About: TSKgel SW Series Size Exclusion Columns

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, 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, SWxL and SuperSW columns are stable from pH 2.0 to 7.5 and have excellent solvent stability up to 100% organic solvent. 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 μ m particles and offer the same resolution in half the time as the 30 cm, 10 μ m 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 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 G3000SWxL columns are the industry standard for quality control of monoclonal antibodies.
 - TSKgel SuperSW3000 columns are utilized when sample is limited or the components of interest are present at very low concentration.
- 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.



			Molar mass of samples		
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 × 10⁵	1,000 – 3 × 10 ⁴	500 – 1.5 x 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	$2 \times 10^4 - 7 \times 10^6$	4,000 – 5 × 10 ⁵	2,000 – 2.5 × 10 ⁵
	т	1			
G2000SWxL, BioAssist G2SWxL, QC-PAK GFC 200	5 µm	12.5 nm	5,000 – 1.5 × 10⁵	1,000 – 3 × 104	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 × 104
G4000SWxL, BioAssist G4SWxL	8 µm	45 nm	$2 \times 10^4 - 7 \times 10^6$	4,000 – 5 × 10⁵	2,000 – 2.5 x 10⁵
SuperSW2000	4 µm	12.5 nm	5,000 – 1.5 × 10⁵	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			
Data generated using the following conditions:					
Columns: TSKgel SuperSW columns in series, 4 µm, 4.6 mm ID × 30 cm × 2 TSKgel SWxL columns in series, 5 µm, 7.8 mm ID × 30 cm × 2					

Table 2: Properties and separation ranges of TSKgel SW, SWxL, SuperSW, and BioAssist DS columns

TSKgel SW columns in series, 10 μm, 7.5 mm ID × 60 cm × 2 Mobile phase: globular proteins: 0.3 mol/L NaCl in 0.1 mol/L (0.05 mol/L for TSKgel SWxL columns) phosphate buffer, pH 7.0; dextrans and polyethylene glycols and oxides (PEOs): distilled water



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 3 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 2 shows the calibration curves for globular proteins, polyethylene oxides and dextrans for each of the three TSKgel SW columns.

Table 3: 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 × 10⁵ Da (globular proteins)	1.0 × 10⁴ - 5.0 × 10⁵ Da (globular proteins)	2.0 × 10 ⁴ - 7.0 × 10 ⁶ Da (globular proteins)	





Separation of E. coli RNA

Separation of four *E. coli* RNAs, shown in Figure 3, 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 3: Separation of total E. coli RNA



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 4 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.





Degradation Products of IgG

High speed is important when analyzing the rate of chemical alteration of proteins (denaturation, condensation, degradation, etc.). Tomono et al¹ tracked the course of enzyme digestion of commercial IgG by pepsin using a TSKgel G3000SW column (Figure 5).

Figure 5: Tracking changes over time



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





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 SW packing material played a role in this isozyme separation. Representative chromatograms are shown in Figure 7.







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 8.

Figure 8: Double stranded DNA fragments





About: TSKgel SWxL Size Exclusion Columns

TSKgel SWxL columns, introduced in 1987, are packed with 5 or 8 μ m 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 μ m particles and offer the same resolution in half the time as the 30 cm, 10 μ m TSKgel G2000SW and G3000SW columns.

Attributes and Applications

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

Table 4: 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 × 10⁵ Da (globular proteins)	1.0 × 10 ⁴ - 5.0 × 10⁵ 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 SWxL columns





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 10A) and glutathione S-transferase (Figure 10B) 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 10A & 10B: Analysis of crude protein samples



Rat Liver Extract

The separation of a crude extract of rat liver using a TSKgel G2000SWxL column is displayed in Figure 11.

Figure 11: Separation of crude extract of rat liver (10 μ L)





lgG

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 12, 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 12: QC test for albumin



DNA Digest

Figure 13 shows the separation of øX174 RF DNA-Hae III digest using a TSKgel G4000SWxL column.

Figure 13: Separation of φX174 RF DNA-Hae III digest (4.5 μg/50 μL)





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 14).

Figure 14: Analysis of various proteins



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 G3000SW_{XL} column was used for the characterization of PEGylated lysozyme, as shown in Figure 15. 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 MM of PEGylated protein and the MM calculated from SEC. This result illustrates the strong effect that PEG has on the hydrodynamic radius of the resulting PEGylated protein.

Figure 15: SEC analysis of reaction mixtures



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 16 demonstrates the purity of an antibody from a cell culture supernatant (Anti TSH). The chromatograms represent the fractions collected from a HIC purification step.







About: TSKgel SuperSW Size Exclusion Columns

TSKgel SuperSW columns, introduced in 1997, contain smaller particles than TSKgel SWxL columns; 4 μ m versus 5 μ m. 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 6 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 5 shows a summary of the product attributes for each of the TSKgel SuperSW columns. The 125 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 250 nm pore size. Since both columns have a 4.6 mm inner diameter, they are ideal for sample-limited applications. Figure 17 shows the calibration curves for protein, polyethylene oxides and glycols for the TSKgel SuperSW columns.

Table 5: 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	2.5-7.5	
Calibration range	5,000 - 1.5 × 10⁵ Da (globular proteins)	1.0 × 10⁴ - 5.0 × 10⁵ Da (globular proteins)	

Figure 17A and 17B: Calibration curves for proteins and polyethylene oxides and glycols for TSKgel SuperSW columns





Table 6: 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 18 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 18: Analysis of standard proteins at low level concentrations



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 requalification 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 19A, 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 19B 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 NaClO₄.





lgG₁

The TSKgel Super SW3000 provides an excellent high resolution separation of IgG_1 from mouse ascites fluid as can be seen in Figure 20.





TOSOH BIOSCIENCE

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





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 22 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 22: Analysis of peptides





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 μ m 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 μ m beads.

*US patent number 7,659,348

Attributes and Applications

Table 7 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	7.	Prod	luct	attri	hutes
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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 23 shows the calibration curve of a 6 mm ID \times 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 23 can be obtained on the commercially available 4.6 mm \times 15 cm and 10 mm ID \times 15 cm TSKgel BioAssist DS columns.

Figure 23: Calibration curve of TSKgel BioAssist DS desalting columns



Desalting of Large Protein Sample Loads

Figure 24 demonstrates the rapid and reproducible desalting of a large number of proteins (see Table 8) at semipreparative scale using a TSKgel BioAssist DS, 10 mm ID \times 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 \times 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 24: Desalting of proteins



Table 8: Proteins

Protein	<i>MM</i> (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