

TSK-gel columns for Gel Filtration Chromatography

Tosoh Bioscience provides TSK-GEL columns for both modes of Size Exclusion Chromatography (SEC), which are Gel Filtration Chromatography (GFC) and Gel Permeation Chromatography (GPC). GFC refers to the SEC separation of water soluble polymers in aqueous mobile phases, while GPC refers to the SEC separation of organic soluble polymers using an organic solvent as mobile phase. From a sample perspective, the TSK-GEL SW type column line, which is based on spherical porous silica particles, is most suitable for analyzing proteins and peptides by gel filtration. Polymer-based TSK-GEL PW columns are best used for GFC analysis of other water soluble polymers, such as oligosaccharides, acrylic acid, etc.

TSKgel SW columns for separation of peptides, proteins, and other molecules solubles in aqueous solutions, are the most used SEC columns all over the world. Reproducibility and outstanding performance are the secret behind this success.

Silica based columns

TSK-GEL SW-type packings are comprised of rigid spherical silica gel chemically bonded with hydrophilic compounds.

SW and SWxl

TSK-GEL SW Size Exclusion Chromatography (SEC) columns are widely used for aqueous separations of biomolecules. The porous silica based SW columns were originally introduced in 10 (G2000SW and G3000SW) and 13 μm (G4000SW). By reducing particle size to 5 μm for G2000SWxl and G3000SWxl and 8 μm for G4000SWxl analysis times were drastically cut down without sacrificing resolution.

SuperSW

Then Tosoh Biosep continued to improve the performance of the TSK-GEL silica SEC columns with the TSKgel SuperSW2000 and TSKgel SuperSW3000. These high performance columns, have a 4 μm particle size silica packing in a 4.6 mm I.D. x 30.0 cm length column which is the same rigid spherical silica gel chemically bonded with hydrophilic compounds that is found in our other TSK-GEL SW-series columns.

The 4 μm particle size and well-defined pore sizes of the TSK-GEL SuperSW columns provide fast separations with guaranteed efficiencies of 30,000 theoretical plates/30 cm column. As demonstrated in Figure 1, the TSKgel SuperSW has shown a 30% improvement in sample resolution and reduced analysis time. The column's three-fold increased sensitivity is especially helpful to researchers with limited sample quantities. Furthermore, the narrow bore column design means less mobile phase consumption and 75% less sample volume. For optimal performance, a low dead volume HPLC system is recommended.

Figure 1 : Comparison of Protein standard mixture separated on TSKgel G2000SW_{XL} and Super SW columns

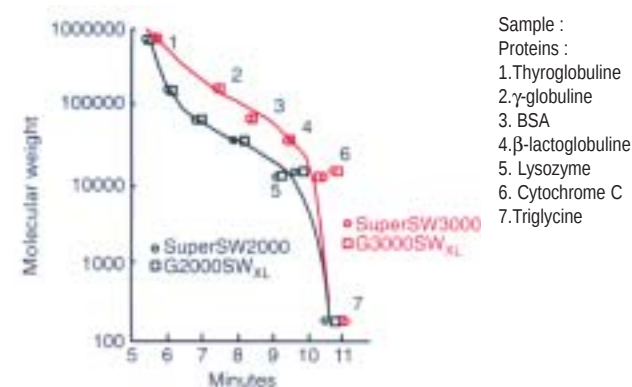
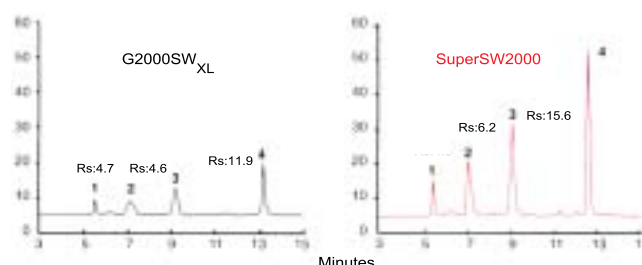


Figure 2 : Calibration curves for the TSKgel SuperSW and SW_{XL} columns



Separation techniques (Proteins)

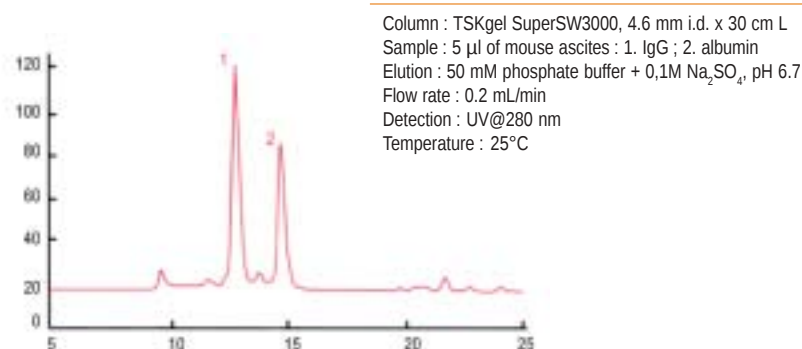
Gel filtration Chromatography TosohBioscience

SEC Analysis of Antibodies

Thermally induced denaturation or aggregation of therapeutic antibodies can be a significant problem during different stages of its production and formulation. Using the TSKgel SW4000 column the amounts of tri-, di- or monomers of human IgM monoclonal antibodies could be monitored after exposure to elevated temperatures. Furthermore, stabilization of this antibody by adding polyvinylpyrrolidone (PVP) and other excipients was examined with the same column.

The SEC separation of IgG from albumin in mouse ascites fluid is shown in figure 4, demonstrating the outstanding performance of the TSKgel SuperSW3000 column.

Figure 4
SuperSW separation of IgG and Albumin



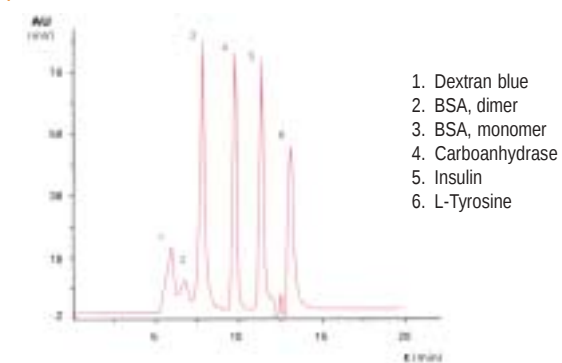
TSK-GEL SuperSW Capillary Columns

Tosoh Biosep is still moving on with developing cutting-edge SEC products. In co-operation with GROM (Herrenberg, D), we offer now our latest generation of high performance SEC-columns : TSKgel SuperSW2000- and TSKgel SuperSW3000-Capillary columns, both available in 0.3 mm ID x 30 cm L dimension (other dimensions are available upon request). Those Capillary columns showed the same performance like the standard 4.6 mm ID HPLC columns (Fig. 5), while increasing sensitivity tremendously.

An application of the TSKgel SuperSW2000 Capillary column is shown in Figure 6 demonstrating its excellent performance in analyzing different Interferon-species³.

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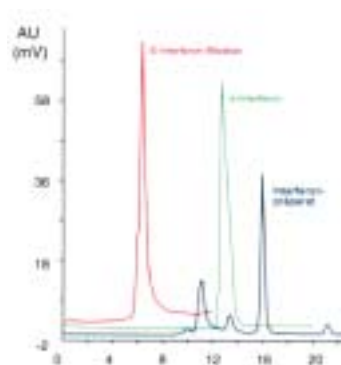
Figure 5
Size exclusion chromatography of a standard protein mixture



Stationary Phase : TSKgel SuperSW2000
 Dimension : 0.5 mm i.d. x 30 cm L
 Eluent : 0.1M Na-Phosphate
 pH 6.8 - 0.1 M NaCl
 Flow rate : 0.35 mm/s

Temperature : RT
 Detection : 206 nm (UV)
 Flow cell : 4 nL / 0.2 mm
 Injection : 300 nl Protein mixture
 (0.1 - 0.4 mg/ml)

Figure 6
Fast screening of interferon formulation with capillary size exclusion chromatography



Stationary Phase :
 TSKgel SuperSW2000
 Dimension :
 0.3 mm i.d. x 30 cm L
 Mobile Phase :
 0.1 M Na-Phosphate
 pH 6.8 +/- 0.1 M NaCl
 Flow rate : 1.48 µl/mn
 Temperature : 21°C
 Detection : 206 nm (UV)
 Flow Cell : 4nL / 0.2mm
 Injection :
 300 nl Protein Mixture

Separation techniques (Proteins)

Gel filtration Chromatography TosohBioscience

Summary : TSKGEL Super SW

Advantages of TSK-GEL Super SW compared to conventional SEC columns :

- ◆ 300% increased Sensitivity
- ◆ 75% reduced Sample Volume
- ◆ 50% reduced Analysis Time / Buffer Consumption
- ◆ 30% increased Efficiency
- ◆ With > 30 000 theoretical plates/column the micro analysis of limited sample amounts is possible

Characteristics of TSKGEL SW Series :

Column Types *)	Pore size (Å)	Particle Size (µm)	Theoretical Plates/Col	PEO (Da)	Exclusion Limits Dextran (Da)	Protein (kDa)
TSKgel G2000SW	125	10	10000	15000	30000	100
TSKgel G2000SWxl	125	5	20000	15000	30000	150
TSKgel G3000SW	250	10	10000	35000	70000	500
TSKgel G3000SWxl	250	5	20000	35000	70000	500
TSKgel G4000SW	450	13	8000	250000	500000	10000
TSKgel G4000 SWxl	450	8	16000	250000	500000	10000
TSKgel Super SW2000	125	4	30 000	15000	30000	150
TSKgel Super SW3000	250	4	30 000	35000	70000	500

*) Available column dimensions : 7.5 mm ID x 30 or 60 cm L for SW columns ; 7.8 mm ID x 30 cm L for SWXL columns

Analytical columns SW

Column	Cat.#	ID (mm)	Length (mm)	Porosity (Å)	Particle size (µm)	Min. Number Theoretical Plates	Flow (ml/mn) Rang.	Max	Max.Pressure Drop (kg/cm²)
TSKgel G2000SW	05788	7.5	300	125	10	> 10 000	0.5-1.0	1.2	20
TSKgel G2000SW	05102	7.5	600	125	10	> 20 000	0.5-1.0	1.2	40
TSKgel G3000SW	05789	7.5	300	250	10	> 10 000	0.5-1.0	1.2	25
TSKgel G3000SW	05103	7.5	600	250	10	> 20 000	0.5-1.0	1.2	50
TSKgel G4000SW	05790	7.5	300	450	13	> 8 000	0.5-1.0	1.2	15
TSKgel G4000SW	05104	7.5	600	450	13	> 16 000	0.5-1.0	1.2	30
TSKgel G2000SW en verre	08799	8.0	300	125	10	> 10 000	0.4-0.8	0.8	20
TSKgel G3000SW en verre	08800	8.0	300	250	10	> 10 000	0.4-0.8	0.8	20
TSKgel G4000SW en verre	08801	8.0	300	450	13	> 8 000	0.4-0.8	0.8	20

Analytical columns SWxl and Super SW

Column	Cat.#	ID (mm)	Length (mm)	Porosity (Å)	Particle size (µm)	Min. Number Theoretical Plates	Flow (ml/mn) Rang.	Max	Max.Pressure Drop (kg/cm²)
TSKgel G2000SWxl	08540	7.8	300	125	5	> 20 000	0.5-1.0	1.2	70
TSKgel G3000SWxl	08541	7.8	300	250	5	> 20 000	0.5-1.0	1.2	70
TSKgel G4000SWxl	08542	7.8	300	450	8	> 16 000	0.5-1.0	1.2	35
TSKgel Super SW2000	18674	4.6	300	125	4	> 30 000	0.1-0.35	0.4	120
TSKgel Super SW3000	18675	4.6	300	250	4	> 30 000	0.1-0.35	0.4	120

Preparatives columns SW

Column	Cat.#	ID (mm)	Length (mm)	Porosity (Å)	Particle size (µm)	Min. Number Theoretical Plates	Flow (ml/mn) Rang.	Max	Max.Pressure Drop (kg/cm²)
TSKgel G2000SW	06727	21.5	300	125	13	> 10 000	3.0-6.0	8.0	10
TSKgel G2000SW	05146	21.5	600	125	13	> 20 000	3.0-6.0	8.0	20
TSKgel G3000SW	06728	21.5	300	250	13	> 10 000	3.0-6.0	8.0	15
TSKgel G3000SW	05147	21.5	600	250	13	> 20 000	3.0-6.0	8.0	30
TSKgel G4000SW	06729	21.5	300	450	17	> 8 000	3.0-6.0	8.0	10
TSKgel G4000SW	05148	21.5	600	450	17	> 16 000	3.0-6.0	8.0	20

Guard columns

Column	Cat.#
Guard column SW	75 x 7.5 mm 05371
Guard column SW en verre	40 x 8 mm 08805
Guard column SWxl	40 x 6 mm 08543
Guard column Super SW	35 x 4.6 mm 18762
Guard preparative column SW	75 x 21.5 mm 05758

Separation techniques (Proteins)

Gel filtration Chromatography TosohBioscience

TSK Gel PW Polymeric based columns

- ◆ Hydrophilic, rigid, spherical, porous methacrylate beads
- ◆ Excellent chemical and mechanical stability
- ◆ pH range of 2 to 12, with up to 50% organic solvent
- ◆ Temperatures up to 80°C (50°C for TSKgel G-DNA-PW)
- ◆ Wide molecular weight separation range up to 8×10^6 Da for linear polymers
- ◆ Available in analytical (7.5 mm & 7.8 mm ID), semi-preparative (21.5 mm ID) and preparative (55 mm and 108 mm ID) columns

TSK-GEL PW 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 TSK-GEL SW columns. While a TSK-GEL SW column is typically the first column to try for biopolymers, TSK-GEL PW columns have demonstrated good results for smaller peptides (<1,000Da), protein aggregates (>10,000,000Da), and DNA fragments.

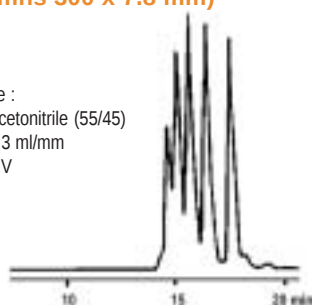
The properties and molecular weight separation ranges for all TSK-GEL PW type columns are summarized in *Table VIII*. Resins with six pore sizes are available. Particle sizes of resins packed in TSK-GEL PW columns are 10μm, 17μm, 20μm, or 22μm. The resins in TSK-GEL PWxl columns are 6μm, 10μm, or 13μm. Thus, PWxl resins are higher performance versions of the TSK-GEL PW resins due to the smaller particle sizes. Specialty resin-based columns include the mixed-bed TSKgel GMPW and TSKgel GMPWXL for samples with a broad molecular weight range. They also include TSKgel G-Oligo-PW and TSKgel G-DNA-PW columns for oligosaccharides and for DNA or RNA respectively.

Molecular weight of sample (Da)

Column Types *)	Pore size (Å)	Particle Size (μm)	PEO (Da)	Exclusion Limits Dextran (Da)	Protein (kDa)
G1000PW	<100	10	up to 1000	—	<2000
G2000PW	125	10, 17, 20	up to 2000	—	<5000
G2500PWXL	<200	6	up to 3000	—	<8000
G2500PW		10, 17, 20			
G3000PWXL	200	6	up to 5×10^4	up to 6×10^4	$500-8 \times 10^5$
G3000PW		10, 17, 20			
G4000PWXL	500	10	$2000-3 \times 10^4$	$1,000-7 \times 10^5$	$1 \times 10^4-1.5 \times 10^6$
G4000PW		17, 22			
G5000PWXL	1000	10	$4000-1 \times 10^6$	$5 \times 10^4-2.5 \times 10^6$	< 1×10^7
G5000PW		17, 20, 22			
G6000PWXL	>1000	13	$4 \times 10^4-8 \times 10^6$	$5 \times 10^5-5 \times 10^7$	< 2×10^8
G6000PW		17, 25			
GMPWXL	<100-1000	13	$500-8 \times 10^6$	< 5×10^7	< 2×10^8
GMPW		17			
G-Oligo-PW	125	6	up to 3 000	—	<3000
G-DNA-PW	>1000	10	$4 \times 10^4-8 \times 10^6$	< 5×10^7	< 2×10^8

Peptides TSKgel G3000PWxl columns (2 columns 300 x 7.8 mm)

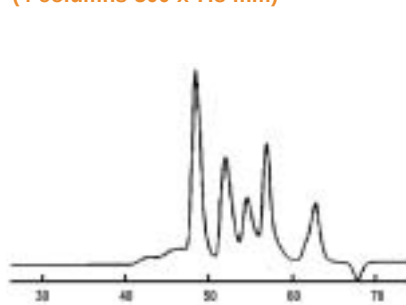
Mobile Phase :
0.1 % TFA/acetonitrile (55/45)
Flow rate : 0.3 ml/min
Detection : UV



Sample
1. Aprotinine
2. Insulin-B-chain
3. α-MSH
4. Bradikinin potentiator C
5. Glutathione

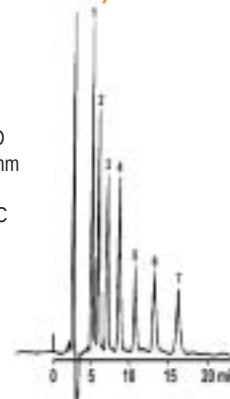
Pullulans TSKgel GMPWxl columns (4 columns 300 x 7.8 mm)

Mobile Phase : 0.1M NaCl
Flow rate : 1.0 ml/min
Detection : RI, LS



Oligosaccharides TSKgel G2000PW columns (2 columns 300 x 7.5 mm)

Mobile Phase : H₂O
Flow rate : 0.7 ml/min
Detection : RI
Temperature : 40°C



Separation techniques (Proteins)

Gel filtration Chromatography TosohBioscience

Cat.#	Column	ID (mm)	Length (mm)	Porosity (Å)	Particle size (µm)	Min. Number Theoretical Plates	Flow (ml/mn) Rang.	Max	Max.Pressure Drop (kg/cm ²)
Analytical columns PW/PWxl (k)									
TSKgel G1000PW	05760	7.5	300	< 100	10	5 000	0.5-1.0	1.2	20
TSKgel G2000PW	05761	7.5	300	125	10	5 000	0.5-1.0	1.2	20
TSKgel G2000PW	05105	7.5	600	125	10	10 000	0.5-1.0	1.2	40
TSKgel G2500PW	08028	7.5	300	< 200	10	5 000	0.5-1.0	1.2	20
TSKgel G2500PW	08029	7.5	600	< 200	10	10 000	0.5-1.0	1.2	40
TSKgel G3000PW	05762	7.5	300	200	10	5 000	0.5-1.0	1.2	20

Analytical columns PW/PWxl

TSKgel G3000PW	05106	7.5	600	200	10	10 000	0.5-1.0	1.2	40
TSKgel G4000PW	05763	7.5	300	500	17	3 000	0.5-1.0	1.2	10
TSKgel G4000PW	05107	7.5	600	500	17	6 000	0.5-1.0	1.2	20
TSKgel G5000PW	05764	7.5	300	1000	17	3 000	0.5-1.0	1.2	10
TSKgel G5000PW	05108	7.5	600	1000	17	6 000	0.5-1.0	1.2	20
TSKgel G6000PW	05765	7.5	300	> 1000	17	3 000	0.5-1.0	1.2	10
TSKgel G6000PW	05109	7.5	600	> 1000	17	6 000	0.5-1.0	1.2	20
TSKgel GMPW*	08026	7.5	300	100-1000	17	3 000	0.5-1.0	1.2	10
TSKgel GMPW*	08027	7.5	600	100-1000	17	6 000	0.5-1.0	1.2	20
TSKgel G2500PWxl	08020	7.8	300	< 200	6	14 000	0.5-0.8	1.0	40
TSKgel G3000PWxl	08021	7.8	300	200	6	14 000	0.5-0.8	1.0	40
TSKgel G4000PWxl	08022	7.8	300	500	10	10 000	0.3-0.8	1.0	20
TSKgel G5000PWxl	08023	7.8	300	1000	10	10 000	0.3-0.8	1.0	20
TSKgel G6000PWxl	08024	7.8	300	> 1000	13	7 000	0.3-0.8	1.0	20
TSKgel GMPWxl*	08025	7.8	300	100-1000	13	7 000	0.3-0.8	1.0	20

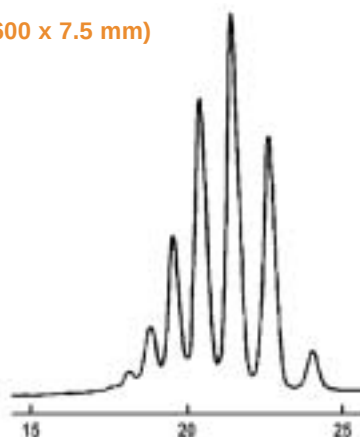
Analytical columns PW/PWxl

TSKgel G2000PW	05150	21.5	600	125	17	10 000	1.0-6.0	8.0	20
TSKgel G2500PW	08030	21.5	600	< 200	17	10 000	1.0-6.0	8.0	20
TSKgel G3000PW	05151	21.5	600	200	17	10 000	1.0-6.0	8.0	20
TSKgel G4000PW	05152	21.5	600	500	22	6 000	1.0-6.0	8.0	20
TSKgel G5000PW	05153	21.5	600	1000	22	6 000	1.0-6.0	8.0	20
TSKgel G6000PW	05154	21.5	600	> 1000	25	6 000	1.0-6.0	8.0	20

* Mixed Bed

Polyethylene glycol 200 Column TSKgel G2000PW (2 columns 600 x 7.5 mm)

Mobile Phase : H₂O
Flow rate : 1.4 ml/min
Detection : RI
Temperature : 55°C



Separation techniques (Proteins)

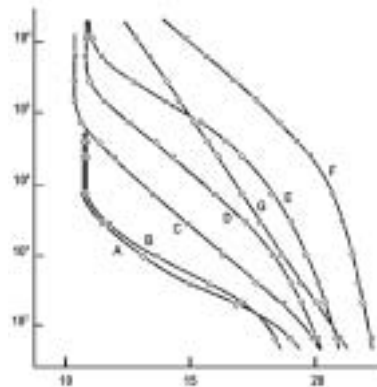
Gel filtration Chromatography TosohBioscience

Column (mm)	Cat.#	ID (mm)	Length (µm)	Particle size	Min. Number Theoretical Plates	Flow (ml/mn) Rang.	Max	Max. Pressure Drop (kg/cm ²)
Stainless steel columns								
G-Oligo-PW	08031	7.8	300	6	14 000	0.5 – 0.8	1.0	40
G-DNA-PW	08032	7.8	300	10	10 000	0.2 – 0.5	0.6	20
G2500PWXL	08020	7.8	300	6	14 000	0.5 – 0.8	1.0	40
G3000PWXL	08021	7.8	300	6	14 000	0.5 – 0.8	1.0	40
G4000PWXL	08022	7.8	300	10	10 000	0.3 – 0.8	1.0	20
G5000PWXL	08023	7.8	30	10	10 000	0.3 – 0.8	1.0	20
G6000PWXL	08024	7.8	30	13	7 000	0.3 – 0.8	1.0	20
GMPWXL	08025	7.8	30	13	7 000	0.3 – 0.8	1.0	20
G1000PW	05760	7.5	30	10	5 000	0.5 – 1.0	1.2	20
G2000PW	05761	7.5	30	10	5 000	0.5 – 1.0	1.2	20
G2500PW	08028	7.5	30	10	5 000	0.5 – 1.0	1.2	20
G3000PW	05762	7.5	30	10	5 000	0.5 – 1.0	1.2	20
G4000PW	05763	7.5	30	17	3 000	0.5 – 1.0	1.2	10
G5000PW	05764	7.5	30	17	3 000	0.5 – 1.0	1.2	10
G6000PW	05765	7.5	30	17	3 000	0.5 – 1.0	1.2	10
GMPW	08026	7.5	30	17	3 000	0.5 – 1.0	1.2	10
G2000PW	05105	7.5	60	10	10 000	0.5 – 1.0	1.2	40
G2500PW	08029	7.5	60	10	10 000	0.5 – 1.0	1.2	40
G3000PW	05106	7.5	60	10	10 000	0.5 – 1.0	1.2	40
G4000PW	05107	7.5	60	17	6 000	0.5 – 1.0	1.2	20
G5000PW	05108	7.5	60	17	6 000	0.5 – 1.0	1.2	20
G6000PW	05109	7.5	60	17	6 000	0.5 – 1.0	1.2	20
GMPW	08027	7.5	60	17	6 000	0.5 – 1.0	1.2	20
G2000PW	05150	21.5	60	17	10 000	1.0 – 6.0	8.0	20
G2500PW	08030	21.5	60	17	10 000	1.0 – 6.0	8.0	20
G3000PW	05151	21.5	60	17	10 000	1.0 – 6.0	8.0	20
G4000PW	05152	21.5	60	22	6 000	1.0 – 6.0	8.0	20
G5000PW	05153	21.5	60	22	6 000	1.0 – 6.0	8.0	20
G6000PW	05154	21.5	60	25	6 000	1.0 – 6.0	8.0	20

PW type Calibration Curve TSKgel PW, 600 x 7.5

Sample :
PEO et PEG

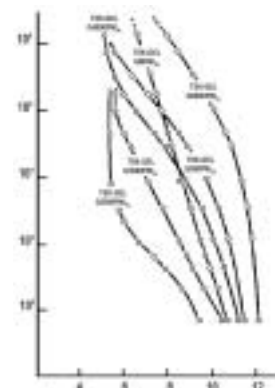
Mobile Phase : water
Flow rate : 1 ml/mm
Detection : RI



PW type_{xl} Calibration Curve TSKgel PW_{xl}, 300 x 7.8

Sample :
PEO et PEG

Mobile Phase : Water
Flow rate : 1 ml/mm
Detection : RI



Gel Filtration Chromatography GF250 & GF450 Columns

TosohBioscienceZorbax GF-250 and GF-450 size exclusion columns are ideal for the size separations of proteins and other biomolecules. The separation range is 4000-900000 for globular proteins when using GF-250 and GF-450 in series. The GF-250/450 size exclusion columns have a hydrophilic diol bonded phase for high recovery of proteins (typically>90%) and a unique zirconia modification of the silica to extend the pH range from 3-8. The GF250 and GF-450 columns are packed with precisely sized porous silica microspheres with narrow pore size and particle size distributions. The result is a highly efficient, rugged and reproducible size exclusion column for separations of proteins with flow rates of up to 3 mL/min. These columns are compatible with organic modifiers and denaturants in the mobile phase to eliminate protein aggregation for proper size determination. Some common applications include separations of protein monomers, dimers and aggregates, desalting, protein molecular weight estimation and separations of modified proteins.

- ◆ High efficiency and reproducibility with short analysis times
- ◆ Hydrophilic diol bonded phase for good protein recovery
- ◆ Compatible with organic modifiers and denaturants
- ◆ Wide usable pH range

Zorbax GF-250 and GF-450 column specifications :

Bonded Phase	Size(mm)	Pore Size	Particle Size	MW Range	Surface area	pH Range	Flow Rate	Max. Pressure	Cat.#
Zorbax GF-250	9.4 x 250	150 Å	4 µm	4 000 - 400 000	140 m ² /g	3.8 - 8.0	< 3.0 ml/min	5000 psi/350 bar	873210
Zorbax GF-250	4.6 x 250	150 Å	4 µm	4 000 - 400 000	140 m ² /g	3.8 - 8.0	< 3.0 ml/min	5000 psi/350 bar	033580
Zorbax GF-450	9.4 x 250	300 Å	6 µm	10 000 - 900 000	50 m ² /g	3.8 - 8.0	< 3.0 ml/min	5000 psi/350 bar	919330

Separation of Protein standards on the ZORBAX GF-250 SEC column

Column : ZORBAX GF-250
 Mobile Phase : 200 mM Sodium Phosphate, pH 7.0
 Flow rate : 2 ml/min
 Temperature : Ambient
 Detection : UV 254 nm
 Sample :
 BioRad Gel filtration Standards for size exclusion

1. Thyroglobulin	670 000 Da
2. Bovine Gamma Globulin	158 000 Da
3. Chicken Ovalbumin	44 000 Da
4. Equine Myoglobin	17 000 Da
5. Vitamin B-12	1 350 Da

The protein standards separated here are a commonly selected set of standards. The ZORBAX GF-250 column shows excellent resolution for this sample. Additional resolution to the thyroglobulin can be obtained by adding the GF-450 column in series.

