



# ION EXCHANGE COLUMNS



**TOSOH BIOSCIENCE**

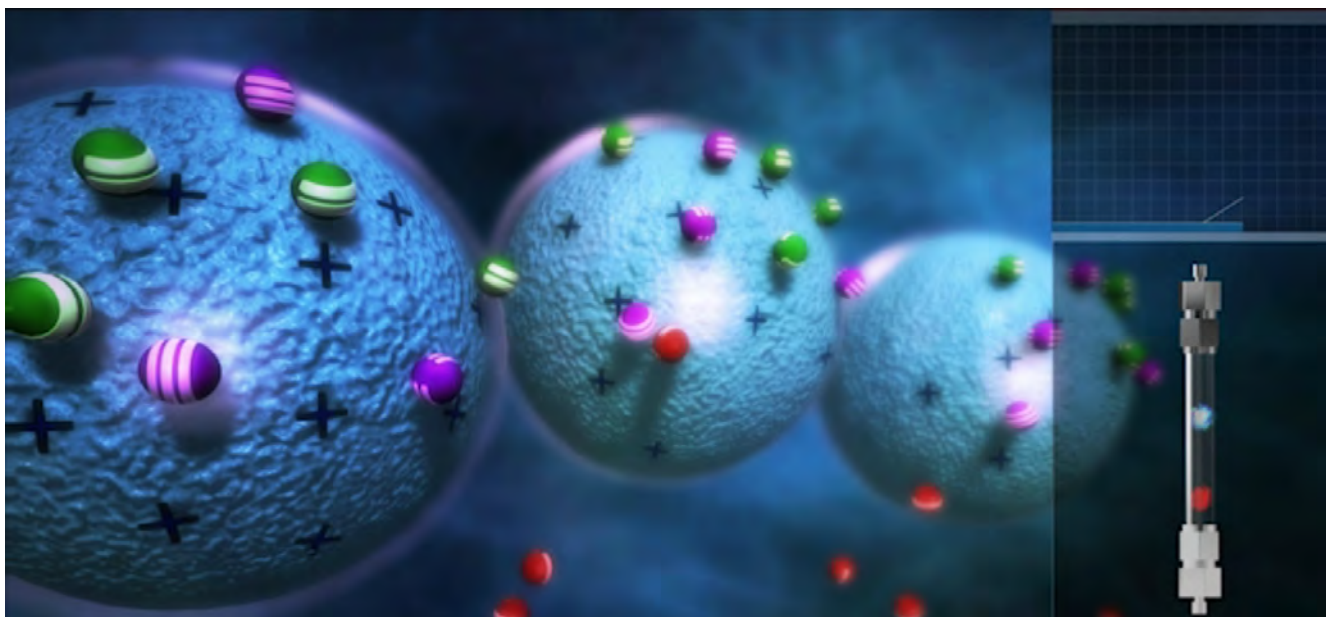
# PRINCIPLES OF CHROMATOGRAPHY

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The analysis, isolation, and purification of biomolecules can be accomplished by a number of chromatographic modes. Each mode is based on specific physical, chemical, or biological interactions between the sample biomolecule and packing material.

Tosoh Bioscience offers a comprehensive line of TSKgel and TOYOPEARL media and pre-packed TSKgel columns for all common modes of liquid chromatography.

The various modes of chromatography involve separations that are based on specific features of the target or sample, like size, charge, hydrophobicity, function or specific content of the molecule. To find out more about general principles of liquid chromatography and on how each of them works, visit us on our YouTube channel.



ION EXCHANGE CHROMATOGRAPHY

# ABOUT US

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## WITH A GLOBAL PERSPECTIVE.

Tosoh Bioscience is a leading manufacturer in the field of liquid chromatography. The portfolio of over 500 specialty products encompasses instruments for size exclusion/gel permeation chromatography and a comprehensive line of media and prepacked (U)HPLC columns for all common modes of liquid chromatography. Over the last 40 years, TSKgel SW columns have become the worldwide industry standard for size exclusion chromatography of biomolecules.

Tosoh manufacturing sites in Japan provide products to the sales and support subsidiaries in the U.S. and Europe, ensuring full global coverage. Our technical specialists in the European Headquarters provide assistance in developing HPLC applications or purification methods, in up-scaling, or packing process columns. We offer chromatographic workshops, on-site training, and are the sole sponsor of the HIC/RPC Bioseparation Conference series.



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➔ **TOSOH HISTORY**

- 1935 FOUNDING OF TOYO SODA MANUFACTURING CO., LTD.
- 1936 OPERATION OF NANYO MANUFACTURING COMPLEX BEGINS
- 1971 SCIENTIFIC INSTRUMENTS DIVISION FORMED, FIRST GPC COLUMN USING TSKgel DEVELOPED BY TOSOH
- 1974 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COLUMN PLANT IS COMPLETED
- 1979 TOSOH DEVELOPS TOYOPEARL MEDIA
- 1983 TOSOH DEVELOPS HYDROPHOBIC INTERACTION MEDIA
- 1987 TOSOH US OPERATIONS FORMED IN MONTGOMERYVILLE
- 1989 TOSOH GMBH OPERATIONS FORMED IN STUTTGART
- 1995 TOSOH NANYO GEL FACILITY RECEIVES ISO 9001
- 2002/2003 ALL TOSOH AFFILIATED SCIENTIFIC & DIAGNOSTIC SYSTEM RELATED COMPANIES IN EUROPE ARE UNIFIED UNDER THE NAME TOSOH BIOSCIENCE.
- 2008 EcoSEC, THE 7TH GENERATION GPC SYSTEM IS INTRODUCED GLOBALLY
- 2010 TOSOH CELEBRATES ITS 75TH YEAR IN BUSINESS WITH THE OPENING OF FIVE NEW PLANTS, AND CONTINUED RAPID EXPANSION IN CHINA
- 2011 TOSOH BIOSCIENCE CELEBRATES 40 YEARS OF OPERATION
- 2012 TOSOH RELEASES FIRST TOYOPEARL MIXED-MODE RESIN TOYOPEARL MX-Trp-650M
- 2013 TOSOH RELEASES A HIGH CAPACITY PROTEIN A CHROMATOGRAPHY RESIN
- 2014 TOSOH BIOSCIENCE GMBH CELEBRATES ITS 25<sup>TH</sup> ANNIVERSARY IN STUTTGART
- 2015 TOSOH BIOSCIENCE SUCCESSFULLY MOVES ITS SALES & MARKETING OFFICES TO GRIESHEIM, DARMSTADT



# IEC ION EXCHANGE CHROMATOGRAPHY



Ion exchange chromatography (IEC) is one of the most frequently used chromatographic modes in the separation and purification of biomolecules. It is a non-denaturing technique that is used for analysis and at all stages and scales of purification: from micro scale purification to industrial scale downstream processing.

Tosoh Bioscience offers analytical and semi preparative ion exchange HPLC columns as well as ion exchange resins for large scale biopurification.

TSKgel HPLC columns are packed with silica or polymer based porous or nonporous beads. They are well suited for a broad range of applications in R&D, quality control or reaction monitoring.

For TSKgel bulk and TOYOPEARL bulk please refer to our Chromatographic Process Media Catalogue.



# IEC HOW IT WORKS

IEC retains molecules based on ionic interactions. The stationary phase surface displays ionic functional groups that interact with analyte ions of opposite charge. IEC is further subdivided into cation exchange and anion exchange chromatography.

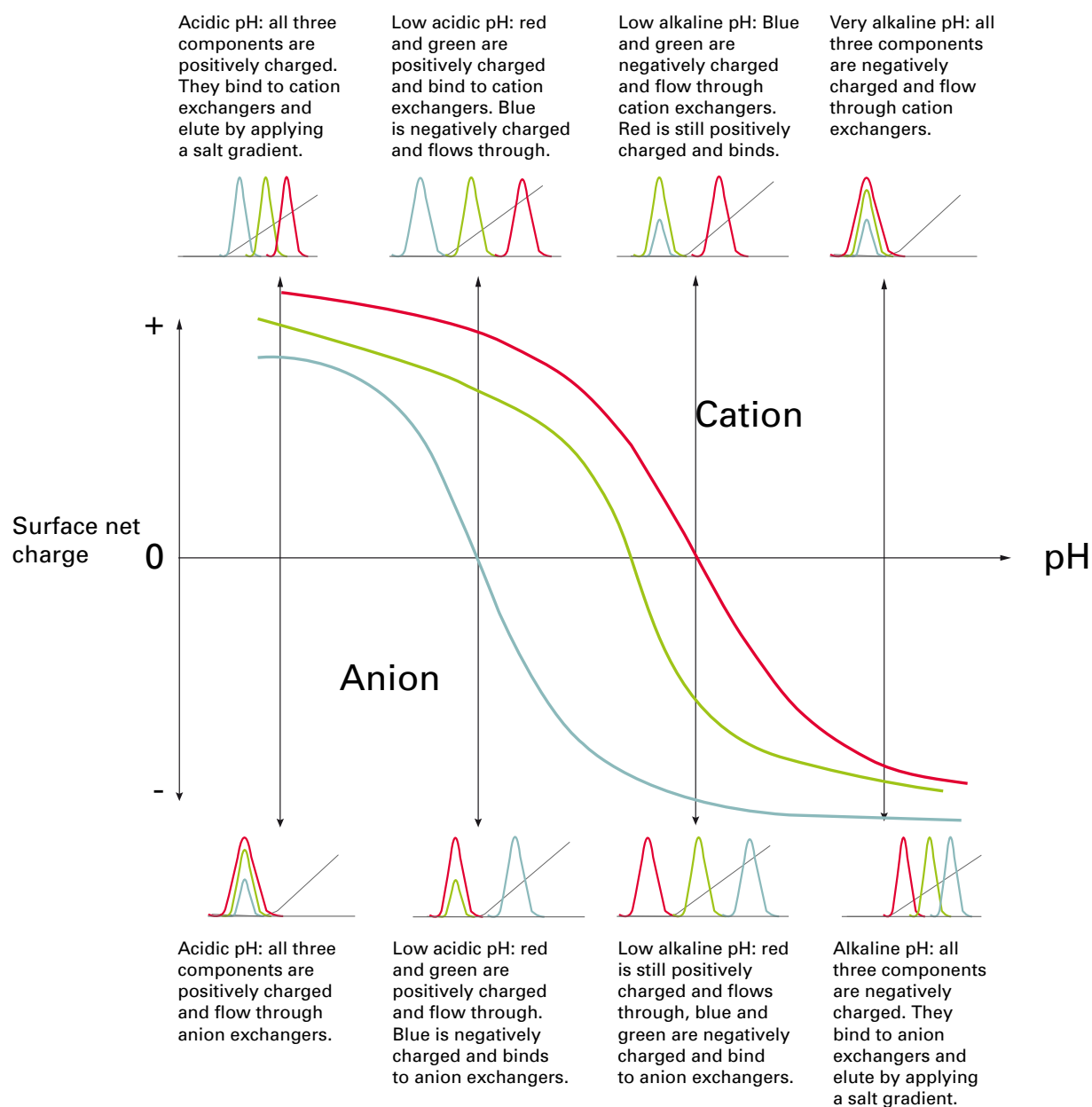
Anion exchange media carry positively charged groups that attract negatively charged anions. Cation exchange resins display negatively charged groups which attract positively charged cations. Charged target molecules are retained on the stationary phase but can be eluted by increasing the concentration of a similarly charged ion that will displace the analyte or target ions from the stationary phase.

Proteins have numerous functional groups that can have both positive and negative charges. IEC separates proteins according to their net surface charge, which is dependent

on the pH and ionic strength of the mobile phase. According to differences in their overall charge and surface charge distribution, proteins can be separated by IEC.

IEC takes advantage of the fact that the relationship between net surface charge and pH is unique for a specific protein. At a pH, equivalent to its isoelectric point, a protein has no net charge and will not interact with the charged stationary phase. At a pH above the pI the protein will have a negative net charge and will therefore bind to a positively charged anion exchanger. At a pH below its pI it will have a positive net charge and will consequently interact with a negatively charged cation exchanger. By adjusting the pH or the salt concentration of the mobile phase, separation can be optimized. For loading, the pH and ionic strength are selected in a way that the targets or analytes bind to the stationary phase (Figure 1).

➤ **FIGURE 1**



# IEC

## INTRODUCTION TO TSK<sub>gel</sub> IEC COLUMNS



Elution is usually performed by changing the ionic strength of the mobile phase by applying a salt gradient. As the salt concentration of the mobile phase increases, the salt ions compete with the bound molecules for the functional groups of the stationary phase. The higher the net charge of the molecule, the higher the salt concentration needed for elution. Very tightly bound compounds are removed at the end of the elution by a wash step with very high salt buffer.

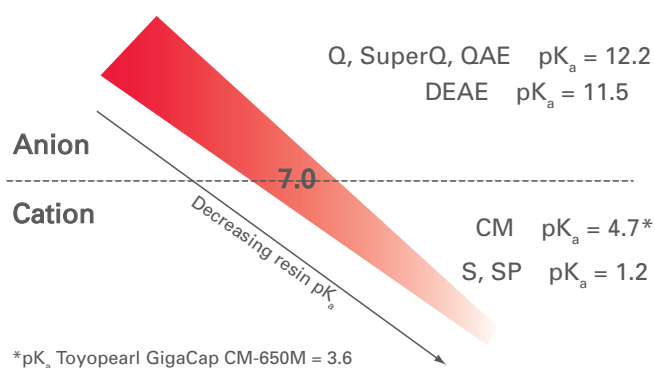
Ion exchange resins are classified as weak or strong ion exchangers. The terms strong and weak do not refer to the performance of the resins or to the strength of interaction between resin and target.

'Strong', respectively 'weak' refers to the extent that the ion exchange capacity varies with change in pH. Strong ion exchange groups have a steep titration curve. They show no variation of their ionization state with the pH and remain fully charged over a broad pH range.

Typical strong anion exchange groups are quaternary amine groups (Q & QAE type), typical strong cation exchange groups are sulfo or sulfopropyl groups (S & SP type). Carboxymethyl (CM, cationic) and diethylaminoethyl (DEAE, anionic) are weak ion exchange groups. Figure 2 shows the pK<sub>a</sub> values for these ligands.

FIGURE 2

PK<sub>a</sub> VALUES FOR ION EXCHANGE GROUPS



The binding buffer pH is selected between the pI of the protein and the pK<sub>a</sub> of the stationary phase.

TABLE 1

CATION EXCHANGE GROUPS		STRUCTURE
Carboxymethyl (CM)	Weak	-O-CH <sub>2</sub> COO <sup>-</sup>
Sulfopropyl (SP)	Strong	-O-R-O-CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -SO <sub>3</sub> <sup>-</sup>
ANION EXCHANGE GROUPS		
Diethylaminoethyl (DEAE)	Weak	-O-CH <sub>2</sub> -CH <sub>2</sub> -N <sup>+</sup> -(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub>
Quarternary Ammonium (Q)	Strong	-O-R-N <sup>+</sup> -(CH <sub>3</sub> ) <sub>3</sub>
Quarternary Aminoethyl (QAE)	Strong	-O-CH <sub>2</sub> -CH <sub>2</sub> -N <sup>+</sup> H-(CH <sub>3</sub> ) <sub>3</sub>



# IEC INTRODUCTION TO TSKgel IEC 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 IEC the product line contains methacrylate and silica based columns. Most of the available chemistries are offered in analytical as well as semi-preparative formats. Particle sizes range from 2.5  $\mu\text{m}$  for fast analysis to 20  $\mu\text{m}$  for preparative purposes. Proteins, peptides, oligonucleotides and nucleic acids are typical samples that are analyzed or isolated on TSKgel IEC columns.

## PACKING MATERIALS AND CHEMISTRIES

Methacrylate, silica and polystyrene are used as matrices for the TSKgel line of ion exchange columns. The base resins are derivatized either with diethylaminoethyl (DEAE), quaternary ammonium (Q), sulfopropyl (SP) or carboxymethyl (CM) functionalities to provide weak anion, strong anion, strong cation and weak cation exchangers, respectively.

Columns from the silica-based TSKgel SW series are typically used in the separation of low molecular weight compounds such as pharmaceuticals, nucleotides or small peptides.

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 G5000 PW base resin of the TSKgel 5PW series is a spherical particle with a mean pore size of 100 nanometer (1.000 Angström).

TSKgel BioAssist columns are also based on methacrylate particles with larger pores (400 nanometer for BioAssist Q and 130 nanometer for BioAssist S) providing better access to the functional groups for large proteins.

The methacrylate chemistry also forms the backbone of non-porous resin columns such as the TSKgel NPR and STAT series. Since rate-limiting pore diffusion 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%.

TSKgel STAT columns are the latest addition to the IEC column line. An innovative bonding chemistry results in columns that show a reasonable sample capacity while traditional non-porous resins usually show limited capacity due to lower surface area. Specific application needs are addressed by offering various column formats and particle sizes.

For special applications polystyrene based columns are offered as well. They are most suitable for analyzing small molecular weight sugars, amino acids, individual nucleic acids and small drug candidates. For detailed information on these special columns please refer to the chromatography catalog.

## FEATURES

### BioAssist Columns

- High capacity even for larger proteins (1 million Da)
- Unique pore structure provides fast mass transfer
- Biocompatible PEEK column hardware
- Available in analytical and semi-prep formats

### Polymer-Based Ion Exchange Columns

- Methacrylate backbone
- Large pore size (100 nm) (excl. limit for proteins ~ 5,000,000 Da)
- Non porous resin-based (STAT and NPR) columns
- Several columns available in 2 mm ID format

### Silica-Based Ion Exchange Columns

- Smaller pore size (2SW = 12.5 nm and 3SW = 25 nm)

## BENEFITS

- Fewer runs to collect required sample amounts
- Sharper peaks improve analysis and isolation
- Less sample loss due to adsorption
- Easy scale-up

- Mechanically and chemically stable (pH 2.0-12.0)
- Withstands repeated cleaning with base, and use of organic solvents, denaturants and surfactants
- Use same column for most biopolymers
- Fast QC analysis and process monitoring
- Reduced solvent consumption and analysis time

- Most suitable for analysing smaller MW samples such as nucleotides, drug candidates, catecholamines and small peptides or proteins



# IEC

## PROPERTIES OF TSKgel IEC COLUMNS



### PROPERTIES OF TSKgel ION EXCHANGE COLUMNS

#### TSKgel ANION EXCHANGE COLUMNS

TSKgel	Matrix*	Particle size (μm)	Pore size (nm)	Functional group	Counter ion	Excl. limit, PEG** (Da)	Capacity (mg BSA/mL)	Small ion capacity meq/mL	pKa	Column hardware***
BioAssist Q	pMA	10, 13	~400	Polyamine	Cl <sup>-</sup>	>5,000,000	70	0.1	9.4	PEEK
SuperQ-5PW	pMA	10,13	100	Trimethyl-amino	Cl <sup>-</sup>	1,000,000	100	> 0.13	12.2	S, G
DEAE-5PW	pMA	10,13, 20	100	DEAE	Cl <sup>-</sup>	1,000,000	30	0.1	11.5	S, G
Q-STAT	pMA	7,10	~ 0	Trimethyl-amino	Cl <sup>-</sup>	500	20	0.27	10.5	S
DNA-STAT	pMA	5	~ 0	Trimethyl-amino	Cl <sup>-</sup>	500	35	0.27	10.5	S
DEAE-NPR	pMA	2.5	~ 0	DEAE	Cl <sup>-</sup>	500	5	> 0.1	11.2	S
DNA-NPR	pMA	2.5	~ 0	Proprietary	ClO <sub>4</sub> <sup>-</sup>	500	5	> 0.1	11.2	S
DEAE-2SW	Silica	5	12.5	DEAE	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	10,000	ND	> 0.3	11.2	S
DEAE-3SW	Silica	10	25	DEAE	Cl <sup>-</sup>	30,000	ND	> 0.3	11.2	S
Sugar AXI	PS-DVB	8	6	Trimethyl-amino	HBO <sub>3</sub> <sup>-</sup>		ND	> 1.2	12.5	S
Sugar AXG	PS-DVB	10	6	Trimethyl-amino	HBO <sub>3</sub> <sup>-</sup>		ND	> 1.2	12.5	S
SAX	PS-DVB	5	6	Trimethyl-amino	Cl <sup>-</sup>		ND	> 1.0	12.5	S

#### TSKgel CATION EXCHANGE COLUMNS

TSKgel	Matrix*	Particle size (μm)	Pore size (nm)	Functional group	Counter ion	Excl. limit, PEG** (Da)	Capacity (mg/mL)	Small ion capacity meq/mL	pKa	Column hardware***
BioAssist S	pMA	7, 13	~130	Sulfopropyl	Na <sup>+</sup>	~4,000,000	70 <sup>(1)</sup>	0.1	2.4	PEEK
SP-5PW	pMA	10, 13, 20	100	Sulfopropyl	Na <sup>+</sup>	1,000,000	40 <sup>(2)</sup>	> 0.1	2.3	S, G
CM-5PW	pMA	10, 13	100	Carboxymethyl	Na <sup>+</sup>	1,000,000	45 <sup>(2)</sup>	> 0.1	4.2	S, G
SP-STAT	pMA	7, 10	~ 0	Sulfopropyl	Na <sup>+</sup>	500	10 <sup>(3)</sup>	> 0.023	2.6	S
CM-STAT	pMA	7, 10	~ 0	Carboxymethyl	Na <sup>+</sup>	500	15 <sup>(3)</sup>	> 0.1	4.9	S
SP-NPR	pMA	2.5	~ 0	Sulfopropyl	Na <sup>+</sup>	500	5 <sup>(2)</sup>	> 0.1	2.3	S
SP-2SW	Silica	5	12.5	Sulfopropyl	Na <sup>+</sup>	10,000	ND	0.3	2.2	S
CM-2SW	Silica	5	12.5	Carboxymethyl	Na <sup>+</sup>	10,000	110 <sup>(2)</sup>	> 0.3	4.2	S
CM-3SW	Silica	10	25	Carboxymethyl	Na <sup>+</sup>	30,000	ND	> 0.3	4.2	S
SCX	PS-DVB	5	6	Sulfonic acid	Na <sup>+</sup> , H <sup>+</sup>		ND	> 1.5		S

\*pMA = poly methacrylate; PS-DVB = polystyrene-divinylbenzene

\*\*Polyethylene glycol

\*\*\*PEEK = polyetheretherketone, S = stainless steel, G = glass (1) γ-globulin; (2) hemoglobin; (3) lysozyme



# IEC

## TSKgel IEC COLUMN SELECTION

SAMPLE TYPE	MW RANGE (Da)	TSKgel COLUMN	pH RANGE
<b>Amino Acids, Peptides and Proteins</b>			
Amino acids	< 2,000	SAX SCX	1.0 - 14.0 1.0 - 14.0
Peptides and small proteins	< 10,000	Q-STAT SP-STAT CM-STAT SCX SP-2SW CM-2SW DEAE-2SW	3.0 - 10.0 3.0 - 10.0 3.0 - 10.0 1.0 - 14.0 2.0 - 7.5 2.0 - 7.5 2.0 - 7.5
Proteins	> 10,000 up to ~ 5,000,000	BioAssist S BioAssist Q Q-STAT SP-5PW DEAE-5PW CM-5PW SP-STAT CM-STAT SP-NPR DEAE-NPR SuperQ-5PW	2.0 - 12.0 2.0 - 12.0 3.0 - 10.0 2.0 - 12.0 2.0 - 12.0 2.0 - 12.0 3.0 - 10.0 3.0 - 10.0 2.0 - 12.0 2.0 - 12.0 2.0 - 12.0
<b>Nucleic Acids</b>			
Purines and pyrimidines		DEAE-2SW SP-2SW	2.0 - 7.5 2.0 - 7.5
Nucleosides		SP-2SW DEAE-2SW	2.0 - 7.5 2.0 - 7.5
Nucleotides		Q-/DNA-STAT DEAE-2SW	3.0 - 10.0 2.0 - 7.5
Oligonucleotides		Q-/DNA-STAT DEAE-5PW DEAE-NPR DNA-NPR SuperQ-5PW	3.0 - 10.0 2.0 - 12.0 2.0 - 12.0 2.0 - 12.0 2.0 - 12.0
DNA, RNA, and PCR products		Q-/DNA-STAT DNA-NPR DEAE-NPR DEAE-5PW DEAE-3SW	3.0 - 10.0 2.0 - 12.0 2.0 - 12.0 2.0 - 12.0 2.0 - 7.5
<b>Other Molecules</b>			
Mono and disaccharides		Sugar AXI, AXG SCX SAX	1.0 - 14.0 1.0 - 14.0 1.0 - 14.0

# IEC

## TSKgel STAT SERIES



TSKgel STAT columns are designed for high efficiency separation of biomolecules and low molecular weight compounds. They provide superior performance at reduced analysis time. STAT columns are available in various formats and sizes of the mono-disperse particles (5, 7 and 10  $\mu\text{m}$ ) to perfectly match specific application needs.

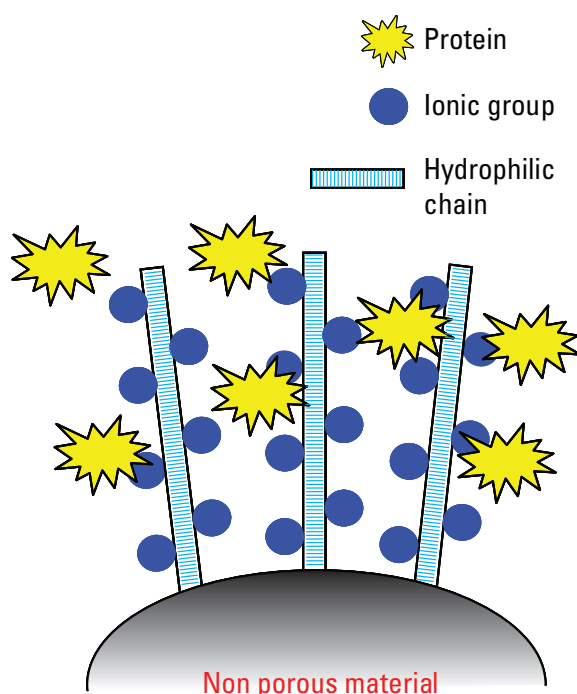
The surface of the hydrophilic non-porous particles consists of an open access network of multi-layered ion exchange groups (carboxymethyl, sulfopropyl or quaternary ammonium groups; see Figure 3). The innovative bonding chemistry results in columns that show a reasonable sample capacity while traditional non-porous resins usually show limited capacity due to lower surface area.

For fast and ultrafast IEC analysis short columns (3 mm ID x 3.5 cm length) are packed with large 10  $\mu\text{m}$  particles. They are ideally suited for rapid candidate screening or process monitoring. Longer columns (4.6 mm ID x 10 cm length) packed with 7  $\mu\text{m}$  particles were designed for high resolution IEC separation. They are perfect for the analysis of nucleic acids, mAb variants, protein aggregates or PEGylated proteins. The DNA-STAT columns (4.6 mm ID x 10 cm length) packed with 5  $\mu\text{m}$  Q-type anion exchange resin are optimized for the analysis of nucleic acids.

The relatively large particle sizes support fast separation at moderate pressure while at the same time the proprietary surface modification technology ensures a high density of functional groups going along with high sample capacity.

FIGURE 3

SCHEMATIC DIAGRAM OF TSKgel STAT SERIES



### PRODUCT HIGHLIGHTS TSKgel STAT SERIES

- Very efficient chromatography for high as well as low MW solutes made possible by novel bonding chemistry and the absence of micro-pores
- High speed and high resolution analysis of Biomolecules in HPLC and UHPLC
- Higher adsorption capacities and lower pressures compared with smaller particle sized TSKgel NPR columns
- 7 or 10  $\mu\text{m}$  particles (TSKgel Q-STAT) and 5  $\mu\text{m}$  particles (TSKgel DNA-STAT)
- 7 or 10  $\mu\text{m}$  particles for SP and CM chemistries

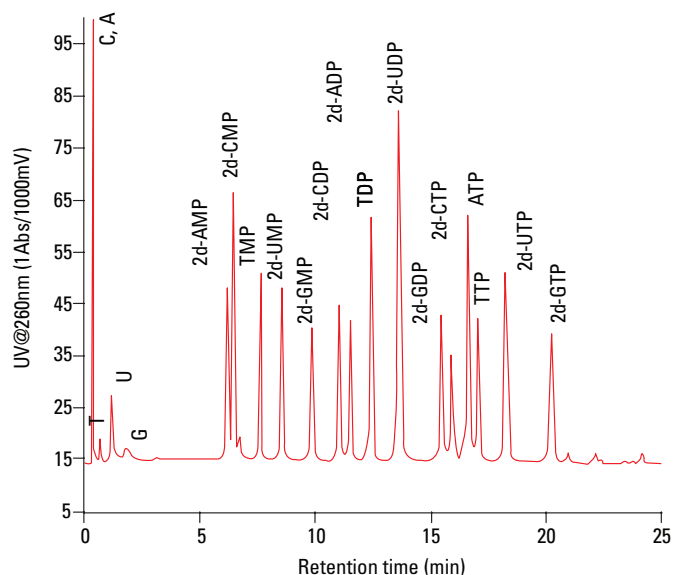
### APPLICATIONS WITH TSKgel STAT ANION EXCHANGE COLUMNS

#### Nucleotides

Mono-, di-, and tri-nucleotides were separated with excellent peak shape on a TSKgel DNA-STAT column. The narrow, symmetrical peaks, as shown in Figure 4, demonstrate the absence of micropores on this new generation of non-porous resin columns. TSKgel DNA-STAT columns are also, as the name implies, first choice for large nucleic acid fragments.

FIGURE 4

HIGH RESOLUTION SEPARATIONS OF NUCLEOTIDES



Column: TSKgel DNA-STAT, 5  $\mu\text{m}$ , 4.6 mm ID x 10.0 cm L;  
 Eluent: A: 20 mmol/L Tris-HCl (pH 8.5); B: 0.75 mol/L NaCl in buffer A;  
 Gradient: 50% B (0 min), 75% B (25 min); Flow rate: 0.8 mL/min;  
 Detection: UV @ 260 nm

# IEC TSKgel STAT SERIES

## APPLICATIONS WITH TSKgel STAT ANION EXCHANGE COLUMNS

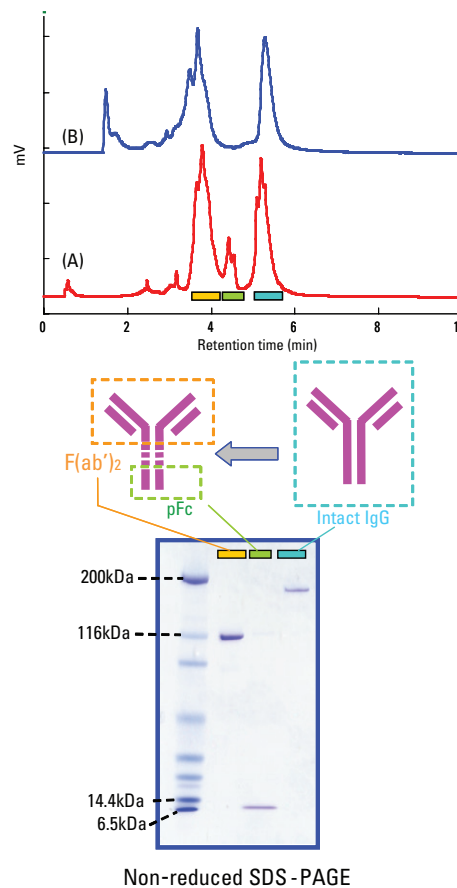
### Monoclonal Antibodies

The monoclonal antibody was digested using pepsin and separated on a TSKgel Q-STAT column and a competitive non-porous WAX column. As shown in Figure 5, three peaks were isolated from the TSKgel Q-STAT column and assigned as F(ab')<sub>2</sub>, pFc and intact IgG by SDS-PAGE. There was no correlation between the peaks obtained on the competitive WAX column and SDS-PAGE.

### High Resolution versus high Throughput Analysis of Nucleotides

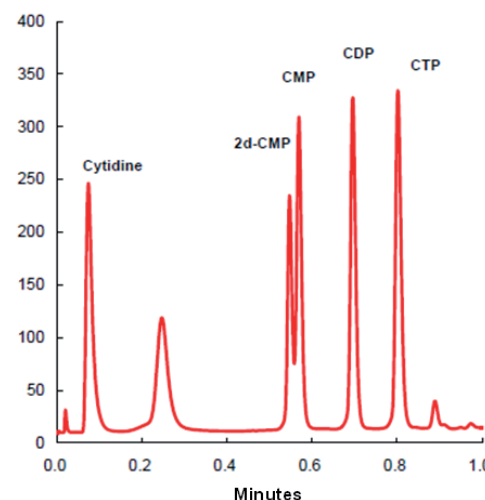
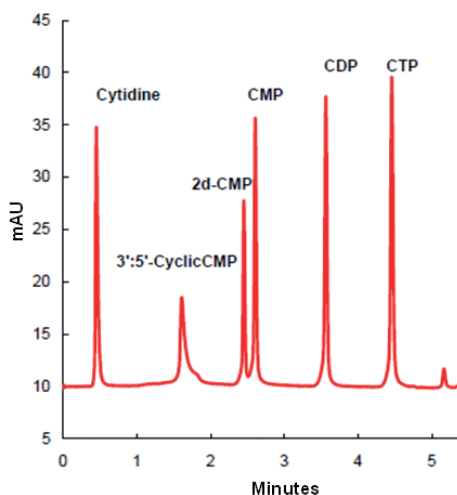
The separation of nucleotides on a 10 cm length Q-STAT column was compared to the separation on a 3.5 cm length column. While the longer column provides a high resolution separation of the nucleotides within five minutes the same sample can be analyzed within one minute on the shorter column allowing high throughput. The chromatograms are shown in Figure 6.

**FIGURE 5**  
ANALYSIS OF IgG FRAGMENTS



Column: A: TSKgel Q-STAT, 7 μm, 4.6 mm ID x 10 cm L; B: Competitor WAX, 10 μm, 4 mm ID x 25 cm L; Eluent: A: 20 mmol/l Tris-HCl (pH 8.5); B: 0.5 mol/L NaCl in buffer A; Gradient: 0% B (0 min), 100% B (10 min); Flow rate: 1.0 mL/min; Detection: UV @ 280 nm; Samples: pepsin digested mAb

**FIGURE 6**  
HIGH RESOLUTION VERSUS HIGH THROUGHPUT ANALYSIS OF NUCLEOTIDES



**High resolution:**

Column: Q-STAT, 4.6 mm ID x 10 cm L (7 μm); Eluent: A) 20 mmol/L Tris-HCl (pH 8.5) B) 0.5 mol/L NaCl in A (pH 8.5); Gradient: 0 to 100% B (10 min.); Flow rate: 1.5 mL/min.; Detection: UV @ 260 nm

**High throughput:**

Column: Q-STAT, 4.6 mm ID x 3.5 cm L (10 μm); Eluent: A) 20 mmol/L Tris-HCl (pH 8.5), B) 0.5 mol/L NaCl in A (pH 8.5); Gradient: 0 to 100% B (1 min.); Flow rate: 4.0 mL/min.; Detection: UV @ 260 nm

# IEC TSKgel STAT SERIES



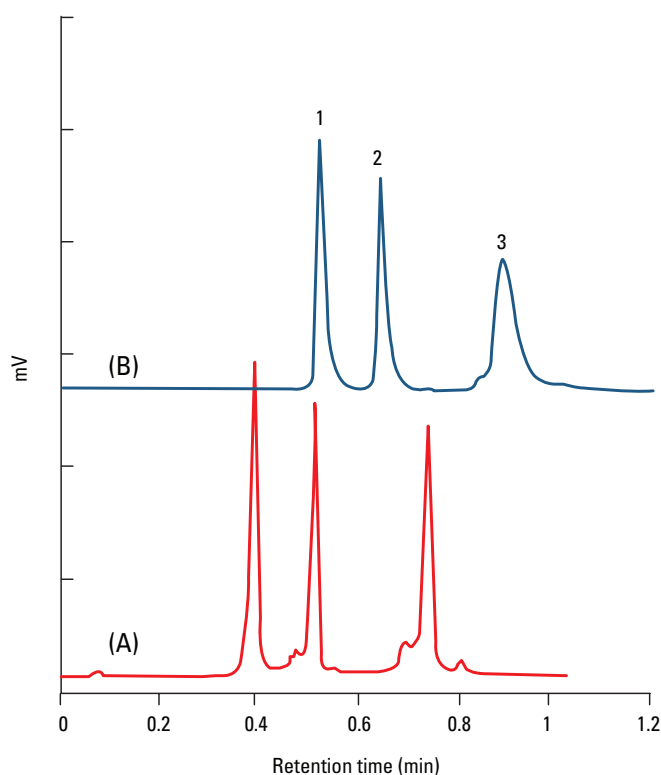
## APPLICATIONS WITH TSKgel STAT CATION EXCHANGE COLUMNS

### Fast Separations

The fast separation of protein standards was investigated using short cation exchange columns (see Figure 7). A TSKgel SP-STAT column shows superior resolution, better peak shape, and shorter analysis time (< 60 seconds) compared to a competitive monolithic SP-type column.

**FIGURE 7**

#### FAST PROTEIN SEPARATION



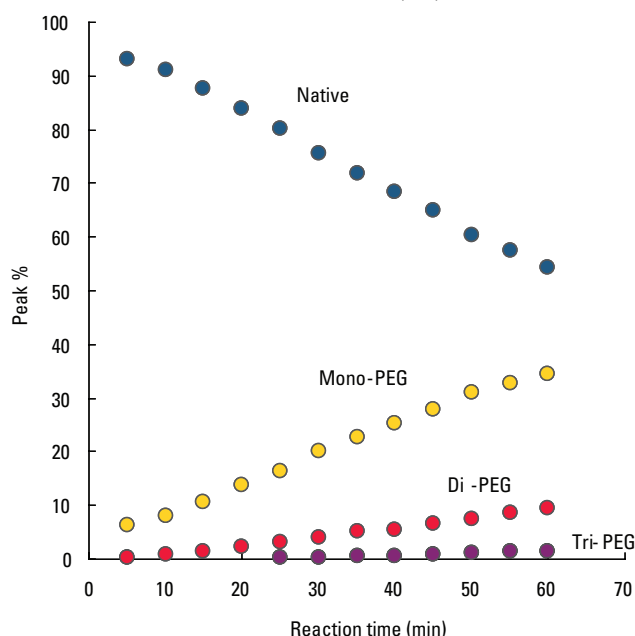
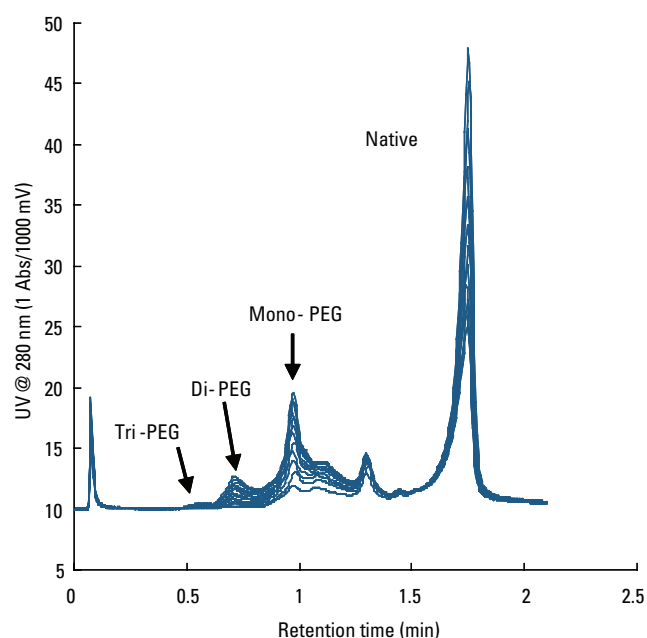
Column: A: TSKgel SP-STAT, 10  $\mu$ m, 3.0 mm ID x 3.5 cm L;  
 B: Competitor column 4.6 mm ID x 5.0 cm L  
 Eluent: A: 20 mmol/L sodium acetate (pH 5.0); B: 1.0 mol/L NaCl in buffer A (pH 5.0) for column A; 1.5 mol/L NaCl in buffer A (pH 5.0) for column B;  
 Gradient: 0% B (0 min), 100% B (1 min);  
 Flow rate: A: 2.0 mL/min; B: 4.73 mL/min; Detection: UV @ 280 nm;  
 Samples: 1.  $\alpha$ -chymotrypsinogen A; 2. cytochrome C; 3. lysozyme

## Reaction Monitoring

A sample of  $\beta$ -lactoglobulin (5 mg/mL) was reacted with polyethylene glycol (5 kDa) in a pH 6.5 phosphate buffer. The formation of PEGylated protein reaction products was monitored in 5 minute intervals on a 3.5 cm L TSKgel SP-STAT column. As demonstrated in Figure 8, peak areas of mono-, di-, and tri- PEGylated  $\beta$ -lactoglobulin increased with reaction time, while the area of unreacted  $\beta$ -lactoglobulin declined.

**FIGURE 8**

#### PEGYLATION MONITORING



Column: TSKgel SP-STAT, 10  $\mu$ m, 3.0 mm ID x 3.5 cm L; Eluent: A: 20 mmol/L sodium acetate (pH 5.0); B: 1.0 mol/L NaCl in buffer A (pH 5.0); Gradient: 0% B (0 min), 100% B (2 min); Flow rate: 2.0 mL/min; Detection: UV @ 280 nm; Samples: PEGylated  $\beta$ -lactoglobulin




**IEC  
TSKgel STAT SERIES**

### Antibody Analysis

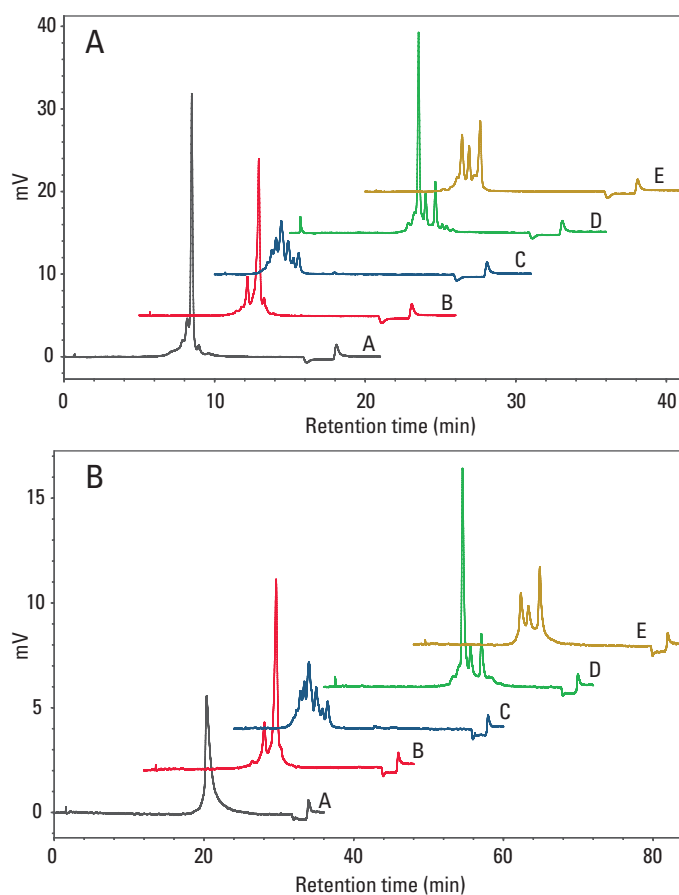
The analysis profiles for five antibodies separated on a TSKgel CM-STAT column were compared with the profiles obtained on a competitive WCX column (Figure 9). Similar or higher resolution profiles were obtained on TSKgel CM-STAT in approximately half the time.

### Column Selection for Antibody Analysis

Different antibodies were analyzed on a CM-STAT and SP-STAT column at pH 7. Figure 10 shows that it is dependent on the antibody which column provides the better resolution. The strong cation exchange column TSKgel SP-STAT shows a better separation of charge variants of mAb A (upper chromatograms) while the weak cation exchange column TSKgel CM-STAT delivers a better separation of a basic variant from the main peak for mAb B.

**FIGURE 9**

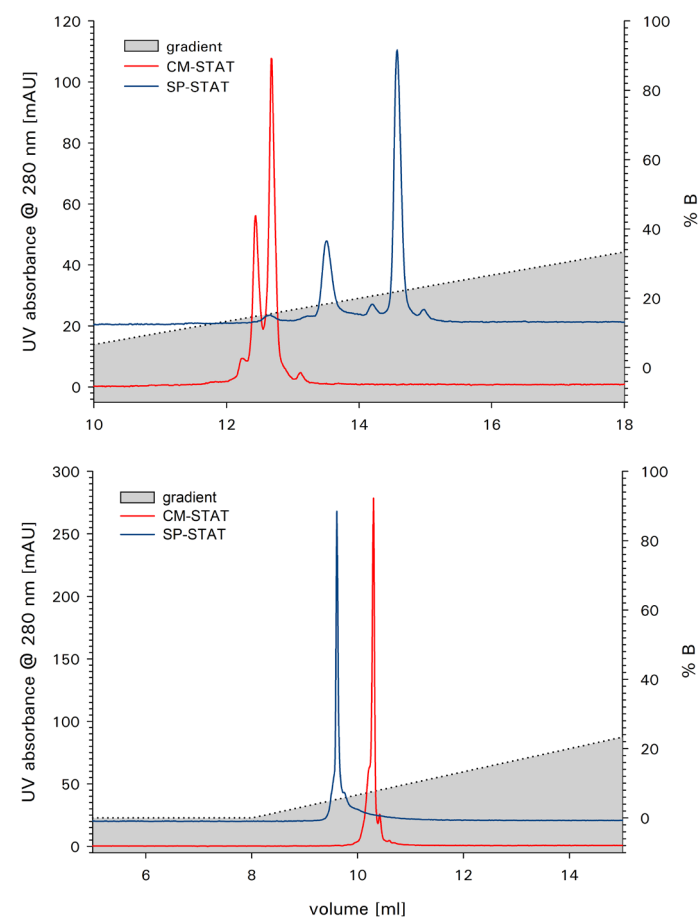
#### ANALYSIS OF CHARGE HETEROGENEITY



Column: A: TSKgel CM-STAT, 7 µm, 4.6 mm ID x 10 cm L; B: Competitor WCX, 10 µm, 4.0 mm ID x 25 cm L;  
 Eluent: A: 20 mmol/L MES (pH 6.0); B: 20 mmol/L MES + 0.5 mol/L NaCl (pH 6.0)  
 Gradient: A: 10% B (0 min), 30% B (15 min), 100% B (15 min), 100% B (17 min), 10% B (17 min), 10% B (21 min); B: 10% B (0 min), 30% B (30 min), 100% B (30 min), 100% B (32 min), 10% B (32 min), 10% B (36 min)  
 Flow rate: A: 1.0 mL/min B: 2.0 mL/min; Temp.: Ambient; Detection: UV @ 280 nm; Inj. Vol.: 20 µL; Sample: monoclonal antibodies (mAb A through E)

**FIGURE 10**

#### COMPARISON OF TSKgel CM- AND SP-STAT COLUMNS



Columns: TSKgel SP-STAT (7 µm, 4.6 mm ID x 10 cm); TSKgel CM-STAT (7 µm, 4.6 mm ID x 10 cm)  
 Mobile phase A: 10 mmol/L sodium phosphate buffer pH 7.0  
 Mobile phase B: 100 mmol/L sodium phosphate pH 7.0 + 500 mmol/L NaCl  
 Gradient: 0-100 % B in 30 min; Flow rate: 1 mL/min; Detection: UV @ 280 nm  
 Injection vol.: 10 µL; Sample: mAb A (2 g/L); mAb B (2 g/L)




**IEC  
TSKgel BioAssist SERIES**
**APPLICATIONS WITH TSKgel BioAssist ANION EXCHANGE COLUMNS**
**Comparison of Dynamic Binding Capacity**

Table 2 shows typical dynamic binding capacities on Bio-Assist Q relative to competitive products.

**TABLE II**
**COMPARISON OF DYNAMIC BINDING CAPACITIES**

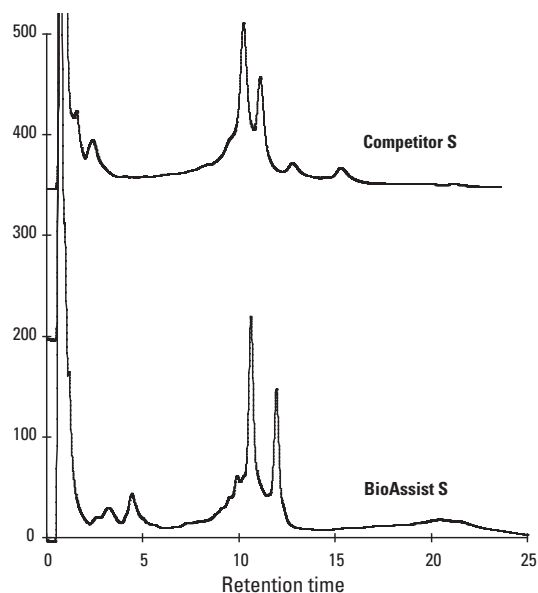
Protein	Binding capacity (mg/mL)			
	BioAssist Q	SuperQ -5PW	Conv. Q type prod. A	Conv. Q type prod. B
Thyroglobulin	77.4	22.9	20.2	1.8
Monoclonal IgG <sub>1</sub>	57.8	43.3	46.7	47.7
Human Serum Albumin	83.1	78.9	48.2	48.8
Trypsin Inhibitor	84.3	92.8	51.8	57.8

Columns: TSKgel BioAssist Q and TSKgel SuperQ-5PW (4.6 mm ID x 1 cm);  
Conventional Q type product A and B (4.6 mm ID x 1 cm)

Solvent: 20 mmol/L Tris-HCl buffer, pH 8.0;

Flow rate: 0.38 mL/min; Det.: UV @ 280 nm

\*Capacity was determined at 10% height of the breakthrough curve; UV 280 nm.

**FIGURE 12**
**BROMELAIN ANALYSIS ON TSKgel BioAssist S & COMPETITOR S COLUMNS**


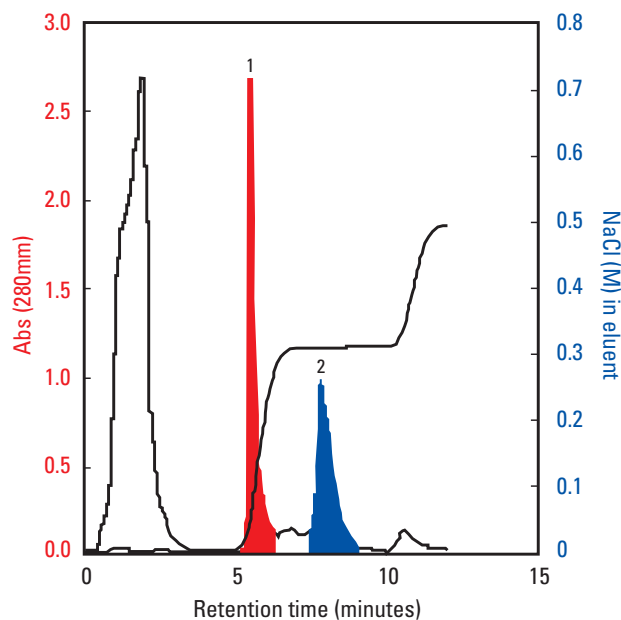
Columns: TSKgel BioAssist S, 4.6 mm ID x 5 cm L, PEEK  
Competitor S 5 mm ID x 5 cm L; Elution: 20 min (TSKgel) or 30 min (Competitor S) linear gradient of NaCl from 0 to 0.5 mol/L in 20 mmol/L sodium phosphate buffer, pH 7.0; Flow rate: 0.8 mL/min for TSKgel; 1.0 mL/min for Competitor S  
Detection: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

**APPLICATIONS WITH TSKgel BioAssist CATION EXCHANGE COLUMNS**
**Bromelain Analysis on TSKgel BioAssist S and Competitor S Column**

Figure 12 shows 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.

**Analysis of IgM**

IgM is known to possess unique and beneficial characteristics relative to other immunoglobulin classes; it is a large molecule comprised of five IgG subunits, resulting in a relatively unstable and difficult to purify protein. Unlike single chain antibodies, IgM cannot be purified by Protein A (affinity material commonly used for its high binding capacity and excellent selectivity for antibodies) due to steric hindrance. Alternative affinity methods have been developed with thiophilic adsorbents but these methods often result in low binding capacity. An alternative purification method of IgM by ion exchange chromatography using a TSKgel BioAssist S column was developed. Figure 13 shows the baseline separation of IgM from other contaminants using a 0.3 mol/L NaCl step gradient after elution of albumin.

**FIGURE 13**
**ANALYSIS OF IgM**


Column: TSKgel BioAssist S, 7  $\mu$ m, 4.6 mm ID x 5 cm L;  
Mobile phase: 20 mmol/L sodium phosphate buffer, pH 6.0;  
Gradient: 0 mol/L - 0.3 mol/L NaCl (5 min), 0.3 mol/L - 0.5 mol/L NaCl (10 min);  
Flow rate: 1 mL/min; Detection: UV @ 280 nm; Sample: 500  $\mu$ L of 9.5 mg/mL IgM in mouse ascites fluid; shaded peaks represent albumin and IgM respectively

# IEC

## TSKgel 5PW SERIES



The G5000 PW base resin of the TSKgel 5PW series is a spherical particle with a mean pore size of 100 nanometer. The chemistries of DEAE, SP and CM functionalities result in standard ion exchangers while the chemistry employed in the manufacturing of TSKgel SuperQ-5PW results in a higher capacity strong anion exchanger by introducing polyamine functional groups. Due to the higher density of anion exchange sites TSKgel SuperQ-5PW has a smaller effective pore size and a higher binding capacity than TSKgel DEAE-5PW.

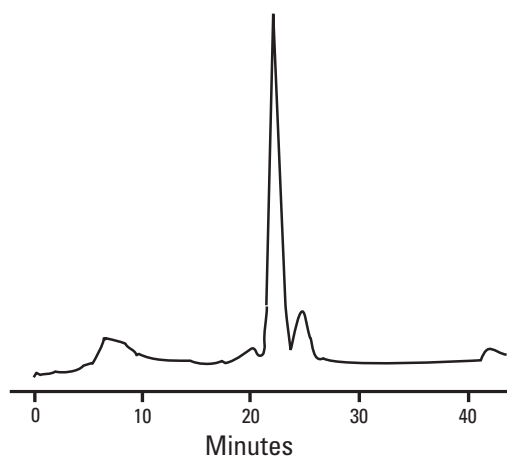
### APPLICATIONS WITH TSKgel 5PW ANION EXCHANGE COLUMNS

#### Analysis of a synthetic Oligonucleotide on TSKgel SuperQ-5PW

Figure 14 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 14**

ANALYSIS OF SYNTHETIC OLIGONUCLEOTIDE ON TSKgel SuperQ-5PW



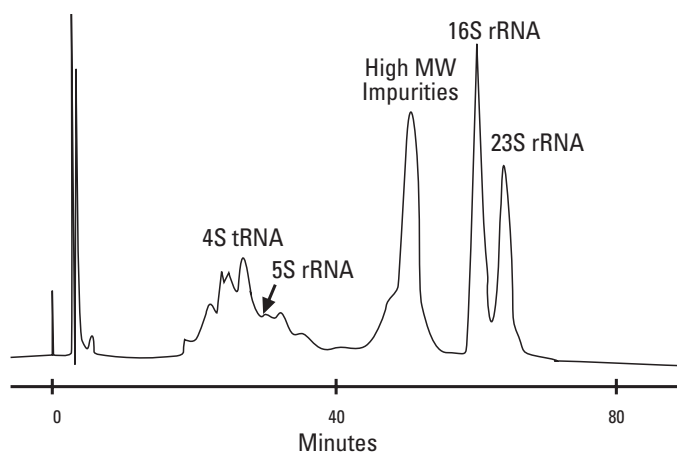
Column: TSKgel SuperQ-5PW, 7.5 mm ID x 7.5 cm L;  
 Sample: 16-mer morpholine oligonucleotide, AAG AAG AAG AGG GGA G;  
 Sample load: 0.5 O.D. (optical density); Mobile phase: A: 10 mmol/L NaOH;  
 B: 10 mmol/L NaOH with 1 mol/L NaCl; Gradient: Initial: 0 % B, 40 min: 50 % B,  
 41 min: 100 % B, 46 min: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

#### Analysis of high MW RNA on TSKgel DEAE-5PW

Figure 15 shows the fractionation of high molecular weight *E. coli* RNA on TSKgel DEAE-5PW, effectively utilizing the large 100 nm pores of this base resin.

**FIGURE 15**

LARGE PORE TSKgel DEAE-5PW RESOLVES HIGH MW RNA



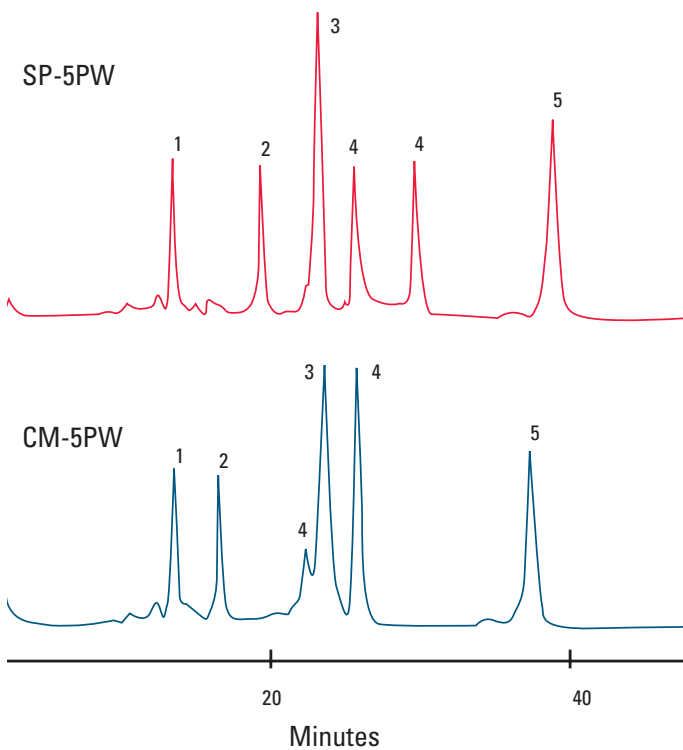
Column: TSKgel DEAE-5PW, 6 mm ID x 15 cm L; Sample: total *E. coli* RNA;  
 Elution: 300 min linear gradient from 0.3 mol/L NaCl in 0.1 mol/L Tris-HCl,  
 pH 7.6; Flow rate: 1.0 mL/min; Detection: UV @ 260 nm


**IEC  
TSKgel 5PW SERIES**
**APPLICATIONS WITH TSKgel 5PW CATION EXCHANGE COLUMNS**
**Comparison of strong and weak Cation Exchangers**

Differences in selectivity between strong (TSKgel SP-5PW) and weak (TSKgel CM-5PW) cation exchangers are demonstrated in Figure 16 which is a separation of globular proteins.

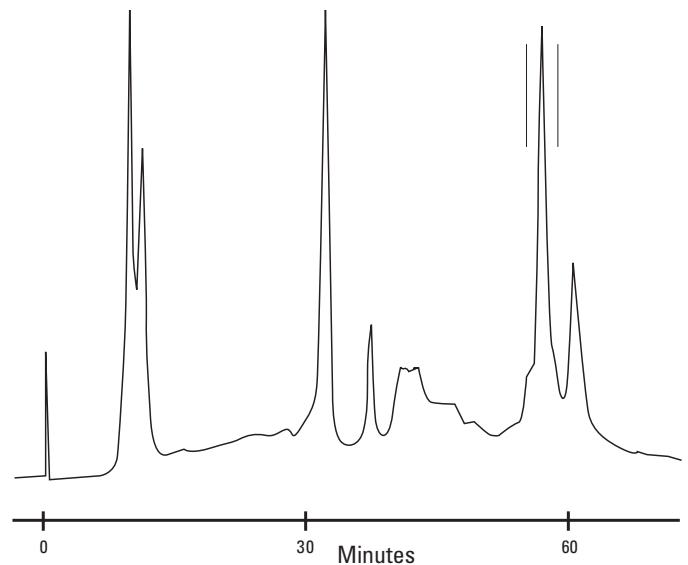
**Semi-preparative Purification of Lipoxidase**

The purification of 200 mg of crude lipoxidase on a 21.5 mm ID TSKgel SP-5PW column is illustrated in Figure 17. Scale-up is simplified as only the particle size changes from 10  $\mu\text{m}$  (7.5 mm ID) to 13  $\mu\text{m}$  (21.5 mm ID) or 20  $\mu\text{m}$  (55 mm ID) column.

**FIGURE 16**
**SELECTIVITY ON TSKgel 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.  $\alpha$ -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

**FIGURE 17**
**SEMI-PREPARATIVE PURIFICATION OF LIPOXIDASE**


Column: TSKgel SP-5PW, 21.5 mm ID x 15 cm L; Sample: crude lipoxidase, 200 mg; Elution: 120 min linear gradient from 0 mol/L to 0.5 mol/L  $\text{Na}_2\text{SO}_4$  in 0.02 mol/L acetate, pH 4.5; Flow rate: 4.0 mL/min; Detection: UV @ 280 nm; Recovery: Lipoxidase activity collected between the two vertical lines was 84%.






**IEC  
TSKgel SW SERIES**

The silica-based ion exchange columns are typically used in the separation of low molecular weight compounds such as pharmaceuticals, nucleotides or small peptides. Silica-based particles are used in pore sizes of 125 nanometer (2SW) and 250 nanometer (3SW) with either diethylaminoethyl (DEAE) or carboxymethyl (CM) functionality. 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.

The increased solubility of the silica backbone at pH above pH 7.5 limits the use of silica based ion exchange columns to acidic or neutral mobile phases.

**APPLICATIONS OF TSKgel SW ION EXCHANGE COLUMNS**
**Silica-based Anion Exchange Columns**

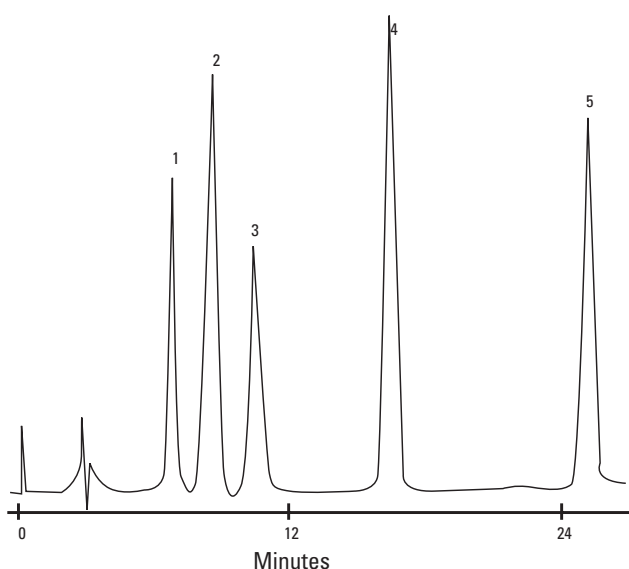
TSKgel 2SW-type columns provide high performance separations of small ionic solutes. High performance analyses of small anionic species are best performed on small pore silica-based anion exchangers, such as TSKgel DEAE-2SW. This is demonstrated in Figure 20. The 250 nanometer pore size TSKgel DEAE-3SW column is used for separating peptides, low MW proteins and DNA fragments.

**Silica-based Cation Exchange Columns**

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 21 shows the separation of nucleosides on TSKgel SP-2SW.

**FIGURE 20**

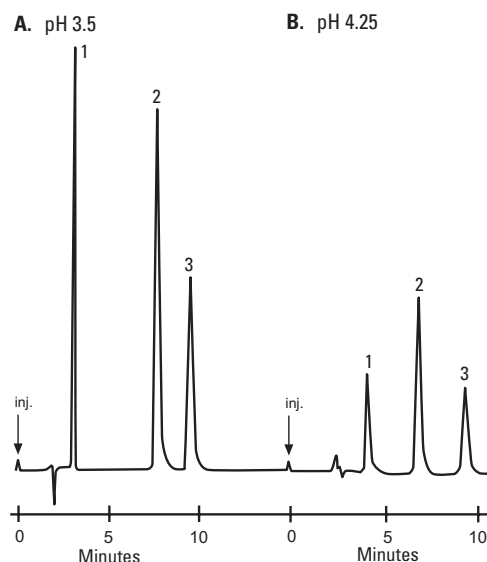
SEPARATION OF NUCLEOTIDES ON TSKgel DEAE-2SW



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

**FIGURE 21**

SEPARATION OF NUCLEOSIDES BY IEC ON TSKgel SP-2SW



Column: TSKgel SP-2SW 4.6 mm ID x 25 cm L  
 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; Temperature 23 °C; Detection: UV @ 260 nm

# TSKgel ANION EXCHANGE COLUMNS

## ORDERING INFORMATION



### ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min) Range	Maximum pressure drop (MPa)
<b>TSKgel GLASS COLUMNS: POLYMER-BASED</b>							
0013061	DEAE-5PW Glass, 100 nm	5.0	5.0	10	≥ 700	0.5 - 0.8	1.5
0008802	DEAE-5PW Glass, 100 nm	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.0
0014016	DEAE-5PW Glass, 100 nm	20.0	15.0	13	≥ 3,000	4.0 - 6.0	1.5
0018386	SuperQ-5PW Glass, 100 nm	8.0	7.5	10	≥ 1,300	0.5 - 1.0	2.0
<b>TSKgel PEEK COLUMNS: POLYMER-BASED</b>							
0019685	BioAssist Q, 400 nm	4.6	5.0	10	≥ 500	0.3 - 1.0	2.5
0021410	BioAssist Q, 400 nm	10.0	10.0	13	≥ 500	1.0 - 5.0	2.5
<b>TSKgel STAINLESS STEEL COLUMNS: POLYMER-BASED</b>							
0021960	Q-STAT, nonporous	3.0	3.5	10	> 200	1.0 - 2.0	10.0
0021961	Q-STAT, nonporous	4.6	10.0	7	> 4,000	0.5 - 1.4	10.0
0021962	DNA-STAT, nonporous	4.6	10.0	5	> 4,000	0.3 - 0.6	15.0
0013075	DEAE-NPR, nonporous	4.6	3.5	2.5	≥ 1,300	1.0 - 1.5	20.0
0018249	DNA-NPR, nonporous	4.6	7.5	2.5	≥ 6,000	0.5 - 1.0	30.0
0018757	DEAE-5PW, 100 nm	2.0	7.5	10	≥ 1,300	0.05 - 0.10	1.5
0007164	DEAE-5PW, 100 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.5
0007574	DEAE-5PW, 100 nm	21.5	15.0	13	≥ 3,000	4.0 - 6.0	2.5
0007930	DEAE-5PW, 100 nm	55.0	20.0	20	≥ 1,500	20.0 - 40.0	0.4
0018257	SuperQ-5PW, 100 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	2.0
0018387	SuperQ-5PW, 100 nm	21.5	15.0	13	≥ 3,000	4.0 - 6.0	2.0
0008639	Sugar AXI, 6 nm	4.6	15.0	8	≥ 3,700	0.2 - 0.4	3.0
0008640	Sugar AXG, 6 nm	4.6	15.0	10	≥ 2,700	0.2 - 0.5	2.0
0007157	SAX, 6 nm	6.0	15.0	5	≥ 2,000	0.5 - 1.0	15.0
<b>TSKgel STAINLESS STEEL COLUMNS: SILICA-BASED</b>							
0018761	DEAE-2SW, 12.5 nm	2.0	25.0	5	≥ 5,000	0.12 - 0.17	13.0
0007168	DEAE-2SW, 12.5 nm	4.6	25.0	5	≥ 5,000	0.6 - 0.8	15.0
0007163	DEAE-3SW, 25 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	2.0
<b>TSKgel GUARD COLUMN PRODUCTS</b>							
0017088	DEAE-NPR Guard column	4.6	0.5	5	For P/N 0013075		
0018253	DNA-NPR Guard column	4.6	0.5	2.5	For P/N 0018249		
0018388	SuperQ-5PW Guardgel Kit			20	For P/N 0018257		
0007210	DEAE-5PW Guardgel Kit			20	For P/N 0007164		
0008806	DEAE-5PW Guardgel Kit, Glass			20	For P/Ns 0013061 and 0008802		
0014466	DEAE-5PW Guardcol., Glass 20.0		2.0	13	For P/N 0014016		
0016092	DEAE-5PW Prep Guardgel Kit			20	For P/N 0007574		
0007928	DEAE-5PW Guard column	45.0	5.0	20	For P/N 0007930		
0007648	DEAE-SW Guardgel Kit			10	For P/Ns 0007168 and 0007163		
0019308	Guard cartridge holder	2.0	1.5		For all 2 mm ID guard cartridges		



# TSKgel CATION EXCHANGE COLUMNS

## ORDERING INFORMATION

### ► ORDERING INFORMATION

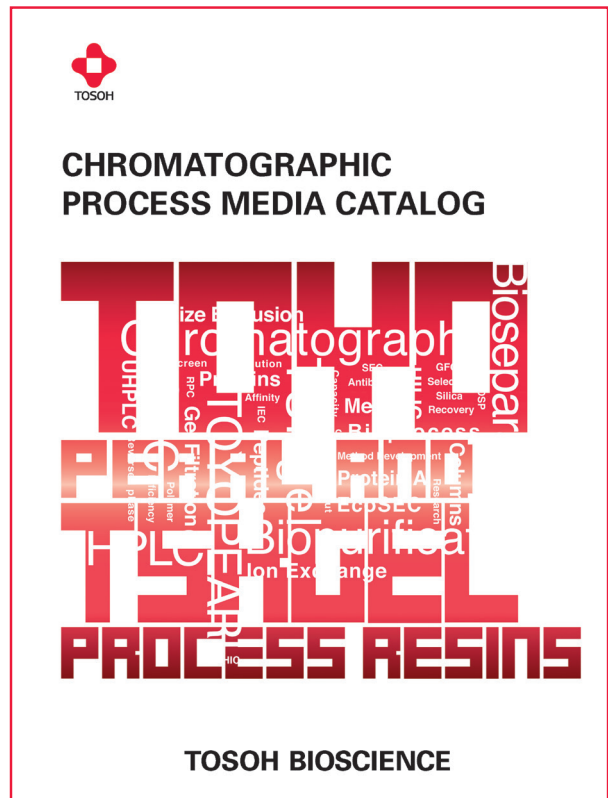
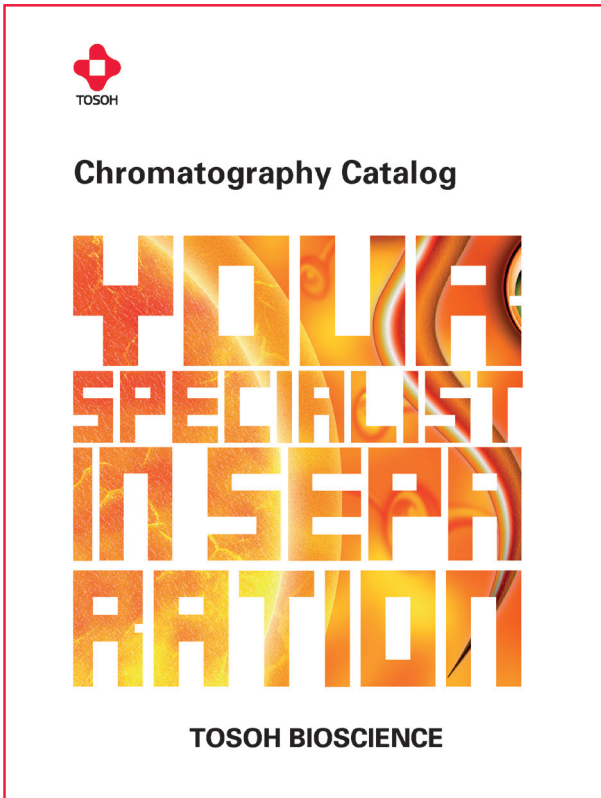
Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min) Range	Maximum pressure drop (MPa)
<b>TSKgel GLASS COLUMNS: POLYMER-BASED</b>							
0014012	CM-5PW Glass, 100 nm	20.0	15.0	13	≥ 2,500	4.0 - 6.0	1.5
0013062	SP-5PW Glass, 100 nm	5.0	5.0	10	≥ 700	0.5 - 0.8	1.5
0008803	SP-5PW Glass, 100 nm	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.0
0014017	SP-5PW Glass, 100 nm	20.0	15.0	13	≥ 3,000	4.0 - 6.0	1.5
<b>TSKgel PEEK COLUMNS: POLYMER-BASED</b>							
0019686	BioAssist S, 130 nm	4.6	5.0	7	≥ 1,500	0.3 - 0.8	2.5
0021411	BioAssist S, 130 nm	10.0	10.0	13	≥ 3,000	1.0 - 5.0	2.5
<b>TSKgel STAINLESS STEEL COLUMNS: POLYMER-BASED</b>							
0021965	CM-STAT, nonporous	3.0	3.5	10	≥ 200	1.0 - 2.0	10.0
0021966	CM-STAT, nonporous	4.6	10.0	7	≥ 2,000	0.5 - 1.0	10.0
0021963	SP-STAT, nonporous	3.0	3.5	10	≥ 200	1.0 - 2.0	10.0
0021964	SP-STAT, nonporous	4.6	10.0	7	≥ 200	0.5 - 1.4	10.0
0013068	CM-5PW, 100 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.5
0018758	SP-5PW, 100 nm	2.0	7.5	10	≥ 1,300	0.05 - 0.10	1.0
0007161	SP-5PW, 100 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.5
0007575	SP-5PW, 100 nm	21.5	15.0	13	≥ 3,000	4.0 - 6.0	2.5
0007934	SP-5PW, 100 nm	55.0	20.0	20	≥ 1,500	20.0 - 40.0	0.4
0013076	SP-NPR, nonporous	4.6	3.5	2.5	≥ 1,300	1.0 - 1.5	20.0
0007156	SCX (Na <sup>+</sup> ), 6 nm	6.0	15.0	5	≥ 2,000	0.5 - 1.0	15.0
0007158	SCX (H <sup>+</sup> ) 6 nm	7.8	30.0	5	≥ 12,000	0.5 - 1.0	5.0
<b>TSKgel STAINLESS STEEL COLUMNS: SILICA-BASED</b>							
0007165	SP-2SW, 12.5 nm	4.6	25.0	5	≥ 5,000	0.6 - 0.8	15.0
0007167	CM-2SW, 12.5 nm	4.6	25.0	5	≥ 5,000	0.6 - 0.8	15.0
0007162	CM-3SW, 25 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	2.0
<b>GUARD COLUMN PRODUCTS</b>							
0013069	CM-5PW Guardgel Kit			10		For P/N 0013068	
0007211	SP-5PW Guardgel Kit			20		For P/N 0007161	
0008807	SP-5PW Guardgel Kit, Glass			20		For P/Ns 0013062 and 0008803	
0016093	SP-5PW Prep Guardgel Kit			20		For P/N 0007575	
0007932	SP-5PW Guard column	45.0	5.0	20		For P/N 0007934	
0007650	CM-SW Guardgel Kit			20		For P/Ns 0007167 and 0007162	

# FURTHER INFORMATION



To get an overview about the whole range of TSKgel columns and small TOYOPEARL and TSKgel bulk media, please request our **Chromatography Catalog**.

For a complete overview of all TOYOPEARL and TSKgel bulk media, refer to our **Chromatographic Process Media Catalog**.



For a deeper insight into applications and questions related to the practical use of TSKgel and TOYOPEARL, check out the website [www.tosohbioscience.de](http://www.tosohbioscience.de)

Our technical experts are happy to discuss your specific separation needs by phone: **+49 (0)6155-70437-36** or mail: [techsupport.tb@tosoh.com](mailto:techsupport.tb@tosoh.com)





**TOSOH**

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