IEC
ION EXCHANGE CHROMATOGRAPHY

IEC PRODUCTS

ANION EXCHANGE

TSKgel Q-STAT -NEW-
TSKgel DNA-STAT -NEW-
TSKgel BioAssist Q
TSKgel SuperQ-5PW
TSKgel DEAE-5PW
TSKgel DEAE-NPR
TSKgel DNA-NPR
TSKgel DEAE-2SW
TSKgel DEAE-3SW
TSKgel Sugar AXI
TSKgel Sugar AXG
TSKgel SAX

CATION EXCHANGE

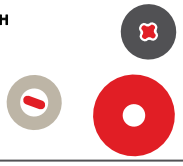
TSKgel SP-STAT -NEW-
TSKgel CM-STAT -NEW-
TSKgel BioAssist S
TSKgel SP-5PW
TSKgel CM-5PW
TSKgel SP-2SW
TSKgel SP-NPR
TSKgel CM-2SW
TSKgel CM-3SW
TSKgel SCX

TOSOH FACT

Tosoh Corporation maintains a large database of HPLC applications utilizing TSK-GEL columns. Sources for this database include articles in journals citing the use of TSK-GEL columns by Tosoh customers as well as technical papers and presentations created by Tosoh scientists.

Tosoh Bioscience offers a large literature library consisting of application notes, instructions manuals, product overviews and separation reports.

Both the literature library and the chromatogram database are available on the website at www.tosohbioscience.com.



INTRODUCTION TO TSK-GEL ION EXCHANGE COLUMNS

Tosoh Bioscience offers a broad line of high efficiency columns for analysis and isolation of biomolecules by anion and cation exchange chromatography. In either mode of Ion Exchange Chromatography (IEC), the product line contains methacrylate-, silica- and polystyrene-based columns. Proteins, peptides, oligonucleotides and other nucleic acid fragments are typical samples that are analyzed or isolated on TSK-GEL ion exchange columns. Most of the available chemistries are offered in analytical as well as semi-preparative column formats. Particle sizes range from 2.5 µm, for fast quality control and process monitoring, to 20 µm and larger particle sizes utilized in process scale separations.

TSK-GEL STAT columns are the latest addition to the IEC column line. They are designed for high effiency separation of biomolecules and low molecular weight compounds. TSK-GEL STAT columns provide superior performance at reduced analysis time. The STAT series encompasses a range of high efficiency anion and cation exchange columns, suitable for various applications from research to quality control.

Also available are a series of ion exchange columns based on a polystyrene matrix. They are most suitable for analyzing small molecular weight sugars, amino acids, individual nucleic acids, and small drug candidates.

Packing Materials and Chemistries

Methacrylate, silica, and polystyrene are used as matrices for the TSK-GEL line of ion exchange columns. The methacrylate backbone chemistry provides a robust, hydrophilic particle that is suitable as a support for high performance analytical and preparative separations of biomolecules.

The polymethacrylate base resin, G5000PW (5PW), is a 10 µm spherical particle with approximately 1000 Å pores. The base resin is derivatized either with diethylaminoethyl (DEAE), sulfopropyl (SP) or carboxymethyl (CM) functionalities to provide a weak anion, a strong cation, and a weak cation exchanger, respectively. While these chemistries result in standard ion exchangers, the chemistry employed in the manufacturing of TSKgel SuperQ-5PW results in a higher capacity strong anion exchanger by introducing polyamine functional groups.

FEATURES	BENEFITS
BioAssist Columns	
High capacity even for larger proteins (1 million Da)sww	Fewer runs to collect required sample amounts
Unique pore structure provides fast mass transfer	Sharper peaks improve analysis and isolation
Biocompatible PEEK column hardware	Less sample loss due to adsorption
Available in analytical and semi-prep formats	Easy scale-up
Polymer-Based Ion Exchange Columns	
Methacrylate backbone	Mechanically and chemically stable (pH 2-12)
	Withstands repeated cleaning with base, and use of organic solvents, denaturants and surfactants
Large pore size (1000 Å) (excl. limit for proteins ~ 5,000,000 Da)	Use same column for most biopolymers
Non porous resin-based (STAT and NPR) columns	Fast QC analysis and process monitoring
Several columns available in 2 mm ID format	Reduced solvent consumption and analysis time
Silica-Based Ion Exchange Columns	
Smaller pore size (2SW = 125 Å and 3SW = 250 Å)	Most suitable for analysing smaller MW samples such as nucleotides, drug candidates, catecholamines and small peptides or proteins

IEC

Due to the higher density of anion exchange sites, TSKgel SuperQ-5PW has a smaller effective pore size than TSKgel DEAE-5PW.

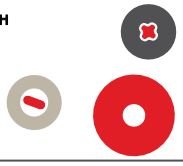
TSK-GEL BioAssist columns are also based on methacrylate particle design technology. TSKgel BioAssist Q contains particles with very large pores (~4000 Å) that are derivatized with a network of polyamine groups. The capacity of TSKgel BioAssist Q has been shown to be high over a wide molecular weight range (up to 1,000,000 Da). TSKgel BioAssist S is packed with particles possessing 1300 Å pores functionalized with sulfopropyl groups. TSKgel BioAssist analytical IEC columns are provided in a 4.6 mm ID x 5 cm L PEEK housing with 7 µm or 10 µm particles for the respective S and Q functionalities. Semi-preparative TSK-GEL BioAssist columns are also available with a 13µm particle size packed in a 10 mm ID x 10 cm L housing. The longer length of the semi-preparative column compensates for the increased particle size, resulting in similar resolution to the analytical column.

The methacrylate chemistry also forms the backbone of non-porous resin columns such as TSK-GEL STAT and NPR columns. Since rate-limiting pore difusion is eliminated with nonporous particles, analysis time is often reduced by as much as 80 % without loss in resolution. Also, recoveries are routinely greater than 90 %. The relatively large particle sizes of nonporous CM-STAT, SP-STAT, Q-STAT, and DNA-STAT columns support fast separations at moderate pressure. Latest surface technology was applied to increase the number of functional groups (carboxymethyl, sulfopropyl or quaternary ammonium group) and reach good sample capacities.

Specific application needs are addressed by offering various column formats and particle sizes: For fast and ultra-fast analysis (e.g. screening or process monitoring) short 3 mm ID columns are packed with 10 µm particles. For high resolution separations longer columns with 4.6 mm ID are packed with 7 µm particles. The DNA-STAT column is packed with smaller particles (5 µm).

TSKgel DEAE-NPR, SP-NPR and DNA-NPR are packed with 2.5 µm particles. High column efficiency coupled with low sample capacity restricts the application of these columns to fast analysis and micro-scale preparative isolation. The DNA-NPR column is a longer version of the DEAE-NPR column that allows improved resolution of oligonucleotides, including those amplified by PCR. Small guard columns are available to protect the DNA-NPR and DEAE-NPR columns.

In the development of new drug candidates, it is often desirable to use the same backbone chemistry throughout the development process. For that reason, the backbone of the 20 µm and 30 µm particle size TSK-GEL PW-type resins and the larger particle size Toyopearl process media are chemically similar to that used in prepacked TSK-GEL PW-type column lines. As a result, TSKgel SuperQ-5PW scales directly to Toyopearl SuperQ-650. Similarly, the TSKgel DEAE-5PW scales directly to TSKgel DEAE-5PW bulk resins, which in turn scales to Toyopearl DEAE-650. The same is true for CM and SP products in the cation exchange column line.



TSK-GEL Anion Exchange Columns

TSK-GEL	Matrix*	Particle Size (µm)	Pore Size (Å)	Functional Group	Counter Ion	Excl. Limit, PEG** (Da)	Capacity (mg BSA/mL)	Small Ion capacity meq/mL	pKa	Column hard-ware***
BioAssist Q	pMA	10, 13	~4000	Polyamine	Cl^-	>5,000,000	70	0.1	9.4	PEEK
SuperQ-5PW	pMA	10,13	1000	Trimethyl-amino	Cl^-	1,000,000	100	> 0.13	12.2	S, G
DEAE-5PW	pMA	10,13, 20	1000	DEAE	Cl^-	1,000,000	30	0.1	11.5	S, G
Q-STAT	pMA	7,10	~ 0	Trimethyl-amino	Cl^-	500	20	0.27	10.5	S
DNA-STAT	pMA	5	~ 0	Trimethyl-amino	Cl^-	500	35	0.27	10.5	S
DEAE-NPR	pMA	2.5	~ 0	DEAE	Cl^-	500	5	> 0.1	11.2	S
DNA-NPR	pMA	2.5	~ 0	Proprietary	ClO_4^-	500	5	> 0.1	11.2	S
DEAE-2SW	Silica	5	125	DEAE	$H_2PO_4^-$	10,000	ND	> 0.3	11.2	S
DEAE-3SW	Silica	10	250	DEAE	Cl^-	30,000	ND	> 0.3	11.2	S
Sugar AXI	PS-DVB	8	60	Trimethyl-amino	HBO_3^-		ND	> 1.2	12.5	S
Sugar AXG	PS-DVB	10	60	Trimethyl-amino	HBO_3^-		ND	> 1.2	12.5	S
SAX	PS-DVB	5	60	Trimethyl-amino	Cl^-		ND	> 1.0	12.5	S

TSK-GEL Cation Exchange Columns

TSK-GEL	Matrix*	Particle Size (µm)	Pore Size (Å)	Functional Group	Counter Ion	Excl. Limit, PEG** (Da)	Capacity (mg BSA/mL)	Small Ion capacity meq/mL	pKa	Column hard-ware***
BioAssist S	pMA	7, 13	~1300	Sulfopropyl	Na^+	~4,000,000	70(1)	0.1	2.4	PEEK
SP-5PW	pMA	10, 13, 20	1000	Sulfopropyl	Na^+	1,000,000	40(2)	> 0.1	2.3	S, G
CM-5PW	pMA	10, 13	1000	Carboxymethyl	Na^+	1,000,000	45(2)	> 0.1	4.2	S, G
SP-STAT	pMA	7, 10	~ 0	Sulfopropyl	Na^+	500	10(3)	> 0.023	4.0	S
CM-STAT	pMA	7, 10	~ 0	Carboxymethyl	Na^+	500	15(3)	> 0.1	4.9	S
SP-NPR	pMA	2.5	~ 0	Sulfopropyl	Na^+	500	5(2)	> 0.1	2.3	S
SP-2SW	Silica	5	125	Sulfopropyl	Na^+	10,000	ND	0.3	2.2	S
CM-2SW	Silica	5	125	Carboxymethyl	Na^+	10,000	110(2)	> 0.3	4.2	S
CM-3SW	Silica	10	250	Carboxymethyl	Na^+	30,000	ND	> 0.3	4.2	S
SCX	PS-DVB	5	60	Sulfonic acid	Na^+, H^+		ND	> 1.5		S

* *pMA = poly methacrylate; PS-DVB = polystyrene-divinylbenzene*
** *Polyethylene glycol*
*** *PEEK = polyethyletherketone, S = stainless steel, G = glass*
(1) γ-globulin; (2) hemoglobin; (3) lysozyme

IEC

Sample Type	MW Range (Da)	TSK-GEL Column	pH Range
Amino Acids, Peptides and Proteins			
Amino acids	< 2000	SAX	1 - 14
		SCX	1 - 14
Peptides and small proteins	< 10,000	Q-STAT	3 - 10
		SP-STAT	3 - 10
		CM-STAT	3 - 10
		SCX	1 - 14
		SP-2SW	2 - 7.5
		CM-2SW	2 - 7.5
		DEAE-2SW	2 - 7.5
Proteins	> 10,000 up to ~ 5,000,000	BioAssist S	2 - 12
		BioAssist Q	2 - 12
		Q-STAT	3 - 10
		SP-5PW	2 - 12
		DEAE-5PW	2 - 12
		CM-5PW	2 - 12
		SP-STAT	3 - 10
		CM-STAT	3 - 10
		SP-NPR	2 - 12
		DEAE-NPR	2 - 12
		SuperQ-5PW	2 - 12
Nucleic Acids			
Purines and pyrimidines		DEAE-2SW	2 - 7.5
		SP-2SW	2 - 7.5
Nucleosides		SP-2SW	2 - 7.5
		DEAE-2SW	2 - 7.5
Nucleotides		Q-/DNA-STAT	3 - 10
		DEAE-2SW	2 - 7.5
Oligonucleotides		Q-/DNA-STAT	3 - 10
		DEAE-5PW	2 - 12
		DEAE-NPR	2 - 12
		DNA-NPR	2 - 12
		SuperQ-5PW	2 - 12
DNA, RNA, and PCR products		Q-/DNA-STAT	3 - 10
		DNA-NPR	2 - 12
		DEAE-NPR	2 - 12
		DEAE-5PW	2 - 12
		DEAE-3SW	2 - 7.5
Other Molecules			
Mono and disaccharides		Sugar AXI, AXG	1 - 14
		SCX	1 - 14
		SAX	1 - 14

TSK-GEL ANION EXCHANGE COLUMNS

HIGHLIGHTS

- TSKgel Q- and DNA-STAT columns provide high efficiency separations at short analysis time.
- TSKgel DNA-NPR columns are ideal for PCR fragment analysis.
- TSKgel SuperQ-5PW columns have higher capacity than TSKgel DEAE-5PW due to novel bonding chemistry, effective pore size is smaller for SuperQ-5PW.
- Pore structure and bonding chemistry of TSKgel BioAssist Q columns provide high capacity for small to very large MW proteins and nucleic acids.
- BioAssist columns are packed in 4.6 mm ID or 10 mm ID PEEK hardware. Other columns are available in glass and stainless steel for analytical, semi-preparative and preparative applications.
- Binding capacity for small to medium size proteins on TSKgel DEAE-3SW is roughly double that of the DEAE-5PW due to the smaller pore size and larger surface area.
- TSKgel DEAE-5PW and DEAE-2SW columns are available in 2 mm ID format for mass spec applications.
- Specialty columns for analysis of mono- and disaccharides and sugar alcohols are also available.

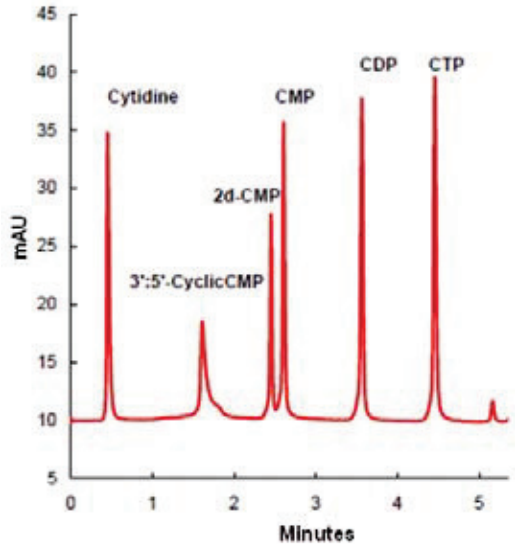
APPLICATIONS

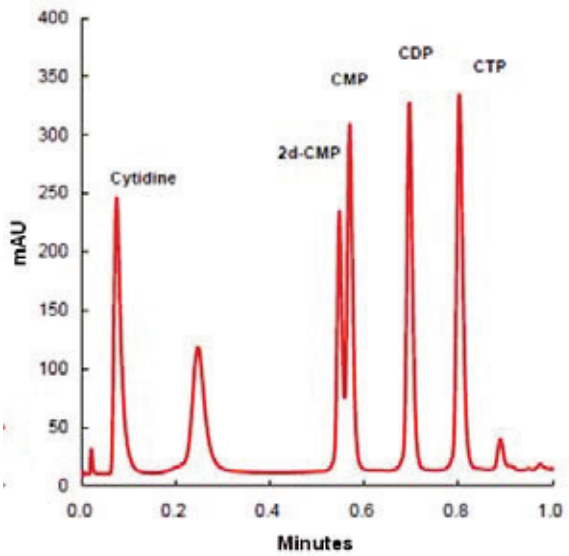
Nonporous TSKgel STAT Anion Exchange Columns

STAT columns are available in various column formats and particle sizes to perfectly match specific application needs. For fast and ultra-fast analysis anion and cation exchange columns in 3 mm ID and 3.5 cm length are packed with 10 µm particles. They are ideally suited for rapid candidate screening or process monitoring. 4.6 mm ID and 10 cm length columns packed with 7 µm particles are designed for high resolution IEC separation for example for the separation of nucleic acids, mAb variants, PEGylated protein or protein aggregates.

FIGURE 1

High resolution versus high throughput analysis of nucleotides





High resolution:

Column:	Prototype Q-STAT 4.6 mm ID x 10 cm L (7 µm)
Eluent:	A) 20 mmol/L Tris-HCl (pH8.5) B) 0.5 mol/L NaCl in A (pH8.5)
Gradient:	0 to 100% B (10 min.)
Flow Rate:	1.5 mL/min.
Detection:	UV @ 260 nm

High throughput:

Column:	Prototype Q-STAT 4.6 mm ID x 3.5 cm L (10 µm)
Eluent:	A) 20 mmol/L Tris-HCl (pH8.5) B) 0.5mol/L NaCl in A (pH8.5)
Gradient:	0 to 100% B (1min.)
Flow Rate:	4.0 mL/min.
Detection:	UV @ 260nm

IEC

The DNA STAT column (4.6 mm ID x 10 cm L) packed with 5 µm Q-type anion exchange resin is ideally suited for the analysis of nucleic acids.

FIGURE 1 compares the high resolution separation of nucleotides on a 10 cm length column to the high throughput separation on a 3.5 cm length column (analysis performed on prototype columns).

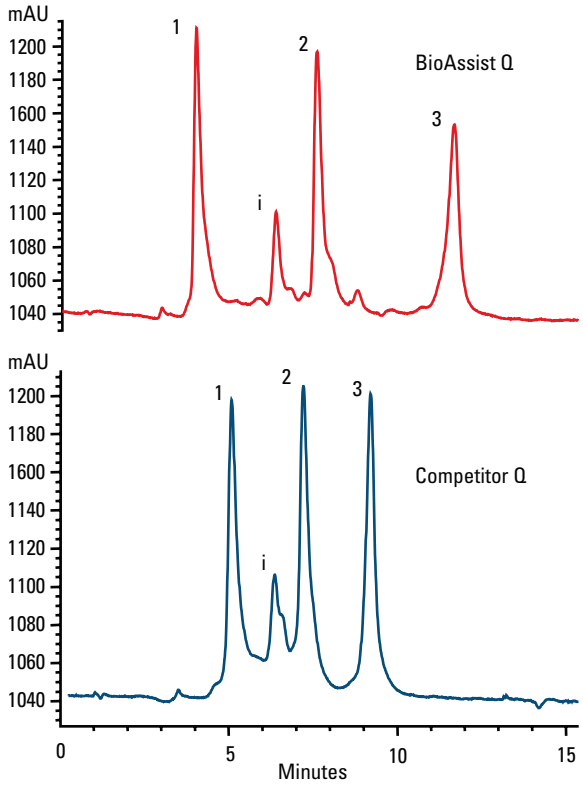
Polymer-based Anion Exchange Columns

BioAssist Q

TSKgel BioAssist Q is suitable for use in systems that are designed for laboratory or semi-preparative applications. FIGURE 2 demonstrates the performance enhancement of TSKgel BioAssist Q over a competitive product when operated side-by-side on an FPLC system. TABLE I shows typical dynamic binding capacities on BioAssist Q relative to competitive products.

FIGURE 2

Performance enhancement on FPLC system



Column:	TSKgel BioAssist Q, 4.6 mm ID x 5 cm (PEEK) Competitor Q, 5.0 mm ID x 5 cm
Elution:	30 min linear gradient from 0 to 1 mol/L NaCl in 20 mmol/L sodium phosphate pH 8.0
Flow Rate:	1.0 mL/min
Detection:	UV @ 280 nm
Sample:	1) conalbumin, i) ovalbumin impurity 2) ovalbumin, 3) trypsin inhibitor

TABLE I

Comparison of dynamic binding capacities

	Binding capacity (mg/mL)			
Protein	BioAssist Q	SuperQ -5PW	Conventional Q type product A	Conventional Q type product B
Thyroglobulin	77.4	22.9	20.2	1.8
Monoclonal IgG_1	57.8	43.3	46.7	47.7
Human Serum Albumin	83.1	78.9	48.2	48.8
Trypsin Inhibitor	84.3	92.8	51.8	57.8

Columns:	TSKgel BioAssist Q (4.6 mmID x 1 cm L) TSKgel SuperQ-5PW (4.6 mmID x 1 cm L) Conventional Q type product A (4.6 mm ID x 1 cm L) Conventional Q type product B (4.6 mm ID x 1 cm L)
Solvent:	20 mmol/L Tris-HCl buffer, pH 8.0
Flow rate:	0.38 mL/min
Detection:	UV (280 nm)

*The capacity was determined at 10% height of the breakthrough curve at UV 280 nm.

SuperQ-5PW and DEAE-5PW

FIGURE 3 shows the analysis of a 16-mer morpholine oligonucleotide on TSKgel SuperQ-5PW column using a NaCl gradient in a 10 mmol/L sodium hydroxide mobile phase.

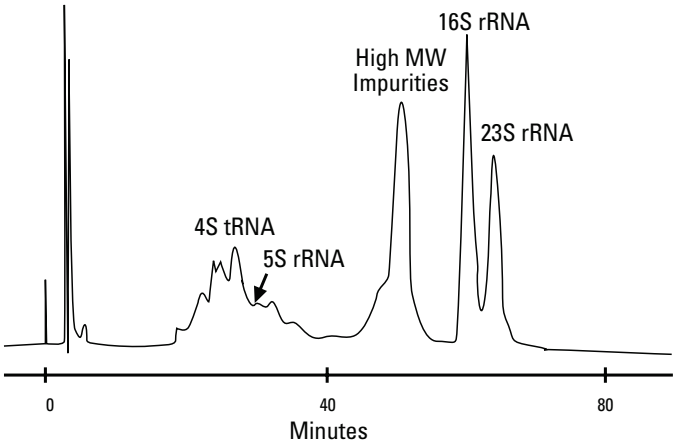
FIGURE 4 shows the fractionation of high molecular weight E. coli RNA on TSKgel DEAE-5PW, effectively utilizing the large 1000 Å pores of this base resin.

DEAE-NPR and DNA-NPR

Because of their small (2.5 µm) particle size, non porous resin (NPR) columns excel in rapid separations of large biomolecules such as DNA digests. A chromatogram of a standard Hae III digest of pBR322 DNA on TSKgel DEAE-NPR, protected by a guard column, is shown in FIGURE 5. To achieve better resolution for PCR fragment analysis we recommend the use of TSK-GEL DNA-NPR columns, which are 7.5 cm long and 4.6 mm wide, providing higher efficiency in a longer column.

FIGURE 4

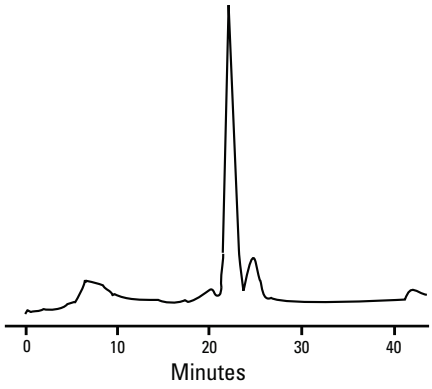
Large pore TSKgel DEAE-5PW resolves high MW RNA



Column:	TSKgel DEAE-5PW, 6mm ID x 15cm
Sample:	total *E. coli* RNA
Elution:	300min linear gradient from 0.3mol/L to 1.0mol/L NaCl in 0.1mol/L Tris-HCl, pH 7.6
Flow Rate:	1.0mL/min
Detection:	UV @ 260nm

FIGURE 3

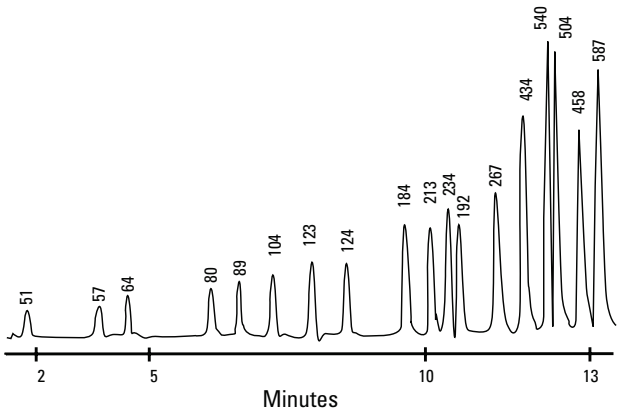
Analysis of synthetic oligonucleotide on TSKgel SuperQ-5PW



Column:	TSKgel SuperQ-5PW, 7.5mm ID x 7.5cm
Sample:	16-mer morpholine oligonucleotide, AAG AAG AAG AGG GGA G
Sample load:	0.5 O.D. (optical density)
Mobile phase:	A: 10mmol/L NaOH B: 10mmol/L NaOH with 1mol/L NaCl
Gradient:	Initial: 0% B 40min: 50% B 41min: 100% B 46min: 100% B
Flow Rate:	1 mL/min
Detection:	UV @ 254nm

FIGURE 5

Higher resolution and faster analysis on TSKgel DEAE-NPR



Column:	TSKgel DEAE-NPR, 4.6mm ID x 3.5cm, with guard column, 4.6mm ID x 0.5cm
Sample:	*Hae* III digest of pBR322 DNA, (base pair number for each peak is indicated)
Buffer A:	0.02mol/L Tris-HCl, pH 9.0
Buffer B:	Buffer A plus 1.0mol/L NaCl
Elution:	15min linear gradient from 48% to 65% buffer B
Flow Rate:	1.5mL/min
Pressure:	2000psi
Temperature:	40°C
Detection:	UV @ 260nm

IEC

Silica-based Anion Exchange Columns

TSK-GEL 2SW-type columns provide high performance separations of small ionic solutes. The increased solubility of the silica backbone above pH 7 limits the use of the TSK-GEL 2SW-type columns to acidic or neutral mobile phases. This restricts method development and requires special cleaning procedures when compared to the more robust TSK-GEL 5PW-type polymer-based columns.

High performance analyses of small anionic species are best performed on small pore silica-based anion exchangers, such as TSKgel DEAE-2SW. This is demonstrated in FIGURE 6.

The 250 Å pore size TSKgel DEAE-3SW column is used for separating peptides, low MW proteins and DNA fragments.

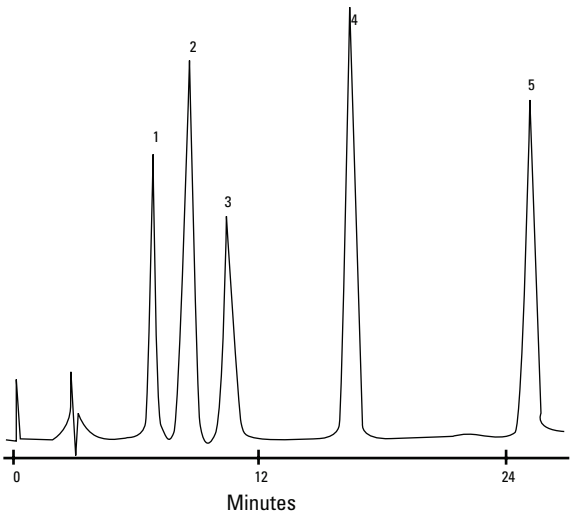
Specialty Columns

Analyses of monosaccharides, disaccharides, and sugar alcohols can be performed on PS-DVB columns, either by isocratic (TSKgel Sugar AXI) or by gradient (TSKgel Sugar AXG) analysis. Saccharides are retained on Sugar AX columns following the formation of negatively charged complexes with boric acid at alkaline pH. FIGURE 7 shows the separation of twelve mono- and di-saccharides.

The strong anion exchange TSKgel SAX column can be used for the separation of isomerized sugars, alcohols, and low molecular weight organic acids.

FIGURE 6

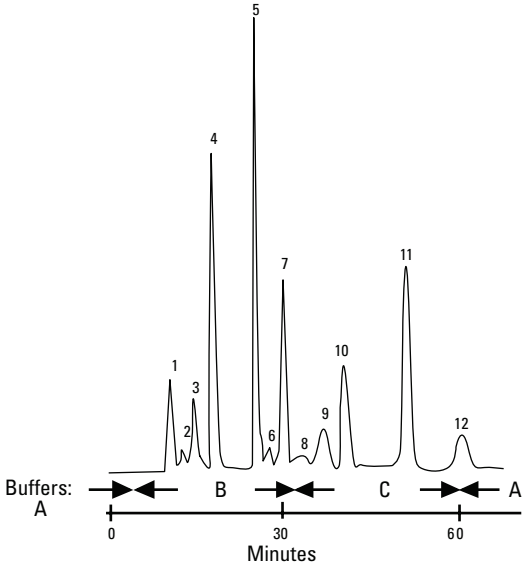
Separation of nucleotides on TSKgel DEAE-2SW



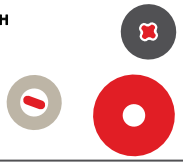
Column:	TSKgel DEAE-2SW, 4.6mm ID x 25cm
Sample:	1. AMP, 2. IMP, 3. GMP, 4.ADP, 5. ATP
Buffer A:	ACN in 0.1mol/L phosphate, pH 3.0, 20/80
Buffer B:	ACN in 0.5mol/L phosphate, pH 3.0, 20/80
Elution:	30min linear gradient from buffer A to B
Flow Rate:	1.0mL/min
Detection:	UV @ 260nm

FIGURE 7

Separation of saccharide mixture on TSKgel Sugar AXG



Column:	TSKgel Sugar AXG, 4.6mm ID x 15cm
Sample:	disaccharides, 25mmol/L; monosaccharides, 50mmol/L: 1. cellobiose, 2. maltose, 3. lactose, 4. rhamnose, 5. lyxose, 6. ribose, 7. mannose, 8. fructose, 9. arabinose, 10. galactose, 11. xylose, 12. glucose
Elution:	step gradient: 6min buffer A, 0.6mol/L boric acid, pH 7.7; then 27min buffer B, 0.7mol/L boric acid, pH 7.25; then 30min buffer C, 0.7mol/L boric acid, pH 8.7
Flow Rate:	0.4mL/min (column and post column reagent solution)
Pressure:	16kg/cm^2
Temperature:	70°C (column), 100°C (post column reactor)
Detection:	fluorescence excitation @ 331nm, emission @ 383nm
PC reagent:	2.5% 2-cyanoacetamide solution



ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Glass columns: polymer-based								
13061	DEAE-5PW Glass, 1000 Å	5.0	5.0	10	≥ 700	0.5 - 0.8	1.0	15
08802	DEAE-5PW Glass, 1000 Å	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.2	10
14016	DEAE-5PW Glass, 1000 Å	20.0	15.0	13	≥ 3,000	4.0 - 6.0	8.0	15
18386	SuperQ-5PW Glass, 1000 Å	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.2	20
PEEK columns: polymer-based								
19685	BioAssist Q, 4000 Å	4.6	5.0	10	≥ 500	0.3 - 1.0	1.2	25
21410	BioAssist Q, 4000 Å	10.0	10.0	13	≥ 500	1.0 - 5.0	7.0	25
Stainless steel columns: polymer-based								
21960	Q-STAT, nonporous -NEW-	3.0	3.5	10	> 200			100
21961	Q-STAT, nonporous -NEW-	4.6	10.0	7	> 2,000			100
21962	DNA-STAT, nonporous -NEW-	4.6	10.0	5	> 4,000			150
13075	DEAE-NPR, nonporous	4.6	3.5	2.5	≥ 1,300	1.0 - 1.5	1.6	200
18249	DNA-NPR, nonporous	4.6	7.5	2.5	≥ 6,000	0.5 - 1.0	1.5	300
18757	DEAE-5PW, 1000 Å	2.0	7.5	10	≥ 1,300	0.05 - 0.10	0.12	15
07164	DEAE-5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	15
07574	DEAE-5PW, 1000 Å	21.5	15.0	13	≥ 3,000	4.0 - 6.0	8.0	25
07930	DEAE-5PW, 1000 Å	55.0	20.0	20	≥ 1,500	20.0 - 40.0	50.0	4
18257	SuperQ-5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	20
18387	SuperQ-5PW, 1000 Å	21.5	15.0	13	≥ 3,000	4.0 - 6.0	8.0	20
08639	Sugar AXI, 60 Å	4.6	15.0	8	≥ 3,700	0.2 - 0.4	0.5	30
08640	Sugar AXG, 60 Å	4.6	15.0	10	≥ 2,700	0.2 - 0.5	0.5	20
07157	SAX	6.0	15.0	5	≥ 2,000	0.5 - 1.0	1.2	150
Stainless steel columns: silica-based								
18761	DEAE-2SW, 125 Å	2.0	25.0	5	≥ 5,000	0.12 - 0.17	0.22	130
07168	DEAE-2SW, 125 Å	4.6	25.0	5	≥ 5,000	0.6 - 0.8	1.0	150
07163	DEAE-3SW, 250 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	20
Guard column products								
17088	DEAE-NPR Guard column	4.6	0.5	5	For P/N 13075			
18253	DNA-NPR Guard column	4.6	0.5	5	For P/N 18249			
18388	SuperQ-5PW Guardgel Kit			20	For P/N 18257			
18389	SuperQ-5PW Guardgel Kit, Glass			20	For P/N 18386			
18390	SuperQ-5PW Guardgel Kit			20	For P/N 18387			
07210	DEAE-5PW Guardgel Kit			20	For P/N 07164			
42152	DEAE-5PW Guard cartridge	2.0	1.0	10	For P/N 18757			
08806	DEAE-5PW Guardgel Kit, Glass			20	For P/Ns 13061 and 08802			
14466	DEAE-5PW Guard column, Glass	20.0	2.0	13	For P/N 14016			
16092	DEAE-5PW Prep Guardgel Kit			20	For P/N 07574			
07928	DEAE-5PW Guard column	45.0	5.0	20	For P/N 07930			
07648	DEAE-SW Guardgel Kit			20	For P/Ns 07168 and 07163			
42154	DEAE-2SW Guard cartridge	2.0	1.0	5	For P/N 18761			
19308	Guard cartridge holder	2.0	1.5		For all 2mm ID guard cartridges			

IEC

TSK-GEL CATION EXCHANGE COLUMNS

HIGHLIGHTS

- TSKgel SP-STAT and CM-STAT nonporous columns provide high efficiency separation at short analysis time.
- Pore structure and bonding chemistry of TSKgel BioAssist S provides high capacity for medium to large MW proteins.
- BioAssist columns are packed in 4.6 mm ID or 10 mm ID PEEK hardware. Other columns are available in glass and stainless steel for analytical, semi-preparative and preparative applications.
- Binding capacity for small to medium size proteins on TSKgel CM-3SW is approximately double that of TSKgel CM-5PW due to the smaller pore size and larger surface area.
- The TSKgel SP-5PW column is available in 2 mm ID format for LC-MS applications.

APPLICATIONS

TSKgel BioAssist S

TSKgel BioAssist S is suitable for use in systems that are designed for HPLC, laboratory, or semi-preparative applications. The large pore size of the TSKgel BioAssist S resin provides high dynamic capacity due to novel bonded phase design (see TABLE II). FIGURE 8 demonstrates these features for the analysis of bromelain, a proteolytic enzyme that is used as a nutritional supplement. Bromelain is a basic glycoprotein with a MW of 33 kDa and a pI of 9.55.

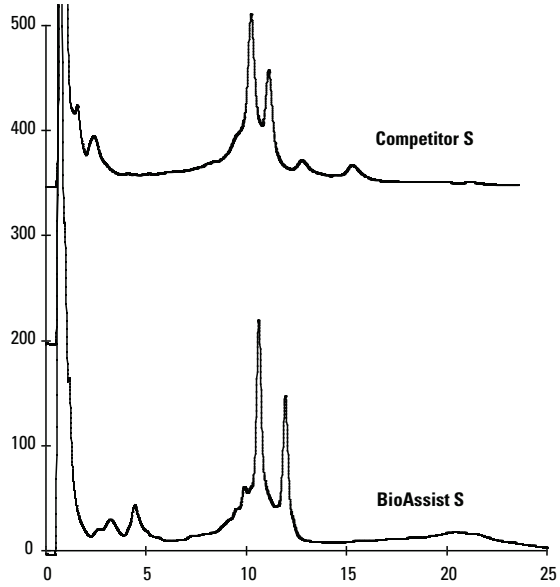
TSKgel SP-5PW and TSKgel CM-5PW

Differences in selectivity between strong (TSKgel SP-5PW) and weak (TSKgel CM-5PW) cation exchangers are demonstrated in FIGURE 9, which is a separation of globular proteins.

The purification of 200mg of crude lipoxidase on a 21.5 mm ID TSKgel SP-5PW column is illustrated in FIGURE 10. Scale-up is simplified as only the particle size changes from 10 µm (7.5 mm ID) to 13 µm (21.5 mm ID) or 20 µm (55 mm ID) columns.

FIGURE 8

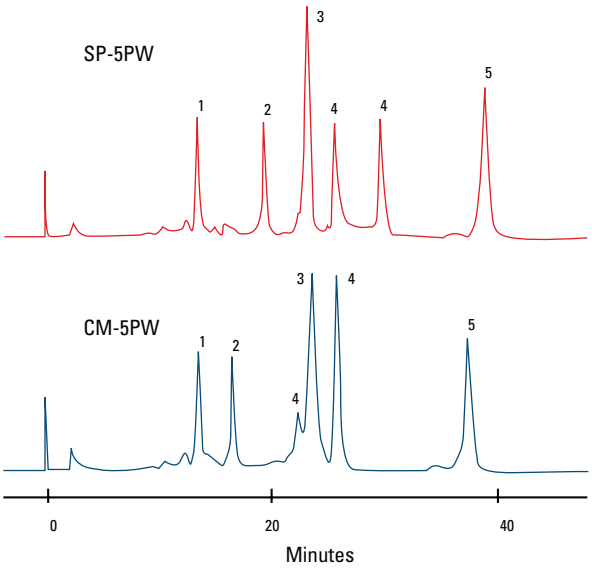
Bromelain Analysis on TSKgel Bioassist S and Competitor S Columns



Columns:	TSKgel BioAssist S, 4.6mm ID x 5cm, PEEK Competitor S 5mm ID x 5cm
Elution:	20 min (TSKgel) or 30 min (Competitor S) linear gradient of NaCl from 0 to 0.5mol/L in 20mmol/L sodium phosphate buffer, pH 7.0
Flow Rate:	0.8mL/min for TSKgel; 1.0mL/min for Competitor S
Detection:	UV @ 280nm
Temperature:	25°C
Sample:	crude bromelain (C4882, Sigma), 1mg in 100µL

FIGURE 9

Selectivity of TSK-GEL strong and weak cation exchangers

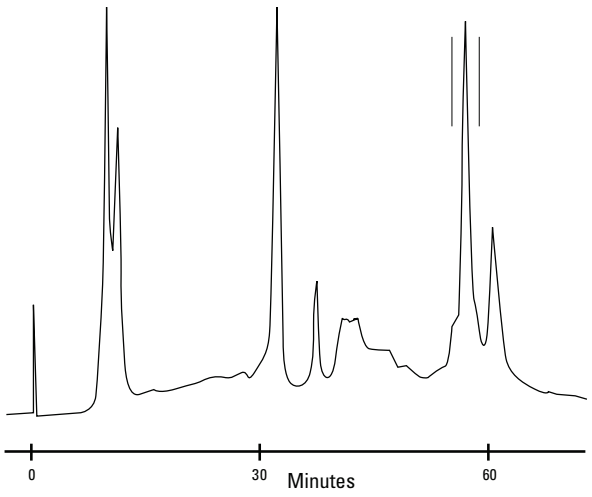


Columns:	TSKgel SP-5PW and TSKgel CM-5PW, 7.5 mm ID x 7.5 cm L
Sample:	1. trypsinogen, 2. ribonuclease A, 3. a-chymotrypsinogen, 4. cytochrome C, 5. lysozyme
Elution:	60 min linear gradient from 0 mol/L to 0.5 mol/L NaCl in 0.02 mol/L phosphate, pH 7.0
Flow Rate:	1.0 mL/min
Detection:	UV @ 280 nm

APPLICATIONS - TSK-GEL CATION EXCHANGE COLUMNS

FIGURE 10

Semi-preparative purification of lipoxidase



Column:	TSKgel SP-5PW, 21.5mm ID x 15cm
Sample:	crude lipoxidase, 200mg
Elution:	120min linear gradient from 0mol/L to 0.5mol/L Na_2SO_4 in 0.02mol/L acetate, pH 4.5
Flow Rate:	4.0mL/min
Detection:	UV @ 280nm
Recovery:	Lipoxidase activity collected between the two vertical lines was 84%

TABLE II

Comparison of dynamic binding capacities

	Binding capacity (mg/mL)*	
Protein	BioAssist S	Conventional S type product
γ-globulin	79	48
Lysozyme	84	63
Cytochrome C	95	43
α-chymotrypsinogen	119	-

Columns:	TSKgel BioAssist Conventional S-type product
Size:	4.6 mm ID x 5 cm L (lysozyme, cytochrome C, α-chymotrypsinogen A) 5.0 mm ID x 1cm L (α-globulin)
Solvent:	20 mmol/L sodium phosphate buffer, pH 6.5 (lysozyme, cytochrome C, α-chymotrypsinogen A) 20 mmol/L sodium phosphate buffer pH 5.0 (α-globulin)
Flow rate:	0.38 mL/min
Temperature:	25°C
Detection:	UV @ 280 nm

*The capacity was determined at 10% height of the breakthrough curve at UV 280nm.

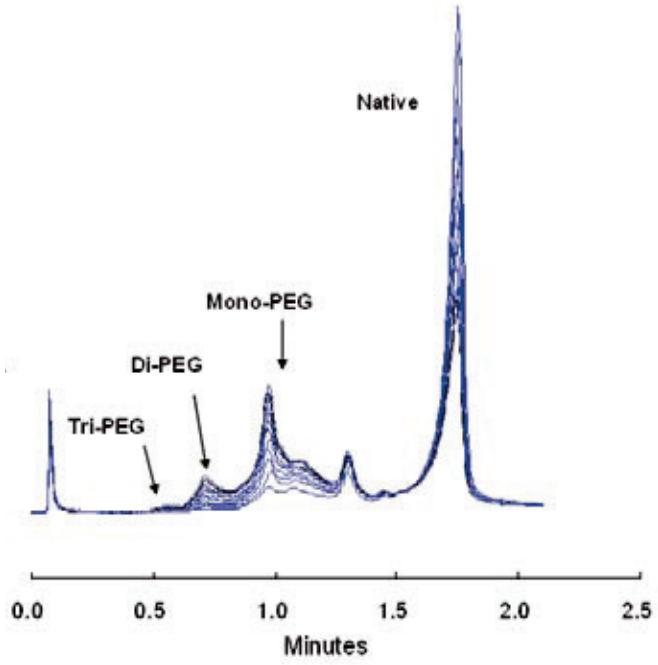
TSKgel SP-STAT, CM-STAT and SP-NPR

Nonporous TSK-GEL STAT columns provide fast, high resolution separations at moderate pressures. FIGURE 11 shows the monitoring of a PEGylation reaction of beta-lactoglobulin on a short SP-STAT column (prototype).

TSKgel SP-NPR columns provide fast separations due to their small (2.5 µm) spherical particles. A purity check of adeno-associated virus, commonly used in gene therapy research, on a TSKgel SP-NPR column is shown in FIGURE 12. This 10 minute HPLC method replaces an existing assay that took two days.

FIGURE 11

Monitoring of PEGylation of ß--lactoglobulin



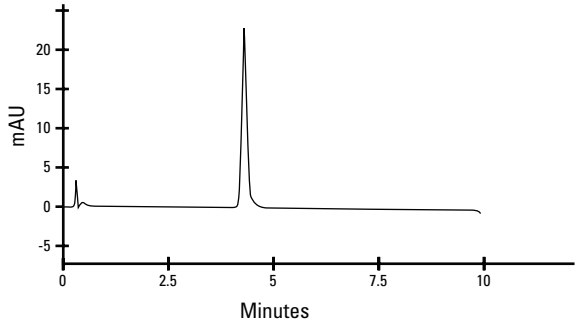
Column:	Prototype SP-STAT, 4.6 mm ID x 3.5 cm L (10 um)
Eluent:	A: 20 mmol/L Na acetate buffer pH 4.5 B: 0.8 mol/L NaCl in A pH 4.5
Gradient:	0 to 30% B (2 min)
Flow Rate:	4.0 mL/min
Detection:	UV @ 280 nm

Real-time analysis of PEGylation reaction (PEG MW=5000) at 5-minutes intervals

IEC

FIGURE 12

Analysis of purified AAV with TSKgel SP-NPR



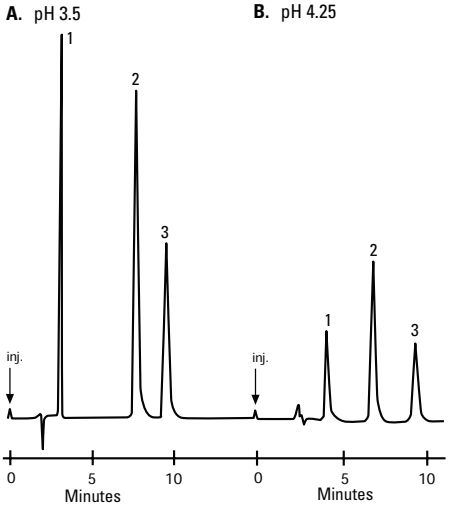
Column: TSKgel SP-NPR, 4.6mm ID x 3.5cm
Sample: purified adeno-associated virus
Elution: A. 50mmol/L HEPES, 1mmol/L EDTA, 5mmol/L MgCl, pH 7.5;
B. 50mmol/L HEPES, 1mmol/L EDTA, 5mmol/L MgCl, pH 7.5 with 0.5mol/L NaCl; linear gradient from 20% to 100% B in 10 column volumes
Flow Rate: 1mL/min
Detection: UV @ 280nm

SKgel SP-2SW, CM-2SW and CM-3SW

Silica-based cation exchangers are typically used in the separation of low molecular weight compounds such as pharmaceuticals, nucleotides, catecholamines, and small peptides. For example, FIGURE 13 shows the separation of nucleosides on the TSKgel SP-2SW column, while FIGURE 14 shows the rapid analysis of the herbicides paraquat and diquat in urine on TSKgel SP-2SW.

FIGURE 13

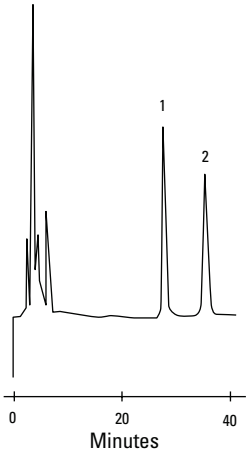
Separation of nucleosides by ion-exchange chromatography on TSKgel SP-2SW



Column: TSKgel SP-2SW 4.6mm ID x 25cm
Sample: Nucleoside Standards: 1) Guanosine, 2) Cytidine, 3) Adenosine
Mobile Phase: A) 0.1 mol/L sodium citrate - phosphoric acid buffer, pH 3.5
B) 0.1 mol/L sodium citrate - acetic acid buffer, pH 4.25
Flow Rate: 0.75 mL/min

FIGURE 14

Rapid Analysis for the Herbicides Paraquat and Diquat



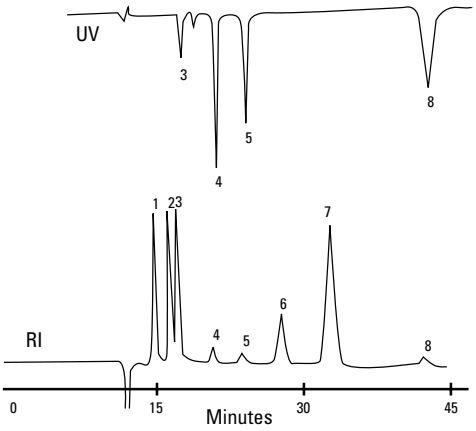
Column: TSKgel SP-2SW, 4.6mm ID x 25cm
Sample: 1. paraquat, 5µg/mL; 2. diquat, 5µg/mL
Elution: 20% ACN in 0.2mol/L phosphate, pH 3.0
Flow Rate: 1.0 mL/min
Detection: UV @ 290nm

Specialty columns

Ion exclusion chromatography can be used as an effective method for separating alcohols. An example of a saccharide, organic acid, and alcohol separation is shown in FIGURE 15 on two TSKgel SCX (H^+) columns in series.

FIGURE 15

Separation of mixture of saccharides, organic acids and alcohols



Column: TSKgel SCX (H^+), two 7.8mm ID x 30cm (in series)
Sample: 1. maltose, 2. glucose, 3. fructose, 4. lactic acid, 5. acetic acid, 6. methanol, 7. ethanol, 8. butyric acid
Elution: 0.05mol/L $HClO_4$
Flow Rate: 0.8mL/min
Detection: UV @ 210nm, Refractive Index

IEC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Glass columns: polymer-based								
14010	CM-5PW Glass, 1000 Å	5.0	5.0	10	≥ 700	0.5 - 0.8	1.0	15
14011	CM-5PW Glass, 1000 Å	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.2	10
14012	CM-5PW Glass, 1000 Å	20.0	15.0	13	≥ 2,500	4.0 - 6.0	8.0	15
13062	SP-5PW Glass, 1000 Å	5.0	5.0	10	≥ 700	0.5 - 0.8	1.0	15
08803	SP-5PW Glass, 1000 Å	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.2	10
14017	SP-5PW Glass, 1000 Å	20.0	15.0	13	≥ 3,000	4.0 - 6.0	8.0	15
PEEK columns: polymer-based								
19686	BioAssist S, 1300 Å	4.6	5.0	7	≥ 1,500	0.3 - 0.8	1.0	25
21411	BioAssist S, 1300 Å	10.0	10.0	13	≥ 3,000	1.0 - 5.0	7.0	25
Stainless steel columns: polymer-based								
21965	CM-STAT, nonporous -NEW-	3.0	3.5	10	≥ 200			100
21966	CM-STAT, nonporous -NEW-	4.6	10.0	7	≥ 2,000			100
21963	SP-STAT, nonporous -NEW-	3.0	3.5	10	≥ 200			100
21964	SP-STAT, nonporous -NEW-	4.6	10.0	7	≥ 2,000			100
13068	CM-5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	15
14021	CM-5PW, 1000 Å	21.5	15.0	13	≥ 2,500	4.0 - 6.0	8.0	25
18758	SP-5PW, 1000 Å	2.0	7.5	10	≥ 1,300	0.05 - 0.10	0.12	10
07161	SP-5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	15
07575	SP-5PW, 1000 Å	21.5	15.0	13	≥ 3,000	4.0 - 6.0	8.0	25
07934	SP-5PW, 1000 Å	55.0	20.0	20	≥ 1,500	20.0 - 40.0	50.0	4
13076	SP-NPR, nonporous	4.6	3.5	2.5	≥ 1,300	1.0 - 1.5	1.6	200
07156	SCX (Na^+)	6.0	15.0	5	≥ 2,000	0.5 - 1.0	1.2	150
07158	SCX (H^+)	7.8	30.0	5	≥ 12,000	0.5 - 1.0	1.2	50
Stainless steel columns: silica-based								
07165	SP-2SW, 125 Å	4.6	25.0	5	≥ 5,000	0.6 - 0.8	1.0	150
07167	CM-2SW, 125 Å	4.6	25.0	5	≥ 5,000	0.6 - 0.8	1.0	150
07162	CM-3SW, 250 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	20
Guard column products								
13069	CM-5PW Guardgel Kit			10	For P/N 13068			
16094	CM-5PW Prep Guardgel Kit			20	For P/N 14021			
14024	CM-5PW Guardgel Kit, Glass			20	For P/Ns 14010 and 14011			
14468	CM-5PW Guard column, Glass	20.0	2.0	13	For P/N 14012			
07211	SP-5PW Guardgel Kit			20	For P/N 07161			
42153	SP-5PW Guard cartridge	2.0	1.0	10	For P/N 18758			
08807	SP-5PW Guardgel Kit, Glass			20	For P/Ns 13062 and 08803			
14467	SP-5PW Guard column, Glass	20.0	2.0	13	For P/N 14017			
16093	SP-5PW Prep Guardgel Kit			20	For P/N 07575			
07932	SP-5PW Guard column	45.0	5.0	20	For P/N 07934			
07650	CM-SW Guardgel Kit			20	For P/Ns 07167 and 07162			
19308	Guard cartridge holder	2.0	1.5		For all 2mm ID Guard cartridges			