## SEC SIZE EXCLUSION CHROMATOGRAPHY

SEC PRODUCTS

## TSK-GEL SW-type

TSKgel SW TSKgel SW<sub>XL</sub> TSKgel SuperSW

#### TSK-GEL PW-type

TSKgel PW TSKgel PW<sub>XL</sub> TSKgel PW<sub>XL</sub>-CP

## TSK-GEL Alpha-type

TSKgel Alpha TSKgel SuperAW

## TSK-GEL H-type

TSKgel H<sub>XL</sub> TSKgel H<sub>HR</sub> TSKgel Super<sub>H</sub> TSKgel Super<sub>HZ</sub> **TOSOH FACT** Tosoh has a long history in size exclusion chromatography (SEC). In 1978 Tosoh first introduced porous silica-based SW columns for the isolation of proteins using LC. These first gels had particle sizes from 10 to 13 µm and were quickly adopted and referred to as the standard for analytical SEC on FPLC and HPLC systems.

As new packing materials were discovered and new bonding chemistries developed, the SEC product line has grown into four major classes of SEC columns. The following pages will help you choose the best column for your application.



SIZE EXCLUSION CHROMATOGRAPHY



## INTRODUCTION TO TSK-GEL SIZE EXCLUSION COLUMNS

**Gel Filtration Chromatography (GFC)** 

GFC is popular among biochemists for the isolation of proteins, for the removal of aggregates, to desalt a protein sample, to separate nucleic acid fractions, or to characterize watersoluble polymers used in food products, paints, pharmaceutical preparations, etc. Available TSK-GEL products are classified by application area and particle composition.

Each of the types below is described in detail in this chapter.

Application Area: Proteins and other biopolymers

Base material: silica

- SW
- SW<sub>x1</sub>
- SuperSW

These columns are ideal for proteins and nucleic acids using an aqueous buffer as mobile phase.

Application Area: Water-soluble polymers

Base material: polymethacrylate

- PW
- PW<sub>x1</sub>
- PW<sub>x1</sub>-CP

These columns are ideal for industrial polymers, oligosaccharides, nucleic acids and small viruses using aqueous buffer or salt solutions as mobile phase. The  $PW_{XL}$ -CP columns are developed to facilitate SEC separation of cationic polymer under low salt conditions.

#### Application Area: Water- and organic-soluble polymers

Base material: polyvinyl

- Alpha
- SuperAW

These columns are ideal for industrial polymers soluble in water, buffers and many organic solvents.

**Gel Permeation Chromatography (GPC)** 

GPC plays an important role in the characterization of organicsoluble polymers in the chemical and petrochemical industries. TSK-GEL GPC columns contain particles prepared from polystyrene crosslinked with divinylbenzene. Available products are grouped according to their relative lack of adsorptive properties and the speed of analysis.

Each of the types below is described in detail in this chapter.

#### Application Area: Organic-soluble polymers

Base material: polystyrene

Ultra-low adsorption columns with limited solvent range

- SuperHZ (high throughput)
- H<sub>x1</sub> (conventional)

Low adsorption columns with expanded solvent range

- SuperH (high throughput)
- H<sub>HR</sub> (conventional)

FEATURES

- Rigid hydrophilic and hydrophobic packings
- Four series of SEC columns with different ranges of solvent compatibility
- Easy scale up

- BENEFITS
- Minimal swelling and excellent physical strength
- Low adsorption resulting in high mass recovery
- Suitable for both types of size exclusion, aqueous (GFC) and nonaqueous (GPC)
- Analytical and preparative pre-packed SEC column

## SUMMARY OF TSK-GEL SIZE EXCLUSION COLUMN LINES

Characteristics of TSK-GEL Size	e Exclusion Column I	Lines		
Column Line	TSK-GEL SW	TSK-GEL PW	TSK-GEL Alpha TSK-GEL SuperAW	TSK-GEL H
Particle Composition	Silica	Methacrylate	Polyvinyl	PS-DVB
No. of Available Pore Sizes	3	6	6	6
pH Stability	2.5 - 7.5	2.0 - 12.0	2.0 - 12.0	1.0 - 14.0
Solvent Compatibility	100% polar	50% polar	100% polar and nonpolar	100% nonpolar, limited polar
Max. Temperature	30°C	80°C*	3°08	60-80°C (H $_{\rm \tiny M}$ and SuperHZ) 140°C (H $_{\rm \tiny \tiny HR}$ and SuperH)
Pressure** (kg/cm²)	10-120	4-40	20-60	15-60
Application Focus	proteins	water-soluble polymers	intermediate polar polymers	organic-soluble polymers

\* Except for the TSKgel G-DNA-PW, which can be operated up to 50°C and the 55 mm ID TSK-GEL PW-type columns, which can be operated up to 60°C. When operating below 10°C, reduce the flow rate to ensure that the maximum pressure is not exceeded.

\*\* Depends on column dimensions and particle size.

Note: The operating conditions and specifications for each column are listed on the Operating Conditions and Specifications sheet (OCS) shipped with the column and in the Ordering Information section at the end of each section.



## **COLUMN SELECTION GUIDE FOR TSK-GEL GEL FILTRATION COLUMNS**

Sample			Column selection	Selection criteria	
			First choice	Alternative	
Carbohydrates	polysaccharides		TSKgel GMPW <sub>xL</sub>	G5000PW <sub>xL</sub> and G3000PW <sub>xL</sub>	large pore size, linear calibration curve, small particles, high resolving power
	oligosaccharides		TSKgel G-Oligo-PW	G2500PW <sub>xL</sub>	small particles, high resolving power
Nucleic acids	DNA fragments	large	TSKgel G-DNA-PW or TSKgel G5000PW <sub>xL</sub>		large pore size, small particles, high resolving power
		medium and small	TSKgel G4000SW <sub>xL</sub> , TSKgel BioAssist G4SW <sub>xL</sub> , TSKgel SuperSW3000, or G3000SW <sub>xL</sub> , TSKgel BioAssist G3SW <sub>xL</sub>		suitable pore sizes
	RNA		TSKgel G4000SW <sub>xL</sub> TSKgel BioAssist G4SW <sub>xL</sub> TSKgel SuperSW3000, or G3000SW <sub>xL</sub> , TSKgel BioAssist G3SW <sub>xL</sub>		suitable pore sizes
	oligonucleotides		TSKgel G2500PW <sub>xL</sub>		small pore size, ionic interaction
Proteins	normal size small-medium proteins		TSKgel SuperSW3000, G3000SW <sub>xL</sub> , SKgel BioAssist G3SW <sub>xL</sub> TSKgel G4000SW <sub>xL</sub> , TSKgel BioAssist G4SW <sub>xL</sub> TSKgel SuperSW2000, or G2000SW <sub>xL</sub> , TSKgel BioAssist G2SW <sub>xL</sub>	G3000PW <sub>XL</sub> or G4000PW <sub>XL</sub>	small particles small to medium range pore sizes
	large proteins	low density lipoprotein	TSKgel G6000PW <sub>xL</sub> or TSKgel G5000PW <sub>xL</sub>		large pore sizes
		gelatin	TSKgel GMPW <sub>xL</sub>	G5000PW <sub>xL</sub> and G3000PW <sub>xL</sub>	large pore size, linear calibration curve
Peptides	large		TSKgel SuperSW3000, G3000SW <sub>xL</sub> , TSKgel BioAssist G3SW <sub>xL</sub> or G2000SW <sub>xL</sub> , TSKgel BioAssist G2SW <sub>xL</sub>	SuperSW2000 or G3000PW <sub>xL</sub>	small to medium range pore size, versatile
	small		TSKgel G2500PW <sub>xL</sub>	SuperSW2000 or G2000SW <sub>xL</sub>	linear calibration curve, high resolving power
Viruses			TSKgel G6000PW <sub>xL</sub> or TSKgel G5000PW <sub>xL</sub>		large pore size, high resolving power
Synthetic polymers			TSKgel GMPW <sub>xL</sub> or TSKgel Alpha-M	G5000PW <sub>xL</sub> and G3000PW <sub>xL</sub> or Alpha-5000 and Alpha-3000	large pore size, low adsorption, linear calibration curve
	cationic		TSKgel G3000PW <sub>xL</sub> -CP, TSKgel G5000PW <sub>xL</sub> -CP TSKgel G6000PW <sub>xL</sub> -CP		medium to large pore size, low adsorption, linear calibration curve
Synthetic oligomers	nonionic		TSKgel G-Oligo-PW, TSKgel G2500PW <sub>xL</sub> or TSKgel Alpha-2500	G2500PW or SuperAW2500	small pore size, high resolving power
	anionic		TSKgel G2500PW <sub>xL</sub> or TSKgel Alpha-2500	G2500PW or SuperAW2500	small pore size, ionic interaction

## TSK-GEL SW, SW<sub>XL</sub> AND SUPERSW GEL FILTRATION COLUMNS

#### HIGHLIGHTS

- TSK-GEL SW-type columns (SW, SW<sub>XL</sub> and SuperSW column lines) are all based on spherical silica particles with very high internal pore volumes.
- Silica particles in SW-type columns are chemically bonded with a stationary phase containing polar diol groups.
- SW-type columns feature low residual adsorption and very high pore volumes, which are essential characteristics of high performance gel filtration columns.
- SW and SW<sub>x1</sub> columns lines are available in three pore size ranges with nominal pore sizes of 125 Å, 250 Å and 450 Å. SuperSW and QC-PAK column lines are available in 125 Å and 250 Å pores.
- SW columns are packed with 10 micron (G2000SW and G3000SW) or 13 micron (G4000SW) particles. SW<sub>xL</sub> columns contain 5 micron (G2000SW<sub>xL</sub> and G3000SW<sub>xL</sub>) or 8 micron (G4000SW<sub>xL</sub>) particles. SuperSW columns contain 4 micron particles (SuperSW2000 and SuperSW3000)
- All SW-type columns are available in stainless steel hardware.
   SW and QC-PAK columns are also available in glass, while SW<sub>x1</sub> columns are also available in PEEK hardware.

#### **Recommendations for TSK-GEL SW series selection** Samples of unknown molecular weight

TSKgel G3000SW<sub>xL</sub> is the ideal scouting column. If the protein of interest elutes near the exclusion volume, then G4000SW<sub>xL</sub> is the logical next step. Conversely, if the protein of interest elutes near the end of the chromatogram, try the G2000SW<sub>xL</sub>.

#### Proteins (general)

Choose one of the TSK-GEL SW<sub>xL</sub> columns using the calibration curves on page 34 to select the appropriate pore size based on knowledge or estimate of protein size.

### Monoclonal antibodies

 $\rm TSKgel~G3000SW_{_{XL}}$  is commonly used for quality control. TSKgel SuperSW3000 is utilized when sample is limited or at very low concentration.

## Peptides

TSKgel G2000SW<sub>xL</sub> is the first selection for the analysis of peptides. TSKgel SuperSW2000 is utilized when sample is limited or at very low concentration.

## Other

The use of TSK-GEL SuperSW columns requires optimization of the HPLC system with respect to extra-column band broadening. Capillary tubing ID, injection volume, detector cell volume, and detector time constant all need to be reduced to fully benefit from the high column efficiency and small peak volumes of the SuperSW columns. Use SW columns when not sample limited or when larger amounts of sample need to be isolated.

Molecular weight of comple (Da)

Properties and separation ranges for TSK-GEL SW-type packings

				cular wergint of sail	inhie (na)
TSK-GEL packing	Particle Size (µm)	Pore Size (Å)	Globular proteins	Dextrans	Polyethylene glycols and oxides
SuperSW2000	4	125	5 x 10³− 1.5 x 10⁵	1 x 10³–3 x 10⁴	5 x 10 <sup>2</sup> –15 x 10 <sup>3</sup>
G2000SW <sub>x1</sub> /BioAssist G2SW <sub>x1</sub>	5	125	5 x 10³− 1.5 x 10⁵	1 x 10³–3 x 10⁴	5 x 10 <sup>2</sup> –15 x 10 <sup>3</sup>
QC-PAK TŠK 200	5	125	5 x 10³− 1.5 x 10⁵	1 x 10³–3 x 10⁴	5 x 10 <sup>2</sup> –15 x 10 <sup>3</sup>
G2000SW	10, 13, 20	125	5 x 10³−1 x 10⁵	1 x 10³–3 x 10⁴	5 x 10 <sup>2</sup> –15 x 10 <sup>3</sup>
SuperSW3000	4	250	1 x 10⁴ – 5 x 10⁵	2 x 10³–7 x 10⁴	1 x 10³–3.5 x 10⁴
G3000SW <sub>x1</sub> /BioAssist G3SW <sub>x1</sub>	5	250	1 x 10⁴ – 5 x 10⁵	2 x 10³–7 x 10⁴	1 x 10³–3.5 x 10⁴
QC-PAK TSK 300	5	250	1 x 10⁴ – 5 x 10⁵	2 x 10³–7 x 10⁴	1 x 10³–3.5 x 10⁴
G3000SW	10, 13, 20	250	1 x 10⁴ – 5 x 10⁵	2 x 10³–7 x 10⁴	1 x 10³–3.5 x 10⁴
G4000SW <sub>x1</sub> /BioAssist G4SW <sub>x1</sub>	8	450	2 x 10⁴ – 7 x 10⁵	4 x 10³–5 x 10⁵	2 x 10³–2.5 x 10⁵
G4000SW	13, 17	450	2 x 10⁴– 7 x 10⁵	4 x 10³–5 x 10⁵	2 x 10³–2.5 x 10⁵

Data generated using the following conditions:

Columns: Two 4 µm, 4.6 mm ID x 30cm L TSK-GEL SuperSW columns in series; two 5 µm, 7.8 mm ID x 30 cm L TSK-GEL SW<sub>xi</sub> columns in series; two 10 µm, 7.5 mm ID x 60 cm L TSK-GEL SW columns in series

Elution: Globular proteins: 0.3 mol/L NaCl in 0.1 mol/L (0.05 mol/L for SW<sub>x</sub> columns) phosphate buffer, pH 7.0 Dextrans and polyethylene glycols and oxides (PEOs): distilled water

2

SE



## CALIBRATION CURVES FOR TSK-GEL SW-TYPE GEL FILTRATION COLUMNS

The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

#### Polyethylene oxide, dextran and protein calibration curves for TSK-GEL SW columns



#### Protein calibration curves for TSK-GEL SW<sub>x1</sub> columns



Calibration curves for TSK-GEL SuperSW and  $SW_{x_1}$ 



UV @ 280 nm (220 nm for triglycine)

Detection:

## COMPARING TSK-GEL SW, SW<sub>xL</sub> AND SUPERSW GEL FILTRATION COLUMNS

**FIGURE 2** 

FIGURE 1 shows the increased resolution and faster separation time with a protein standard mixture on the TSK-GEL SW<sub>xL</sub> compared to TSK-GEL SW. This is due to the smaller particle size (5 vs. 10  $\mu$ m) of the TSK-GEL SW<sub>xL</sub> packing material.

FIGURE 2 & FIGURE 3 show the increased resolution and sensitivity of the TSK-GEL SuperSW columns compared to TSK-GEL SW<sub>xL</sub> columns. This is due to the smaller particle size (4 vs. 5  $\mu$ m) coupled with a narrow column (4.6 mm ID).

#### FIGURE 1 ...

## Higher resolution with 5 $\mu m$ TSK-GEL SW\_{xL} compared with 10 $\mu m$ TSK-GEL SW columns



Comparison of TSKgel SuperSW3000 and TSKgel G3000SW $_{\rm XL}$  for the separation of proteins



FIGURE 3 :

Comparison of TSKgel SuperSW2000 and TSKgel G2000SW $_{\rm XL}$  for the separation of proteins



35



## **APPLICATIONS OF TSK-GEL SW-TYPE GEL FILTRATION COLUMNS**

### **Proteins**

The effect of different concentrations of surfactant on the separation of membrane proteins is seen in FIGURE 4. As the concentration of octaethyleneglycol dodecylether increases to 0.05%, the main peak becomes sharper and recovery increases. The TSKgel SuperSW3000 provides an excellent high resolution separation of IgG, from mouse ascites fluid as can be seen in FIGURE 5.

#### FIGURE 4 🚍 =

## Separation of membrane protein by SEC with different surfactant concentration in the eluent



**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 and glutathione S-transferase were separated in only 15 minutes on a TSKgel  $G3000SW_{x1}$  column and activity recovery was 98% and 89%, respectively.

The elution profiles of the separations in FIGURE 6 show that all of the activity eluted in a narrow band of about 1.5 mL.

## FIGURE 6

Separation of crude protein samples on TSKgel G3000SW<sub>XI</sub>







Column:	TSKgel G3000SW <sub>XL</sub> , 5µm, 7.8mm ID x 30cm
Sample:	A. crude peroxidase from Japanese radish,
	0.15mg in 0.1mL
	B. crude glutathione S-transferase from guinea
	pig liver extract, 0.7mg in 0.1mL
Elution:	0.3mol/L NaCl in 0.05mol/L phosphate buffer, pH 7
Flow Rate:	1.0mL/min
Detection:	UV @ 220nm (solid line) and enzyme assay
	tests (dashed line)
Recovery:	enzymatic activity recovered was 98% in A and 89% in B



=



### 

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	l Th	Number eoretical	<u>Flow Ra</u> Range	<u>ite (mL/min)</u> Max.	Maximum Pressure Drop (kg (om²)
Glass	columns					Flates			Drop (kg/cm²)
16214	QC-PAK GFC 200GL	8.0	15	5	$\geq$	10,000	0.5 - 1.0	1.2	40
16216	QC-PAK GFC 300GL	8.0	15	5	$\geq$	10,000	0.5 - 1.0	1.2	40
08799	G2000SW, Glass	8.0	30	10	$\geq$	10,000	0.4 - 0.8	0.8	20
08800	G3000SW, Glass	8.0	30	10	$\geq$	10,000	0.4 - 0.8	0.8	20
08801	G4000SW, Glass	8.0	30	13	$\geq$	8,000	0.4 - 0.8	0.8	20
14464	G3000SW, Glass	20.0	30	13	≥	6,000	3.0 -6.0	8.0	8
Stainle	ess steel columns								
18674	SuperSW2000	4.6	30	4	$\geq$	30,000	0.1 -0.35	0.4	120
21845	SuperSW3000 -NEW-	1.0	30	4	$\geq$	18,000	0.016	0.02	120
21485	SuperSW3000 -NEW-	2.0	30	4	$\geq$	25,000	0.065	0.075	120
18675	SuperSW3000	4.6	30	4	$\geq$	30,000	0.1 - 0.35	0.4	120
08540	G2000SW <sub>xL</sub>	7.8	30	5	≥	20,000	0.5 -1.0	1.2	70
08541	G3000SW <sub>xL</sub>	7.8	30	5	$\geq$	20,000	0.5 - 1.0	1.2	70
08542	G4000SW <sub>xL</sub>	7.8	30	8	≥	16,000	0.5 - 1.0	1.2	35
16215	QC-PAK GFC 200	7.8	15	5	≥	10,000	0.5 -1.0	1.2	40
16049	QC-PAK GFC 300	7.8	15	5	$\geq$	10,000	0.5 -1.0	1.2	40
05788	G2000SW	7.5	30	10	$\geq$	10,000	0.5 -1.0	1.2	20
05789	G3000SW	7.5	30	10	$\geq$	10,000	0.5 -1.0	1.2	25
05790	G4000SW	7.5	30	13	$\geq$	8,000	0.5 -1.0	1.2	15
05102	G2000SW	7.5	60	10	$\geq$	20,000	0.5 -1.0	1.2	40
05103	G3000SW	7.5	60	10	$\geq$	20,000	0.5 -1.0	1.2	50
05104	G4000SW	7.5	60	13	$\geq$	16,000	0.5 -1.0	1.2	30
06727	G2000SW	21.5	30	10	$\geq$	10,000	3.0 -6.0	8.0	10
06728	G3000SW	21.5	30	10	$\geq$	10,000	3.0 -6.0	8.0	15
06729	G4000SW	21.5	30	13	≥	8,000	3.0 - 6.0	8.0	10
05146	G2000SW	21.5	60	13	≥	20,000	3.0 -6.0	8.0	20
05147	G3000SW	21.5	60	13	≥	20,000	3.0 -6.0	8.0	30
05148	G4000SW	21.5	60	17	2	16,000	3.0 -6.0	8.0	20
07428	G2000SW	55.0	30	20	≥	400	25.0 - 40.0	50.0	10
07481	G3000SW	55.0	30	20	≥	4,000	15.0 -25.0	50.0	10
07429	G2000SW	55.0	60	20	≥	750	20.0 -30.0	35.0	15
07482	G3000SW	55.0	60	20	≥	9,000	15.0 - 25.0	50.0	15
PEEK (	Columns								
20027	BioAssist G2SW <sub>xL</sub>	7.8	30	5	$\geq$	20,000	0.5 - 1.0	1.2	70
20026 20025	BioAssist G3SW <sub>xL</sub> BioAssist G4SW <sub>xL</sub>	7.8 7.8	30 30	5 8	≥ ≥	20,000 16,000	0.5 - 1.0 0.5 - 1.0	1.2 1.2	70 35

38

SEC



### ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	
Guard	column products				
08805	SW Guard column, Glass	8.0	4.0		For all 8 mm ID SW glass columns
14465	SW Guard column, Glass	20.0	4.0		For P/N 14464 SW glass columns
18762	SuperSW Guard column	4.6	3.5		For 4.6 mm ID SuperSW columns
					(contains SuperSW3000 packing)
08543	SW <sub>xi</sub> Guard column	6.0	4.0		For all SW $_{_{ m XI}}$ columns and P/Ns 16215 and 16049
					(contains 3000SW <sub>x</sub> packing)
18008	SW <sub>x1</sub> Guard column, PEEK	6.0	4.0		For all BioAssist SW <sub>xt</sub> , PEEK columns
05371	SW Guard column	7.5	7.5		For all 7.5 mm ID SW columns (contains 3000SW packing)
05758	SW Guard column	21.5	7.5		For all 21.5 mm ID SW columns
07427	SW Guard column	45.0	5.0		For 55 mm ID SW columns
Bulk p	acking				1
08544	$SW_{xL}$ Top-Off, 1g wet gel			5	For SW <sub>xL</sub> and QC-PAK columns
06819	SW Top-Off, 1g wet gel			10	For all 7.5 mm ID SW columns



## **TSK-GEL PW and TSK-GEL PW**<sub>xL</sub> columns Gel Filtration Chromatography of water soluble polymers

#### HIGHLIGHTS

- 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 separation range up to 8 x 10<sup>6</sup> Da for linear polymers
- PEEK column hardware available for G6000PW packings for ultra-low sample adsorption during virus analysis
- Available in analytical, semi-preparative and preparative stainless steel columns TSK-GEL
- New PW<sub>xL</sub>-CP columns designed for low salt SEC separations of cationic polymers.

Polymeric TSK-GEL PW and TSK-GEL PW<sub>xL</sub> columns are designed for GFC of water soluble organic polymers, polysaccharides, oligosaccharides, DNA and RNA. For the analysis of proteins and peptides SW type columns are recommended.

A number of specialty columns include columns for samples with a broad molecular weight range, oligosaccharides, DNA, and RNA. For analytical purposes the TSK-GEL  $PW_{xL}$  columns are preferred. For preparative work, or for other cases in which large amounts of sample must be used, the 60 cm TSK-GEL PW columns are recommended because of their increased loading capacity. TSK-GEL  $PW_{xL}$ -CP columns are especially suited for the separation of cationic polymers.

#### **PROPERTIES AND SEPARATION RANGES FOR TEK-GEL PW-TYPE PACKINGS**

			Mole	ecular weight of sam	ple (Da)
TSK-GEL packing	Particle Size* (µm)	Pore Size (Å)	Polyethylene glycols and oxides	Dextrans**	Globular proteins**
G2000PW	10	125	< 2 x 10 <sup>3</sup>		< 5 x 10 <sup>3</sup>
G2500PW <sub>xL</sub>	7	< 200		< 3 x 10 <sup>3</sup>	< 8 x 10 <sup>3</sup>
G2500PW	12, 17, 20	< 200	< 3 x 10 <sup>3</sup>		
G3000PW <sub>xL</sub>	7	200	< 5 x 10 <sup>4</sup>	< 6 x 10 <sup>4</sup>	5 x 10 <sup>2</sup> - 8 x 10 <sup>5</sup>
G3000PW	10, 17, 20	200	< 5 x 10 <sup>4</sup>		
G3000PW <sub>XL</sub> -CP	7	< 200	< 5 x 10 <sup>4</sup>		
G4000PW <sub>xL</sub>	10	500	< 3 x 10⁵	1 x 10³ - 7 x 10⁵	1 x 104 - 1.5 x 10
G4000PW	17, 20	500	< 3 x 10⁵		
G5000PW <sub>xL</sub>	10	1000	< 1 x 10 <sup>6</sup>	5 x 10⁴ - 2.5 x 10⁵	< 1 x 10
G5000PW	17, 20	1000	< 1 x 10 <sup>6</sup>		
G5000PW <sub>xl</sub> -CP	10	< 1000	< 5 x 10 <sup>4</sup>		
G6000PW <sub>xL</sub>	13	> 1000	< 8 x 10 <sup>6</sup>	5 x 10⁵ - 5 x 10 <sup>7</sup>	< 2 x10 <sup>8</sup>
G6000PW / BioAssist G6PW	17	> 1000	< 8 x 10 <sup>6</sup>		
G6000PW <sub>XL</sub> -CP	13	> 1000	< 8 x 10 <sup>6</sup>		
GMPW <sub>xL</sub>	13	< 100 - 1000	5 x 10 <sup>2</sup> - 8 x 10 <sup>6</sup>	< 5 x 10 <sup>7</sup>	< 2 x 10 <sup>8</sup>
GMPW	17	< 100 - 1000	5 x 10 <sup>2</sup> - 8 x 10 <sup>6</sup>		
G-Oligo-PW	7	125	< 3 x 10 <sup>3</sup>		< 3 x 10 <sup>3</sup>
G-DNA-PW	10	> 1000	< 8 x 10 <sup>6</sup>	<5 x 10 <sup>7</sup>	< 2 x 10 <sup>8</sup>

Column:TSK-GEL PW columns, 7.5 mm ID x 60 cm L; TSKgel  $PW_{xt}$ , TSKgel  $PW_{xt}$ -CP, G-Oligo-PW & G-DNA-PW, 7.8 mm ID x 30 cm LElution:Polyethylene glycols and oxides: distilled water; dextrans and proteins: 0.2 mol/L phosphate buffer, pH 6.8Flow Rate:1.0 ml/min

Note: \*Larger particle sizes of each group are for 21.5 mm ID x 60 cm L semi-preparative and 55 mm or 108 mm ID x 60 cm L preparative columns. \*\*Maximum separation range determined from estimated exclusion limits.

3

ы С



## CALIBRATION CURVES FOR TSK-GEL PW-TYP COLUMNS The best results are obtained when selecting a column with

The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

## Polyethylene glycol and oxide calibration curves on TSK-GEL PW and TSK-GEL $\text{PW}_{\text{XL}}$ columns



## Protein calibration curves on TSK-GEL PW $_{XL}$ columns



Detection: UV @ 280 nm

**COMPARISON BETWEEN TSK-GEL PW AND TSK-GEL PWXL** The smaller particle sizes of the TSK-GEL  $PW_{xL}$  columns provide almost 1.5 times the resolution of their TSK-GEL PW counterparts. With shorter TSK-GEL  $PW_{xL}$  columns, similar or higher resolution separations are possible in less than half the time.

### Faster analysis and higher resolution with TSK-GEL $\text{PW}_{\text{xL}}$ columns



Column: A. TSKgel G2500PW, two 10µm, 7.5mm ID x 60cm columns in series B. TSKgel G2500PW<sub>x1</sub>, two 6µm, 7.5mm ID x 60cm columns in series C. TSKgel G4000PW, 17µm, 7.5mm ID x 60cm D. TSKgel G4000PW<sub>x1</sub>, 10µm, 7.8mm ID x 30cm

- Sample: A. & B.: polyethylene glycol 200
  - C. & D.: polyethylene oxide standards: SE-150, SE-15 and SE-2 in 100µL
- Elution: A. & B.: distilled water; C. & D.: 0.1mol/L NaCl
- Flow Rate: 1.0mL/min
- Temp.: A. & B.: 25°C; C. & D.: 50°C

Detection: RI

## **COLUMNS FOR SPECIFIC APPLICATIONS**

### **TSK-GEL PWXL-CP**

The new TSK-GEL PW<sub>xL</sub>-CP columns are designed to facilitate the separation of cationic polymers by SEC at low salt conditions. They are based on the well known PW-type of polymeric resins for aqueous SEC. Cationic surface modification enables low salt elution of cationic polymers with high recoveries. The columns show high theoretical plate numbers, linear calibration curves and high durability. They are produced with three pore sizes for diffrent ranges (G3000-, G5000- and G6000PW<sub>xL</sub>-CP). FIGURE 7 shows the analysis of various cationic polymers on a series of TSKgel PW<sub>xL</sub>-CP columns.

### **TSK-GEL G-Oligo-PW**

The specialty column TSKgel G-Oligo-PW is designed for high resolution separations of nonionic and cationic oligomers. Because of the presence of residual cationic groups, this column is not recommended for separating anionic materials. The polyethylene glycol and polythylene oxide calibration curves for TSKgel G-Oligo-PW (not shown) are identical to the calibration curve for TSKgel G2500PW<sub>x1</sub> (shown on the previous page).

### **TSK-GEL G-DNA-PW**

The TSKgel G-DNA-PW column is dedicated to the separation of large polynucleotides, such as DNA and RNA fragments of 500 to 5,000 base pairs. The exclusion limits for double-stranded DNA fragments are lower than those for rRNAs, indicating that double-stranded DNA fragments have a larger molecular size in solution than rRNAs of the same molecular weight. The packing of the TSKgel G-DNA-PW column has very large pores (>1000 Å) and a small particle size (10  $\mu$ m).

For the separation of large DNA fragments greater than 1,000 base pairs, a four-column system is typically required. Baseline resolution of DNA fragments up to 7,000 base pairs can be achieved, provided there is a two-fold difference in the chain length of the fragments.

## FIGURE 7

## Separation of cationic polymers



Columns:	TSKgel G3000PW <sub>x1</sub> -CP, 7µm (7.8mm ID x 30cm),
	TSKgel G5000PW <sub>x1</sub> -CP, 10µm (7.8mm ID x 30cm)
	TSKgel G6000PW <sub>v1</sub> -CP, 13µm (7.8mm ID x 30cm)
Eluent:	0.1mol/L NaNO
Flow Rate:	1mL/min
Detection:	RI
Temperature:	25°C
Sample Load:	3g/L, 100µL

### **TSK-GEL GMPW and TSK-GEL GMPWXL**

When the molecular weight range of the sample is broad or unknown, Tosoh Bioscience offers two mixed-bed columns, TSKgel GMPW and TSKgel GMPW<sub>xL</sub>, for analysis. The TSKgel GMPW column and its high resolution counterpart, TSKgel GMPW<sub>xL</sub>, are packed with the G2500, G3000 and G6000 PW or corresponding PW<sub>xL</sub> resins. They offer a broad molecular weight separation range. As shown on the previous page, the calibration curve for polyethylene glycols and oxides on these mixed-bed columns is fairly shallow and is linear over the range of 100-1,000,000 Da.

The introduction of mixed-bed columns has made the problems of analyzing polydisperse samples much easier. Previously, many two-column systems such as TSKgel G3000PW and TSKgel G6000PW, were required to achieve good resolution with wide MW-range samples. The substitution of a TSK-GEL GMPW series column can save both time and money compared with multi-column systems. B



## OPTIMIZING GEL FILTRATION WITH TSK-GEL PW AND TSK-GEL $\mathsf{PW}_{\mathsf{xL}}$ COLUMNS

## **Selecting Mobile Phase Buffers**

SEC separation is based on the difference of apparent molecular size with no additional interaction between the column matrix and the sample molecules. In practice, however, a small number of weakly charged groups on the surface of PW-type packings can cause changes in elution order from that of an ideal system. The eluent composition can vary greatly with TSK-GEL PW columns to be compatible with a wide range of neutral, polar, anionic, and cationic samples. The table below lists appropriate eluents for GFC of major polymer types.

For some nonionic, nonpolar polymers, such as polyethylene glycols, ideal size exclusion behavior can be obtained by using distilled water. More polar ionic polymers may exhibit abnormal peak shapes or minor peaks near the void volume when eluted with distilled water, due to ionic interactions between the sample and residual charged groups on the resin surface. To eliminate ionic interactions, a neutral salt such as sodium nitrate or sodium sulfate should be added. Generally, a salt concentration of 0.1 to 0.5 mol/L is sufficient to overcome undesirable ionic interactions.

### **Hydrophobic Samples**

TSK-GEL PW-type resins are more hydrophobic than polysaccharide gels such as cross-linked dextran. Depending on the sample, this can lead to hydrophobic interaction as a secondary retention mechanism. The extent of hydrophobic interaction increases as the salt concentration of the eluent increases, but it can be reduced by the addition of an organic modifier such as acetonitrile. Water-soluble organic solvents are frequently used as modifiers to suppress hydrophobic interactions between the sample and the resin surface.

Modifiers are also used for optimizing the elution of both charged and neutral hydrophobic polymers. Typical examples for a variety of sample types are given in the table below. All TSK-GEL PW-type column packings are compatible with 20 % aqueous solutions of methanol, ethanol, propanol, acetonitrile, dimethyl formamide, dimethyl sulfoxide, formic acid, and acetic acid. In addition, these columns can be operated in 50 % aqueous acetone.

Type of polymer	Typical sample	Suitable eluent
Nonionic hydrophilic	polyethylene glycol soluble starch, methyl cellulose, pullulan dextran, hydroxyethyl cellulose, polyvinyl alcohol, polyacrylamide	distilled water 0.01N NaOH 20% DMSO Buffer or salt solution (e.g., 0.1–0.5 mol/L NaNO <sub>3</sub> )
Nonionic hydrophobic	polyvinylpyrrolidone	Buffer or salt solution with organic solvent (e.g., 20% ACN in 0.1mol/L NaNO <sub>3</sub> )
Anionic hydrophilic	sodium chondroitin sulfate, sodium alginate, carboxymethyl cellulose, sodium polyacrylate, sodium hyaluronate	Buffer or salt solution (e.g., 0.1 mol/L NaNO $_3$ )
Anionic hydrophobic	sulfonated lignin sodium salt, sodium polystyrenesulfonate	Buffer or salt solution with organic solvent (e.g., 20% ACN in 0.1 mol/L NaNO <sub>3</sub> )
Cationic hydrophilic	glycol chitosan, DEAE-dextran, poly(ethyleneimine), poly(trimethylaminoethyl methacrylate) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L Na <sub>2</sub> SO <sub>4</sub> , or 0.8 mol/L NaNO <sub>3</sub> (0.1 mol/L NaNO <sub>3</sub> for PW <sub>xL</sub> -CP type)
Cationic hydrophobic	poly(4-vinylbenzyltrimethylammonium chloride), poly(N-methyl-2-vinylpyridinium) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L $\rm Na_2SO_4$
Amphoteric hydrophilic	peptides, proteins, poly-and oligosaccharides, DNA, RNA	Buffer or salt solution (e.g., 0.1 mol/L NaNO <sub>3</sub> )
Amphoteric hydrophobic	blue dextran, collagen, gelatin, hydrophobic proteins hydrophobic peptides	Buffer or salt solution with organic solvent (e.g., 20% ACN in 0.1 mol/L NaNO <sub>3</sub> or 35–45% ACN in 0.1% TFA)

## **RECOMMENDED ELUENTS FOR GFC OF WATER-SOLUBLE POLYMER ON TSK-GEL PW-TYPE COLUMNS**

## **APPLICATIONS OF TSK-GEL PW-TYPE GEL FILTRATION COLUMNS**

#### **Nucleic acids**

Desalting of nucleosides can be accomplished using TSKgel G2500PW<sub>xL</sub>, as depicted in FIGURE 8. Clearly, adenosine elutes after the void volume in the unbuffered water mobile phase.

### **Polysaccharides**

TSK-GEL PW columns are recommended for polysaccharide analysis due to their ability to separate a wide molecular weight distribution. Nonionic polysaccharides are the least complicated molecules to analyze by SEC because they seldom exhibit secondary interactions with the solid support. TSKgel G5000PW and TSKgel G3000PW in series are effective for the characterization of clinical dextran. Cationic samples can be adsorbed on the resin by electrostatic interaction. If the polymer is strongly cationic, a fairly high salt concentration is required to prevent ionic interactions with conventional SEC packings. A mobile phase of 0.5 mol/L acetic acid with 0.3 mol/L Na<sub>2</sub>SO<sub>4</sub> can also be used.

The new TSKgel  $PW_{xL}$ -CP series enables elution of water soluble, cationic polymers under low salt conditions (e.g. 0.1 mol/L NaNO<sub>2</sub>).

An effective separation of the anionic hydrophilic glucosaminoglycan, hydraluronic acid, is shown in FIGURE 9 on a TSKgel G6000PW and TSKgel G4000PW column in series with a 0.2 mol/L sodium chloride mobile phase.



43



= FIGURE 10 .....

## Faster analysis and higher resolution of chito-oligosaccharides on a TSKgel G-Oligo-PW column



Column: A. Tskyel G-Oligo-PW, two fupin, 7.5mm ID x ooch columns in series
B. TSKgel G-Oligo-PW, two 6μm, 7.8mm ID x 30cm columns in series
Sample: 1. chitohexaose, 2. chitopentaose, 3. chitotetraose, 4. chitotriose, 5. chitobiose.
Elution: distilled water
Flow Rate: 1.0mL/min
Detection: RI

#### ORDERING INFORMATION

#### **Oligomers**

The TSKgel G-Oligo-PW column is designed for high resolution separations of nonionic and cationic oligomers. FIGURE 10 demonstrates excellent resolution of chito-oligosaccharides obtained by using the smaller, 6 µm particle size packing in TSKgel G-Oligo-PW columns as compared with the resolution obtained with a TSKgel G2000PW column. The pore sizes in both TSKgel G-Oligo-PW and TSKgel G2000PW columns are about 125 Å and both resins bear approximately 0.2 µeq/mL of cationic groups. Because of the presence of cationic groups, neither column is recommended for separating anionic materials. However, for nonionic oligomers, TSKgel G-Oligo-PW columns provide higher resolution than TSKgel G2500PW<sub>x1</sub> columns.

Part #	Description	ID	Length	Particle	Number	<b>Flow Rate</b>	(mL/min)	Maximum
		(mm)	(cm)	Size (µm)	Theoretical Plates	Range	Max.	Pressure Drop (kg/cm²)
Stainle	ess steel columns							
08031	G-Oligo-PW	7.8	30	7	≥ 14,000	0.5 - 0.8	1.0	40
08032	G-DNA-PW	7.8	30	10	≥ 10,000	0.2 - 0.5	0.6	20
08020	G2500PW <sub>xL</sub>	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	40
08021	G3000PW <sub>xL</sub>	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	40
08022	G4000PW <sub>xL</sub>	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	20
08023	G5000PW <sub>xL</sub>	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	20
08024	G6000PW <sub>xL</sub>	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	20
08025	GMPW <sub>xL</sub>	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	20
21873	G3000PW <sub>xl</sub> -CP	7.8	30	7	≥ 16,000		1.0	55
21874	G5000PW <sub>xL</sub> -CP	7.8	30	10	≥ 10,000		1.0	25
21875	G6000PW <sub>xL</sub> -CP	7.8	30	13	≥ 7,000		1.0	20
05761	G2000PW	7.5	30	12	≥ 5,000	0.5 - 1.0	1.2	20
08028	G2500PW	7.5	30	12	≥ 5,000	0.5 - 1.0	1.2	20
05762	G3000PW	7.5	30	12	≥ 5,000	0.5 - 1.0	1.2	20

• 0	RDERING INFORM	ATION						
Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	<u>Flow Rate (</u> Range	<u>mL/min)</u> Max.	Maximum Pressure Drop (kg/cm²)
05763	G4000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.2	10
05764	G5000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.2	10
05765	G6000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.2	10
08026	GMPW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.2	10
05105	G2000PW	7.5	60	12	≥ 10,000	0.5 - 1.0	1.2	40
08029	G2500PW	7.5	60	12	≥ 10,000	0.5 - 1.0	1.2	40
05106	G3000PW	7.5	60	12	≥ 10,000	0.5 - 1.0	1.2	40
05107	G4000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	1.2	20
05108	G5000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	1.2	20
05109	G6000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	1.2	20
08027	GMPW	7.5	60	17	≥ 6,000	0.5 - 1.0	1.2	20
08030	G2500PW	21.5	60	17	≥ 10,000	1.6 - 6.0	8.0	20
05151	G3000PW	21.5	60	17	≥ 10,000	1.6 - 6.0	8.0	20
05152	G4000PW	21.5	60	20	≥ 6,000	1.6 - 6.0	8.0	10
05153	G5000PW	21.5	60	20	≥ 6,000	1.6 - 6.0	8.0	10
07926	G3000PW	55.0	60	20	ND	15.0 - 25.0	30.0	15
07927	G5000PW	55.0	60	20	≥ 5,000	15.0 - 25.0	30.0	15
PEEK o	olumns							
20024	BioAssist G6PW	7.8	30	17	≥ 3,000	0.5 - 1.0	1.2	10
Guard	columns							
08034	Oligo Guard column	6.0	4.0	13	For 7.8 mm ID G-Olig	o-PW columns		
08033	$PW_{XL}Guard\ column$	6.0	4.0	12	For 7.8 mm ID $PW_{XL}$ a	and G-DNA-PW co	lumns	
					(contains TSKgel G3	000PW packing)		
21876	$PW_{XL}$ -CP Guard colur	mn 6.0	4.0	13	For 7.8 mm ID $PW_{XL}$ -	·CP columns		
06763	PW Guard column	7.5	7.5	13	For 7.5 mm ID G1000	PW and G2000PW	columns	
06762	PW Guard column	7.5	7.5	13	For 7.5 mm ID G2500	PW through GMP	N columns	
06758	PW Guard column	21.5	7.5	17	For 21.5 mm ID G250	0PW through G500	00PW columns	
07924	PW Guard column	45.0	5.0	20	For 55 mm ID G3000F	PW through G5000	PW columns	
Bulk p	acking							

08035 PW<sub>xL</sub>Top-Off, 1 g wet resin

10

For all  $\ensuremath{\mathsf{PW}_{\mathsf{xL}}}\xspace$  and <code>G-DNA-PW</code> columns

45 



## TSK-GEL ALPHA AND SUPERAW GEL FILTRATION COLUMNS Gel Filtration and Gel Permeation Chromatography of water-soluble and polar organic-soluble polymers

## HIGHLIGHTS

- A unique hydrophilic, polyvinyl resin is available in conventional column dimensions (Alpha) and high throughput column format (SuperAW).
- Exhibits strong mechanical stability and minimal swelling characteristics
- A wide range of solvent compatibility, from 100% water to 100% non-polar organic solvents
- The reduced particle size and shorter column length of TSK-GEL SuperAW columns provide equivalent resolution in one half the time for high throughput applications.
- Unlike polystyrene-divinylbenzene (PS-DVB) resins that may adsorb polymers due to hydrophobic interaction, both the TSK-GEL Alpha and SuperAW columns allow for the separation of polymers soluble in methanol.
- Provide accurate molecular weight determination of samples in dimethyl formamide and exhibit normal retention of polystyrene polymers
- System peaks from salts in the eluent elute away from the oligomer of interest, providing accurate MW determinations.

#### **Column Selection**

The TSK-GEL Alpha Series consists of six columns with three particle sizes: 7, 10, and 13  $\mu$ m. These columns span a wide MW separation range from 100 to more than 1 x 10<sup>6</sup> Da when using polyethylene oxide (PEO) as a MW standard. Exclusion limits for the TSK-GEL Alpha columns for polyethylene oxide (PEO), polyethylene glycols (PEG) and polystyrenes (PS) are shown in the table below. Calibration curves for the TSK-GEL Alpha Series columns are shown on the next page for polyethylene oxide, polyethylene glycol and polystyrene standards.

The TSK-GEL SuperAW series contains a similar chemistry as the TSK-GEL Alpha series but offers the benefit of smaller particle sizes (4  $\mu$ m to 9  $\mu$ m) and smaller column dimensions. Reductions in analysis time and mobile phase consumption make SuperAW columns ideal for high throughput applications. TSK-GEL Alpha and SuperAW columns are offered in 5 discrete exclusion ranges and 1 mixed bed. Both column types can accommodate polymer standards up to several million Dalton molecular weight (see calibration curves on the next page).

Column	Particle Size (µm)	Exclusion limit (Da) for various standards and eluents					
		PEO <sup>®</sup> /H <sub>2</sub> O	PS <sup>b</sup> /10mmol/L LiBr in DMF	PEG°/10mmol/L LiBr in MeOH			
Alpha-2500	7	5 x 10 <sup>3</sup>	1 x 10 <sup>4</sup>	1 x 10 <sup>4</sup>			
Alpha-3000	7	9 x 10⁴	1 x 10 <sup>5</sup>	6 x 10⁴			
Alpha-4000	10	4 x 10⁵	1 x 10 <sup>6</sup>	3 x 10 <sup>6</sup>			
Alpha-5000	10	1 x 10 <sup>6</sup>	7 x 10 <sup>6</sup>	N.D.			
Alpha-6000	13	> 1 x 10 <sup>7</sup>	> 1 x 10 <sup>7</sup>	N.D.			
Alpha-M	13	> 1 x 10 <sup>7</sup>	> 1 x 10 <sup>7</sup>	N.D.			
SuperAW2500	4	5 x 10 <sup>3</sup>	8 x 10 <sup>3</sup>	1 x 10 <sup>4</sup>			
SuperAW3000	4	9 x 10 <sup>4</sup>	8 x 104	1 x 10⁵			
SuperAW4000	6	1 x 10 <sup>6</sup>	6 x 10 <sup>5</sup>	6 x 10⁵			
SuperAW5000	7	1 x 10 <sup>6*</sup>	N.D.	N.D.			
SuperAW6000	9	1 x 10 <sup>7*</sup>	N.D.	N.D.			
SuperAWM-H	9	1 x 10 <sup>7*</sup>	N.D.	N.D.			

#### **Exclusion limits for TSK-GEL Alpha Series and SuperAW Series columns**

N.D. = not determined a Polyethylene oxide b Polystyrene divinyl benzene c Polyethylene glycol

\* Exclusion limit for SuperAW5000, SuperAW6000, and SuperAWM-H are estimated, respectively

## CALIBRATION CURVES FOR TSK-GEL ALPHA AND SUPERAW GEL FILTRATION COLUMNS The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

### Polyethylene oxide (PEO), polyethylene glycol (PEG) and polystyrene (PS) calibration curves for TSK-GEL Alpha columns



 Eluent:
 A. H<sub>2</sub>O; B. 10 mmol/L LiBr in Methanol; C. 10 mmol/L LiBr in DMF

 Flow Rate:
 1.0 mL/min

 Temperature:
 A. 25°C; B. 25°C; C. 40°C

 Detection:
 RI

### Calibration curves for TSK-GEL SuperAW Series in different solvents with different polarity





Samples: Standard polyethylene oxide, polyethylene glycol, ethylene glycol



## APPLICATIONS OF TSK-GEL ALPHA AND SUPERAW GEL FILTRATION COLUMNS

3

The versatility of using TSK-GEL Alpha columns with various polar solvents is illustrated in FIGURE 11 for the analysis of cellulose derivatives. A TSKgel Alpha-M column was used to separate ethylcellulose with the polar solvent DMF and ethylhydroxyethyl cellulose with methanol.

The separation of polyvinylalcohol with different degrees of saponification is shown in FIGURE 12. This separation was performed with a TSKgel Alpha-5000 and a TSKgel Alpha-3000 column in series using a hexafluoroisopropanol mobile phase.

FIGURE 13 shows that the column efficiency of TSK-GEL SuperAW series columns is maintained in a wide variety of polar organic solvents.

#### **E** FIGURE 11

#### **TSKgel Alpha-M separation of cellulose derivatives**



 

 Sample:
 A. 50μL ethylcellulose, 0.1%; B. 50μL ethylhydroxyethylcellulose, 0.1%

 Elution:
 A. 10mmol/L LiBr in DMF; B. 10mmol/L LiBr in methanol

 Flow Rate:
 0.5mL/min

 Temperature:
 40°C

 Detection:
 RI

 FIGURE 12

Polyvinylalcohol characterization using TSK-GEL Alpha-5000 and Alpha-3000 columns in series



 Column:
 TSKgel Alpha-5000 and Alpha-3000, 7.8mm ID x 30cm in series

 Sample:
 degree of saponification of polyvinyl alcohol:

 A. 75%; B. 88%; C. 100%
 hexafluoroisopropanol (HFIP)

 Flow Rate:
 0.5mL/min

 Temperature:
 40°C

 Detection:
 RI

#### FIGURE 13 \_\_\_\_\_\_



48

Part #	Description	ID (mm)	Length (cm)	Particle Size (μm)	Number Theoretical Plates	<u>Flow Rate (r</u> Range	<u>nL/min)</u> Max.	Maximum Pressure Drop (kg/cm²)
Stainle	ess steel columns							
18339	Alpha-2500	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	40
18340	Alpha-3000	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	40
18341	Alpha-4000	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	30
18342	Alpha-5000	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	30
18343	Alpha-6000	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	20
18344	Alpha-M (mixed bed)	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	20
Guard	columns							
18345	Alpha Guard column	6	4	13	For all Alph	na columns		
VMpal	c columns*							
20011	VMpak-25	2.0	5	7	≥ 1,000	0.1 - 0.2	0.25	20
20012	VMpak-25	2.0	15	7	≥ 3,000	0.1 - 0.2	0.25	60
Stainle	ess steel columns							
19315	SuperAW2500	6.0	15	4	≥ 16,000	0.3 - 0.6	0.6	60
19316	SuperAW3000	6.0	15	4	≥ 16,000	0.3 - 0.6	0.6	60
19317	SuperAW4000	6.0	15	6	≥ 10,000	0.3 - 0.6	0.6	40
19318	SuperAW5000	6.0	15	7	>10,000	0.3 - 0.6	0.6	30
19319	SuperAW6000	6.0	15	9	>7,000	0.3 - 0.6	0.6	20
19320	SuperAWM-H	6.0	15	9	>7,000	0.3 - 0.6	0.6	20
Guard	columns							
19321	SuperAW-L Guard Columr	4.6	3.5	7	For SuperAV	W2500-4000 colum	ins.	
19322	SuperAW-H Guard Colum	n 4.6	3.5	23	For SuperAW5000-AWM-H columns			

\*TSK-GEL VMpak-25 series contains a similar packing as TSKgel Alpha-2500. It can be used for multimodal LC/LC-MS separations.



## TSK-GEL H<sub>xt</sub>, H<sub>HR</sub>, SUPERH AND SUPERHZ GEL PERMEATION COLUMNS Polymer-based columns for Gel Permeation Chromatography of organic-soluble polymers

## HIGHLIGHTS \_\_\_\_\_

- Porous, highly cross-linked, spherical polystyrene divinylbenzene (PS-DVB) resin.
- Four different TSK-GEL H-type columns are available. Each of these are packed with different particle sizes (see table below).
- H-type packings are available in eight pore sizes.
- Expanded molecular weight ranges with exclusion limits from 1,000 Da to an estimated 4 x 108 Da
- Minimal shrinking and swelling of the column bed
- Chemically and thermally stable
- Use 4.6 mm ID SuperH and SuperHZ columns for reduced solvent consumption in high throughput analysis.
- Novel multi-pore distribution in the TSKgel MultiporeH<sub>vi</sub>-M column provides linear calibration curves over a wider MW range.
- Mixed bed columns with optimized particle and pore sizes to prevent polymer sheering.
- Semi-micro SuperHZ columns now available as multipore columns with linear calibration curves.

TSK-GEL H-type packings are available in eight pore sizes and span four different column chemistries. For polymer samples with a broad molecular range, packing of several pore sizes are provided in the mixed bed columns: TSK-GEL SuperHZM series, TSK-GEL SuperHM series, TSKgel  $\text{GMH}_{_{\!\!\text{Xl}}}$ , TSKgel  $\text{GMH}_{_{\!\!\text{HR}}}$ , and selected high temperature versions provide linear calibration curves up to several million Daltons (see page 51).

#### **Column Selection**

The Super prefix refers to the efficiency of the column. The Super series columns contain ultra efficient particles as small as 3 µm, housed in 15 cm length columns. The smaller particle allows for equivalent resolution to conventional  $H_{x_1}$  columns, with 50% less run time due to the shorter column length. The Super series columns are an excellent choice for high throughput polymer analysis.

Series Type	SuperHZ	H <sub>xL</sub>	SuperH	H <sub>HR</sub>	
Application focus	High-throughput polymer analysis with ultra low polymer adsorption. Limited solvent compatibility range.	Conventional polymer analysis with ultra low polymer adsorption. Limited solvent compatibility range.	High-throughput polymer analysis with expanded solvent compatibility.	Conventional polymer analysis with expanded solvent compatibility range.	
Particle size         3, 5 and 10 μm, depending on pore size         5		5, 6 and 9 µm, depending on pore size	3 and 5 µm, depending on pore size	5 µm	
Theoretical plates <sup>1</sup>	16,000/15 cm column	16,000/30 cm column	16,000/15 cm column	16,000/30 cm column	
Maximum temperature	G1000 - G4000 60°C G5000 - mixed 80°C	G1000 - G4000 60°C G5000 - mixed 80°C	140°C	140°C	
Standard shipping solvent	THF	THF <sup>2</sup>	THF <sup>2</sup>	THF <sup>2</sup>	
THF can be switched to	benzene, chloroform, toluene, xyle dicholoroethane³	ene, dichloromethane <sup>3</sup> and	see our website for detailed information		
Other shipping solvents available?	yes⁴		no		
Number of solvent substitutions	One time only		Several⁵		
Solvent exchange instructions	Linear gradient with a 2%/min rate of change at a flow rate <0.25 mL/min.	Linear gradient with a 2%/ min rate of change at a flow rate <0.5 mL/min.	Linear gradient with a 2%/min rate of change according to flow rates listed on our website.		

1) Theoretical plates listed are based on smallest particle size listed 2) High-temperature columns (HT) are shipped with OCDB (Orthochlorodivinylbenzene) as standard

shipping solvent. 3) Switching from THF to dichloromethane and dichloroethane is not recommended for G1000 pore size columns

4) See our website for available shipping solvents 5) After switching to a very polar solvent such as acetone, switching back to a nonpolar solvent is

not recommended

## **CALIBRATION CURVES FOR TSK-GEL H-TYPE GELPERMEATION COLUMNS**



The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

Calibration curves for TSK-GEL H<sub>XL</sub> columns with polystyrene standards







### Calibration curves for TSK-GEL SuperH columns with polystyrene standards





0+

0.01

0.1

Pore size (um)

52

SEC

## MULTI-PORE SIZE DISTRIBUTION IN A POLYSTERENE PACKING MATERIAL Novel approach to GPC of samples with a wide range of molecular weights

The TSKgel MultiporeH<sub>x1</sub>-M column offers a unique packing material and a novel strategy for the precise analysis of polymers by Gel Permeation Chromatography (GPC). Until now, the GPC separation of a sample containing a wide range of molecular weight polymers was performed by one of two strategies. One strategy combines columns with different pore sizes of packing material in series. The other strategy employs a single column with a blend of different pore sizes of packing materials, commonly referred to as a mixed bed. Mixed bed columns do not always provide linear calibration curves, which may result in broad or split peaks. With the introduction of the TSKgel MultiporeH<sub>v1</sub>-M column, a novel strategy was introduced using a single column containing a novel polystyrene packing material with a multi-pore size distribution. FIGURE 14 illustrates the three strategies. The TSKgel Multipore column has several different pore sizes with continuous distribution in every bead. This results

in sharper peaks without inflections that may be observed using mixed-bed columns.

The pore size distributions of the TSKgel MultiporeH<sub>xL</sub>-M column and a mixed-bed column are shown in FIGURE 15. The mixed-bed column shows a sharp maximum for pores with a diameter of 0.08  $\mu$ m, though the overall pore size distribution ranges from 0.006 to 0.6  $\mu$ m in diameter. In the case of the TSKgel MultiporeH<sub>xL</sub>-M column, the pore size distribution exhibits a wider maximum range from 0.02 to 0.1  $\mu$ m in diameter. This difference in pore size distribution may explain the reason for the inflection phenomenon. A comparison of calibration curves for polystyrene standards on the TSKgel MultiporeH<sub>xL</sub>-M column, the TSKgel GMH<sub>HR</sub>-H and PLgel Mixed-C, are shown in FIGURE 16. Both the TSKgel GMH<sub>HR</sub>-H and PLgel Mixed-C columns are mixed-bed columns.



10

## APPLICATIONS OF TSK-GEL H-TYPE GEL PERMEATION COLUMNS

### Phthalate esters

FIGURE 17 demonstrates the high efficiency separation on a TSKgel G1000 $H_{xL}$  column for low molecular weight phthalate esters. Resolution was close to baseline, even though the molecular weights of the esters differed by less than 50 Da.

#### **Phenol resin**

The TSKgel GMH<sub>x1</sub>-L column has been designed to provide a complete profile for high molecular weight samples that contain low molecular weight additives. The calibration curve for this mixed-bed column is shallow in the low molecular weight range





- 5. diethylphthalate (222Da), 6. dimethylphthalate (194Da),
- 7. n-propylbenzene (120Da), 8. ethylbenzene (116Da),
- 9. toluene (92Da), 10. benzene (78Da)

Elution: THF

Column:

Sample:

Flow Rate: 1.0mL/min

Detection: UV @ 254nm

of oligomers. Sample adsorption is not observed. For example, the complete profile of a phenol resin, with high resolution of the low molecular weight components, is shown in FIGURE 18. Other applications for the TSKgel GMH<sub>x1</sub>-L column include analyses of paint materials, bond and adhesive components and synthetic polymer additives.

#### **Fatty acids**

In FIGURE 19, two TSKgel G2000 $H_{x_1}$  columns in series separate a mixture of fatty acids ranging from C4 to C30.





Detection:

RI

53 2



## **APPLICATIONS OF TSK-GEL H-TYPE GEL PERMEATION COLUMNS**

#### **Acrylic polymer**

FIGURE 20 shows the separation of an acrylic polymer on the TSKgel MultiporeH<sub>xL</sub>-M column compared with two commercially available mixed-bed columns. The arrows illustrate the inflections seen in the chromatograms from mixed-bed columns and the improvement achieved when using the TSKgel MultiporeH<sub>xL</sub>-M column.

#### Polymethylmethacrylate

The effect of different pore size distributions in the mixed beds of TSKgel  $GMH_{HR}$ -H and TSKgel  $GMH_{HR}$ -M is illustrated in FIGURE 21. The TSKgel  $GMH_{HR}$ -M produces better resolution in the 8 x 10<sup>5</sup> to 1 x 10<sup>4</sup> Da range.

#### **Epoxy resin**

FIGURE 22 demonstrates the excellent fingerprint obtained using small diameter SuperHZM-M mixed bed columns.

#### 

## Separation of acrylic resin by SEC on TSKgel MultiporeH<sub>XL</sub>-M and mixed-bed type columns



- Column:
   A. TSKgel MultiporeH<sub>XL</sub>-M, two 7.8 mm ID x 30 cm L columns in series;

   B. Competitor P, two 7.5 mm ID x 30 cm L columns in series, mixed-bed type;

   C. Competitor S, two 8.0 mm ID x 30 cm L columns in series, mixed-bed type

   Sample:
   acrylic polymer (0.1%, 50 µL)

   Elution:
   THF

   Flow Rate:
   1.0 mL/min

   Temperature:
   40°C
- Detection: RI

#### Chromatogram of epoxy resin

FIGURE 22



## maaricon of TSKaal CMU

FIGURE 21

2

Comparison of TSKgel GMH\_{\rm HR}-H and -M columns with polymethylmethacrylate standards



## ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	<u>Flow Rate (n</u> Range	n <u>L/min)</u> Max.	Maximum Pressure Drop (ka/cm²)
Stainle	ess steel columns							
17352	G1000H <sub>HR</sub>	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17353	G2000H <sub>HR</sub>	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17354	G2500H <sub>HR</sub>	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17355	G3000H <sub>HR</sub>	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17356	G4000H <sub>HR</sub>	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17357	G5000H <sub>HR</sub>	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17358	G6000H <sub>HR</sub>	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17359	G7000H <sub>HR</sub>	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17362	$GMH_{_{HR}}\text{-}L$ mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
18055	$GMH_{{}_{HR}}-N$ mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17392	$GMH_{{}_{HR}}-M$ mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
17360	$GMH_{_{HR}}-H\ mixed-bed$	7.8	30	5	≥ 16,000	0.5 - 1.0	2.0	50
18393	GMH <sub>HR</sub> -H(S)HT mixed-bed	7.8	30	13	≥ 8,0000.	5 - 1.0	2.5	20
16131	G1000H <sub>xL</sub>	7.8	30	5	≥ 16,000	0.5 - 1.0	1.0	50
16134	G2000H <sub>xL</sub>	7.8	30	5	≥ 16,000	0.5 - 1.0	1.2	50
16135	G2500H <sub>xL</sub>	7.8	30	5	≥ 16,000	0.5 - 1.0	1.2	50
16136	G3000H <sub>xL</sub>	7.8	30	6	≥ 16,000	0.5 - 1.0	1.2	35
16137	G4000H <sub>xL</sub>	7.8	30	6	≥ 16,000	0.5 - 1.0	1.2	35
16138	G5000H <sub>xL</sub>	7.8	30	9	≥ 14,000	0.5 - 1.0	1.2	15
16139	G6000H <sub>xL</sub>	7.8	30	9	≥ 14,000	0.5 - 1.0	1.2	15
16140	G7000H <sub>xL</sub>	7.8	30	9	≥ 14,000	0.5 - 1.0	1.2	15
16141	GMH <sub>xL</sub> mixed-bed	7.8	30	9	≥ 16,000	0.5 - 1.0	1.2	15
07112	GMH <sub>x∟</sub> -HT	7.8	30	13	≥ <b>5</b> ,5000.	5 - 1.0	1.2	15
16652	GMH <sub>x⊥</sub> -L mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	1.2	35
18403	Multipore H <sub>xL</sub> -M	7.8	30	5	≥ 16,000	0.5 - 1.0	1.0	35
17990	TSKgel SuperH1000	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	60
17991	TSKgel SuperH2000	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	60
17992	TSKgel SuperH2500	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	60
17993	TSKgel SuperH3000	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40
17994	TSKgel SuperH4000	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40
17995	TSKgel SuperH5000	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40
17996	TSKgel SuperH6000	6.0	15	5	≥ 16,000	0.3 - 0.6	0.8	40
17997	TSKgel SuperH7000	6.0	15	5	≥ 16,000	0.3 - 0.6	0.8	40
17998	TSKgel SuperHM-L	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40
17999	TSKgel SuperHM-N	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40
18000	TSKgel SuperHM-M	6.0	15	3	≥ 16,000	0.3 - 0.6	0.8	40
18001	TSKgel SuperHM-H	6.0	15	3	≥ 16.000	0.3 - 0.6	0.8	40
	<b>S</b> 1							





## ORDERING INFORMATION

Part #	Description	ID	Lenath	Particle	Number	Flow Rate (mL/min)		Maximum
		(mm)	(cm)	Size (µm)	Theoretical Plates	Range	Max.	Pressure Drop (kg/cm²)
Stainle	ess steel columns							
19309	TSKgel SuperHZ1000	4.6	15	3	≥ 16,000	0.15 - 0.35	0.4	56
19302	TSKgel SuperHZ1000	6.0	15	3	≥ 16,000	0.25 - 0.6	0.7	56
19310	TSKgel SuperHZ2000	4.6	15	3	≥ 16,000	0.15 - 0.35	0.4	50
19303	TSKgel SuperHZ2000	6.0	15	3	≥ 16,000	0.25 - 0.6	0.7	50
19311	TSKgel SuperHZ2500	4.6	15	3	≥ 16,000	0.15 - 0.35	0.4	40
19304	TSKgel SuperHZ2500	6.0	15	3	≥ 16,000	0.25 - 0.6	0.7	40
19312	TSKgel SuperHZ3000	4.6	15	3	≥ 16,000	0.15 - 0.35	0.4	30
19305	TSKgel SuperHZ3000	6.0	15	3	≥ 16,000	0.25 - 0.6	0.7	30
19313	TSKgel SuperHZ4000	4.6	15	3	≥ 16,000	0.15 - 0.35	0.4	35
19306	TSKgel SuperHZ4000	6.0	15	3	≥ 16,000	0.25 - 0.6	0.7	35
19960	TSKgel SuperHZM-N	4.6	15	3	≥ 16,000	0.15 - 0.35	0.4	35
19661	TSKgel SuperHZM-N	6.0	15	3	≥ 16,000	0.25 - 0.6	0.7	35
19662	TSKgel SuperHZM-M	4.6	15	3 and 5	≥ 16,000	0.15 - 0.35	0.4	20
19663	TSKgel SuperHZM-M	6.0	15	3 and 5	≥ 16,000	0.25 - 0.6	0.7	20
19664	TSKgel SuperHZM-H	4.6	15	10	≥ 9,000	0.15 - 0.35	0.4	10
19665	TSKgel SuperHZM-H	6.0	15	10	≥ 9,000	0.25 - 0.6	0.7	10
21488	SuperMultiporeHZ-M	4.6	15	4	≥ 16,000			24
21815	SuperMultiporeHZ-N	4.6	15	3	≥ 20,000			40
21885	SuperMultiporeHZ-H	4.6	15	6	≥ 11,000			10

### **Guard columns**

18404	MultiporeH <sub>xL</sub> -M Guard	6.0	4.0	5	For P/N 18403
07113	H <sub>xL</sub> Guard Column	6.0	4.0		For G1000H <sub>xL</sub> through G4000H <sub>xL</sub> columns
13727	H <sub>xL</sub> Guard Column	6.0	4.0		For G5000H <sub>xL</sub> through GMH <sub>xL</sub> -L mixed-bed columns
17368	H <sub>HR</sub> Guard Column	6.0	4.0	5	For G1000-4000H <sub>HR</sub> and GMHhr-L columns
17369	H <sub>HR</sub> Guard Column	6.0	4.0	5	For G5000-7000H <sub>HR</sub> and and GMH <sub>HR</sub> -M; -N; -H columns
18002	SuperH Guard Column	4.6	3.5	3	For SuperH1000-4000
18003	SuperH Guard Column	4.6	3.5	3	For SuperH5000-7000 and HM-L;-N;-M;-H columns
19314	SuperHZ Guard Column	4.6	2.0	3	For 4.6 mm ID SuperHZ1000-4000 and HZM-N &-M columns
19668	SuperHZ Guard Column	4.6	2.0	10	For 4.6 mm ID SuperHZM-H columns
19666	SuperHZ Guard Column	4.6	3.5	3	For 6.0 mm ID SuperHZ1000-4000 and HZM-N &-M columns
19667	SuperHZ Guard Column	4.6	3.5	10	For 6.0 mm ID SuperHZM-H columns
21489	SuperMP-M Guard	4.6	2.0	4	For SuperMultipore HZ-M P/N 21488
21816	SuperMP-N Guard	4.6	2.0	3	For SuperMultipore HZ-N P/N 21815
21886	SuperMP-H Guard	4.6	2.0	6	For SuperMultipore HZ-H P/N 21887

56

TOSOH

## **ECOSEC GPC SYSTEM - BASED ON 35 YEARS OF EXPERIENCE IN GPC**

EcoSEC is a compact, all-in-one GPC system for fast, high resolution, semi-micro GPC. Comprising a precision solvent delivery system, automatic injector, column oven and a high performance refractive index detector, the design of the system components, their configuration and the optimized flow line provides outstanding performance with minimized dead volume. This makes EcoSEC the ideal instrument to be used in combination with the well respected TSK-GEL semi-micro GPC/SEC columns. In Europe, EcoSEC is offered in cooperation with Polymer Standards Service (PSS), an acknowledged leader in the field of polymer analysis.

