TOSOH

TSKgel® SW-type Column Guide

For Size Exclusion HPLC

Setting the standard in SEC since 1977,
with new columns optimized for your applications.

Please contact your Fisher Scientific Representative for more information.

Fisher Scientific

About Tosoh Bioscience

Tosoh Bioscience LLC is a major supplier of chromatography products. Located in King of Prussia, PA, Tosoh Bioscience provides sales and service to pharmaceutical and biotechnology customers in North and South America. European operations are in Griesheim, Germany, while Asia is served by Tosoh Corporation in Tokyo, Japan, Shanghai, China and Singapore.

Tosoh's portfolio of over 500 specialty products encompasses all common modes of liquid chromatography, and coupled with our expertise, we can help you purify any protein, peptide, enzyme, nucleic acid, antibiotic, or small molecule. In addition to our extensive line of analytical HPLC columns, Tosoh provides bulk chromatographic resins for the purification of biopharmaceutical drugs in commercial manufacturing processes. Our TOYOPEARL® and TSKgel® chromatographic resins and TSKgel columns are renowned for their quality and reliability. The EcoSEC® GPC system, introduced in the U.S. in 2008 and a market leader in Japan for more than thirty years, is a top-of-the-line dedicated gel permeation chromatography instrument for the analysis of polymers in organic and aqueous/organic solvents.

TSKgel SW-type columns are the industry standard for size exclusion chromatography of proteins. Our expertise in size exclusion chromatography is based on a fundamental understanding of the role played by pore diameter and molecular size in chromatographic separations. This knowledge, coupled with a thorough understanding of transport phenomena and wide-ranging experience in polymer chemistry and surface modifications, allows us to design higher performance polymeric resins for other modes of chromatography, including ion exchange, hydrophobic interaction, affinity, and reversed phase.

Tosoh Bioscience: More than SEC

This Product Guide will introduce you to our world-renowned line of TSKgel SW-type columns for size exclusion chromatography. In addition to SEC columns, Tosoh Bioscience is a leader in all modes of HPLC! Visit us at www.tosohbioscience.com to learn more about our TSKgel columns for the following modes of chromatography:

Affinity

Three group-specific ligands and one chemically active functionality

Hydrophobic Interaction (HIC)

Three ligands with high to low hydrophobicity

Ion Exchange

Wide variety of columns for both anion and cation exchange

Normal Phase/HILIC

Amide and amino-bonded column choices for retention of polar compounds

Reversed Phase

14 distinct columns based on methacrylate and silica particles

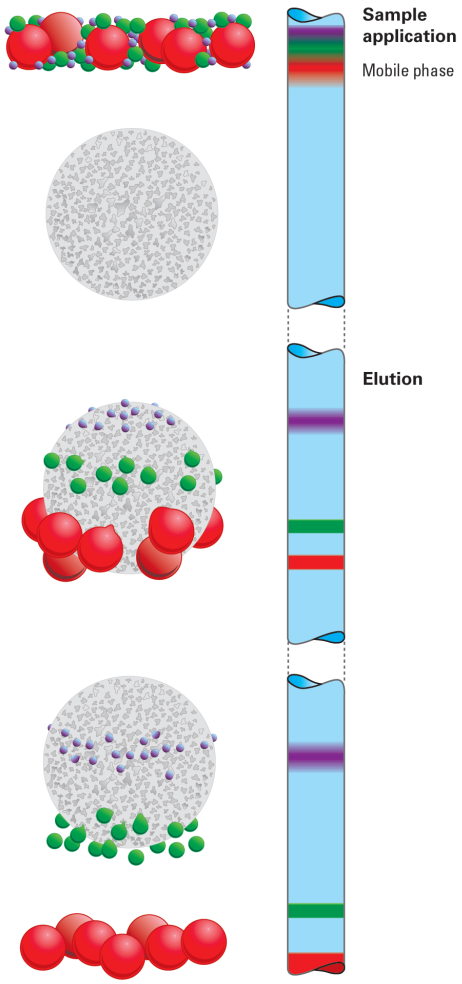
What is Size Exclusion Chromatography?

Size exclusion chromatography (SEC) is the general name for the separation mode based on the size of the molecule, or its hydrodynamic volume. SEC is based on the discrimination of individual sample components by the pores of the packing material. It is the dominant mode of separation for proteins and polymers. Large sample molecules cannot or can only partially penetrate the pores, whereas smaller molecules can access all or a larger number of pores.

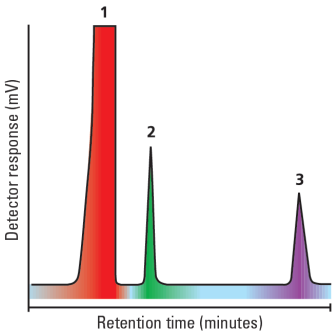
In SEC, large molecules elute from the column first, smaller molecules elute later, and the smallest molecules that can access all the pores elute last from the column. Size exclusion chromatography is the only mode of chromatography that does not involve interaction with a stationary phase by means of adsorption or partitioning of the solutes.

GFC and GPC are used interchangeably as terms for size exclusion chromatography. GFC stands for Gel Filtration Chromatography, the term most widely used by chemists when working with proteins, and GPC is Gel Permeation Chromatography, a method for working with polymers soluble in polar organic or organic solvents. Tosoh Bioscience carries TSKgel size exclusion columns for both GFC and GPC.

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.

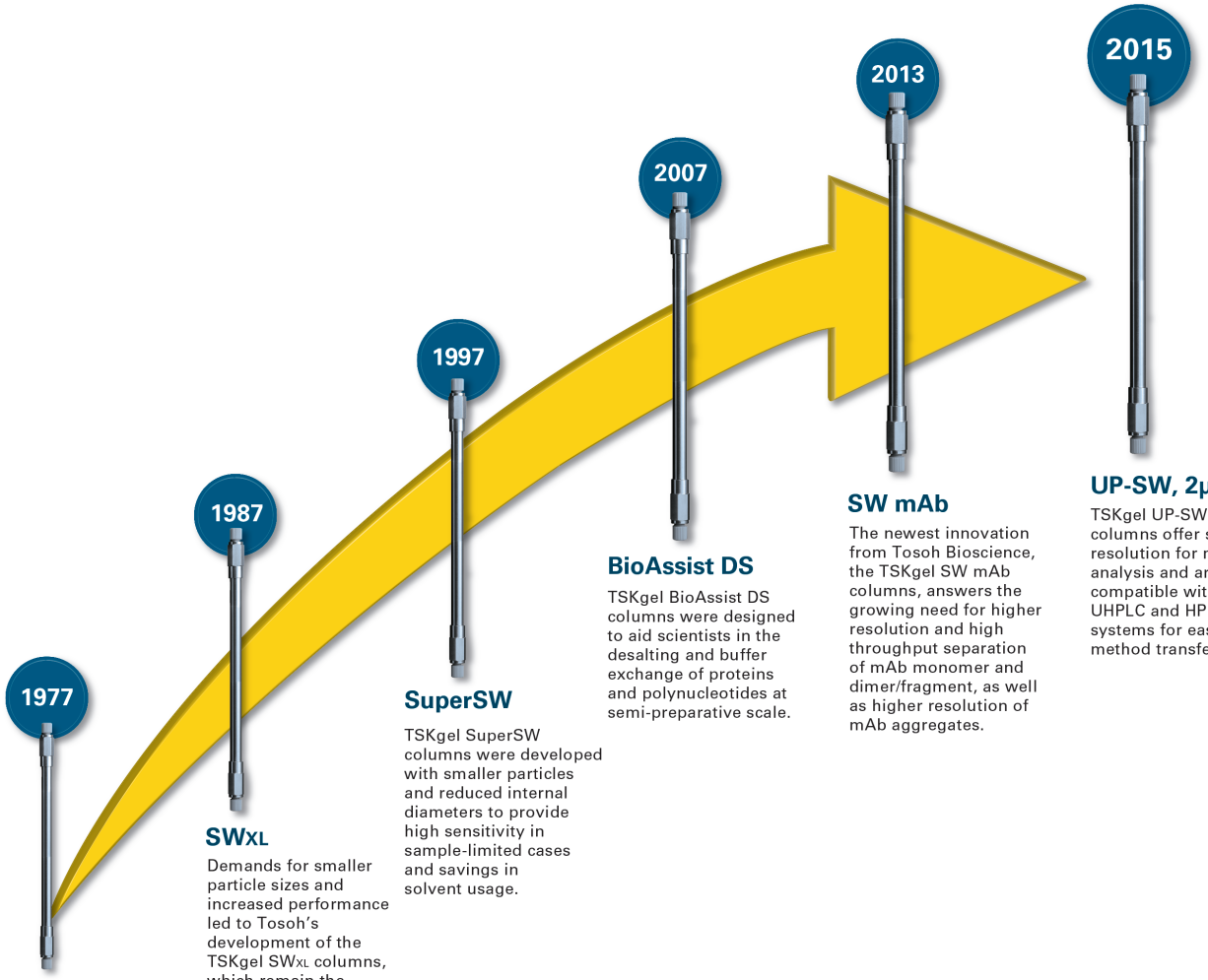


typical chromatogram



TSKgel SEC SW-type Column History and Overview

For more than thirty five years, Tosoh Bioscience has been the world leader in the analysis and purification of proteins with our TSKgel SW-type columns. More than **100,000** TSKgel SW-type columns have been used in U.S. laboratories over the last ten years. With the latest addition of the TSKgel UP-SW3000 columns to the TSKgel SW-type column line, we are confident scientists will continue to prefer these columns for protein analysis.



SW

The first TSKgel SW columns were developed by Tosoh Bioscience for the analysis of biomolecules.

SWXL

Demands for smaller particle sizes and increased performance led to Tosoh's development of the TSKgel SWXL columns, which remain the leaders in new SEC method development.

SuperSW

TSKgel SuperSW columns were developed with smaller particles and reduced internal diameters to provide high sensitivity in sample-limited cases and savings in solvent usage.

BioAssist DS

TSKgel BioAssist DS columns were designed to aid scientists in the desalting and buffer exchange of proteins and polynucleotides at semi-preparative scale.

SW mAb

The newest innovation from Tosoh Bioscience, the TSKgel SW mAb columns, answers the growing need for higher resolution and high throughput separation of mAb monomer and dimer/fragment, as well as higher resolution of mAb aggregates.

UP-SW, 2µm

TSKgel UP-SW3000 columns offer superior resolution for mAb analysis and are compatible with both UHPLC and HPLC systems for ease of method transfer.

TSKgel SW-type Columns Include:

- **TSKgel UP-SW3000**
- **TSKgel SW mAb**
- **TSKgel SWXL**
- **TSKgel SuperSW**
- **TSKgel SW**
- **TSKgel BioAssist DS**

The nomenclature of the TSKgel SW-type columns are simple:

S: stands for silica based material
W: stands for water-loving

Every column in the TSKgel SW-type line is **silica based** and can be used in **100% aqueous conditions**. The hydrophilic diol-type bonded phase shields the silica surface from interacting with protein samples. Because they are silica based, the optimum pH range for use is 2.5 - 7.5.

TSKgel SW-type SEC columns stand out from competitive columns because they contain a large pore volume per unit column volume. By having a large pore volume per unit column volume, they provide ultimate separation for proteins of different sizes with higher molar mass selectivity, better resolution, and more well-defined peaks.

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 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 TSKgel SW mAb column line consists of three specialized columns designed for the separation and analysis of monoclonal antibodies (mAb). 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 UltraSW Aggregate column is a smaller particle size, 3 µm, and offers high resolution separation of mAb multimers and aggregates.

The original TSKgel SW columns are well established in thousands of methods worldwide and provide excellent, highly reproducible results. These columns are 10, 13 and 17 µm particle size, and are available in stainless steel and glass hardware. In addition, the TSKgel SW columns are available in a 60 cm length for higher resolution and in semi-prep dimensions (21.5 mm ID x 30 cm and 60 cm).

While the TSKgel SW columns continue to perform in their established methods, the TSKgel SWXL columns offer improved sample resolution due to a smaller 5 and 8 µm particle size. Analysis time can be reduced with the use of the 15 cm TSKgel QC-PAK SWXL columns. In addition to stainless steel, TSKgel SWXL columns are available in PEEK housing to reduce sample adsorption to stainless steel.

TSKgel SuperSW columns, the third generation of Tosoh’s TSKgel SW-type columns, contain 4 µm particles. These columns have an internal diameter of 4.6 mm ID to provide higher sensitivity in sample-limited cases and to cut down on solvent use.

The TSKgel BioAssist DS columns are designed for the desalting and buffer exchange of proteins and polynucleotides at semi-preparative scale.

TSKgel SW-type Columns

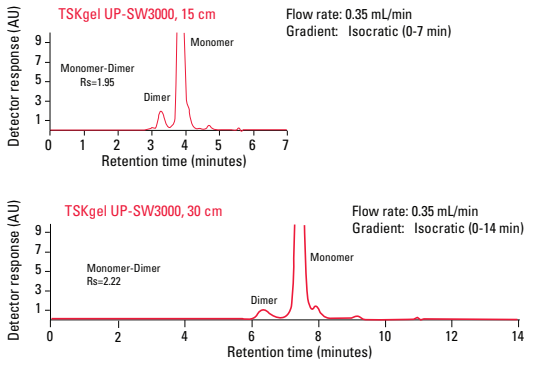
			Molar mass of samples		
TSKgel column	**Particle size**	**Pore size**	**Globular proteins**	**Dextrans**	**Polyethylene glycols & oxides**
G2000SW	10 µm and 13 µm	12.5 nm	5,000 – 1.5 × 10^5	1,000 – 3 × 10^4	500 – 1.5 × 10^4
G3000SW	10 µm and 13 µm	25 nm	1 × 10^4 – 5 × 10^5	2,000 – 7 × 10^4	1,000 – 3.5 × 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 x 10^4 - 5 x 10^5	–	–
SuperSW mAb HTP	4 µm	25 nm	1 x 10^4 - 5 x 10^5	–	–
UltraSW Aggregate	3 µm	30 nm	1 x 10^4 - 2 x 10^6	–	–
UP-SW3000	2 µm	25 nm	1 x 10^4 - 5 x 10^5	–	–

TSKgel UP-SW3000 Columns: In Action

TSKgel UP-SW3000, 2 µm 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. These columns are designed to be operated with a simple and well established method (sodium phosphate mobile phase, pH 6.8) and are packed for use in both HPLC and UHPLC systems.

Figure 1 shows the separation comparison data for mAb between a 30 cm TSKgel UP-SW3000 column and a 15 cm length. Both columns were 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. 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 1: Comparison of mAb aggregates analysis

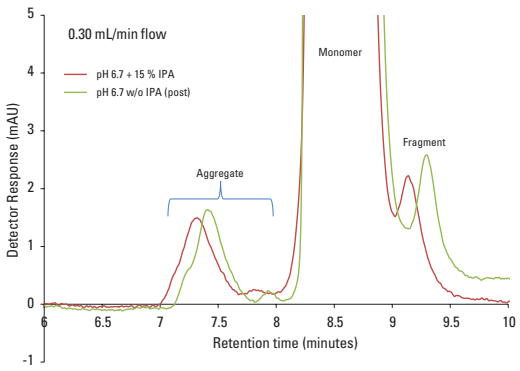


Nonspecific absorption of antibodies onto a column gel matrix poses a challenge, with some newly engineered antibodies possessing a high degree of hydrophobicity. The use of organic solvents such as isopropyl alcohol (IPA) or salts can decrease this interaction as reported by many scientists. However, the additives may alter the diffusion of these molecules, which results in retention time shift and poor peak resolution that did not occur in a typical aqueous buffer system, such as sodium phosphate buffer at neutral pH.

A TSKgel UP-SW3000, 30 cm column was used for analyzing mAbs with the addition of 15% IPA in sodium phosphate buffer, pH 6.7. As demonstrated in Figure 2, peak resolution and retention time shift were not impacted. The figure shows 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.

At 0.3 mL/min, the pressure of the column is slightly higher when IPA is added to the mobile phase compared to when the column is operated without IPA. However, the pressure 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 2: Separation of USP mAb Standard with Sodium Phosphate Buffer, pH 6.7, with and without 15% IPA Added



TSKgel SW mAb Columns: In Action

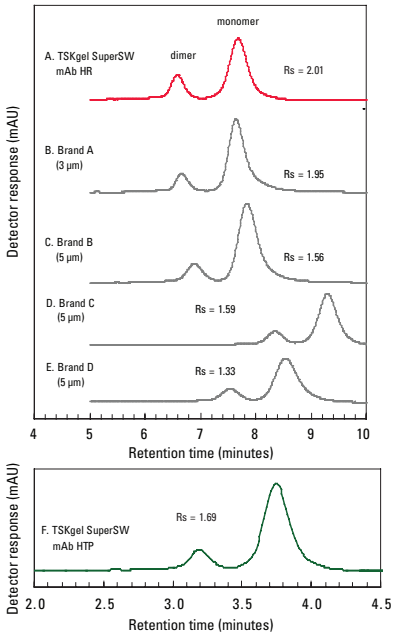
TSKgel SW mAb columns meet the growing demand for the higher resolution and high throughput separation of monoclonal antibody monomer and dimer/fragment, as well as higher resolution of mAb aggregates. TSKgel SuperSW mAb HR and SuperSW mAb HTP both contain 4 µm particles. The HR designation represents high resolution while the HTP stands for "high throughput" due to the smaller dimensions (4.6 mm ID × 15 cm). The TSKgel UltraSW Aggregate column is a smaller particle size, 3 µm, and offers high resolution separation of mAb multimers and aggregates.

Figure 3 shows the superior resolution of the TSKgel SuperSW mAb HR and the TSKgel SuperSW mAb HTP columns compared to four competitive columns in the analysis of a mAb monomer and dimer. TSKgel SuperSW mAb HR shows superior resolution of gamma-globulin dimer and monomer, while TSKgel SuperSW mAb HTP separates the gamma-globulin dimer and monomer in half the analysis time of the conventional columns.

The TSKgel SuperSW mAb HR utilizes a unique pore-controlled technology which produces a shallow calibration curve in the molecular weight region of a typical monoclonal antibody, resulting in high resolution separations. The TSKgel SuperSW mAb HTP is designed with the same pore controlled technology as the TSKgel SuperSW mAb HR column and is ideal for fast and efficient run times.

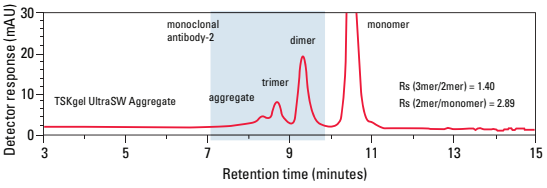
Figure 4 shows the analysis of a mouse-human chimeric IgG using the TSKgel UltraSW Aggregate column. Superior resolution of the mAb trimer and dimer is obtained. The smaller particle size (3 µm) and higher molecular weight exclusion limit (2,500 kDa, globular proteins) of the TSKgel UltraSW Aggregate column, compared to the TSKgel SuperSW mAb HR and HTP columns, allows for high resolution separation of mAb multimers and aggregates.

Figure 3: Comparison of resolution of mAb dimer and monomer



Column:	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 F. TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm
Mobile phase:	200 mmol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate:	A-E. 1.0 mL/min; F. 0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	A-E. 10 µL; F. 3.5 µL
Samples:	IgG (human polyclonal) (A-E: 1.0 g/L, F: 4.5 g/L)

Figure 4: Separation of a therapeutic mAb



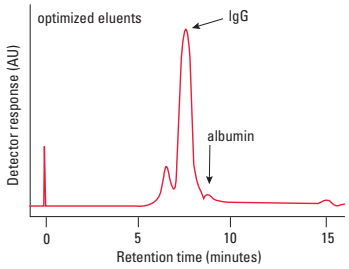
Column:	TSKgel UltraSW Aggregate, 3 µm, 7.8 mm ID × 30 cm
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate:	0.8 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Sample:	monoclonal antibody-2 (mouse-human chimeric IgG, *Erbitux*), 10 µL

TSKgel SWXL Columns: In Action

TSKgel SWXL columns are commonly used in the quality control of monoclonal antibodies and other biopharmaceutical products. The TSKgel SWXL column line consists of stainless steel columns in 30 cm length, QC-PAK columns in 15 cm length, for faster analysis, and BioAssist columns in PEEK housing material.

A therapeutic solution of intravenous IgG may contain albumin as a stabilizer, and both proteins must be quantified following manufacture. A method developed on a TSKgel G3000SWXL column provides quantitative separation of the two proteins in 15 minutes with an isocratic elution system. As shown in Figure 5, 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.

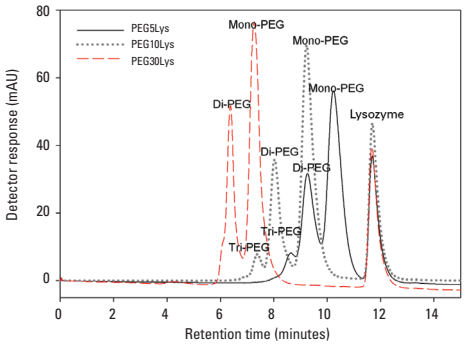
Figure 5: QC test for albumin



Column:	TSKgel G3000SWXL, 5 µm, 7.8 mm × 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

A TSKgel G3000SWXL column was used for the characterization of PEGylated lysozyme, as shown in Figure 6. 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.

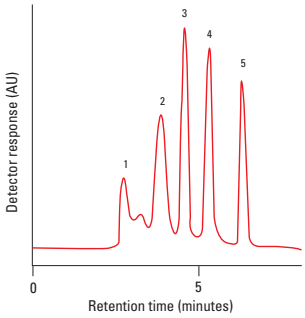
Figure 6: SEC analysis of reaction mixtures



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

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, as shown in Figure 7.

Figure 7: Analysis of various proteins



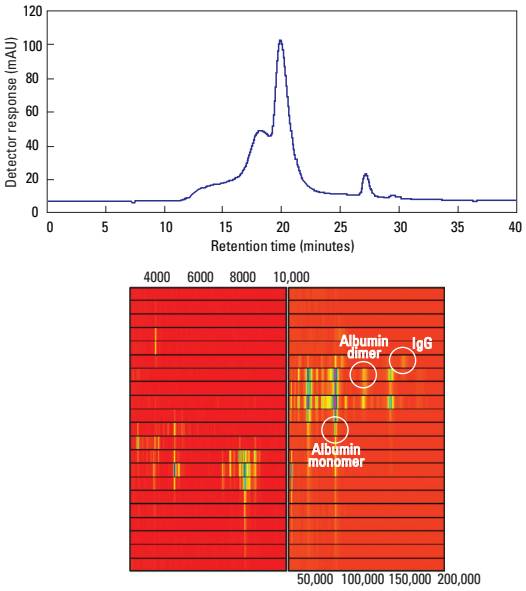
Column:	TSKgel QC-PAK 300GL, 5 µm, 8 mm × 15 cm
Mobile phase:	0.1 mol/L Na_2SO_4 in 0.1 mol/L phosphate buffer, pH 7 and 0.05% NaN_3
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Samples:	1. thyroglobulin 2. IgG 3. ovalbumin 4. ribonuclease 5. p-aminobenzoic acid

TSKgel SuperSW Columns: In Action

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.

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

Figure 8: Analysis of proteins in human serum



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

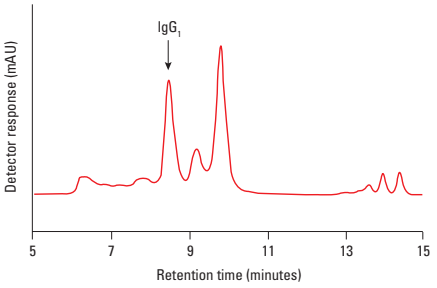
Column:	TSKgel SuperSW3000, 1 mm ID × 30 cm
Mobile phase:	50 mmol/L NaH_2PO_4 + 0.5 mol/L NaCl, pH 7.0
Flow rate:	8 µL/min
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	human serum (× 10), 1 µL

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.

The TSKgel SuperSW3000 provides an excellent high resolution separation of IgG_1 from mouse ascites fluid, as can be seen in Figure 9.

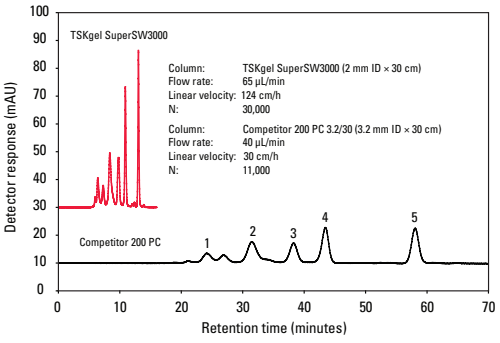
Figure 9: Separation of monoclonal antibody



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)

Figure 10 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 10: 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)

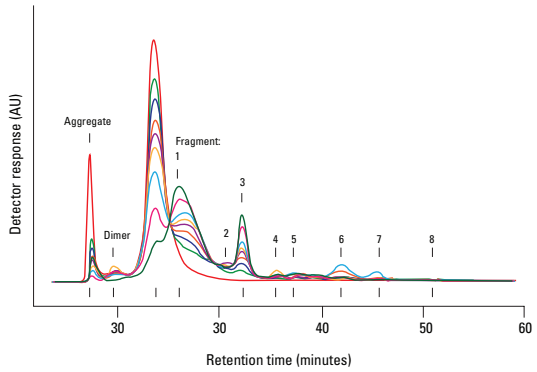
TSKgel SW Columns: In Action

The TSKgel SW column line features 10 or 13 µm particle sizes for improved sample capacity and are available in stainless steel and glass hardware. Additionally, the TSKgel SW columns are available in a 60 cm length for higher resolution and in semi-prep dimensions (21.5 mm ID × 30 cm and 60 cm).

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 11).

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 12.

Figure 11: Tracking changes over time

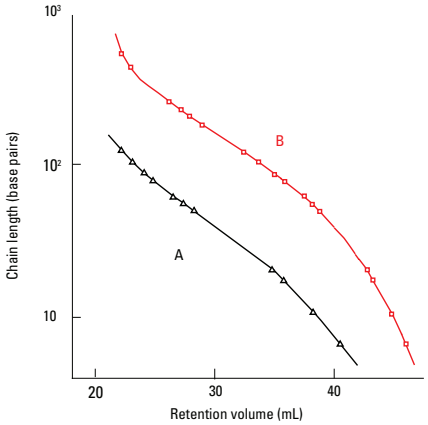


Column:	TSKgel G3000SW, 10 µm, 7.5 mm ID × 60 cm
Mobile phase:	0.1 mol/L acetate buffer, pH 5.0 + 0.1 mol/L sodium sulfate
Samples*:	100 µL solutions produced by digestion of IgG (20 g/L) by pepsin after 0, 2, 4, 6, 8, 10, 15, 30 and 60 minutes

*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)

Figure 12: Double stranded DNA fragments



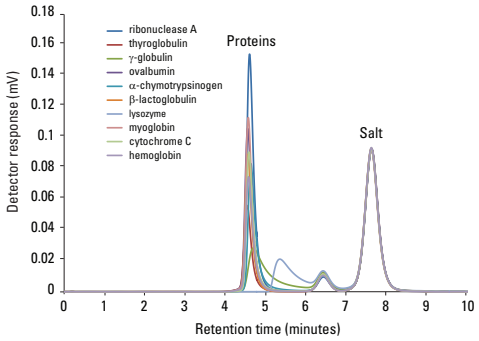
Columns:	A: TSKgel G3000SW, 10 µm, 7.5 mm ID × 60 cm × 2 B: TSKgel G4000SW, 13 µm, 7.5 mm ID × 60 cm × 2
Mobile phase:	0.05 mol/L Tris-HCl, 0.2 mol/L NaCl, 1 mmol/L EDTA, pH 7.5
Flow rate:	A: 1 mL/min, B: 0.33 mL/min
Detection:	UV @ 260 nm
Temperature:	25 °C
Sample:	Hae III-cleaved pBR322 DNA
Sample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

TSKgel BioAssist DS Columns: In Action

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. Packed with 15 µm polyacrylamide beads in PEEK hardware, these columns show excellent desalting performance.

Figure 13 demonstrates the rapid and reproducible desalting of a large number of proteins 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.

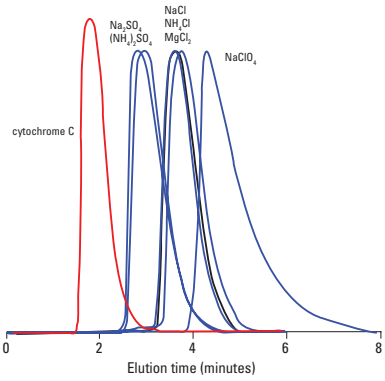
Figure 13: Desalting of proteins



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 mpl/L phosphate buffer, pH 6.7
Flow rate:	0.8 mL/min (4.6 mm ID) and 1.0 mL/min (10.0 mm ID)
Detection:	UV @ 80 nm and RI
Temperature:	ambient
Injection vol.:	10 µL

Figure 14 demonstrates the separation of mobile phase additives such as salts, surfactants, and denaturants. These additives are used for protein stabilization during different stages of the protein purification process and which need to be removed at the final stage of the process.

Figure 14: Elution profiles of high salt concentration



Column:	TSKgel BioAssist DS, 10 mm ID × 15 cm, PEEK
Mobile phase:	DI H_2O
Flow rate:	3.0 mL/min
Temperature:	4 °C
Injection vol.:	2 mL
Each salt concentration:	0.5 mol/L

TSKgel SW-type Column Selection

With all of these great choices, it can be difficult to select the right TSKgel SW-type column for your application. Outlined below are a few recommendations and factors to consider when selecting a TSKgel size exclusion column.

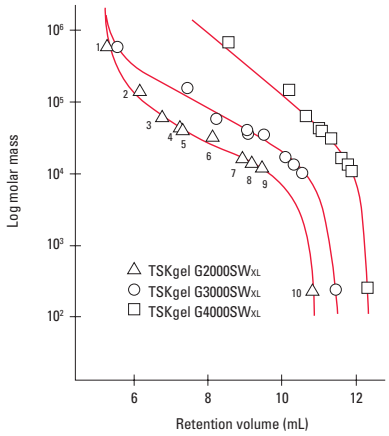
Samples of known molar mass

Calibration curves for each TSKgel SW-type column are provided on our website (www.tosohbioscience.com) 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 GFC 300 column to develop the method (scouting) and the TSKgel G3000SWXL column to obtain the highest resolution when working with a sample of unknown molar mass. If the compound of interest elutes near the exclusion volume, then a TSKgel G4000SWXL column is the logical next step.

Figure 15: displays the protein calibration curves for the TSKgel SWXL columns, for example.



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 (660,000 Da) 2. IgG (156,000 Da) 3. bovine serum albumin (67,000 Da) 4. ovalbumin (43,000 Da) 5. peroxidase (40,200 Da) 6. β-lactoglobulin (35,000 Da) 7. myoglobin (16,900 Da) 8. ribonuclease A (13,700 Da) 9. cytochrome C (12,400 Da) 10. glycine tetramer (246 Da)

TSKgel SW-type Column Selection

Monoclonal antibodies

TSKgel UP-SW3000 columns are ideal for the analysis of monoclonal antibodies and can be used on both UHPLC and HPLC systems. Alternatives include the TSKgel G3000SW$_{XL}$, and SuperSW mAb columns. In addition, SuperSW3000 columns can be used when sample is limited or the components of interest are present at very low concentrations.

Peptides

TSKgel G2000SW$_{XL}$ 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 concentrations.

Other sample types

Use TSKgel SW columns when not sample limited or when larger amounts of sample need to be isolated.

Solvent Solubility

- Consider what mobile phase is required and whether the column is compatible with this solvent. For most protein chemists, it is generally a phosphate based buffer in 100% aqueous condition and at pH <7.
- Some proteins with hydrophobic sites may require a small amount of organic solvent in the mobile phase, such as 5% methanol. For peptide applications, you may need to use 45% ACN with 0.1% TFA.

Secondary Interactions

There may be situations where BSA, SDS, non-ionic detergents, arginine, etc. may be needed as an additive to prevent secondary interactions from occurring between the sample and the column. TSKgel SW-type columns are compatible with these additives. Please note: the column should be dedicated for these types of applications.

Hardware

Secondary interactions can also occur with the use of stainless steel hardware. In this case, TSKgel SW-type columns made of PEEK should be used.

Ordering Information

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
50-104-9799	TSKgel UP-SW3000, 2 um, 25 nm	silica	Stainless Steel	4.6	30
50-104-9800	TSKgel UP-SW3000, 2um, 25 nm	silica	Stainless Steel	4.6	15
50-104-9801	Guard Column TSKgel UP-SW3000	silica	Stainless Steel	4.6	2
50-104-9802	Guard Column DC* for TSKgel UP-SW3000	silica	Stainless Steel	4.6	2
50-851-404	QC-PAK GFC 200GL, 5 µm, 12.5 nm	silica	Glass	8	15
50-851-406	QC-PAK GFC 300GL, 5 µm, 25 nm	silica	Glass	8	15
50-851-405	QC-PAK GFC 200, 5 µm, 12.5 nm	silica	Stainless Steel	7.8	15
50-851-380	QC-PAK GFC 300, 5 µm, 25 nm	silica	Stainless Steel	7.8	15
50-851-271	Guard Column for 7.8 mm ID TSKgel QC-PAK GFC columns, 7 µm	silica	Stainless Steel	6	4
50-851-284	TSKgel G3000SW Glass, 10 µm, 25 nm	silica	Glass	8	30
50-851-285	TSKgel G4000SW Glass, 13 µm, 45 nm	silica	Glass	8	30
50-851-136	TSKgel G2000SW, 10 µm, 12.5 nm	silica	Stainless Steel	7.5	30
50-851-092	TSKgel G2000SW, 10 µm, 12.5 nm	silica	Stainless Steel	7.5	60
50-851-137	TSKgel G3000SW, 10 µm, 25 nm	silica	Stainless Steel	7.5	30
50-851-093	TSKgel G3000SW, 10 µm, 25 nm	silica	Stainless Steel	7.5	60
50-851-138	TSKgel G4000SW, 13 µm, 45 nm	silica	Stainless Steel	7.5	30
50-851-094	TSKgel G4000SW, 13 µm, 45 nm	silica	Stainless Steel	7.5	60
50-851-150	TSKgel G2000SW, 13 µm, 12.5 nm	silica	Stainless Steel	21.5	30
50-851-100	TSKgel G2000SW, 13 µm, 12.5 nm	silica	Stainless Steel	21.5	60
50-851-151	TSKgel G3000SW, 13 µm, 25 nm	silica	Stainless Steel	21.5	30
50-851-101	TSKgel G3000SW, 13 µm, 25 nm	silica	Stainless Steel	21.5	60
50-851-152	TSKgel G4000SW, 17 µm, 45 nm	silica	Stainless Steel	21.5	30
50-851-102	TSKgel G4000SW, 17 µm, 45 nm	silica	Stainless Steel	21.5	60
50-851-289	Guard Column for 8 mm ID TSKgel G3000SW-G4000SW glass columns, 10 µm	silica	Glass	8	4
50-851-126	Guard Column for 7.5 mm ID TSKgel G2000SW-G4000SW columns, 10 µm	silica	Stainless Steel	7.5	7.5
50-851-128	Guard Column for 21.5 mm ID TSKgel G2000SW-G4000SW columns, 13 µm	silica	Stainless Steel	21.5	7.5

Ordering Information

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
50-550-524	TSKgel BioAssist DS, 15 µm for Desalting Applications	silica	PEEK	4.6	15
50-550-525	TSKgel BioAssist DS, 15 µm for Desalting Applications	silica	PEEK	10	15
50-851-628	TSKgel BioAssist G2SWXL, 5 µm, 12.5 nm	silica	PEEK	7.8	30
50-851-627	TSKgel BioAssist G3SWXL, 5 µm, 25 nm	silica	PEEK	7.8	30
50-851-626	TSKgel BioAssist G4SWXL, 8 µm, 45 nm	silica	PEEK	7.8	30
50-851-268	TSKgel G2000SWXL, 5 µm, 12.5 nm	silica	Stainless Steel	7.8	30
50-851-269	TSKgel G3000SWXL, 5 µm, 25 nm	silica	Stainless Steel	7.8	30
50-851-270	TSKgel G4000SWXL, 8 µm, 45 nm	silica	Stainless Steel	7.8	30
50-851-472	Guard Column for 7.8 mm ID TSKgel G2SWXL-G4SWXL BioAssist columns, 7 µm	silica	PEEK	6	4
50-851-271	Guard Column for 7.8 mm ID TSKgel G2000SWXL-G4000SWXL columns, 7 µm	silica	Stainless Steel	6	4
NC0147343	TSKgel SuperSW3000, 4 µm, 25 nm	silica	Stainless Steel	1	30
50-851-689	TSKgel SuperSW3000, 4 µm, 25 nm	silica	Stainless Steel	2	30
50-851-511	TSKgel SuperSW2000, 4 µm, 12.5 nm	silica	Stainless Steel	4.6	30
50-851-512	TSKgel SuperSW3000, 4 µm, 25 nm	silica	Stainless Steel	4.6	30
50-851-521	Guard Column for 4.6 mm ID TSKgel SuperSW2000 & SuperSW3000 columns, 4 µm	silica	Stainless Steel	4.6	3.5
50-851-272	TSKgel SWXL Top-Off, 5 µm, for TSKgel SWXL and QC-PAK columns, 1 g	silica			
50-851-156	TSKgel SW Top-Off, 10 µm, for 7.5 mm ID TSKgel SW columns, 1 g	silica			
50-486-523	TSKgel SuperSW mAb HR, 4 µm	silica	Stainless Steel	7.8	30
50-550-504	TSKgel SuperSW mAb HTP, 4 µm	silica	Stainless Steel	4.6	15
50-486-525	TSKgel UltraSW Aggregate, 3 µm	silica	Stainless Steel	7.8	30
50-486-524	TSKgel guard column for TSKgel SuperSW mAb HR, 4 µm	silica	Stainless Steel	6	4
50-550-505	TSKgel guard column for TSKgel SuperSW mAb HTP, 4 µm	silica	Stainless Steel	3	2
50-486-526	TSKgel guard column for TSKgel UltraSW Aggregate, 3 µm	silica	Stainless Steel	6	4

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