TSK-GEL PW Brochure

Table of Contents

TSK-GEL PW Series

HPLC columns for the size separation of biological macromolecules

TSK-GEL PW columns are designed for aqueous, Size Exclusion Chromatography (SEC) of proteins, polysaccharides, oligosaccharides, DNA and RNA. The column packing materials are porous, hydrophilic, rigid, polymer beads with particle sizes ranging from 6 µm to 22 µm. They exhibit excellent chemical and mechanical stability, have been used from pH 2.0 to 12.0, and can be cleaned with 0.5 M NaOH.
They are particularly useful at pH extremes which would adversely affect silica SEC resins (TSK-GEL SW Series). Depending on which of the six pore size ranges is selected, these columns have 10,000 to 30,000 plates/m and are available in both analytical and preparative housings.

TSK-GEL PWXL columns are a smaller particle size version of the TSK-GEL PW polymer packing. These columns are used where the highest resolution of sample peaks is required. There are five pore size ranges available with chromatographic efficiencies varying from 23,000 to 50,000 plates/m.

TSK-GEL PW specialty columns

TSK-GEL GMPW is a mixed-bed SEC column comprised of various pore sizes and 17 µm particles. This maximizes the range of molecular weights that will elute between the excluded volume and the permeation volume.

Polyethylene glycol and oxide calibration curves for TSK-GEL PW and TSK-GEL PWXL columns

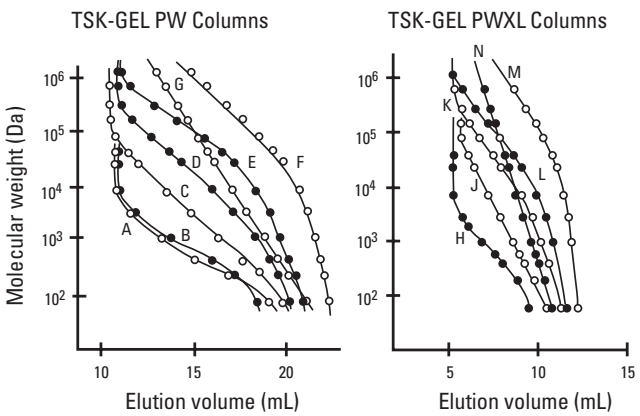

Figure 1
Column: TSK-GEL PW columns: A. G2000PW, B. G2500PW, C. G3000PW, D. G4000PW, E. G5000PW, F. G6000PW, G. GMPW, all 7.5 mm L x 60 cm L
TSK-GEL PWXL columns: H. G2500PWXL, J. G3000PWXL, K. G4000PWXL, L. G5000PWXL, M. G6000PWxL, N. GMPWXL, all 7.8 mm ID x 30 cm L
Sample: PEO and PEG
Elution: distilled water
Flow rate: 1.0 ml/min
Detection: RI

TSK-GEL GMPWXL is the 13 µm high resolution version of TSK-GEL GMPW (an example is shown in Figure 9).

TSK-GEL G-Oligo-PW is specially designed for oligosaccharides and other aqueous nonionic polar oligomers of less than 3,000 Daltons. This column is based on a 6 µm version of the TSK-GEL G2000PW packing material (see Figure 3, 7, 6).

TSK-GEL G-DNA-PW is also a specially designed column but for the separation of DNA and RNA molecules of less than 7,000 base pairs and other large molecules (see Figure 4 and 8).

Protein calibration curves on TSK-GEL PWXL columns

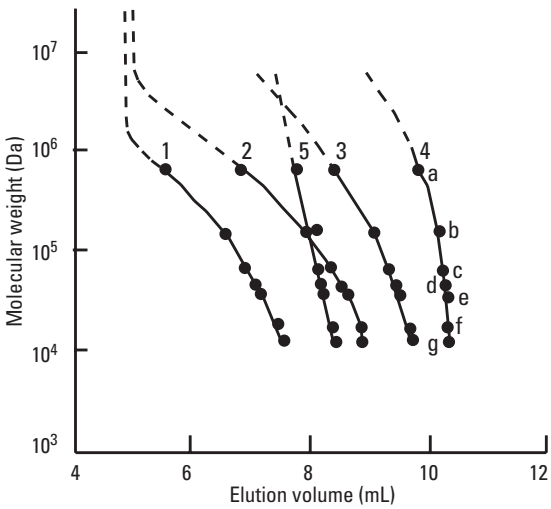

Figure 2
Column: 1. G3000PWXL, 2. G4000PWXL, 3. G5000PWXL 4. G6000PWXL, 5. GMPWXL
Sample: a. thyroglobulin, b. y-globulin, c. albumin, d. ovalbumin, e. ß-lactoglobulin, f. myoglobin, g. cytochrome C
Elution: 0.2 M phosphate buffer, pH 6.8
Flow rate: 1.0 ml/min
Detection: UV @ 280 nm

TSK-GEL PW Series

Properties and molecular weight separation ranges for TSK-GEL PW packings

TSKgel column	Particle Size* (µm)	Average pore Size (Å)	Molecular weight of sample (Da)		
			Polyethylene glycols & oxides	Dextrans**	Globular proteins**
G2000PW	12, 17, 20	125	up to 2,000	—	<5,000
$G2500PW_{XL}$ G2500PW	6 12, 17, 20	<200	up to 3,000	—	<8,000
$G3000PW_{XL}$ G3000PW	6 12, 17, 20	200	up to 5×10^4	up to 6×10^4	$500 - 8 \times 10^5$
$G4000PW_{XL}$ G4000PW	10 17, 22	500	$2{,}000–3 \times 10^4$	$1{,}000 – 7 \times 10^5$	$1 \times 10^4 – 1.5 \times 10^6$
$G5000PW_{XL}$ G5000PW	10 17, 20, 22	1000	$4{,}000–1 \times 10^6$	$5 \times 10^4 – 2.5 \times 10^6$	$<1 \times 10^7$
$G6000PW_{XL}$/BioAssist $G6PW_{XL}$ G6000PW	13 17, 25	>1000	$4 \times 10^4 – 8 \times 10^6$	$5 \times 10^5 – 5 \times 10^7$	$<2 \times 10^8$
$GMPW_{XL}$ GMPW	13 17	<100–1000	$500–8 \times 10^6$	$<5 \times 10^7$	$<2 \times 10^8$
G-Oligo-PW G-DNA-PW	6 10	125 >1000	up to 3,000 $4 \times 10^4 – 8 \times 10^6$	— $<5 \times 10^7$	<3,000 $< 2 \times 10^8$

Column: TSK-GEL PW columns, 7.5 mm ID x 60 cm L; TSKgel PW_{XL}, G-Oligo-PW & G-DNA-PW, 7.8 mm ID x 30 cm L
Elution: Polyethylene glycols and oxides: distilled water; dextrans and proteins: 0.2 M phosphate buffer, pH 6.8
Flow 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.

Table I

Calibration curve for TSK-GEL G-Oligo-PW

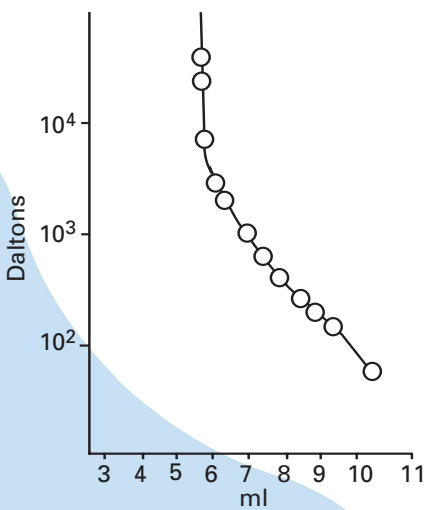

Figure 3
Column: TSKgel G-Oligo-PW, 7.8 mm ID X 30 cm L
Sample: PEG and PEO standards
Flow rate: 1.0 ml/min
Detection: RI

Calibration curve for double-stranded DNA

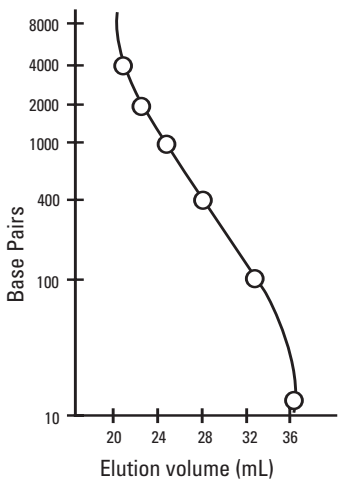

Figure 4
Column: TSKgel G-DNA-PW, four 10 µm, 7.8 mm ID X 30 cm L columns in series
Sample: double-stranded DNA fragments: EcoR I and BstN I cleaved pBR322 DNA, void volume determined with DNA
Elution: 0.3 M NaCl in 0.1 M Tris-HCl, pH 7.5
Flow rate: 15 ml/min
Detection: UV @ 260 nm

TSK-GEL PW Series

Recommended eluents for GFC of water-soluble polymers on TSK-GEL PW type columns

Type of polymer	Typical sample	Suitable eluent
Nonionic hydrophilic	polyethylene glycol soluble starch, methyl cellulose, pullulan dextran, hydroxyethyl cellulose, polyvinyl alcohol, polyacrylamide	distilled water 0.01 N NaOH 20% DMSO Buffer or salt solution (e.g., 0.1–0.5 M $NaNO_3$)
Nonionic hydrophobic	polyvinylpyrrolidone	Buffer or salt solution with organic solvent (e.g., 20% CH_3CN in 0.1 M $NaNO_3$)
Anionic hydrophilic	sodium chondroitin sulfate, sodium alginate, carboxymethyl cellulose, sodium polyacrylate, sodium hyaluronate	Buffer or salt solution (e.g., 0.1 M $NaNO_3$)
Anionic hydrophobic	sulfonated lignin sodium salt, sodium polystyrenesulfonate	Buffer or salt solution with organic solvent (e.g., 20% CH_3CN in 0.1 M $NaNO_3$)
Cationic hydrophilic	glycol chitosan, DEAE-dextran, poly(ethyleneimine), poly(trimethylaminoethyl methacrylate) iodide salt	0.5 M acetic acid with 0.3 M Na_2SO_4, or 0.8 M $NaNO_3$
Cationic hydrophobic	poly(4-vinylbenzyltrimethylammonium chloride), poly(N-methyl-2-vinylpyridinium) iodide salt	0.5 M acetic acid with 0.3 M Na_2SO_4
Amphoteric hydrophilic	peptides, proteins, poly-and oligosaccharides, DNA, RNA	Buffer or salt solution (e.g., 0.1 M $NaNO_3$)
Amphoteric hydrophobic	blue dextran, collagen, gelatin, hydrophobic proteins, hydrophobic peptides	Buffer or salt solution with organic solvent (e.g., 20% CH_3CN in 0.1 M $NaNO_3$ or 35–45% CH_3CN in 0.1% TFA)

Table II

PW Columns are compatible with*:

Up to 50% Polar Organics

Acetonitrile
Acetone
Isopropanol
Methanol
Ethanol
DMF
DMSO

Up to 20% Nonpolar Organics

THF

*Switch to these buffers at 50% of the standard flow rate.

Detergents

up to 0.1% SDS
up to 1% Tween, Triton

Chaotropic Agents

8 M Urea
6 M Guanidine

Not Compatible

Toluene (not water soluble)
and other not water soluble solvents

TSK-GEL PW Series

Elution curves for PEG standards on TSK-GEL G25000PWXL

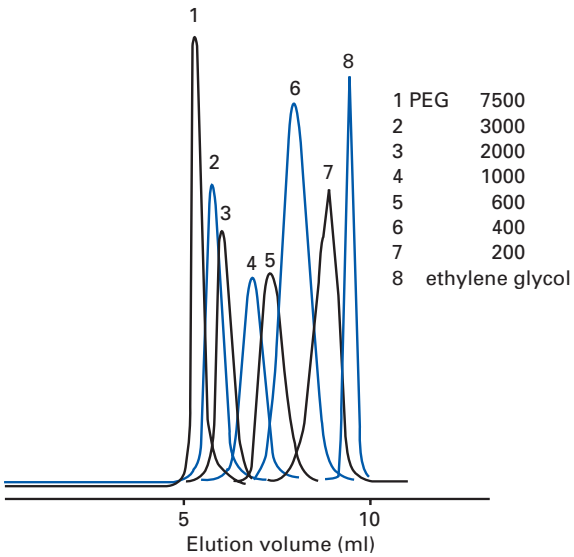

Figure 5
Column: TSKgel G2500PWXL, 7.8 mm ID X 30 cm L
Sample: polyethylene glycols
Elution: destilled water
Flow rate: 1.0 ml/min
Detection: RI

Separation of chito oligosaccharides

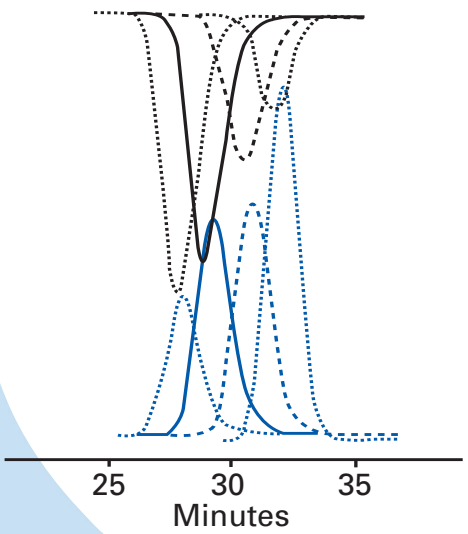

Figure 6
Column: TSKgel G-Oligo-PW, four 7.8 mm ID X 30 cm L in series
Sample: chito oligosaccharides
Elution: destilled water
Detection: RI

Separation on hydrolyzed ß-cyclodextrin

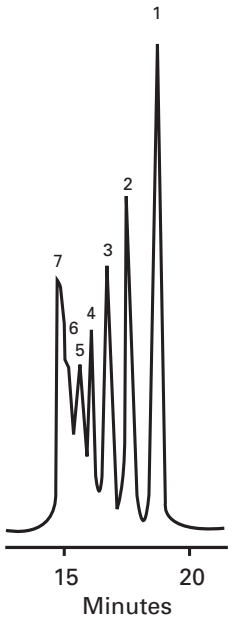

Figure 7
Column: TSKgel G-Oligo-PW, two 6 µm, 7.8 mm ID X 30 cm L columns in series
Sample: hydrolyzed ß-cyclodextrin
Elution: destilled water
Flow rate: 1.0 ml/min
Temperature: 60 °C
Detection: RI

Separation of EcoR I and BstN I cleaved pBR322 DNA

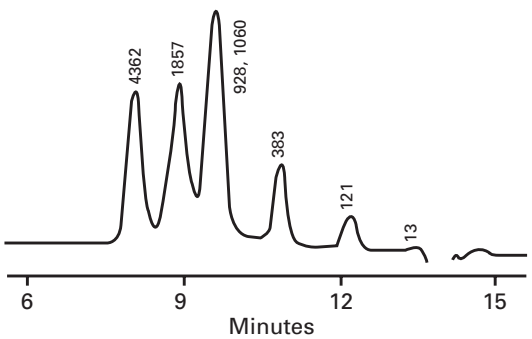

Figure 8
Column: TSKgel G-DNA-PW, four 10 µm, 7.8 mm ID X 30 cm L in series
Sample: 1.7 µg of EcoR I cleaved pBR322 DNA and 8.0 µg of BstN I cleaved pBR322 DNA
Elution: 0.3 M NaCl in 0.1 Tris-HCl, pH 7.5 and 1 mM EDTA
Flow Rate: 0.3 ml/min
Detection: UV @ 260 nm

Separation of pullulan

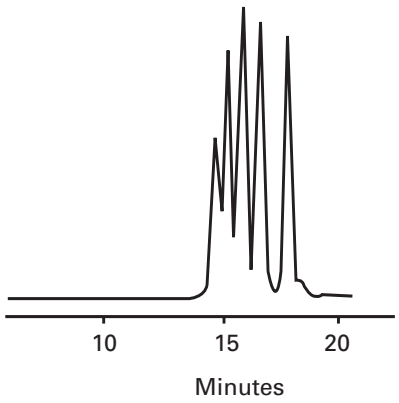

Figure 9
Column: TSKgel GMPWXL, four 7.8 mm ID X 30 cm L in series
Sample: pullulan
Elution: 0.1 M NaCl
Flow Rate: 1.0 ml/min
Detection: RI, LS

Separation of gelatin

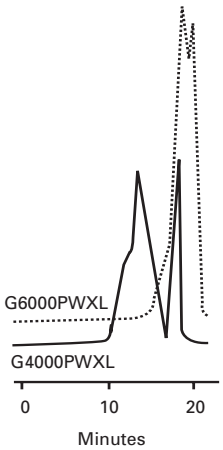

Figure 10
Column: ——— TSKgel G4000PWXL
..... TSKgel G6000PWXL
Sample: gelatin
Elution: 0.2 mM phosphate buffer, pH 6.9
Flow Rate: 1.0 ml/min
Detection: RI

Troubleshooting and cleaning TSK-GEL PW columns

Selecting mobile phase buffers

In an ideal SEC separation, the mechanism is purely sieving, with no chemical interaction between the column matrix and the sample molecules. In practice, however, a small number of weakly charged groups on the surface of all TSK-GEL PW type packings can cause changes in elution order from that of an ideal system. Fortunately, the eluent composition can be varied greatly with TSK-GEL PW columns, to be compatible with a wide range of neutral, polar, anionic, and cationic samples. Table II lists appropriate eluents for GFC of all polymer types on TSK-GEL PW type columns.

For some nonionic, nonpolar polymers, such as poly-ethylene glycols, normal chromatograms can be obtained by using distilled water. Some more polar nonionic polymers exhibit abnormal peak shapes or minor peaks near the void volume when eluted with distilled water, due to ionic interactions between the sample and the charged groups on the resin surface. To eliminate ionic interactions, a neutral salt, such as sodium nitrate or sodium sulfate, is added to the aqueous eluent. Generally, a salt concentration of 0.1 M to 0.5 M is sufficient to overcome undesired ionic interactions.

TSK-GEL PW-type resins are more hydrophobic than polysaccharide gels such as cross-linked dextran. The hydrophobic interaction increases as the salt concentration of the eluent increases. This hydrophobic interaction between the sample and the resin surface can be suppressed by the addition of a water-soluble organic solvents modifier (e.g. acetonitrile, isopropanol).

Modifiers are used for proper elution of both charged and neutral hydrophobic polymers. Typical examples for a variety of sample types are given in Table II. All TSK-GEL PW-type column packings are compatible with up to 50% aqueous solutions of methanol, ethanol, propanol, acetonitrile, formic acid, acetic acid, dimethyl formamide, dimethyl sulfoxide, or acetone. Solvent exchange must be carried out slowly.

Improving column performance

Listed below are the four most common causes for poor column performance and the methods you can use to prevent these problems:

1. Void or dead volume at the column inlet, channeling, denaturation of the packing surface

Sudden pressure surges and higher than recommended flow rates can compress the column packing and cause a void. We recommend continuous flow injection with a loop injector and installation of a pulse damper to suppress the sudden pressure surges encountered with quick return pumps. Bulk packing is available to refill voids in the analytical and semi-preparative columns. A guard column will protect your analytical column from pressure surges and irreversibly binding contaminants. Upon injection, the sample can cause a change in the eluent pH. The guard column will allow the pH to equilibrate with the mobile phase before it reaches the analytical column.

2. Air on the column

The column should be tightly capped when not in use. Mobile phases should be sparged to prevent air from entering the column. If air does enter the column, follow the rehydration method.

3. Column contamination or incomplete recovery of the sample

The cleaning directions which follow give suggestions for removing ionic, hydrophobic or absorbed contaminants. Refer to "Selecting mobile phase buffers" for advice on modifiers that will prevent nonspecific binding to the media matrix.

4. Frit plugging and high pressure

Solvents and samples should be filtered though a 0.45 µm filter to prevent clogging of the column frits. If the frit becomes partially plugged, the result may be split peaks or high pressure. The entire end-fitting can be removed and sonicated in 6 M nitric acid. Be careful not to disturb the packing and rinse well after cleaning. Alternatively, we stock replace-ment end-fittings according to the column ID. Furthermore, we recommend placing an in-line filter before the injector, to prevent particles created by pump seal wear from reaching the analytical column.

5. Column overload

Column overload can cause peak splitting and poor resolution. Analytical work necessitates an injection volume less than 1% of the total column volume with a sample concentration less than 10 mg/ml.

Improving resolution

1. Verify that the column is not being overloaded.

2. Decrease the dead volume in your HPLC system by using the shortest tubing lengths and the smallest tubing ID possible without exceeding the maximum pressure for the column.

3. Decrease the flow rate, but not lower than 0.3 ml/min because diffusion will increase.

4. If using a 30 cm PW column, add an additional 30 cm column or switch to an XL-type column which has a smaller particle size and will improve resolution more than two fold.

Cleaning

If the column becomes contaminated, the following cleaning procedures are suggested with the column in reverse flow at half the recommended maximum flow rate (see also ODS-sheet that comes with the column or the TSK-GEL Instruction Manual):

1. If the column is contaminated with basic com-pounds, rinse with 3-5 column volumes (CV) of 0.5-1.0 M neutral salt or a buffered solution at low pH (pH 2-3) or high pH (0.1-0.2 N NaOH,pH 13.0).

2. If the column is contaminated with hydrophobic compounds, rinse with 3-5 CV of 20% organic methanol, acetonitrile, THF etc.)
3. For precipitated protein, chaotropic reagents such as 0.1 % SDS, 8 M urea or 6 M guanidine, or proteolytic enzymes such as pepsin may be used. However, an extended washing with buffer is required to remove SDS and guanidine.

Unexpected elution behavior can occur if these reagents are not completely removed.
Rinse with 5 CV of D. I. water after each cleaning step before proceeding to the next step.

Storage

When the column will be used the next day, allow it to run overnight at a low flow rate in a buffer that does not contain a halide. When the column will not be used for more than a day, flush salt from the column and store in 0.05% sodium azide or 20% ethanol. Seal tightly to prevent drying out.

Rehydration

Dehydration of the column may occur after long term storage or from inadvertently pumping air over a column. Rehydrate the column with the following procedure:

1. Connect the column to your LC pump, but not to the detector, in reverse flow direction.

2. Pump a filtered mobile phase of 20% methanol in distilled, deionized water over the column at half the recommended maximum flow rate listed in the ordering information for your column.

3. Continue this procedure for several hours until you are confident that the column has been rehydrated.

4. Reconnect the column to your LC in the proper flow direction.

5. Rinse well with distilled, deionized water then equilibrate with your normal mobile phase.

Perform the recommernded QC test to ensure that the column is performing properly.

TSK-GEL PW Series

Ordering Information

Analytical and preparative TSK-GEL Size Exclusion polymer-based column products: typical properties

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Min. Number Theoretical Plates	Flow Rate (mL/min) Range	Max.	Maximum Pressure Drop (kg/cm2)
Stainless steel columns								
08031	G-Oligo-PW, 125 Å	7.8	30	6	14,000	0.5 – 0.8	1.0	40
08032	G-DNA-PW, >1.000 Å	7.8	30	10	10,000	0.2 – 0.5	0.6	20
08020	G2500PWXL, <200 Å	7.8	30	6	14,000	0.5 – 0.8	1.0	40
08021	G3000PWXL, 200 Å	7.8	30	6	14,000	0.5 – 0.8	1.0	40
08022	G4000PWXL, 500 Å	7.8	30	10	10,000	0.3 – 0.8	1.0	20
08023	G5000PWXL, 1.000 Å	7.8	30	10	10,000	0.3 – 0.8	1.0	20
08024	G6000PWXL, >1.000 Å	7.8	30	13	7,000	0.3 – 0.8	1.0	20
08025	GMPWXL, 100-1.000 Å	7.8	30	13	7,000	0.3 – 0.8	1.0	20
05761	G2000PW, 125 Å	7.5	30	12	5,000	0.5 – 1.0	1.2	20
08028	G2500PW, <200 Å	7.5	30	12	5,000	0.5 – 1.0	1.2	20
05762	G3000PW, 200 Å	7.5	30	12	5,000	0.5 – 1.0	1.2	20
05763	G4000PW, 500 Å	7.5	30	17	3,000	0.5 – 1.0	1.2	10
05764	G5000PW, 1.000 Å	7.5	30	17	3,000	0.5 – 1.0	1.2	10
05765	G6000PW, >1.000 Å	7.5	30	17	3,000	0.5 – 1.0	1.2	10
08026	GMPW, 100-1.000 Å	7.5	30	17	3,000	0.5 – 1.0	1.2	10
05105	G2000PW, 125 Å	7.5	60	12	10,000	0.5 – 1.0	1.2	40
08029	G2500PW, <200 Å	7.5	60	12	10,000	0.5 – 1.0	1.2	40
05106	G3000PW, 200 Å	7.5	60	12	10,000	0.5 – 1.0	1.2	40
05107	G4000PW, 500 Å	7.5	60	17	6,000	0.5 – 1.0	1.2	20
05108	G5000PW, 1.000 Å	7.5	60	17	6,000	0.5 – 1.0	1.2	20
05109	G6000PW, >1.000 Å	7.5	60	17	6,000	0.5 – 1.0	1.2	20
08027	GMPW, 100-1.000 Å	7.5	60	17	6,000	0.5 – 1.0	1.2	20
05150	G2000PW, 125 Å	21.5	60	17	10,000	1.0 – 6.0	8.0	20
08030	G2500PW, <200 Å	21.5	60	17	10,000	1.0 – 6.0	8.0	20
05151	G3000PW, 200 Å	21.5	60	17	10,000	1.0 – 6.0	8.0	20
05152	G4000PW, 500 Å	21.5	60	22	6,000	1.0 – 6.0	8.0	20
05153	G5000PW, 1.000 Å	21.5	60	22	6,000	1.0 – 6.0	8.0	20
05154	G6000PW, >1.000 Å	21.5	60	25	6,000	1.0 – 6.0	8.0	20
07926	G3000PW, 200 Å	55.0	60	20	4,500	15.0 – 25.0	30.0	15
07927	G5000PW , 1.000 Å	55.0	60	20	4,500	15.0 – 25.0	30.0	15
Guard columns								
08034	Oligo Guard column	6.0	4.0	13	For G-Oligo-PW			
08033	PWXL Guard column	6.0	4.0	12	For 7.8 mm ID PWXL and G-DNA-PW (contains 3000PW packing)			
06763	PW-L Guard column	7.5	7.5	13	For 7.5 mm ID G2000PW, (contains 2000PW packing)			
06762	PW-H Guard column	7.5	7.5	13	For 7.5 mm ID G2500PW-G6000PW + GMPW (contains 3000PW packing)			
06757	PW-L Guard column	21.5	7.5	17	For 21.5 mm ID G2000PW			
06758	PW-H Guard colum	21.5	7.5	17	For 21.5 mm ID G2500PW through G6000PW			
07924	PW Guard colum	45.0	5.0	20	For 55 mm ID G3000PW + G5000PW			
Bulk packing								
08035	PWXL Top-Off, 1g wet resin			10	For all PWXL and G-DNA-PW			