 1.0 mL/min
Detection: UV @ 280 nm
Samples: 1. cytidine
2. uridine
3. guanosine
4. adenosine

Glycated Proteins in Human Serum Albumin

Glycated (GA) and non-glycated (NGA) proteins in human serum albumin (HSA) were analyzed using a TSKgel Boronate-5PW column (Figure 4). The amount of glycated human serum albumin provides useful information on short term blood glucose control in diabetic patients. By comparison of GA levels in serum between a healthy person and a diabetic patient, the percentage of GA in total HSA increased from 10.1% to 26.8%. The significant elevation of the GA level was commonly observed for diabetic patients, as a consequence of high blood glucose level.

Figure 4: Separation of albumin from healthy person (A) and diabetic patient (B) using a TSKgel Boronate-5PW column



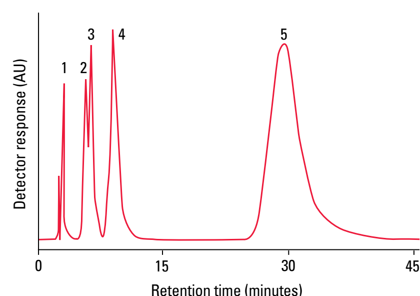
Column:	**TSKgel Boronate-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	A: 250 mmol/L ammonium acetate + 50 mmol/L magnesium chloride + 5% ethanol, pH 8.5 B: 200 mmol/L sorbitol + 100 mmol/L Tris + 50 mmol/L EDTA-2Na, pH 8.5
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Injection vol.:	100 µL
Samples:	serum from euglycemic healthy person (A), patient with type 2 diabetes (B)

Bai, X.; Wang, Z.; Huang, C.; Wang, Z.; Chi, L. Investigation of non-enzymatic glycosylation of human serum albumin using ion trap-time of flight mass spectrometry. *Molecules.* 2012, 17, 8782-8794. (http://creativecommons.org/licenses/by/3.0/). No changes were made to the figure.

Catecholamines

Catecholamines are “fight-or-flight” hormones that are released by the adrenal glands in response to stress. They are called catecholamines because they contain a catechol group and are derived from the amino acid tyrosine. Figure 5 demonstrates the analysis of catecholamines on a TSKgel Boronate-5PW column.

Figure 5: Analysis of catecholamines



Column:	**TSKgel Boronate-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	0.1 mol/L phosphate buffer, pH 6.5
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Samples:	1. tyrosine 2. normetanephrine 3. metanephrine 4. DOPA 5. epinephrine

About: TSKgel Chelate-5PW Affinity Chromatography Columns

TSKgel Chelate-5PW columns contain iminodiacetic acid (IDA) groups that are covalently bonded to the TSKgel G5000PW polymeric support. Prior to chromatography, a metal ion, such as Zn^{2+}, Ni^{2+} or Cu^{2+}, is chelated to the IDA group. The selected metal ion is fixed at three coordinating sites on the IDA group. Therefore the target molecule can be tightly bound at three free binding sites at the metal ion. Because of the high concentration of the fixed metal ion (20 µmol metal ion per mL gel), the TSKgel Chelate-5PW column has high binding capacity for the target molecules.

Peptides and proteins containing histidine residues will normally adsorb to these chelated ions at neutral pH. A buffer containing imidazole or glycine is used for protein desorption. The key to making successful use of this retention mechanism is selecting the proper metal ion and elution buffer. Cu^{2+} interacts more strongly with proteins, while Zn^{2+} usually enhances resolution. With Zn^{2+} the column is loaded to saturation, while stronger binding Cu^{2+} is loaded to about half the total capacity. Gradients of increasing imidazole or glycine concentrations or decreasing pH are often used for protein elution. Glycine (in HEPES buffer) is a strong eluent that also desorbs the metal ion, making it necessary to regenerate the column after each run. Imidazole (in phosphate buffer) is a weak eluent, allowing several runs before column regeneration is necessary.

Attributes and Applications

Table 4 lists the attributes of TSKgel Chelate-5PW columns. Applications for TSKgel Chelate-5PW columns include: the analysis of lectins, serum proteins such as immunoglobulins and transferrin, milk proteins, membrane proteins, and peptides.

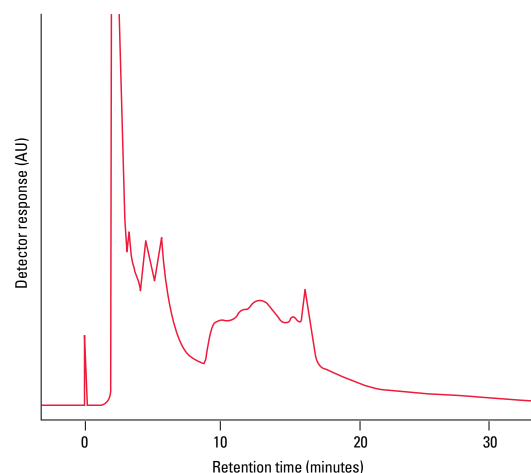
Table 4: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Exclusion limit (base resin, estimate)	<1.0×10^7 Da globular proteins
Ligand concentration	20 µmol/L resin
Particle size	10 µm and 13 µm
pH stability	2.0-12.0
Functional group	iminodiacetic acid

Monoclonal Antibody

Figure 6 demonstrates monoclonal antibody (mAb) purification from culture supernatant on a TSKgel Chelate-5PW column loaded with Zn^{2+} ion. This figure shows that the mAb (IgG_1) is eluted in about 32 minutes by pH gradient elution and is well separated from other impurities.

Figure 6: Monoclonal antibody purification

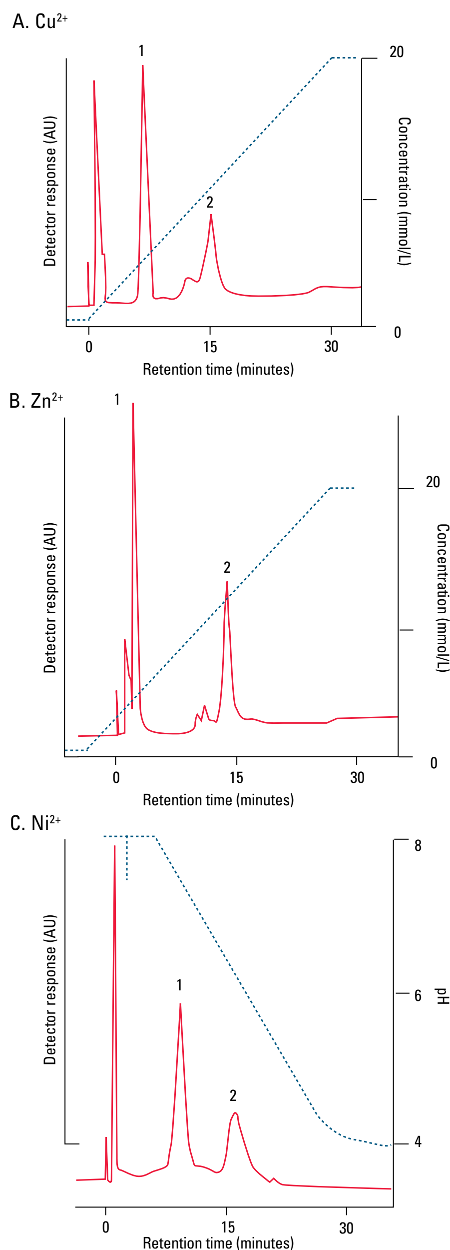


Column:	**TSKgel Chelate-5PW (Zn^{2+}), 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	A: 20 mmol/L Tris-HCl, pH 8.0, + 0.5 mol/L NaCl B: A + 200 mmol/L glycine
Gradient:	30 min (A→B), linear
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Sample:	Anti-HLA-A, B, C (IgG_1), NS-1 cultured supernatant

Standard Proteins

Retention and peak shape of two globular proteins on Zn-, Cu-, and Ni-loaded TSKgel Chelate-5PW columns are compared in Figure 7.

Figure 7: Analyses of ribonuclease A and transferrin

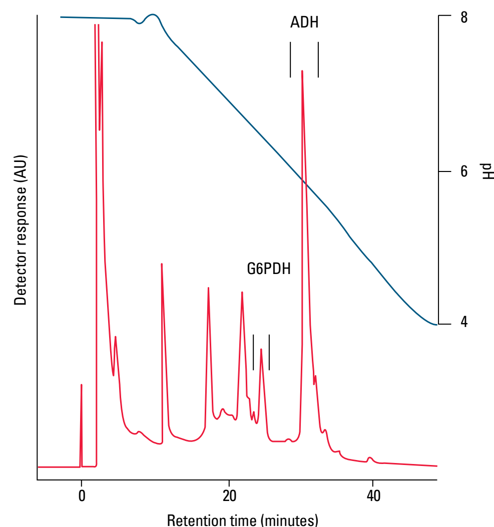


Column:	**TSKgel Chelate-5PW, 10 µm, 5 mm ID × 5 cm, glass**
Metal ions:	A: Cu^{2+} B: Zn^{2+} C: Ni^{2+}, all saturated
Mobile phase:	A and B: 30 min linear gradient from 1 mmol/L to 20 mmol/L imidazole in 20 mmol/L HEPES-NaOH buffer, pH 8.0, containing 0.5 mol/L NaCl C: 30 min linear pH gradient from 20 mmol/L HEPES-MES-acetic acid, pH 8.0, to 20 mmol/L HEPES-MES-acetic acid, pH 4.0, both in 0.5 mol/L NaCl
Flow rate:	0.8 mL/min
Detection:	UV @ 280 nm
Samples:	1. ribonuclease A (bovine) 2. transferrin (human)

Yeast Enzymes

A TSKgel Chelate-5PW column was used in Figure 8 to recover yeast enzymes with high activity and yield.

Figure 8: Analysis of yeast enzymes



Column:	**TSKgel Chelate-5PW, 10 µm, 8 mm ID × 7.5 cm, glass**
Metal ions:	Zn^{2+}, saturated
Mobile phase:	40 min linear pH gradient from 20 mmol/L HEPES-MES-acetic acid, pH 8.0, to 20 mmol/L HEPES-MES-acetic acid, pH 4.0, both in 0.5 mol/L NaCl
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Recovery:	G6PDH*: 90%, and ADH*: 97% of enzymatic activity
Sample:	yeast enzyme concentrate
Purification:	G6PDH: 8.7-fold, and ADH: 3.9-fold

*G6PDH: glucose-6-phosphate dehydrogenase
*ADH: alcohol dehydrogenase

About: TSKgel Tresyl-5PW Affinity Chromatography Columns

Unlike other TSKgel affinity columns, the TSKgel Tresyl-5PW columns which are derivatized with the 2,2,2-trifluroethanesulfonyl ligand, require 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 columns 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 g/L 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 g/L resin.

Attributes and Applications

Table 5 lists the attributes of TSKgel Tresyl-5PW columns. Examples of the wide range of applications using TSKgel Tresyl-5PW columns include the binding of such ligands as concanavalin A (a lipoprotein lectin that binds to glycoproteins), numerous antibodies, and enzymes.

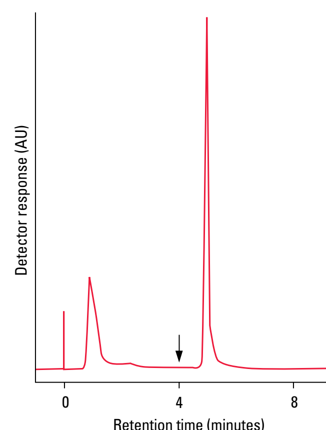
Table 5: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Particle size (mean)	10 µm
pH stability	2.0-12.0
Ligand concentration	ca. 20 µmol/L resin
Adsorption capacity	>60 mg/g dry resin (coupling capacity with soybean tryspin inhibitor)
Exclusion limit (base resin, estimate)	<1.0 × 10^7 Da globular proteins
Active group	tresyl

Peroxidase on Concanavalin A

Concanavalin A is a lipoprotein lectin that binds to glycoproteins such as peroxidase. TSKgel Tresyl-5PW is activated by binding concanavalin A to the resin. The chromatogram in Figure 9 shows the purification of peroxidase by the concanavalin A ligand coupled to the TSKgel Tresyl-5PW affinity support resin.

Figure 9: Purification of peroxidase

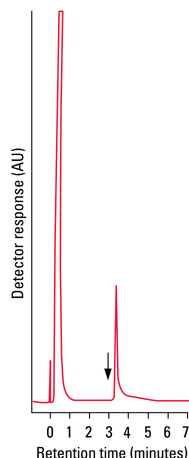


Column:	**TSKgel Tresyl-5PW, 10 µm, 6 mm ID × 4 cm, modified with concanavalin A**
Binding:	0.05 mol/L acetate buffer, pH 5.0, containing 0.5 mol/L NaCl, and 1 mmol/L each of $CaCl_2$, $MnCl_2$ and $MgCl_2$
Mobile phase:	Step gradient at 4 min (see arrow on diagram) to 25 mmol/L α-methyl-D-glucoside in binding buffer
Flow rate:	1.0 mL/min
Detection:	UV @ 403 nm
Sample:	crude peroxidase, 0.5 mg
Washing step:	Wash TSKgel Tresyl-5PW, 6 mm ID × 4 cm, with dissolved H_2O
Ligand solution:	Dissolve 40 mg of concanavalin A in 10 mL of 0.1 mol/L $NaHCO_3$, pH 8.0, containing 0.5 mol/L NaCl
Coupling step:	Recycle the ligand solution overnight through the column at 0.2 mL/min at 25 °C
Blocking step:	Block the residual tresyl groups with 0.1 mol/L Tris-HCl, pH 8.0, at 1.0 mL/min for 1 hr at 25 °C

Human Transferrin

Human transferrin is a plasma protein for iron ion delivery. When human transferrin loaded with iron encounters a transferrin receptor on the surface of a cell, it binds to it and is consequently transported into the cell in a vesicle. The cell will acidify the vesicle, causing human transferrin (TF) to release its iron ions. The purification of human transferrin using a TSKgel Tresyl-5PW column immobilized with an anti-human transferrin antibody is shown in Figure 10.

Figure 10: Purification of human transferrin



Column:	**TSKgel Tresyl-5PW, 10 µm, 6 mm ID × 10 cm**
Mobile phase:	0.1 mol/L phosphate buffer, pH 7.4 eluted by pulse method with 0.1 mol/L citrate-hydrochloride buffer, pH 1.6
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Injection vol.:	20 µL
Sample:	human transferrin in serum

Ordering Information

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
14449	TSKgel Boronate-5PW Glass, 10 µm, 100 nm	Polymer	Glass	5	5
13066	TSKgel Boronate-5PW, 10 µm, 100 nm	Polymer	Stainless Steel	7.5	7.5
14451	TSKgel Glass Guardgel Kit for 5 mm ID TSKgel Boronate-5PW columns, 20 µm	Polymer	Glass		
13125	TSKgel Guardgel Kit for 7.5 mm ID TSKgel Boronate-5PW column, 20 µm	Polymer	Stainless Steel		
20022	TSKgel BioAssist Chelate, 10 µm, 100 nm	Polymer	PEEK	7.8	5
14440	TSKgel Chelate-5PW Glass, 10 µm, 100 nm	Polymer	Glass	5	5
08645	TSKgel Chelate-5PW, 10 µm, 100 nm	Polymer	Stainless Steel	7.5	7.5
08646	TSKgel Chelate-5PW, 13 µm, 100 nm	Polymer	Stainless Steel	21.5	15
08647	TSKgel Guardgel Kit for 7.5 mm ID TSKgel Chelate-5PW column, 20 µm	Polymer	Stainless Steel		
14455	TSKgel Tresyl-5PW, 10 µm, 100 nm	Polymer	Stainless Steel	6	4
14456	TSKgel Tresyl-5PW, 10 µm, 100 nm	Polymer	Stainless Steel	7.5	7.5
16208	TSKgel Tresyl-5PW Guardgel, 10 µm, 2 g	Polymer			

TSKgel FcR-IIIA-NPR

Fc Receptor Tips:

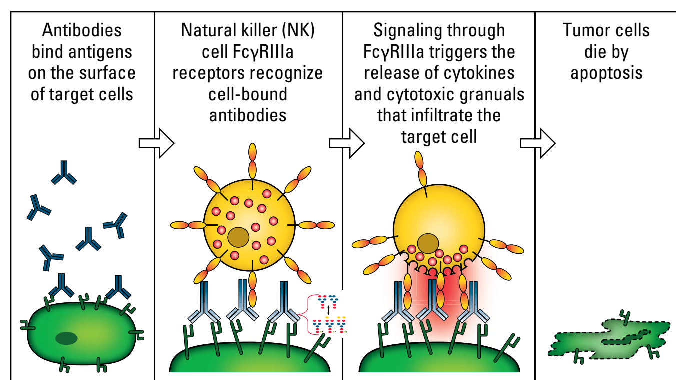
- The TSKgel FcR-IIIA-NPR column is offered in PEEK hardware to promote low adsorption of biomolecules. The ligand is bonded to a nonporous stationary phase allowing high throughput affinity chromatography at the analytical scale.
- As with all columns used with gradient elution, affinity columns should be washed with final elution buffer prior to their re-equilibration with initial (binding) buffer. Always wash the column and the LC system with halide-free buffer at the end of the day.
- Column shipping solvent is an aqueous solution of 10 mmol/L citrate buffer, pH 6.5, with 0.025% ProClin300.
- The TSKgel FcR-IIIA-NPR column is supplied with an Inspection Data Sheet, which includes a QC chromatogram, an Analysis Report, which includes gel batch data, and an OCS Sheet summarizing the recommended operating conditions for optimum column performance.
- A separate TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems, can be downloaded from the Tosoh Bioscience LLC website (www.tosohbioscience.com).

About: Fc Receptor Affinity Chromatography

Monoclonal antibodies (mAbs) comprise the largest class of glycosylated protein therapeutics currently on the market and glycosylation is known to be a major source of mAb heterogeneity[1]. N-glycosylation of IgG-Fc of mAbs is known to impact drug therapeutic mechanism of action (MOA), thus monitoring glycan critical quality attributes (CQAs) is essential for maintaining drug product safety and efficacy[2-5].

Antibody-dependent cell mediated cytotoxicity (ADCC) has been recognized as a therapeutic MOA for several mAbs. ADCC begins when the Fab region of an antibody binds to an antigen on a target cell and the Fc domain binds Fcγ receptors on the surface of an effector cell. Signaling through the Fcγ receptor triggers degranulation into a lytic synapse which ultimately leads to apoptosis (Figure 1). In particular, FcγRIIIa expressed on peripheral blood mononuclear cells (PBMC) or natural killer (NK) cells have been shown to play an essential role in ADCC[6].

Figure 1: Representation of Mechanism of Action for ADCC Activity



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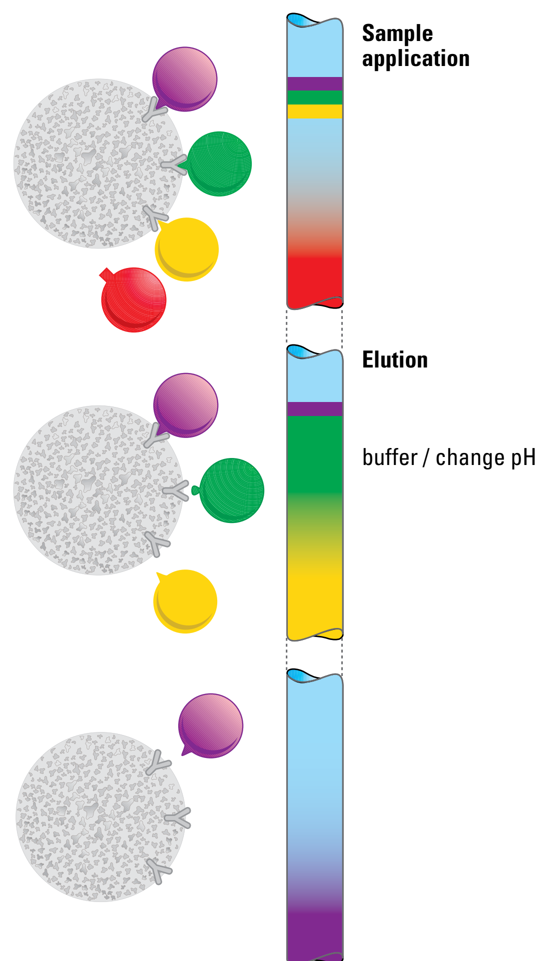
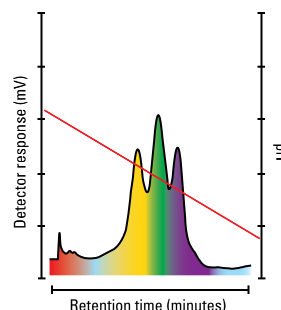
In FcγRIIIa chromatography, purified drug product or crude feed stock is passed through a column under conditions that promote binding of N-glycosylated mAbs to the immobilized recombinant FcγRIIIa ligand on the surface of the particle. mAbs which do not possess glycosylation elute in the void volume. Elution of mAb glycoforms present in the sample is performed by altering the pH of the mobile phase in order to disrupt the target/ligand interactions. Glycoforms are eluted as multiple peaks, correlating to the affinity of the N-glycosylation of IgG-Fc for the recombinant FcγRIIIa ligand (Figure 2).

Figure 2: FcγRIIIa Affinity Chromatography





About: TSKgel FcR-IIIA-NPR Affinity Chromatography Column

TSKgel FcR-IIIA-NPR is a 5 µm, 4.6 mm ID x 7.5 cm PEEK column for high performance affinity chromatography. This column is designed for the separation of mAb efficacy variants on the basis of affinity of the N-linked glycosylation in the Fc Region of IgG_1-Fc for the recombinant FcγRIIIa stationary phase. The ligand is bonded to nonporous polymethrylate beads, providing efficient and rapid separation of mAb glycoforms. The rugged nature of the column facilitates analysis both prior to or after purification.

TSKgel FcR-IIIA-NPR can be utilized for the following applications:

- Comparison between biosimilar/biobetter and innovator reference product
- QC Analysis of lot-to-lot difference for mAb drug products
- Monitoring fermentation stage of cell culture media
- Screening the potential of cell lines for ADCC activity

Attributes

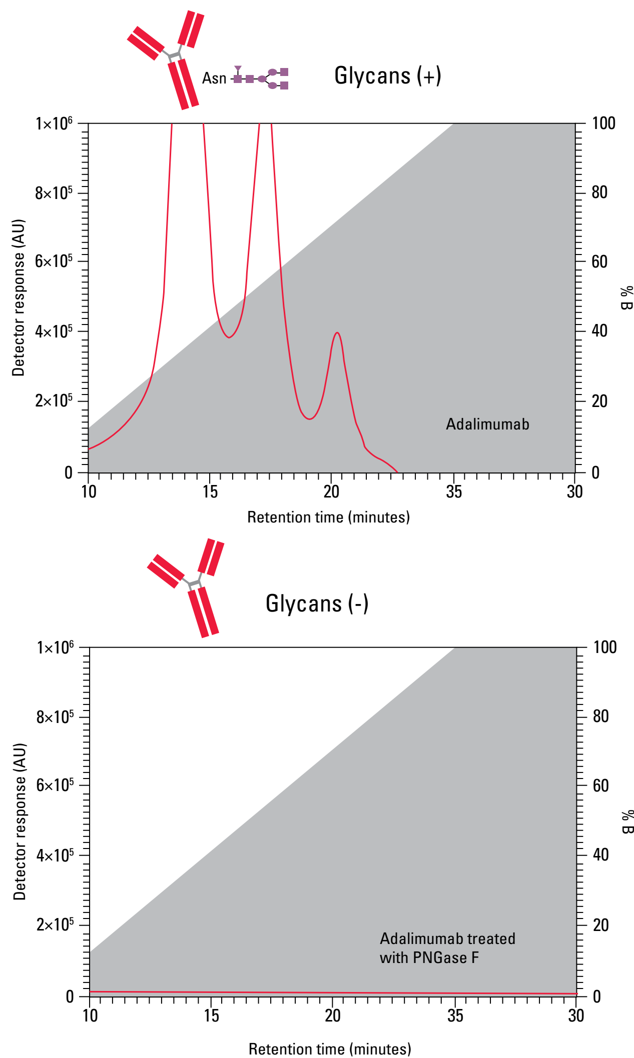
Table 1: Product Attributes

Ligand	Modified recombinant FcγRIIIA (*E. Coli* expression system, non-glycosylated)
Base matrix	Nonporous resin, 5 µm
Column	4.6 mm ID x 7.5 cm, PEEK
Sample mass	5 – 50 µg of IgG (recommended)
Flow rate	Max 1.0 mL/min
Recommended temperature	15 °C ~ 25 °C (column oven)
pH stability	pH 4 - 8 (short term) pH 5 - 7 (long term)
Recommended buffer system	A: 50 mmol/L citrate buffer, 150 mmol/L NaCl, pH6.5 B: 50 mmol/L citrate buffer, 150 mmol/L NaCl, pH4.5
Maximum pressure	9 MPa

Affinity for N-glycosylated mAbs

Figure 3 shows the specificity of the recombinant FcγRIIIA ligand for mAbs which contain N-glycans. When adalimumab is injected onto the column, three peaks are able to be resolved, corresponding with the molecule's glycan heterogeneity. Treatment of adalimumab with PNGase F results in the de-glycosylation of the sample. Upon injection of the de-glycosylated sample onto TSKgel FcR-IIIA-NPR, the sample is not retained. These results show the affinity of the FcγRIIIA ligand for mAb glycoforms.

Figure 3: HPLC Analysis of adalimumab with and without PNGase F treatment using TSKgel FcR-IIIA-NPR

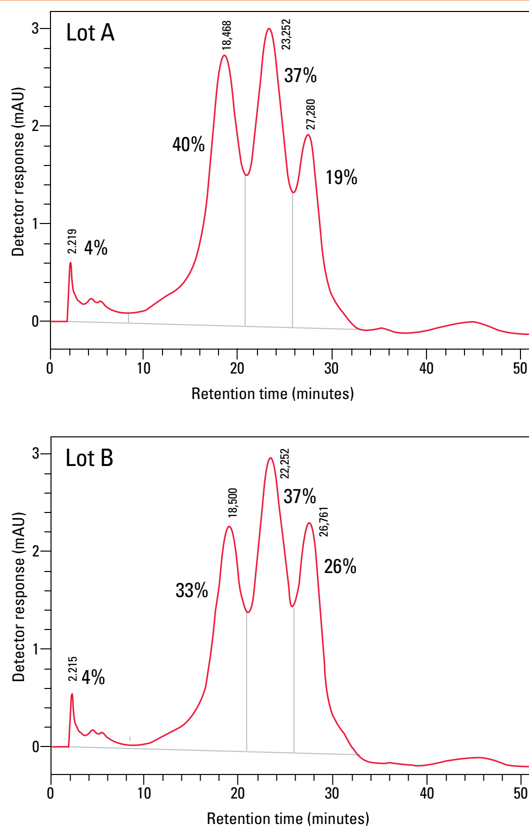


Column:	**TSKgel FcR-IIIA-NPR**
Mobile phase A:	50 mmol/L citrate, pH 6.5
Mobile phase B:	50 mmol/L citrate, pH 4.5
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	50 µL of adalimumab or PNGase F treated adalimumab (1 µg/µL)

mAb Quality Control

Figure 4 shows the utility of the TSKgel FcR-IIIA-NPR column for mAb quality control. Two lots of the same monoclonal antibody-based biotherapeutic were injected onto the column for analysis. Differences in relative peak area percentages indicate that lot-to-lot variations are present. This column can provide a fast and effective way to detect differences in mAb glycoform prevalence in drug product.

Figure 4: TSKgel FcR-IIIA-NPR elucidates lot-to-lot variation of a mAb biotherapeutic

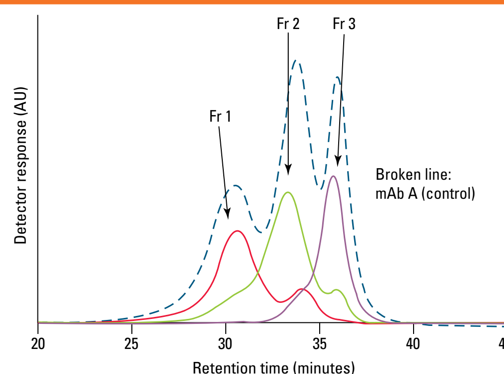


Column:	**TSKgel FcR-IIIA-NPR**
Mobile phase A:	50 mmol/L citrate, pH 6.5
Mobile phase B:	50 mmol/L citrate, pH 4.5
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	mAb based biotherapeutic, Lot A and B

ADCC Efficacy

Affinity of a mAb glycoform for FcγRIIIa is correlated to increased ADCC activity. Peak fractions from a typical separation of mAb A were collected and pooled as shown in Figure 5. Figure 6 shows the corresponding ADCC activity of each sample. As indicated, the most retentive component, fraction 3, displays the highest level of ADCC activity. Each individual fraction shows a different level of ADCC activity than calculated for the unfractioned mAb sample. The TSKgel FcR-IIIA-NPR column allows the generation of additional insight regarding ADCC activity than can be identified by analyzing the mAb alone.

Figure 5: Pooled fractions of mAb A for ADCC analysis

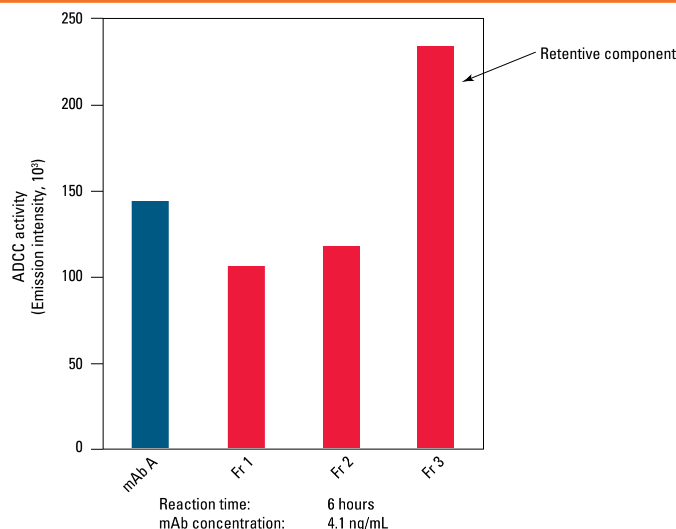


Content (%)

	Peak 1	Peak 2	Peak 3
Control	22	44	34
Fr 1	83	17	0
Fr 2	12	80	8
Fr 3	0	9	91

Column:	**TSKgel FcR-IIIA-NPR**
Mobile phase A:	50 mmol/L citrate, pH 6.5
Mobile phase B:	50 mmol/L citrate, pH 4.5
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	mAb-based biotherapeutic

Figure 6: ADCC activities of each fraction

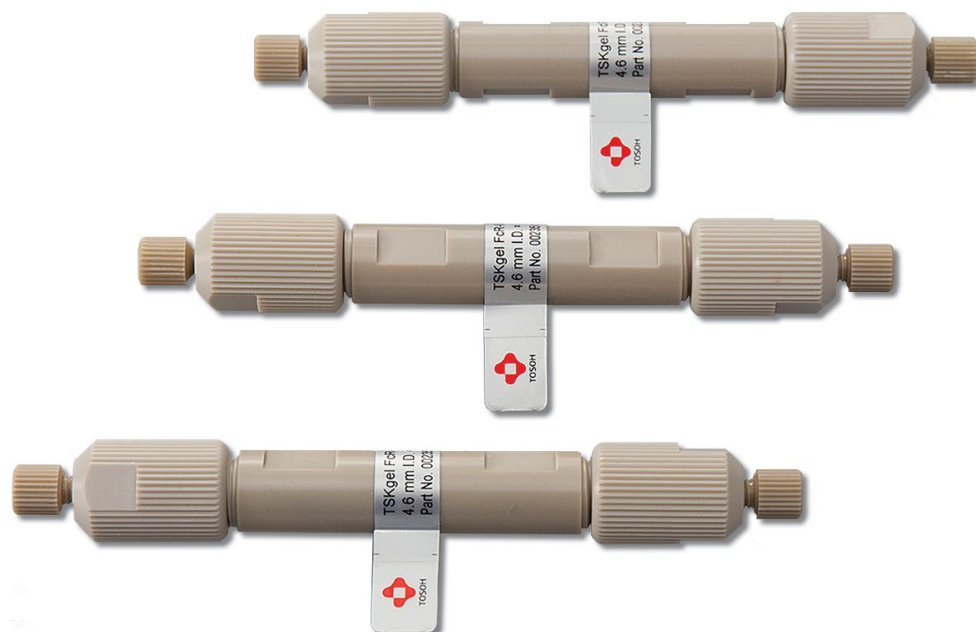


Ordering Information

Part #	Description	Matrix	Housing	ID (mm)	Lenght (cm)
23513	TSKgel FcR-IIIA-NPR	Polymer	PEEK	4.6	7.5

References

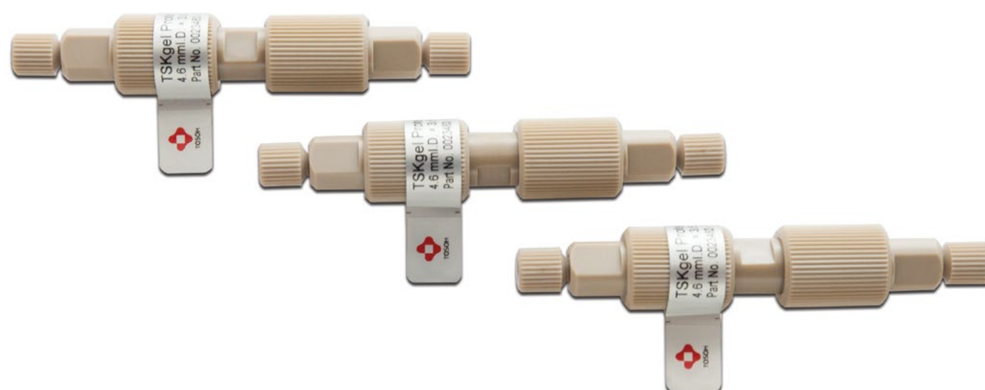
1. Ecker et al; *mAbs*; 2015, 7, 9-14
2. de Val and Kontoravdi; *Biotechnol Prog*; 2010, 26, 1505-1527
3. Arnold et al; *Annu Rev Immunol*; 2007, 25, 21-50
4. Vidarsson et al; *Front Immunol*; 2014, 5, 520
5. Kiyoshi et al; *Nature/Scientific Reports*; 2018, 8, 3955
6. Shields et al; *J Biol Chem*; 2001, 276, 6591-6604



TSKgel Protein A-5PW

Protein A Tips:

- The TSKgel Protein A-5PW column is offered in PEEK hardware.
- As with all columns used with gradient elution, affinity columns should be washed with final elution buffer prior to their re-equilibration with initial (binding) buffer. Always wash the column and the LC system with halide-free buffer at the end of the day.
- Column shipping solvent is an aqueous solution containing 20% ethanol.
- The TSKgel Protein A-5PW column is supplied with an Inspection Data Sheet, which includes a QC chromatogram and an OCS Sheet summarizing the recommended operating conditions for optimum column performance.
- A separate TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems, can be downloaded from the Tosoh Bioscience LLC website (www.tosohbioscience.com).

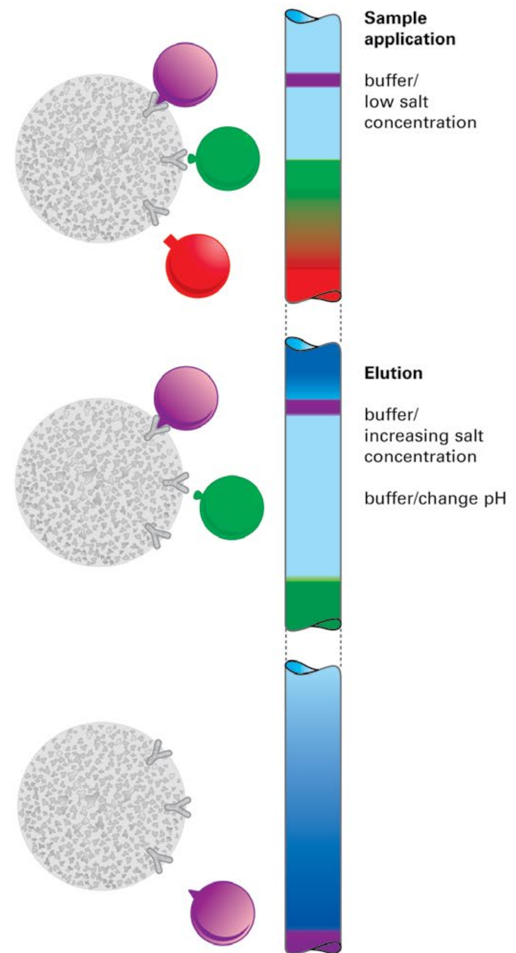


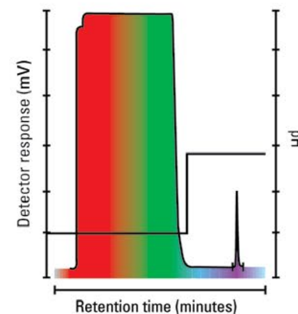
About: Protein A Chromatography

Protein A chromatography, the most widely used type of affinity chromatography, relies on the specific and reversible binding of antibodies to an immobilized ligand; in this case protein A. Protein A is a 56 kDa surface protein native to the cell wall of the bacterium *Staphylococcus aureus*. It is composed of five immunoglobulin-binding domains, each of which are able to bind proteins from many mammalian species, most notably Immunoglobulin G (IgG) through the heavy chain within the Fc region. While the native form of Protein A was used as the ligand for first generation Protein A resins, the recombinant form (rProtein A) produced in *E. coli* is the most prevalent today. The protein A ligand can either bind directly to the Fc region of an antibody or to an Fc tag that has been fused to the target of interest.

In protein A chromatography, crude feed stock is passed through a column under conditions that promote binding. After loading is complete, the column is washed under conditions that do not interrupt the specific interaction between the target and ligand, but that will disrupt any non-specific interactions between process impurities (host cell proteins, etc.) and the stationary phase. The bound protein is then eluted with mobile phase conditions that disrupt the target/ligand interactions. Elution of the target molecule from protein A resin is most commonly accomplished by lowering the pH of the mobile phase, creating an environment whereby the structure of the target molecule is altered in such a way as to inhibit binding. Low pH elution can have a negative effect on protein stability and it is advised that the eluted protein solution be neutralized to minimize aggregation and denaturation.

Figure 1: Protein A Chromatography





About: TSKgel Protein A-5PW Affinity Chromatography Column

TSKgel Protein A-5PW is a 20 µm, 4.6 mm ID × 3.5 cm column for high performance affinity chromatography. Made of PEEK hardware, this column has been designed for the rapid separation and robust quantification of a variety of antibodies. Monoclonal antibodies from harvested cell culture media can be captured and accurately quantitated in less than 2 minutes per injection.

The TSKgel Protein A-5PW column can be used for more than 2,000 injections without regeneration or cleaning. Packed with hydroxylated methacrylic polymer beads, this column is designed with a high degree of crosslinking, which allows a high flow rate for chromatography while still maintaining chromatographic efficiency, peak width and resolution. The recombinant protein A ligand is a code-modified hexamer of the C domain. An enhanced rProtein A ligand is bound to the TSKgel 5PW base bead via multipoint attachment resulting in excellent base stability in 0.1 mol/L NaOH.

The wide range loading capacity of the TSKgel Protein A-5PW column can accurately determine the titer of mAb at various stages of cell culture media processing. The low level of protein A leaching makes this column a good candidate for small scale purification of mAbs for initial characterization. Its reproducibility of injection-after-injection allows the users to accurately monitor the titer of mAb with high confidence.

Attributes

Products attributes of the TSKgel Protein A-5PW column is listed in Table 1.

Table 1: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Particle size	20 µm
pH stability	2.5-7.5
Exclusion limit	1,000 kDa
Ligand	Recombinant protein A, hexamer of C domain

Affinity for Various Antibodies

Because the recombinant protein A ligand of the TSKgel Protein A-5PW column is a code-modified hexamer of the C domain, this column has an affinity for various antibodies that the native protein A and some other recombinant protein A ligands do not possess. For example, it has high affinity for different subclasses of antibodies from rat and goat which native protein A does not have any affinity for, as demonstrated in Table 2.

Table 2: Affinity of protein A to various antibodies

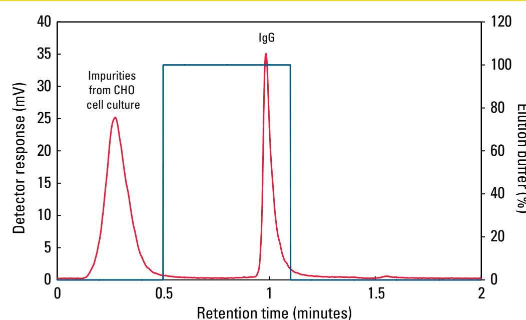
Species	Subclass	Protein A ligand of Protein A-5PW	Native Protein A
Human	IgG_1 IgG_2 IgG_3 IgG_4	+++++ +++++ - +++++	++++ ++++ - ++++
Mouse	IgG_1 IgG_{2a} IgG_{2b} IgG_3	++++ +++++ +++++ ++++	+ ++++ +++ ++
Rat	IgG_1 IgG_{2a} IgG_{2b} IgG_{2c}	++++ - +++ ++++	- - - -
Goat	IgG_s	++++	-
Chicken	IgY	-	-
Rabbit	IgG	+++++	++++

Titer Analysis

Figure 2 shows the fast capture of mAb (human IgG) using a TSKgel Protein A-5PW column. After harvesting, the supernatant from a CHO cell culture is briefly spun to remove cell debris and to concentrate the sample. It is then injected onto a protein A column for titer analysis.

The run was completed within 2 minutes, including bind, wash, elution, and re-equilibration steps. Host cell proteins from the supernatant were not absorbed by the column and so eluted as a flow-through peak. Only IgG was captured and then eluted from the column at approximately a 1 minute retention time. The IgG peak fraction was subjected to size exclusion chromatography using a TSKgel UP-SW3000 column for aggregate and monomer analysis. The result of that analysis indicated that the collected IgG consisted of more than 98% monomer (data not shown).

Figure 2: Fast capture of IgG in the mixture of CHO cell supernatant spiked with IgG

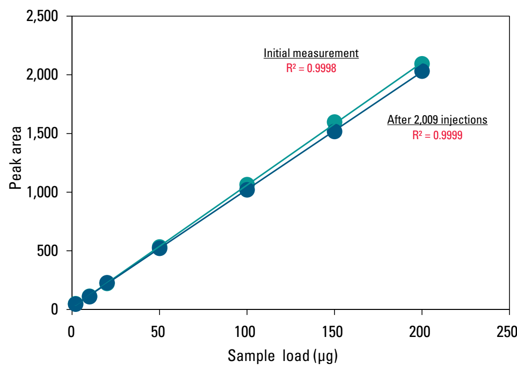


Column: **TSKgel Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm**
Binding buffer: 20 mmol/L sodium phosphate buffer, pH 7.4
Elution buffer: 20 mmol/L sodium phosphate buffer, pH 2.5
Stepwise gradient: 0 – 0.5 min: binding buffer
0.5 – 1.1 min: elution buffer
1.1 – 2.0 min: binding buffer
Flow rate: 2 mL/min
Detection: UV @ 280 nm
Sample: 20 µL of CHO cell culture supernatant spiked with polyclonal IgG (0.5 mg/mL)

Durability and Wide Dynamic Range

The high durability and wide dynamic range of the TSKgel Protein A-5PW column is demonstrated in Figure 3. The column was subjected to a linearity analysis test. Purified IgG was initially injected onto the column with subsequent injections of IgG made at different volumes. The column was then used up to 2,009 injections without being cleaned. A linearity analysis test was then repeated. No significant change in the calibration curve for IgG was seen. The column still maintained its high loading capacity with an excellent linearity ($R^2 = 0.9999$).

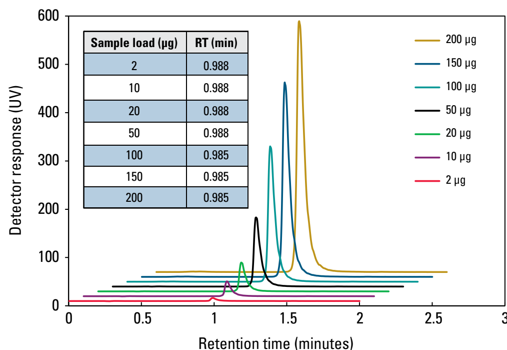
Figure 3: Durability and dynamic range of TSKgel Protein A-5PW column



Wide Dynamic Range and Sensitivity of Detection

Determination of mAb concentration from harvested cell culture supernatant requires a column with good linearity over a wide dynamic range so that the concentrations of mAb can be accurately determined. Similar chromatograms from 2 to 200 µg of load without any change of peak profile or retention are produced by this column (Figure 4).

Figure 4: Wide range of loading concentrations of purified IgG

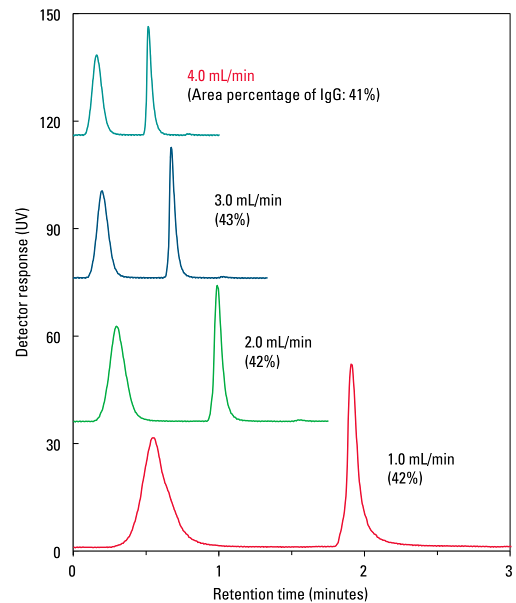


Column: **TSKgel Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm**
Binding and washing buffer: 20 mmol/L sodium phosphate buffer, pH 7.4
Elution buffer: 20 mmol/L sodium phosphate buffer, pH 2.5
Stepwise gradient: 0 - 0.5 min: binding buffer
0.5 - 1.1 min: elution buffer
1.1 - 2.0 min: binding buffer
Flow rate: 2 mL/min
Detection: UV @ 280 nm
Sample: CHO supernatant and IgG

High Flow Rate Tolerance for High Throughput

Four different flow rates (1, 2, 3 and 4 mL/min) were used to demonstrate the high flow rate performance of the TSKgel Protein A-5PW column. Figure 5 shows there is a minimal effect of flow rate on IgG binding or absorbing onto the column. The relative peak area percentages of the unbound (flow-through) protein peak and the bound IgG remained unchanged at different flow rates. Less than 1 minute analysis was available at 4.0 mL/min with a similar peak profile. At 4.0 mL/min, the TSKgel Protein A-5PW column showed a wide dynamic range (2~200 µg) with good linearity ($R^2 = 1.0000$) for IgG (Figure 6).

Figure 5: Effect of flow rate on separation

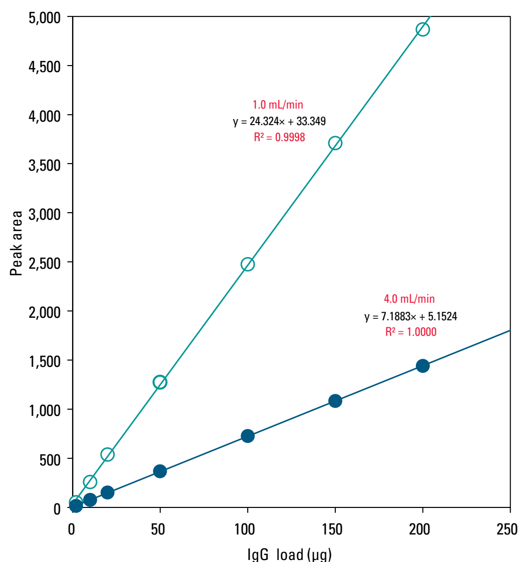


Gradient conditions

Flow rate (mL/min)	Binding buffer (min)	Elution buffer (min)	Binding buffer (min)
4.0	0-0.25	0.25-0.55	0.55-1.00
3.0	0-0.33	0.33-0.73	0.73-1.33
2.0	0-0.50	0.50-1.10	1.10-2.00
1.0	0-1.00	1.00-2.20	2.20-4.00

20 µL of CHO cell supernatant spiked with polyclonal antibody (0.5 mg/mL)

Figure 6: Effect of flow rate on calibration curve

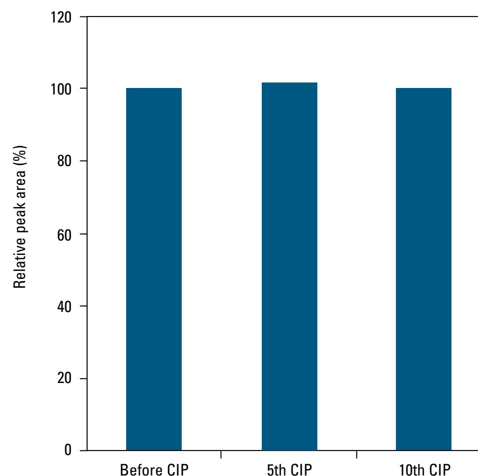


Column:	**TSKgel Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm**
Binding and washing buffer:	20 mmol/L sodium phosphate buffer, pH 7.4
Elution buffer:	20 mmol/L sodium phosphate buffer, pH 2.5
Stepwise gradient:	0 - 0.5 min: binding buffer 0.5 - 1.1 min: elution buffer 1.1 - 2.0 min: binding buffer
Flow rate:	1.0, 2.0, 3.0, 4.0 mL/min
Detection:	UV @ 280 nm
Sample:	CHO supernatant containing 0.5 g/L IgG

Alkaline Stability

A clean-in-place (CIP) study using a polyclonal IgG sample (10 g/L, dissolved in binding buffer) was conducted to test the alkaline stability of the TSKgel Protein A-5PW column. Prior to CIP, IgG was injected onto the column to establish the efficacy of the column for IgG capture. Following this step, 500 µL of 0.1 mol/L NaOH solution was injected onto the column. After the 5th and 10th CIP cycle, the column was injected with polyclonal IgG and the peak area of IgG was integrated and compared to the data prior to the CIP being performed. As demonstrated in Figure 7, the TSKgel Protein A-5PW column shows alkaline stability up to 10 cycles of CIP.

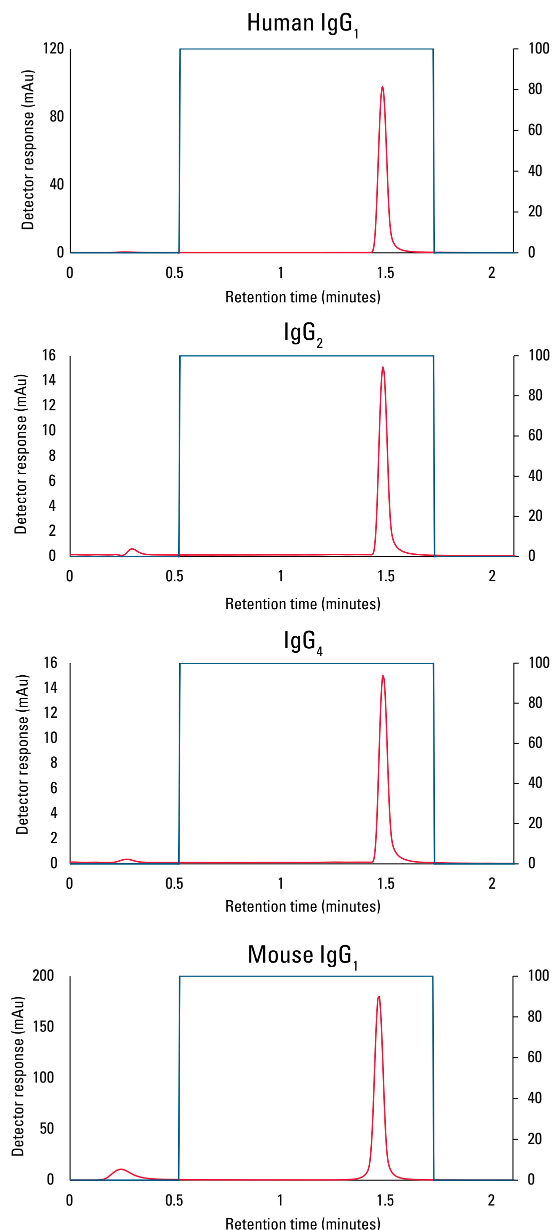
Figure 7: Alkaline stability



Fast Capture of Various IgG Subclasses and Species

Human IgG_1, IgG_2, and IgG_4 and Mouse IgG_1 were subsequently injected onto a TSKgel Protein A-5PW column for titer analysis. Figure 8 shows the fast capture of the various IgG subclasses using this column. The run was completed in less than 2.2 minutes, including bind, wash and re-equilibration steps. The peaks were eluted within the elution step of the chromatographic conditions as shown in the figures.

Figure 8. Fast capture of various IgG species and subclasses using TSKgel Protein A-5PW column

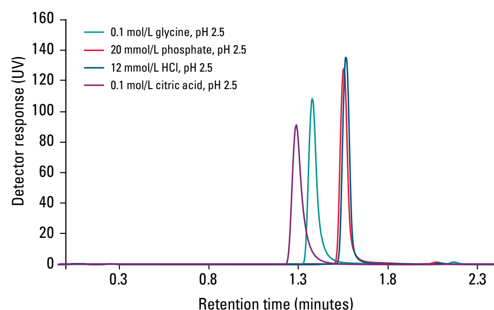


Column:	**TSKgel Protein A-5PW, 4.6 mm ID × 3.5 cm, 20 µm**
Mobile phase:	A. 20 mmol/L sodium phosphate, pH 7.4 B. 12 mmol/L HCl, pH 2.5
Gradient:	equilibration: mobile phase A: 0.0-0.5 min A elution: mobile phase B: 0.51-1.7 min re-equilibration: mobile phase A 1.71-2.2 min
Flow rate:	2.0 mL/min
Detection:	UV @280nm
Temperature:	ambient
Injection vol.:	5 µL
Sample:	as listed

Compatibility with Various Mobile Phases

The TSKgel Protein A-5PW column can be successfully used to analyze mAbs within 2.2 minutes under a variety of mobile phase conditions. A number of elution conditions, specifically 12 mmol/L HCl, pH 2.5; 100 mmol/L citric acid, pH 2.5; 100 mmol/L glycine, pH 2.5; 20 mmol/L phosphate, pH 2.5 were used for eluting the IgGs bound to the TSKgel Protein A-5PW column. The peaks were eluted within the elution step of the chromatographic conditions as shown in Figure 9.

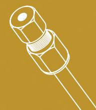
Figure 9. Compatibility with multiple elution buffers



Column:	**TSKgel Protein A-5PW, 4.6 mm ID × 3.5 cm, 20 µm**
Mobile phase:	A. 20 mmol/L sodium phosphate; pH 7.4 B. 12 mmol/L HCl, pH 2.5 100 mmol/L citric acid, pH 2.5 100 mmol/L glycine, pH 2.5 20 mmol/L phosphate, pH 2.5
Gradient:	equilibration: mobile phase A: 0.0-0.5 min A elution: mobile phase B: 0.51-1.7 min re-equilibration: mobile phase A 1.71-2.2 min
Flow rate:	2.0 mL/min
Detection:	UV @280nm
Temperature:	ambient
Injection vol.:	5 µL
Sample:	IgG_1 (5mg/mL)

Ordering Information

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
23483	TSKgel Protein A-5PW, 20 µm, 100 nm	Polymer	PEEK	4.6	3.5



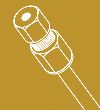
Calibration Standards

TSKgel standards polystyrene
TSKgel standards poly(ethylene oxide)
PStQuick polystyrene calibration standards

Replacement Parts

TSKgel guard column products
Fittings
Pre-injector filters
Pre-column filters
Tubing

有機溶媒系サイズ排除クロマトグラフィー用
標準ポリスチレンキット
PStQuick シリーズ
Standard Polystyrene Kits for Organic
Solvent High Performance SEC
PStQuick series
PStQuick Kit-M
Aluminum Sheet Type
Part No.: 21895
TOSOH
東ソー株式会社
TOSOH CORPORATION



About: Calibration Standards

The TSKgel standards product line consists of bulk quantities of polystyrene and polyethylene oxide standards of varying molar mass and pre-mixed quantities of polystyrene polymers for calibration of GPC columns (PStQuick). All standards are tested to certify quality and reliability. Testing is done by laser light scattering, except for the standards below 1,000 Da, which are tested by size exclusion chromatography.

TSKgel polystyrene bulk calibration standards are used to calibrate size exclusion columns for subsequent analysis of unknown samples. The standards range from 400 to 2.1×10^7 Da.

TSKgel polyethylene oxide calibration standards were developed to meet the demand for a high molar mass aqueous standard polymer. Synthesized by living anionic polymerization, this product, like its sister product TSKgel polystyrene calibration standards, has an extremely sharp molar mass distribution. The polymer is available in seven types, with molar mass values between 1.8×10^4 and 1.0×10^6 Da. The polymer may be used as a test sample for liquid chromatography, such as SEC using water (buffer) or alcohol as a solvent for evaluating the performance of a separation membrane, or for calibrating light-scattering photometers and other molar mass analysis devices. It may also be used for biochemical research.

PStQuick polystyrene calibration standards contain pre-mixed quantities of polystyrene polymers in autosampler vials for calibration of GPC columns. Addition of solvent is all that is required for easy preparation and analysis. Twelve different kits containing polystyrene polymers of various molar masses are available. Of the 12 kits, 9 are individual kits, each containing 3 to 5 polystyrene polymers. The remaining 3 are composite kits containing 2 or 3 of the individual kits.

Ordering Information - TSKgel polystyrene calibration standards

Part #	Description	Weight
05202	A-300, 400 Da	10 g
05203	A-500, 530 Da	10 g
05204	A-1000, 950 Da	10 g
05205	A-2500, 2,800 Da	5 g
05206	A-5000, 6,200 Da	5 g
05207	F-1, 1.0×10^4 Da	5 g
05208	F-2, 1.7×10^4 Da	5 g
05209	F-4, 4.4×10^4 Da	5 g
05210	F-10, 1.0×10^5 Da	5 g
05211	F-20, 1.9×10^5 Da	5 g
05212	F-40, 4.2×10^5 Da	5 g
05213	F-80, 7.8×10^5 Da	5 g
05214	F-128, 1.3×10^6 Da	1 g
05215	F-288, 2.9×10^6 Da	1 g
05216	F-380, 3.8×10^6 Da	1 g
05217	F-450, 4.5×10^6 Da	1 g
05218	F-550, 5.5×10^6 Da	1 g
05219	F-700, 6.8×10^6 Da	1 g
05220	F-850, 8.4×10^6 Da	1 g
05221	F-2000, 2.1×10^7 Da	1 g
06476	Oligomer Kit, A-500 thru F-128	12 × 1 g
06477	High MW Kit, F-10 thru F-2000	12 × 1 g

Ordering Information - TSKgel polyethylene oxide calibration standards

Part #	Description	Weight
06211	SE-2, 1.8 × 10^4 Da	0.5 g
06212	SE-5, 3.9 × 10^4 Da	0.5 g
06213	SE-8, 8.6 × 10^4 Da	0.5 g
06214	SE-15, 1.5 × 10^5 Da	0.5 g
06215	SE-30, 2.5 × 10^5 Da	0.5 g
06216	SE-70, 5.9 × 10^5 Da	0.5 g
06217	SE-150, 1.0 × 10^6 Da	0.5 g
05773	Polyethylene Oxide Kit, SE-2 thru SE-150	7 × 0.2 g

Ordering Information - PStQuick polystyrene calibration standards

To calibrate TSKgel SuperMultiporeHZ columns

Part #	Description	Remarks	Calibration Range	Contents	Vials
21912	PStQuick MP-N	For SuperMultiporeHZ-N	530 to 4.4 × 10^4	A-500, A-5000, F-4	60
21913	PStQuick MP-M	For SuperMultiporeHZ-M	530 to 8.0 × 10^5	A-500, A-5000, F-10, F-80	60
21914	PStQuick MP-H	For SuperMultiporeHZ-H	950 to 5.5 × 10^6	A-1000, F-1, F-10, F-80, F-550	60

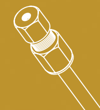
To calibrate TSKgel H-type mixed bed columns

Part #	Description	Remarks	Calibration Range	Contents	Vials
21915	PStQuick Kit-L	For H-type – N grade	530 to 4.2 x 10^5	PStQuick E, F	40**
21916	PStQuick Kit-M	For H-type – M grade	530 to 2.9 x 10^6	PStQuick C, D	40**
21917	PStQuick Kit-H	For H-type – H grade	530 to 8.4 x 10^6	PStQuick A, B, C	60*

*20 of each type × 3, **20 of each type × 2

To calibrate other TSKgel GPC columns

Part #	Description	Remarks	Calibration Range	Contents	Vials
21911	PStQuick A	For other GPC columns	2,800 to 8.4 × 10^6	A-2500, F-2, F-20, F-128, F-850	20
21910	PStQuick B	For other GPC columns	950 to 5.5 × 10^6	A-1000, F-1, F-10, F-80, F-550	20
21909	PStQuick C	For other GPC columns	530 to 2.9 × 10^6	A-500, A-5000, F-4, F-40, F-288	20
21908	PStQuick D	For other GPC columns	2,800 to 1.3 × 10^6	A-2500, F-2, F-20, F-128	20
21907	PStQuick E	For other GPC columns	950 to 4.2 × 10^5	A-1000, A-5000, F-4, F-40	20
21906	PStQuick F	For other GPC columns	530 to 1.9 × 10^5	A-500, A-2500, F-2, F-20	20



About: Parts and Accessories

Tosoh Bioscience offers a limited line of replacement parts for its columns and guard columns. With the introduction of TSKgel BioAssist series, the TSKgel column line consists of stainless steel, glass and PEEK columns.

The tubing, fittings and frits in the stainless steel TSKgel columns are manufactured from 316-grade stainless steel. The top and bottom end-fittings are engraved with the pore size of the frit. If the top frit plugs, the entire end-fitting can be removed and replaced. Order the appropriate replacement fitting according to the ID of the column. Stainless steel end-fittings will connect to the 10-32 PEEK or stainless steel nuts and ferrules on a standard HPLC system.

The end-fittings on the glass columns contain Pyrex frits with a nominal 10 µm pore size. The materials in the glass column fittings that come into contact with the sample and the mobile phase are chlorotrifluoroethylene and tetrafluoroethylene. The end-fittings will connect to 1/4-28 and also to M6 nuts and ferrules.

Tosoh Bioscience recommends that each column be protected by a guard column and filter. Guard columns are listed with corresponding columns in each section of this catalog. We recommend installing a pre-column filter in front of the (guard) column to avoid clogging the top frit, particularly when working with columns that contain smaller than 5 µm particles. In addition, when suspecting debris from the breakdown of pump seals, we have found it beneficial to use a pre-injector membrane filter to prevent any particulates from reaching the injector and/or (guard) column.

Ordering Information - TSKgel guard holders

Part #	Description	ID (mm)	Length (cm)
07093	TSKgel Guardgel Holder for 7.5 mm ID SS columns	6	1
08809	TSKgel Guardgel Holder for 5 mm ID and 8 mm ID glass columns	8	1
16106	TSKgel Guardgel Holder for 21.5 mm ID SS columns	20	1
18206	TSKgel Guardfilter Holder for TSKgel Super-ODS, -Octyl, -Phenyl guard filters		
19018	TSKgel Guard Cartridge Holder for 3.2 mm ID cartridges	3.2	1.5
19308	TSKgel Cartridge Guard Holder for 2 mm ID columns	2	1

Ordering Information - fittings

Part #	Description
16566	One-Piece Fingertight Fitting, 1/16", 10-32, PEEK, ea
05748	End-fitting w/ Fixed 10 µm Frit for 7.5 mm ID SS columns
06191	End-fitting w/ Fixed 10 µm Frit for 21.5 mm ID SS columns
07619	End-fitting w/ Fixed 2 µm Frit for 4.6 mm ID SS columns
08092	End-fitting w/ Fixed 2 µm Frit for all 6 mm ID SS columns
08095	End-fitting w/ Fixed 2 µm Frit for all 7.8 mm ID SS columns
08600	End-fitting w/ 10 µm Pyrex Frit for all 8 mm ID Glass columns
13998	End-fitting w/ Fixed 1 µm Frit for NPR columns
16104	End-fitting w/ 10 µm Pyrex Frit for all 5 mm ID Glass columns
16105	End-fitting w/ 10 µm Pyrex Frit for all 20 mm ID Glass columns
18254	End-fitting w/ Fixed 1 µm Frit for TSKgel SuperH columns
18255	End-fitting w/ Fixed 1 µm Frit for TSKgel Super Series columns
18544	End-fitting w/ Fixed 2 µm Frit for TSKgel ODS-80Ts QA columns
19834	End-fitting w/5 µm Frit for 4.6 mm ID TSKgel BioAssist columns
19838	End-fitting w/5 µm Frit for TSKgel BioAssist PEEK SWxl columns

Ordering Information - filter

Part #	Description
14594	Pre-Injector Membrane Filter Holder, SS

有機溶媒系サイズ排除クロマトグラフィー用
標準ポリスチレンキット
PStQuick シリーズ
Standard Polystyrene Kits for Organic
Solvent High Performance SEC
PStQuick series
PStQuick Kit-M
Aluminum Sheet Type
TOSOH
東ソー株式会社
TOSOH CORPORATION

Appendix

Tosoh Corporation closely monitors all stages of the manufacturing process for chromatographic media that are used to pack TSKgel columns. Packing materials are produced in large 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 bonded lot is again tested to ensure that it meets the specifications for parameters such as ligand density, retention, selectivity, etc.

TSKgel 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 enable consistency in retention time and selectivity irrespective of the use of different lots of the same column type.

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.

Guard Product Options

GLP procedures often specify that the separation column be protected by a guard column. The guard column is installed between the injector and the analytical column. It is designed to protect the analytical column from unwanted materials, such as highly retained or irreversibly adsorbed compounds and particulate matter. Tosoh Bioscience supplies an assortment of packed guard columns, guardgel kits, guard cartridges, and guardfilters.

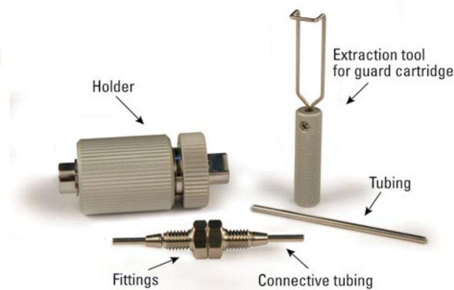
Guardgel kits contain the hardware and the gel packing material to fill a guard column using an aspirator. Detailed instructions on how to pack a stainless steel and a glass guardgel column are available from the Tosoh Bioscience website in the Literature section, under the heading "Instruction Manuals". The manual is entitled "Instruction Manual -- Guardgel Kits for TSKgel HPLC Columns" (IM04). In addition, step-by-step instructions are avaible on the Tosoh Bioscience YouTube channel (www.youtube.com/tosohbiosciencellc). Figure 1 is an example of a guardgel kit, in this case for a TSKgel DEAE-5PW column.

Figure 1: Guardgel kit

Guard cartridges (Figure 2) are pre-packed, small replaceable columns easily inserted into a hand-tight guard cartridge holder (Figure 3).

Figure 2: Guard cartridges

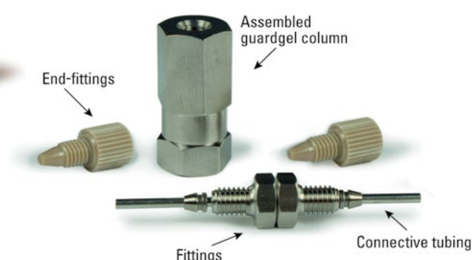
Figure 3: Guard cartridge holder



Guardfilters (Figure 4) are pre-packed, small replaceable columns easily inserted into a hand-tight guardfilter holder (Figure 5).

Figure 4: Guardfilter

Figure 5: Guardfilter holder



For those columns where a guard product is not available, Tosoh Bioscience recommends the use of an in-line filter with a 0.5 μm cutoff to avoid frequent plugging of the 1.0 μm pores in the column frit of TSKgel ODS-140HTP, Super-ODS, Super-Octyl, and Super-Phenyl columns. A pre-injector membrane filter is also recommended to prevent particles generated by pump seal wear from reaching the column. Consult the Replacement Parts chapter in this Product Guide for these and other hardware products.

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. The pH of the sample (if different from the mobile phase pH) will be adjusted to the pH of the mobile phase before entering into the analytical column provided a guard column is used. This is particularly important in the silica-based SW 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 can be removed by sparging with helium, mobile phase filtration or other degassing procedures. This is particularly important for polymer-based columns. If air does enter the column, follow the rehydration procedure described later in this appendix.

3. Column contamination or incomplete sample recovery
   Cleaning conditions for all column types are provided on the Tosoh Bioscience website in the resource section of the technical support channel. 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 mol/L 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 Replacement Parts chapter within this Product Guide for these and other hardware products.

5. Peak splitting
   Column overload, whether in volume or concentration, can cause peak splitting and poor resolution. The appropriate concentration and volume of analyte will vary on the loading capacity of the column.

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.

An Inspection Data Sheet and an Operating Conditions and Specifications (OCS) Sheet accompanies all TSKgel 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 Inspection Data 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 TSKgel 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 TSKgel column has a recommended set of cleaning solutions specific to the column, as described below and on the Tosoh Bioscience website in the resource section of the technical support channel. Choose a cleaning solution based upon the column and sample 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).

Cleaning Solutions

Size Exclusion: TSKgel SW and SWXL columns

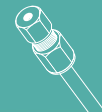
1. Turn the column in reverse flow direction and run at half the maximum flow rate.
2. Clean with 5 column volumes (CV) of 1 mol/L NaCl, pH 7.0
3. Clean with 5 CV of ultra-pure water.
4. Clean with 5 CV of 20% acetonitrile.
5. Clean with 5 CV of ultra-pure water.
6. Turn column in normal flow direction and equilibrate in mobile phase for at least 45 minutes.

Size Exclusion: TSKgel PW and PWXL columns

1. Turn the column in reverse flow direction and run at half the maximum flow rate.
2. Clean with 5 column volumes (CV) of 1 mol/L NaCl, pH 7.0
3. Clean with 5 CV of ultra-pure water.
4. Clean with 5 CV of 20% acetonitrile.
5. Clean with 5 CV of ultra-pure water.
6. Turn column in normal flow direction and equilibrate in mobile phase for at least 45 minutes.

Ion Exchange, TSKgel SW-type columns

1. High concentration salt (e.g. 0.5 mol/L - 1.0 mol/L Na_2S0_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. Urea* (8 mol/L) or non-ionic surfactant in buffer solution. *Note: caution for chaotropic agents.



Ion Exchange, TSKgel PW-type columns

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. Inject up to 1 CV in 250 µL increments of 20% - 40% aqueous acetic acid on analytical columns. (Since acid can precipitate protein it should be used after other cleaning methods.) Inject proportionally larger volumes on semi-preparative columns.
3. Water soluble organic (MeOH, ACN, EtOH, 10% - 20%) in aqueous buffer
4. Urea* (8 mol/L) or non-ionic surfactant in buffer solution. *Note: caution for chaotropic agents.

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 columns

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. Inject up to 1 CV in 250 µL increments of 20% - 40% aqueous acetic acid on analytical columns. (Since acid can precipitate protein it should be used after other cleaning methods.) Inject proportionally larger volumes on semi-preparative columns.

Reversed Phase, silica-based columns

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

Reversed Phase, polymer-based columns

1. 100% acetonitrile or methanol
2. 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.
3. Inject up to 1 CV in 250 µL increments of 20% - 40% aqueous acetic acid on analytical columns. (Since acid can precipitate protein it should be used after other cleaning methods.) Inject proportionally larger volumes on semi-preparative columns.

HILIC columns

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 and Protein A-5PW columns

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

Rehydration

Dehydration of TSKgel 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 ultrapure 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.

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.

Beware of Extra-Column Band Broadening

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 × 30 cm to 6 mm ID × 15 cm, this results in a reduction of peak volume by a factor of (7.8/6)*(30/15) = 3.4. Similarly, the reductions in peak volume are 5.8 when going from 7.8 mm ID × 30 cm to 4.6 mm ID × 15 cm, and 21.1 when replacing a 4.6 mm ID × 30 cm column by one that is 1 mm ID × 30 cm. 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.

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. Neglecting to optimize the HPLC system can seriously detract from the true column efficiency, which ultimately can result in unacceptable analysis results.

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

USP number	USP Description	Recommended columns
L1	Octadecyl silane chemically bonded to porous or non-porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter, or a monolithic rod.	TSKgel ODS-100V, ODS-100Z, Super-ODS, ODS-80TM, ODS-80TS, ODS-120A, ODS-120T, ODS-140HTP, OligoDNA-RP, ODS-100S
L3	Porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod.	TSKgel Silica-60
L7	Octylsilane chemically bonded to totally or superficially porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod.	TSKgel Super-Octyl, Octyl-80TS
L8	An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5 to 10 µm in diameter.	TSKgel NH_2-100, TSKgel NH_2-100 DC
L9	Irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation exchange coating, 3 to 10 µm in diameter.	TSKgel SP-2SW
L10	Nitrile groups chemically bonded to porous silica particles, 3 to 10 µm in diameter.	TSKgel CN-80TS
L11	Phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter.	TSKgel Super-Phenyl
L13	Trimethylsilane chemically bonded to porous silica particles, 3 to 10 µm in diameter.	TSKgel TMS-250
L14	Silica gel having a chemically bonded, strongly basic quaternary ammonium anion exchange coating, 5 to 10 µm in diameter.	TSKgel QAE-2SW
L20	Dihydroxypropane groups chemically bonded to porous silica or hybrid particles, 1.5 to 10 µm in diameter.	TSKgel QC-PAK GFC, SWXL, SW, SuperSW, and SW mAb series
L21	A rigid, spherical styrene-divinylbenzene copolymer, 3 to 30 µm in diameter.	TSKgel HXL, HHR, SuperH, SuperHZ, and SuperMultiporeHZ series
L22	A cation exchange resin made of porous polystyrene gel with sulfonic acid groups, about 10 µm in size.	TSKgel SCX
L23	An anion exchange resin made of porous polymethacrylate or polyacrylate gel with quartenary ammonium groups, about 10 µm in size.	TSKgel SuperQ-5PW, BioAssist Q, Q-STAT, DNA-STAT, IC-Anion-PW
L25	Packing having the capacity to separate compounds with a molecular weight range from 100-5,000 Da (as determined by polyethylene oxide), applied to neutral, anionic, and cationic water-soluble polymers.	TSKgel G1000PW, G2000PW, G2500PW, G2500PWXL
L26	Butyl silane chemically bonded to totally porous silica particles, 1.5 to 10 µm in diameter	TSKgel Protein C_4-300
L33	Packing having the capacity to separate dextrans by molecular size over a range of 4,000 to 5.0 × 10^5 Da. It is spherical, silica-based, and processed to provide pH stability.	TSKgel SuperSW, SWXL, QC-PAK GFC, SW, and SW mAb series
L37	Packing having the capacity to separate proteins by molecular size over a range of 2,000 to 4.0 × 10^4 Da. It is a polymethacrylate gel.	TSKgel G3000PWXL, G3000PW, G3000PWXL-CP
L38	A methacrylate-based size exclusion packing for water-soluble samples.	TSKgel PWXL, PW, PWXL-CP, Alpha, SuperAW series, and SuperMultiporePW
L39	A hydrophilic polyhydroxymethacrylate gel of totally porous spherical resin.	TSKgel PW, PWXL, PWXL-CP, Alpha, and SuperAW, SuperMultipore series
L52	A strong cation exchange resin made of porous silica with sulfopropyl groups, 5 to 10 µm in diameter.	TSKgel SP-2SW, IC-Cation SW
L58	Strong cation exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the sodium form, about 6 to 30 µm diameter.	TSKgel SCX (Na^+)
L59	Packing for the size exclusion separations of proteins (separation by molecular weight)over the range of 5 to 7,000 kDa. The packing is spherical, 1.5 - 10 µm, silica or hybrid packing with a hydrophilic coating.	TSKgel SuperSW, SWXL, SW, and SW mAb series
L68	Spherical, porous silica, 10 µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and not endcapped	TSKgel Amide-80
L89	Packing having the capacity to separate compounds with a molecular weight range from 100 - 3000 (as determined by polyethylene oxide), applied to neutral and anionic water-soluble polymers.	TSKgel G-Oligo-PW, SuperOligoPW

Call customer service: 866-527-3587, technical service: 800-366-4875

Molecule(s) of Interest

Antibodies

Antibody drug conjugates (ADCs)
SEC ... 26

Bispecific antibody (bsAb)
SEC/MS ... 13

Chimeric IgG, mouse-human
SEC ... 19

Glycan profile
HILIC ... 129

Monoclonal antibodies (mAbs), general
SEC ... 9-12, 16, 17-19, 22, 27, 29, 32
SEC/MS ... 14
AEX ... 92, 97
CEX ... 101, 107-108
HIC ... 119-121
HILIC ... 129
RPC ... 147
Protein A ... 212-216
Affinity ... 197

Monoclonal antibodies (mAbs), glycoforms
FcR ... 206-207

Crude Extracts

Mouse ascites fluid
AEX ... 86, 96

Rat liver extract
SEC ... 27

Root extract
RPC ... 151

Nucleic Acids

DNA
SEC ... 24, 28, 42
AEX ... 89-90, 93, 96

Nucleic acids
SEC ... 23, 45

Nucleobases
HILIC ... 18

Nucleosides
SEC ... 23
CEX ... 103
Affinity ... 195

Nucleotides and oligonucleotides
AEX ... 88-89, 91, 94
HILIC ... 130
RPC ... 162, 175

RNA
SEC ... 21
AEX ... 90
HIC ... 122

Oligosaccharides

Glycans
HILIC ... 129

Oligosaccharides, general
SEC ... 39, 42-43
AEX ... 98
CEX ... 109
HILIC ... 133, 136

Polyphosphates
AEX ... 99

Sugar alcohol
AEX ... 99
HILIC ... 131

Organic Soluble Polymers (GPC)

Polymers, general
SEC ... 53-54, 59-60, 63-65, 67-68, 70-74

Peptides

Amino acids
RPC ... 162

Peptide mixtures
SEC ... 33, 44
CEX ... 102, 105
HILIC ... 132
RPC .. 149, 151, 154, 161-162, 168-169, 173, 177, 180-182

Proteins

Glycated proteins
Affinity ... 196

Metalloproteins
SEC ... 20, 23

PEGylated protein
SEC ... 29
CEX ... 108

Proteins, general
SEC ... 10-11, 17-18, 22, 26, 28, 31, 33
AEX ... 87
CEX ... 100-101, 104-105, 107, 111
HIC ... 120, 122-123
RPC ... 146-147, 163-164, 176, 181, 183
Affinity ... 198-200

Molecule(s) of Interest: Cont.

Small Molecule Analytes

Antioxidants
RPC 153

Aromatics
RPC 165

Caffeine
RPC 150

Catecholamines
RPC 174
Affinity 196

Cyanuric acid
HILIC 131

Food products
RPC 167, 169

Herbal extract
RPC 150

Melamine
HILIC 131

Organic acids
CEX 110
RPC 155

Pharmaceuticals
RPC .. 134, 152, 156-157, 160, 166, 168, 170-171, 178-179

Pollutants
RPC 159

Polyalcohols
HILIC 134

Polynuclear aromatic hydrocarbons (PAH)
RPC 172

Polyphenols
RPC 159

Surfactants
RPC 152

Vitamins
RPC 155, 160

Water Soluble Polymers (GFC)

Cationic polymers
SEC 46-47

Polymers, general
SEC 39, 43-45, 47, 49

Where to Order

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Freedom Evo is a trademark of Tecan.

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