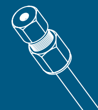


Silica-based for protein analysis:

TSKgel SW mAb
TSKgel SW
TSKgel SW_{XL}
TSKgel SuperSW

Polymer-based for desalting:

TSKgel BioAssist DS Columns

Polymethacrylate-based for water-soluble polymers analysis:

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

Polymethacrylate-based for polar organic-soluble polymers analysis:

TSKgel Alpha
TSKgel SuperAW

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

TSKgel H_{XL}
TSKgel H_{HR}
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.

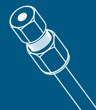
- Halide salts corrode stainless steel tubing, fitting, and frits. Do not store SS columns in a mobile phase containing NaCl and, where possible, use another salt in the operating buffer. Chlorotrifluorethylene and tetrafluorethylene are the materials in the glass column fittings that come into contact 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 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) is the dominant mode of separation for polymers. SEC is the general name for the chromatographic mode in which components of a mixture are separated according to their molecular size, based on the flow of the sample through a column packed with porous particles. Large sample molecules cannot or can only partially penetrate the pores, whereas smaller molecules can access all or a larger number of pores (as demonstrated in Figure 1). In SEC, large molecules elute from the column first followed by smaller molecules, and the smallest molecules that can access all the pores elute last from the column. Size exclusion chromatography 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.

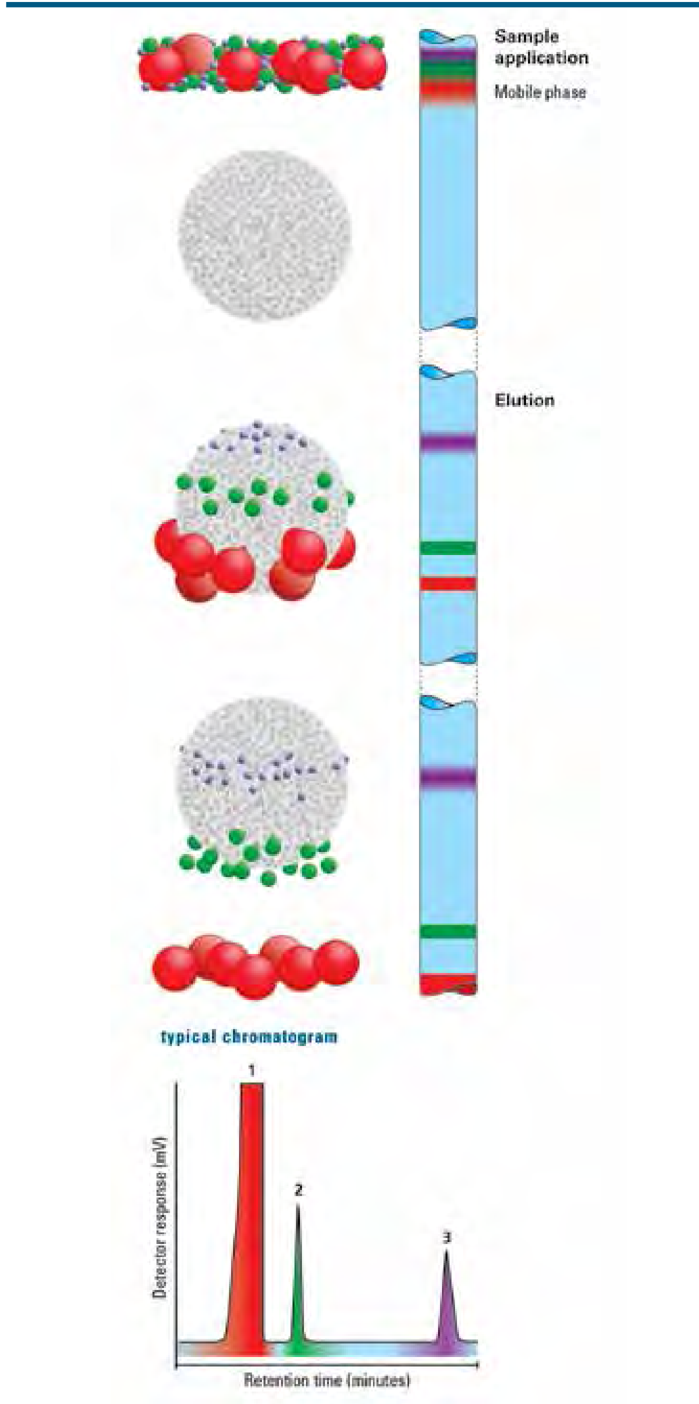
TSKgel Gel Filtration Chromatography Columns: GFC

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. 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.

Figure 1: Size Exclusion Chromatography



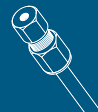
TSKgel Gel Permeation Chromatography Columns: GPC

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

Tosoh recently added three TSKgel SW mAb columns to the renowned line of TSKgel SW series SEC columns. 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 mAb 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 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 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.

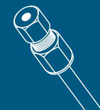
TSKgel BioAssist DS desalting columns are designed to reduce the concentration of salt and buffer of protein or polynucleotide sample solutions at semi-preparative scale. Packed with 15 µm polyacrylamide beads in PEEK hardware, TSKgel BioAssist DS columns show excellent desalting performance.

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 G3000SWXL and TSKgel 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 SW mAb, SW, SWXL, 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
G2000SWXL, BioAssist G2SWXL, 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
G3000SWXL, BioAssist G3SWXL, QC-PAK GFC 300	5 µm	25 nm	1×10^4 – 5×10^5	2,000 – 7×10^4	100 – 3.5×10^4
G4000SWXL, BioAssist G4SWXL	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	–	–



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.

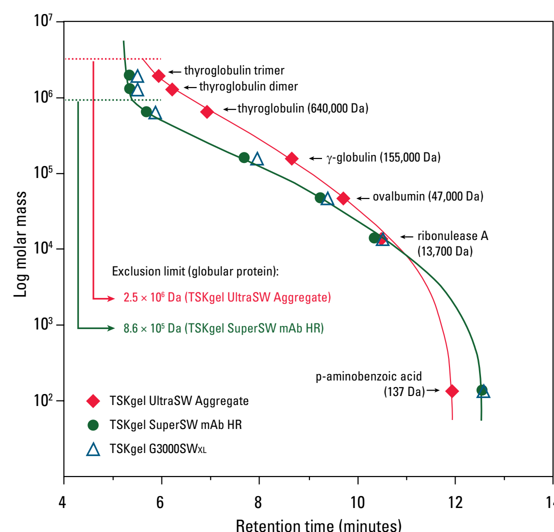
Attributes and Applications

Table 3 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 2, 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 3: 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)

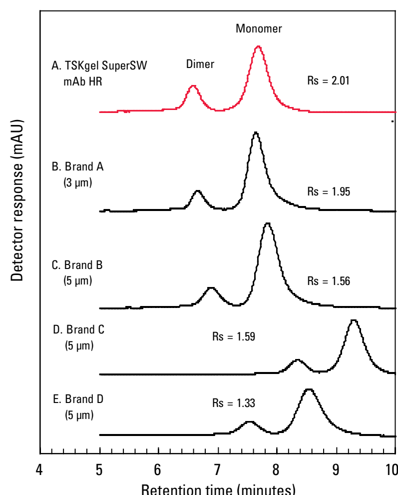
Figure 2: Protein calibration curves for TSKgel SW mAb columns



mAb Monomer and Dimer

Figure 3 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 3: Comparison of resolution of mAb monomer and dimer

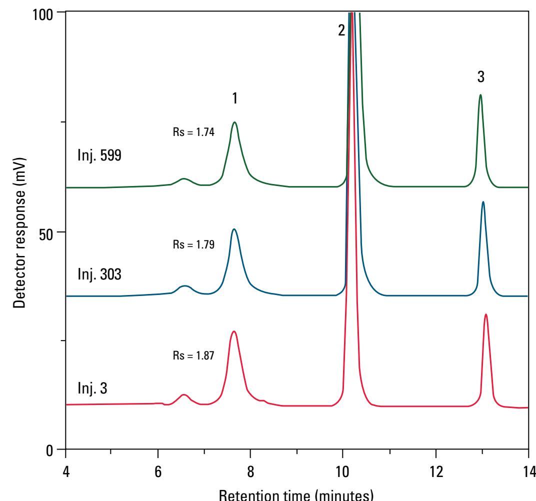


Columns:	**A. TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm**
	B. Brand A, 3 µm, 7.8 mm ID × 30 cm
	C. Brand B, 5 µm, 7.8 mm ID × 30 cm
	D. Brand C, 5 µm, 8.0 mm ID × 30 cm
	E. Brand D, 5 µm, 8.0 mm ID × 30 cm
Mobile phase:	200 mmol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate:	1.0 mL/min
Detection: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

Durability

Figure 4 demonstrates the good durability of the TSKgel SuperSW mAb HR column through the reproducibility of Rs for a γ-globulin sample injection.

Figure 4: High durability of TSKgel SuperSW mAb HR column

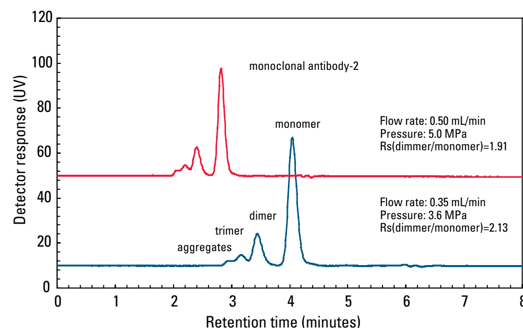


Column:	**TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate:	0.8 mL/min
Detection:	UV @ 280 nm
Injection vol.:	10 µL
Samples:	1. g-Globulin
	2. Cytochrome C
	3. DNP-L-Alanine

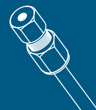
Therapeutic mAb

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 5 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 5: 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

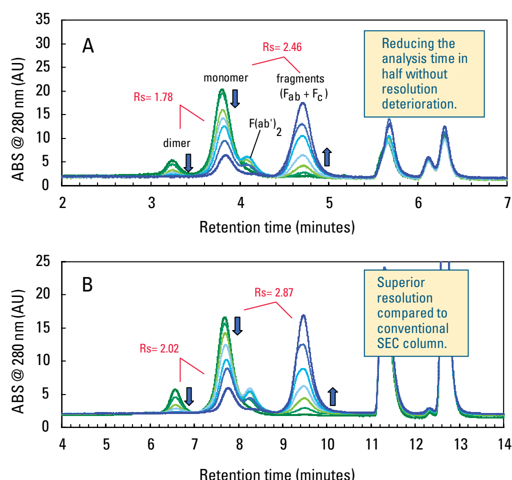


Papain digested IgG

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 6). 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 6: Analysis of IgG monomer, dimer and fragments

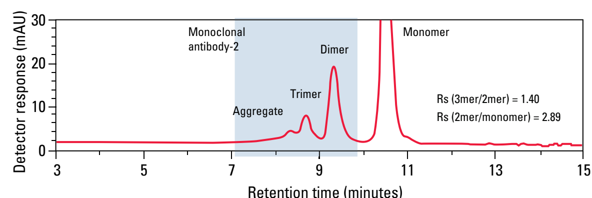


Columns:	**A. TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm**
	B. TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm
Mobile phase:	200 mmol/L phosphate buffer + 0.05% NaN_3, pH 6.7
Flow rate:	A: 0.35 mL/min; B: 1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	A: 5 µL; B: 10 µL
Sample: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

Mouse-human Chimeric IgG

Figure 7 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 molecular weight 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 7: Separation of mAb trimer and dimer

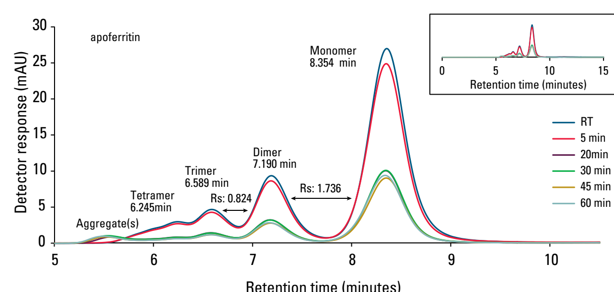


Column:	**TSKgel UltraSW Aggregate, 3 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate:	0.8 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Sample:	monoclonal antibody-2 (mouse-human chimeric IgG, Erbitux), 10 µL

Metalloprotein

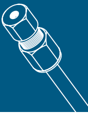
The analysis of a heat denatured, large hydrophobic metalloprotein, apoferritin, is shown in Figure 8. A set of six, 0.3 mL HPLC vials each containing 100 µL stock solution of apoferritin was used for protein thermal denaturation. Thermal denaturation was carried out at 60 °C using an electric heating 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 8: 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

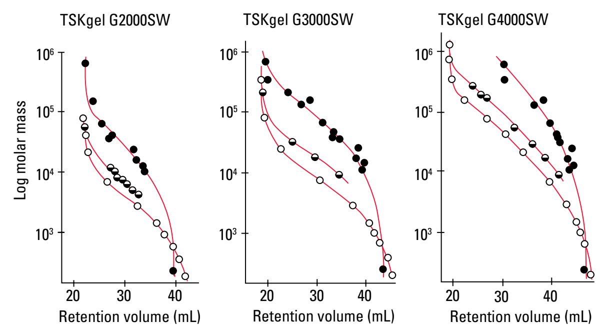
Attributes and Applications

Table 4 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 9 shows the calibration curves for globular proteins, polyethylene oxides and dextrans for each of the three TSKgel SW columns.

Table 4: 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 9: Calibration curves for globular proteins, polyethylene oxides and dextrans for TSKgel SW columns

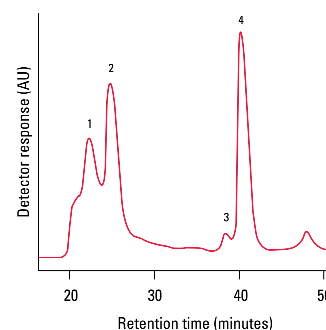


Column:	**TSKgel SW columns, 7.5 mm ID × 60 cm × 2**
Mobile phase:	dextrans and polyethylene oxides: distilled water; proteins: 0.3 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.0
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm and RI
Samples:	● proteins, ○ polyethylene oxides, ◒ dextrans

Separation of *E. coli* RNA

Separation of four *E. coli* RNAs, shown in Figure 10, 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 10: Separation of total E. coli RNA

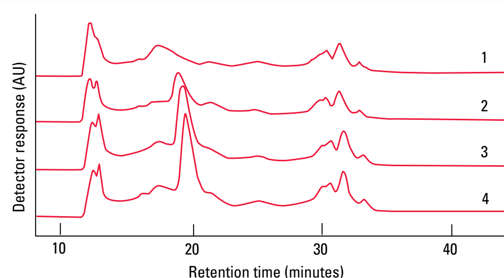


Columns:	**TSKgel G4000SW, 13 µm, 7.5 mm ID × 30 cm × 2** **TSKgel G4000SW, 17µm, 7.5 mm ID × 30 cm × 2**
Mobile phase:	0.13 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.0, + 1 mmol/L EDTA
Flow rate:	1.0 mL/min
Detection:	UV @ 260 nm
Injection vol.:	5 µg
Sample:	0.1 mL of 1:10 diluted solution of total *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 11 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 11: Analysis of membrane protein with differing surfactant concentrations in the mobile phase

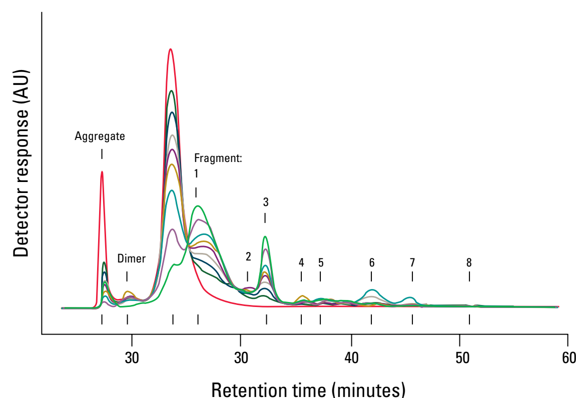


Column:	**TSKgel G3000SW, 10 µm, 7.5 mm ID × 60 cm**
Mobile phase:	(0.2 mol/L sodium chloride + 20% glycerol + octaethylene glycol dodecylether) in 50 mmol/L phosphate buffer, pH 7.0 Note: concentration of surfactant: 1. 0.005% 2. 0.01% 3. 0.025% 4. 0.05%
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
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 12).

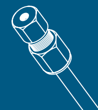
Figure 12: Tracking changes over time



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

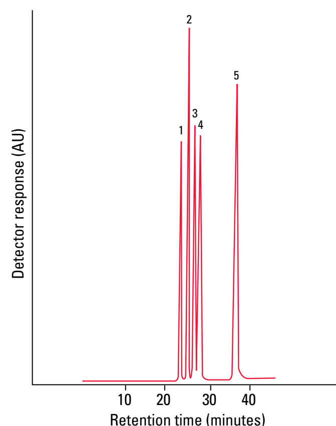
1. a) T. Tomono, T. Suzuki, and E. Tokunaga, Anal, Biochem., 123, 394 (1982)
b) T. Tomono, T.Suzuki, and E. Tokunaga, Bio, Bio. Phys. Acta., 660, 186 (1981)



Nucleic Acid

Figure 13 shows the separation of nucleic acid bases and nucleosides using a TSKgel G2000SW column.

Figure 13: Separation of nucleic acid

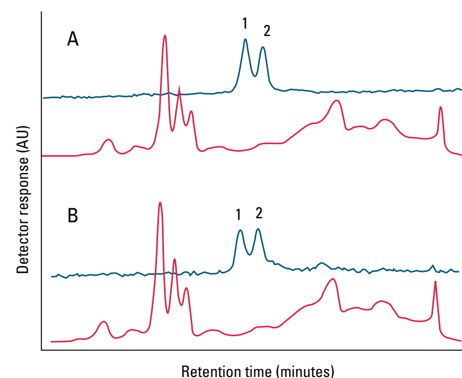


Column:	**TSKgel G2000SW, 10 µm, 7.5 mm ID × 60 cm**
Mobile phase:	acetic acid/triethylamine/H_2O = 3/3/94
Flow rate:	0.74 mL/min
Detection:	UV @ 260 nm
Samples:	1. uridine
	2. uracil
	3. thymine
	4. adenosine
	5. adenine

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 14.

Figure 14: Analysis of metallothionein



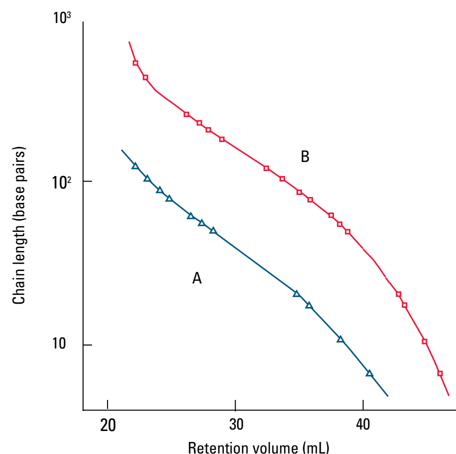
Column:	**TSKgel G3000SW, 13 µm, 21.5 mm ID × 60 cm**
Mobile phase:	50 mmol/L Tris-HCl buffer
Detection:	atomic absorption (Cd, Zn) + UV @ 280 nm
	A: Cd
	B: Zn
Samples*:	rat liver supernatant
	1.metallothionein I
	2.metallothionein II

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

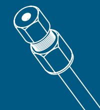
DNA Fragments

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 15.

Figure 15: 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.

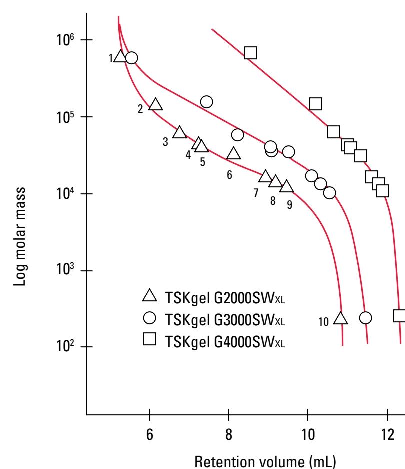
Attributes and Applications

Table 5 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 16 shows the calibration curves for globular proteins, polyethylene oxides, and dextrans for each of the three TSKgel SWXL columns.

Table 5: 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 16: Calibration curves for proteins for TSKgel SWXL columns

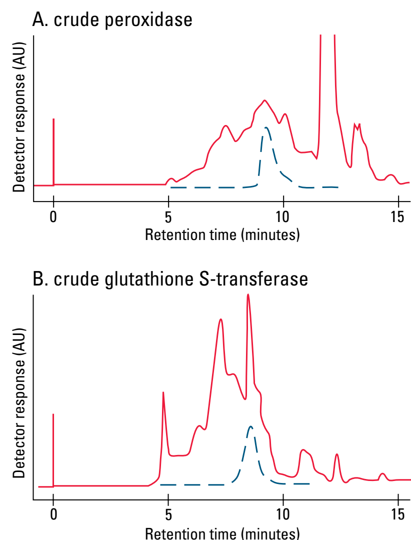


Column:	**TSKgel SWXL columns, 7.8 mm ID × 30 cm**
Mobile phase:	0.3 mol/L NaCl in 0.1 mol/L sodium phosphate buffer, pH 7.0
Detection:	UV @ 220 nm
Samples:	1. thyroglobulin (6.6 × 10^5 Da) 2. IgG (1.56 × 10^5 Da) 3. bovine serum albumin (6.7 × 10^4 Da) 4. ovalbumin (4.3 × 10^4 Da) 5. peroxidase (4.02 × 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)

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 17A) and glutathione S-transferase (Figure 17B) 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 17A and 17B: Analysis of crude protein samples

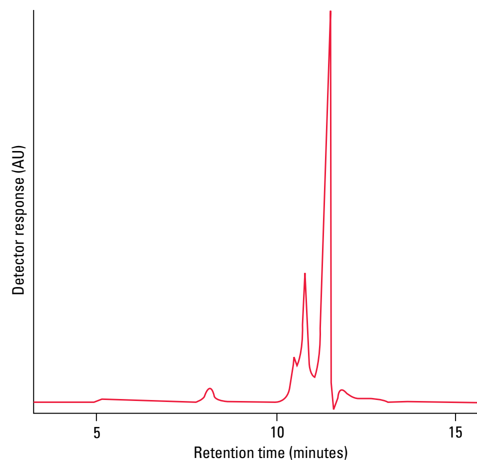


Column:	**TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.3 mol/L NaCl in 0.05 mol/L phosphate buffer, pH 7.0
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm (solid line) and enzyme assay tests (dashed line)
Recovery:	enzymatic activity recovered was 98% in A and 89% in B
Samples:	A. crude peroxidase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

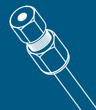
Rat Liver Extract

The separation of a crude extract of rat liver using a TSKgel G2000SWXL column is displayed in Figure 18.

Figure 18: Separation of crude extract of rat liver (10 µL)



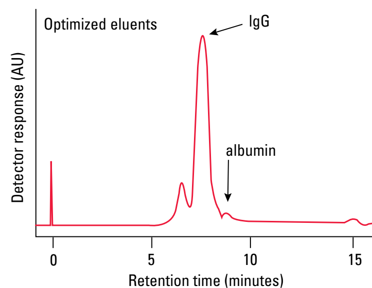
Column:	**TSKgel G2000SWXL, 5 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.05 mol/L phosphate buffer, pH 7.0 + 0.3 mol/L NaCl
Flow rate:	1 mL/min
Detection:	UV @ 220 nm
Temperature:	25 °C



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 19, 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 19: QC test for albumin

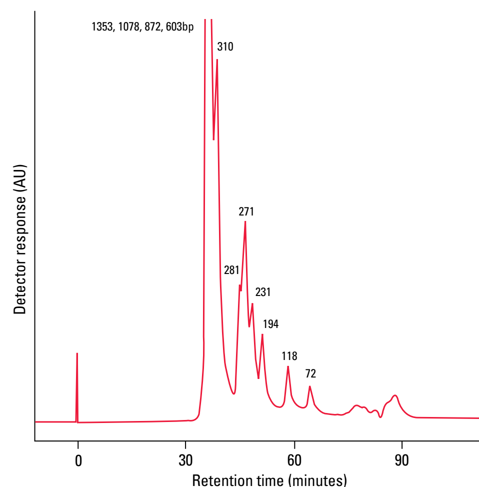


Column:	**TSKgel G3000SWXL, 5 µm, 7.8 mm ID x 30 cm**
Mobile phase:	0.1 mol/L Na_2SO_4 in 0.05 mol/L sodium phosphate buffer, pH 5.0
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Sample:	5 µL of venilon, containing 237.5 mg of IgG and 12.5 mg of albumin

DNA Digest

Figure 20 shows the separation of øX174 RF DNA-Hae III digest using a TSKgel G4000SWXL column.

Figure 20: Separation of ϕX174 RF DNA-Hae III digest (4.5 µg/50 µL)

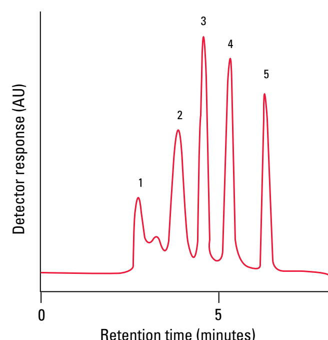


Column:	**TSKgel G4000SWXL, 8 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.05 mol/L phosphate buffer, pH 7.0 + 0.3 mol/L NaCl + 1 mmol/L EDTA
Flow rate:	0.15 mL/min
Detection:	UV @ 260 nm
Temperature:	25 °C

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 21).

Figure 21: Analysis of various proteins



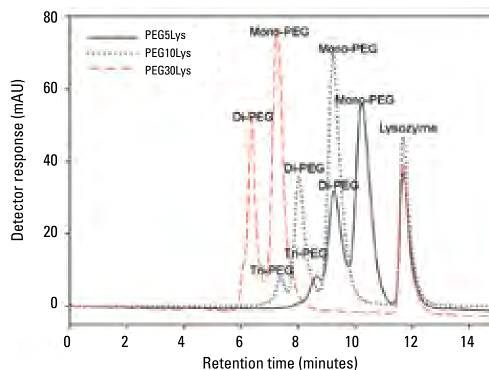
Column:	**TSKgel QC-PAK 300GL, 5 µm, 8 mm ID × 15 cm**
Mobile phase:	0.1 mol/L Na_2SO_4 in 0.1 mol/L phosphate buffer, pH 7.0 and 0.05% NaN_3
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Samples:	1. thyroglobulin 2. IgG 3. ovalbumin 4. ribonuclease 5. p-aminobenzoic acid

Characterization Studies of PEGylated Lysozyme

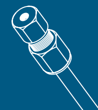
Chemical modification of therapeutic proteins in order to enhance their biological activity is of increasing interest. One of the most frequently used protein modification methods, PEGylation, changes the biochemical and physicochemical properties of the protein, which can result in several important 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 22. 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 22: SEC analysis of reaction mixtures



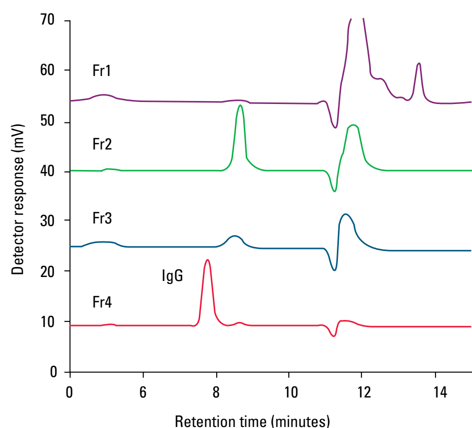
Column:	**TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.1 mol/L phosphate buffer, 0.1 mol/L Na_2SO_4, pH 6.7
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Injection vol.:	20 µL
Sample:	5, 10, 30 kDa methoxy PEG aldehyde



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 23 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 23: 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 SW_{XL} 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 7 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

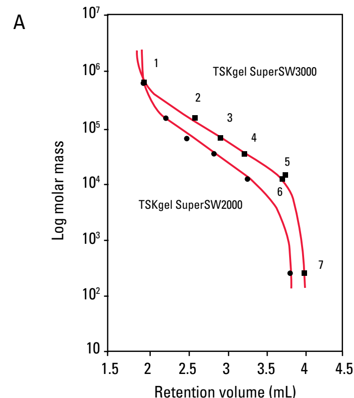
Table 6 shows a summary of the product attributes for each of the TSKgel SuperSW columns. The 12.5 nm pore size of the TSKgel SuperSW2000 columns results in a fractionation range up to 1.5×10^5 Da for globular proteins. The TSKgel SuperSW3000 columns have a fractionation range up to 5.0×10^5 Da for globular 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 24A and 24B show the calibration curves for protein, polyethylene oxides and glycols for the TSKgel SuperSW columns.

Table 6: 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)

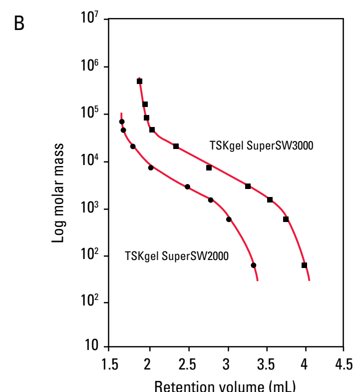
Figure 24A and 24B: Calibration curves for proteins and polyethylene oxides and glycols for TSKgel SuperSW columns

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)

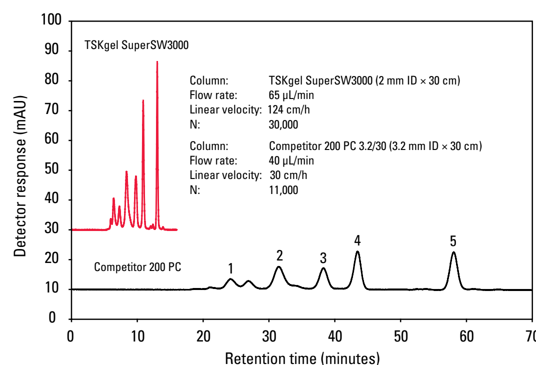
Table 7: Operating instructions when using TSKgel SuperSW columns

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 25 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 25: Analysis of standard proteins at low level concentrations



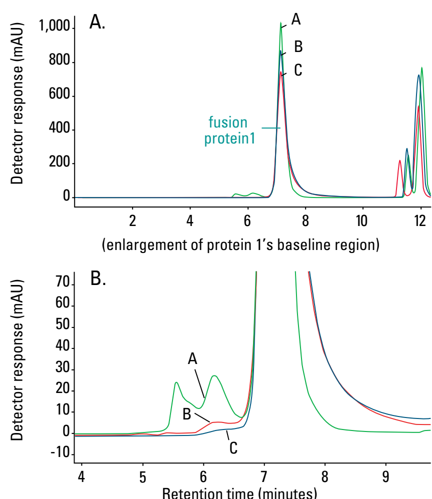
Columns: **TSKgel SuperSW3000, 4 μm, 2 mm ID × 30 cm**
Competitor 200 PC 3.2/30, 13 μm, 3.2 mm ID × 30 cm
Mobile phase: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3, pH 6.7
Detection: UV @ 280 nm
Temperature: 25 °C
Injection vol.: 0.2 μL
Samples: 1. thyroglobulin (1.0 g/L)
2. β-globulin (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 26A, 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 26B 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 26A and 26B: Overlays of monoclonal antibody separation

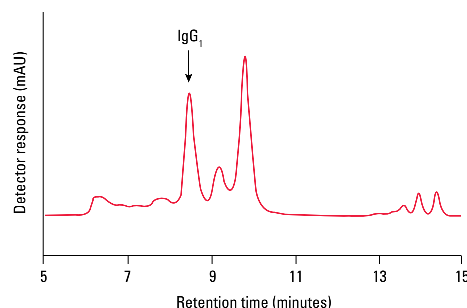


Column:	**TSKgel SuperSW3000, 4 µm, 4.6 mm ID × 30 cm**
Mobile phase:	A: 0.4 mol/L $NaClO_4$, 0.05 mol/L NaH_2PO_4, B: 0.2 mol/L $NaClO_4$, 0.05 mol/L NaH_2PO_4, C: 0.2 mol/L NaCl, 0.1 mol/L NaH_2PO_4
Flow rate:	0.35 mL/min
Detection:	UV @ 214 nm
Injection vol.:	5 µL
Samples:	monoclonal antibodies

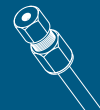
IgG_1

The TSKgel Super SW3000 provides an excellent high resolution separation of IgG_1 from mouse ascites fluid as can be seen in Figure 27.

Figure 27: Separation of monoclonal antibody



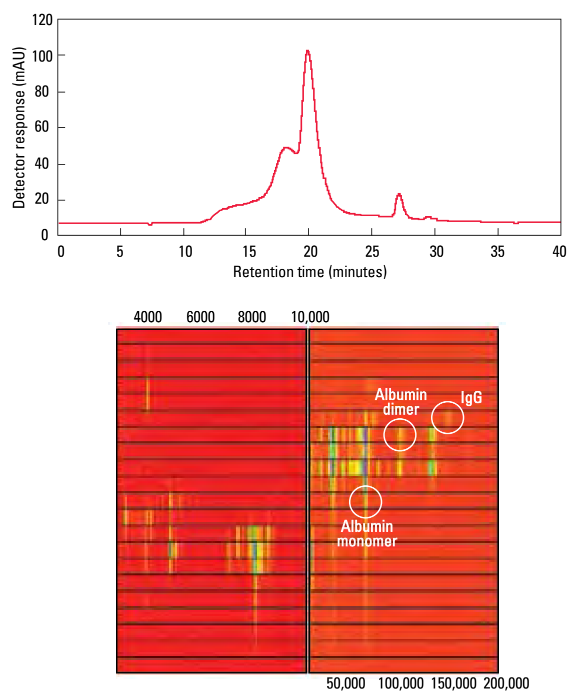
Column:	**TSKgel SuperSW3000, 4 µm, 4.6 mm ID × 30 cm**
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.7
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm, micro flow cell
Sample:	mouse ascites (5 µL)



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 28 demonstrates the applicability of TSKgel SuperSW3000 columns for the trace analysis of biological components by LC/MS analysis.

Figure 28: 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

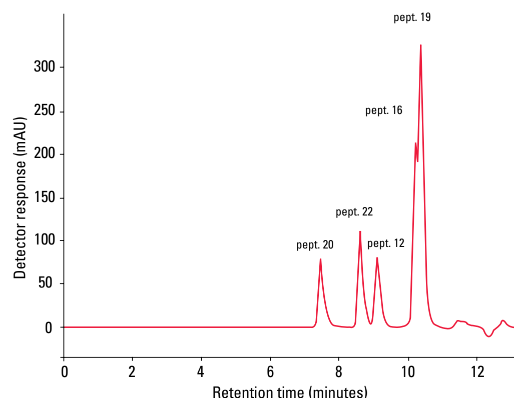
Fraction (1 mL) was directly loaded to SELDI chip H50. The chip was washed and desalted then applied to MS.

This data is courtesy of Dr. Majima, Protenova.

Peptide Mixture

Figure 29 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 29: 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 8 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.

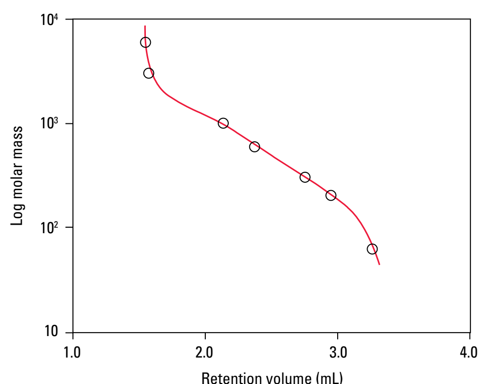
Table 8: Product attributes

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 30 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 30 can be obtained on the commercially available 4.6 mm ID × 15 cm and 10 mm ID × 15 cm TSKgel BioAssist DS columns.

Figure 30: Calibration curve of TSKgel BioAssist DS desalting columns



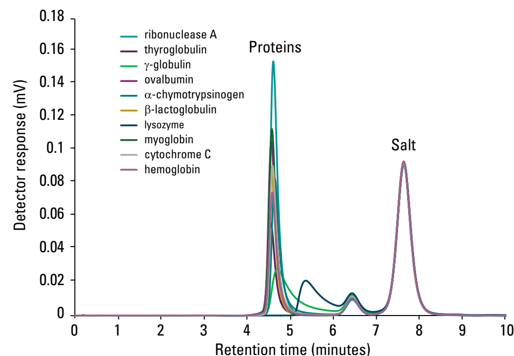
Column: **Custom TSKgel BioAssist DS column, 6 mm ID x 15 cm**
Mobile phase: distilled H_2O
Flow rate: 0.5 mL/min
Sample: ethylene glycol, PEGs

Desalting of Large Protein Sample Loads

Figure 31 demonstrates the rapid and reproducible desalting of a large number of proteins (see Table 9) 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 31: 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 9: Proteins

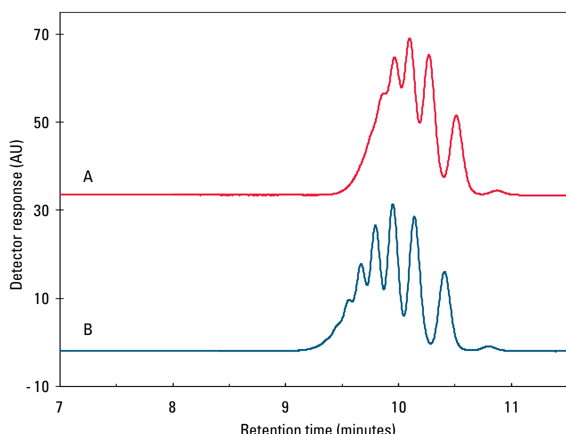
Protein	*MM* (kDa)	Concentration* (g/L approx.)
ribonuclease A	14.7	19.5
thyroglobulin	670	11.3
γ-globulin	150	14.5
ovalbumin	45	13.1
α-chymotrypsinogen	25.6	13.1
β-lactoglobulin	18.4	10.8
lysozyme	14.7	11.6
myoglobin	16.7	14.5
cytochrome C	12.3	11.0
hemoglobin	68	11.9

*in 100 mmol/L phosphate buffer, pH 6.7

Standard Polystyrene

Figure 97 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 97: Analysis of standard polystyrene

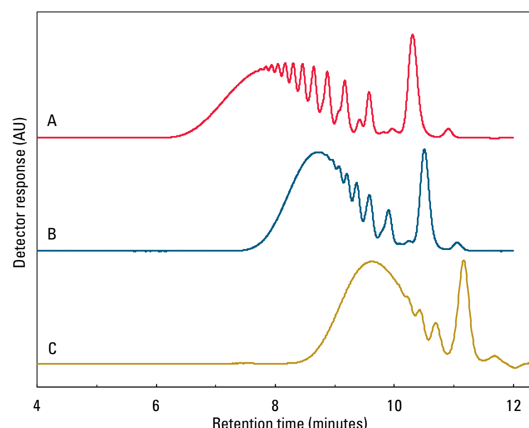


Columns:	**A. TSKgel SuperMultiporeHZ-M, 4 µm, 4.6 mm ID × 15 cm × 2**
	B. TSKgel SuperMultiporeHZ-N, 3 µm, 4.6 mm ID × 15 cm × 2
Mobile phase:	THF
Flow rate:	0.35 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Injection vol.:	5 µL
Sample:	standard polystyrene oligomer (TSKgel standard polystyrene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

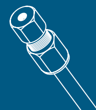
Epoxy Resin

Figure 98 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 98: Analysis of epoxy resin



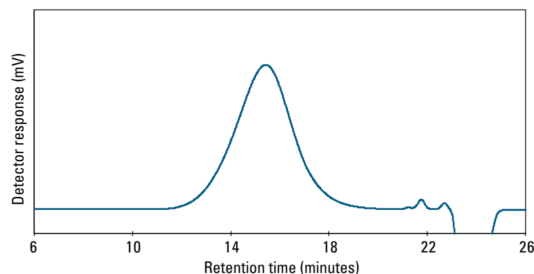
Columns:	**A. TSKgel SuperMultiporeHZ-N, 3 µm, 4.6 mm ID × 15 cm × 2**
	B. TSKgel SuperMultiporeHZ-M, 4 µm, 4.6 mm ID × 15 cm × 2
	C. TSKgel SuperMultiporeHZ-H, 6 µm, 4.6 mm ID × 15 cm × 2
Mobile phase:	THF
Flow rate:	0.35 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample: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



Polyvinylbutyral

The analysis of polyvinylbutyral using a TSKgel SuperMultiporeHZ-H column is show in Figure 99. A smooth chromatogram without any distortion is obtained.

Figure 99: 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)