About: TSKgel BioAssist Q Anion Exchange Columns

Especially designed for the separation of large biomolecules, such as antibodies, the large pores of the TSKgel BioAssist Q anion exchange column offer superior capacity and resolution at a modest column back pressure. The anion exchange functionality of BioAssist Q columns is introduced via a special graft polymerization technique that results in a high density of ionic exchange groups in the large particle pores that normally could only be achieved by using particles containing a much smaller pore size.

TSKgel BioAssist Q anion exchange columns are offered in a 4.6 mm ID × 5 cm format and a 10 mm ID × 10 cm semi-preparative column for scale-up. The hardware for both columns is made of PEEK to reduce protein adsorption.

Attributes and Applications:

Table 3 lists the product attributes of TSKgel BioAssist Q columns. The capacity of these columns is high over a wide molecular weight range (up to 1.0 × 10^6 Da) and they are an excellent choice for high throughput applications.

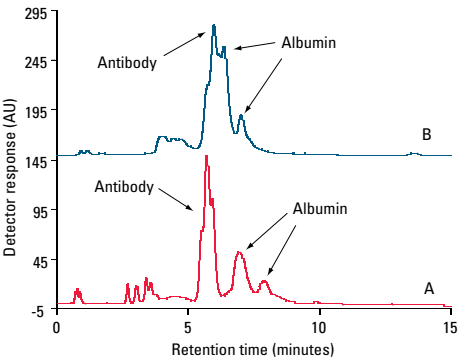
Table 3: Product attributes

Attribute	Value
Matrix	polymethacrylate
Particle size (mean)	10 µm and 13 µm
Pore size (mean)	400 nm
Functional group	polyamine
Counter ion	Cl^-
pH stability	2.0-12.0
Capacity (mg BSA/mL)	70
Small ion capacity	0.1 eq/L
pKa	9.4

Mouse Ascites

Figure 2 compares chromatograms of mouse ascites fluid. Excellent separation between the antibody and albumin has been obtained using a TSKgel BioAssist Q column versus a competitive Q-type product.

Figure 2: Analysis of mouse ascites fluid

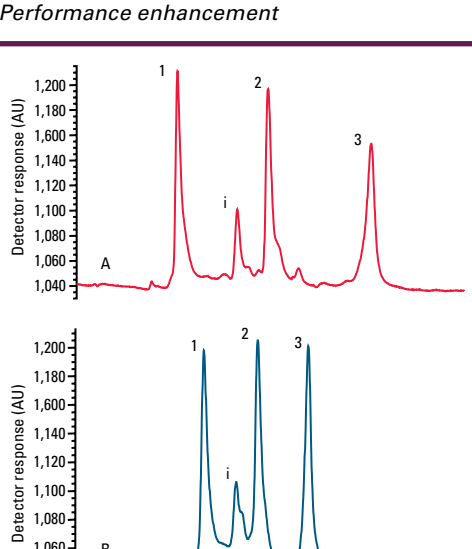


Columns:	**A: TSKgel BioAssist Q, 10 µm, 4.6 mm ID × 5 cm**
	B: Commercial Q-type product A, 5.0 mm ID × 5 cm
Mobile phase:	15 min linear gradient of NaCl from 0 to 1.0 mol/L in 20 mmol/L Tris-HCl buffer, pH 8.0
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	5 µL
Sample:	mouse ascites fluid (3-fold dilution with initial eluent)

Performance Enhancement on FPLC System

Figure 3 demonstrates the performance enhancement of a TSKgel BioAssist Q column over a competitive product when operated side-by-side on an FPLC system.

Figure 3: Performance enhancement



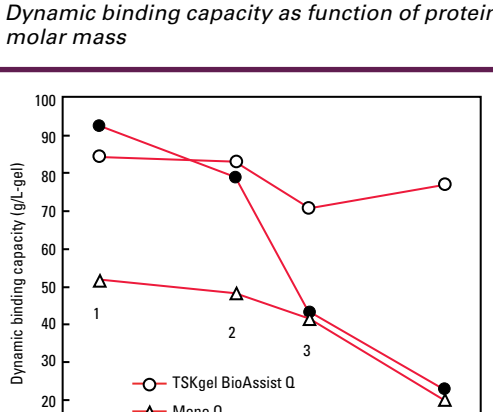
Columns:	**A: TSKgel BioAssist Q, 10 µm, 4.6 mm × 5 cm**
	B: Competitor Q, 5.0 mm ID × 5 cm
Mobile phase:	30 min linear gradient from 0 to 1 mol/L NaCl in 20 mmol/L sodium phosphate, pH 8.0
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Samples:	1. conalbumin
	i. ovalbumin impurity
	2. ovalbumin
	3. trypsin inhibitor

Dynamic Binding Capacity

The dynamic binding capacity for a TSKgel BioAssist Q column and two commercially available columns is shown in Figure 4 as a function of protein molar mass. Dynamic capacity is plotted against the molar mass of 4 proteins varying in molar mass from 2.0×10^4 Da to 6.7×10^5 Da and is determined by continuously loading the column with the protein solution and calculating the amount of protein adsorbed at 10% height of the breakthrough curve.

The binding capacity on TSKgel BioAssist Q is uniformly high for all proteins, while that of Mono Q (80 nm pores) and TSKgel SuperQ-5PW (100 nm pores) is distinctly lower for the larger proteins. It is evident that neither material is optimized for the analysis of monoclonal antibodies, which have a molar mass of 1.5×10^5 Da. Antibodies are blood components and as such are most stable at pH 7.35; they become more labile at acidic pH. Their excess positive charge makes anion exchange chromatography the method of choice for their chromatographic analysis.

Figure 4: Dynamic binding capacity as function of protein molar mass



Columns:	**TSKgel BioAssist Q, 10 µm, 4.6 mm ID × 1 cm**
	Conventional Q-type product A, 5.0 mm ID × 1 cm
	TSKgel SuperQ-5PW, 4.6 mm ID × 1 cm
Mobile phase:	20 mmol/L Tris-HCl buffer, pH 8.0
Flow rate:	0.38 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Samples:	1. trypsin inhibitor, 10 g/L
	2. human serum albumin, 10 g/L
	3. IgG_1, 2.3 g/L
	4. thyroglobulin, 5 g/L

About: TSKgel DEAE-2SW, DEAE-3SW and QAE-2SW Anion Exchange Columns

TSKgel DEAE-2SW, DEAE-3SW, and QAE-2SW columns are packed with porous spherical silica beads which are chemically modified with a weak anion exchange group. These columns are for analyzing smaller molar mass samples such as nucleotides, drug candidates, catecholamines, and small peptides or proteins.

Attributes and Applications:

Table 4 lists the product attributes of the TSKgel DEAE-2SW, DEAE-3SW, and QAE-2SW columns. These columns are packed with particles composed of silica with 12.5 nm and 25 nm pores and are stable in a pH range from 2.0 – 7.5.

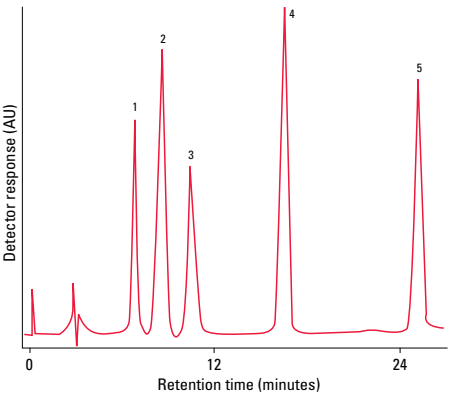
Table 4: Product attributes

TSKgel column	DEAE-2SW	DEAE-3SW	QAE-2SW
Matrix	Silica		
Particle size (mean)	5 µm	10 µm	5 µm
Pore size (mean)	12.5 nm	25 nm	12.5 nm
Functional group	$CH_2CH_2N^+(C_2H_5)_3$	$CH_2CH_2N^+(C_2H_5)_3$	trimethylamino
Counter ion	$H_2PO_4^-$	Cl^-	$H_2PO_4^-$
pH stability	2.0-7.5		
Small ion capacity	>0.3 eq/L		
pKa	11.2		

Nucleotides

High performance analyses of small anionic species are best performed on small pore silica-based anion exchange columns, such as TSKgel DEAE-2SW. This is demonstrated in Figure 5.

Figure 5: Separation of nucleotides

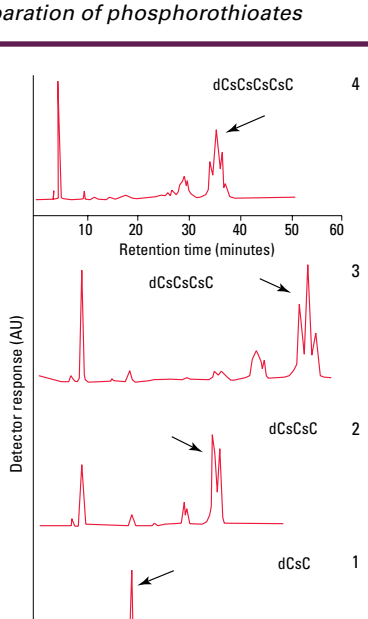


Column:	**TSKgel DEAE-2SW, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase:	A: CH_3CN in 0.1 mol/L phosphate, pH 3.0, 20/80
	B: CH_3CN in 0.5 mol/L phosphate, pH 3.0, 20/80
Gradient:	30 min linear gradient from buffer A to B
Flow rate:	1.0 mL/min
Detection:	UV @ 260 nm
Samples:	1. AMP 2. IMP 3. GMP 4. ADP 5. ATP

Oligonucleotides

Backbone-modified oligonucleotides are increasingly used for antisense therapy. These novel oligos have the benefit of longer half-lives due to resistance to endogenous nucleases. One common type of backbone-modified oligonucleotides is phosphorothioates where one of the two nonbridged oxygen atoms in the phosphate linkage has been replaced by a sulfur atom. The separation of several phosphorothioates on a TSKgel DEAE-2SW column is shown in Figure 6.

Figure 6: Separation of phosphorothioates

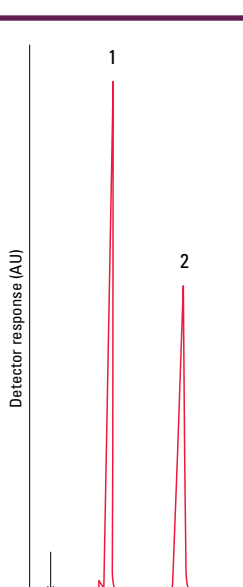


Column:	**TSKgel DEAE-2SW, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase:	A: 50 mmol/L ammonium acetate B: 1.5 mol/L ammonium acetate
Gradient:	linear, 0-100% B in 60 minutes
Flow rate:	1 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. 2 base phosphorothioate oligonucleotide 2. 3 base phosphorothioate oligonucleotide 3. 4 base phosphorothioate oligonucleotide 4. 5 base phosphorothioate oligonucleotide

Deoxyribonucleic Acids

Figure 7 demonstrates the successful separation of adenosine triphosphate (ATP) and deoxyribonucleic acid (DNA) using a TSKgel DEAE-3SW column.

Figure 7: Separation of ATP and DNA



Column:	**TSKgel DEAE-3SW, 10 µm, 6 mm ID × 15 cm**
Mobile phase:	ACN/0.6 mol/L ammonium formate buffer, pH 7.0 = 20/80
Flow rate:	0.7 mL/min
Detection:	UV @ 260 nm
Temperature:	23 °C
Samples:	1. adenosine triphosphate (ATP) 2. deoxyribonucleic acid (d-TCGAGCATAATA), DNA

About: TSKgel DEAE-5PW and SuperQ-5PW Anion Exchange Columns

The polymethacrylate-based resin, TSKgel 5PW, is a spherical 10 µm particle with approximately 100 nm pores. It is derivatized with a diethylaminoethyl (DEAE) functionality to provide the weak anion exchange column, TSKgel DEAE-5PW, and with a polyamine functionality to provide the strong anion exchange column, TSKgel SuperQ-5PW. The polyamine network chemistry employed in TSKgel SuperQ-5PW columns results in a much higher capacity, but also a smaller effective pore size than TSKgel DEAE-5PW columns.

The TSKgel SuperQ-5PW columns are used for the separation and analysis of proteins, oligonucleotides, and other biomolecules. These columns are offered in a stainless steel housing in dimensions of 7.5 mm ID × 7.5 cm and 21.5 mm ID × 15 cm and in an 8 mm ID × 7.5 cm glass format.

TSKgel DEAE-5PW columns are also used for the separation and analysis of proteins, along with nucleotides, nucleosides, and other biomolecules. These columns are available in internal diameters varying from 2 mm to 21.5 mm and in column housings of either glass or stainless steel.

Attributes and Applications:

Table 5 lists the product attributes of TSKgel SuperQ-5PW and DEAE-5PW columns. These columns are an excellent choice for biologically active molecules. TSKgel SuperQ-5PW and DEAE-5PW columns are stable over a pH range from 2.0 – 12.0 and have a mean pore size of 100 nm.

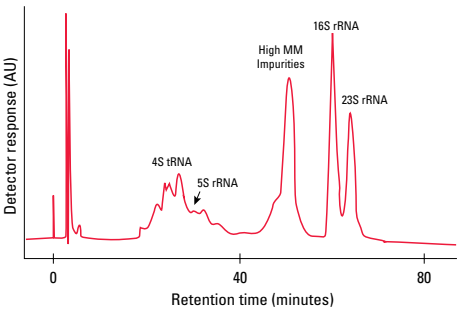
Table 5: Product attributes

TSKgel column	SuperQ-5PW	DEAE-5PW
Matrix	polymethacrylate	
Particle size (mean)	10 µm and 13 µm	10 µm, 13 µm, and 20 µm
Pore size (mean)	100 nm	
Functional group	trimethylamino	$CH_2CH_2N^+(C_2H_5)_3$
Counter ion	Cl^-	
pH stability	2.0-12.0	
Capacity (mg BSA/mL)	100	30
Small ion capacity	>0.13 eq/L	0.1 eq/L
pKa	12.2	11.5

E. coli RNA

Figure 8 shows the fractionation of high molar mass *E. coli* RNA on TSKgel DEAE-5PW, effectively utilizing the large 100 nm pores of the TSKgel 5PW resin.

Figure 8: Analysis of high MM RNA

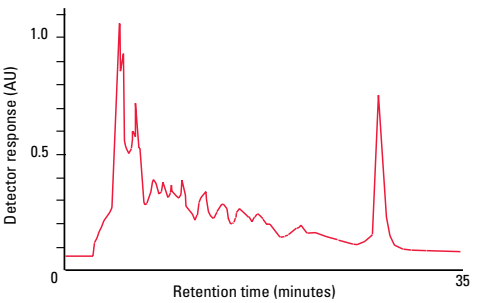


Column:	**TSKgel DEAE-5PW, 10 µm, 6 mm ID × 15 cm (custom)**
Mobile phase:	300 min linear gradient from 0.3 mol/L to 1.0 mol/L NaCl in 0.1 mol/L Tris-HCl, pH 7.6
Flow rate:	1.0 mL/min
Detection:	UV @ 260 nm
Sample:	total *E. coli* RNA

Plasmid

Figure 9 illustrates the separation of crude pBR322 plasmid on a TSKgel DEAE-5PW column. This chromatographic separation provides purified plasmid in one hour, as opposed to a conventional Cs-Cl density gradient ultracentrifugation, which can take up to three days.

Figure 9: Detection of HIV-1 PCR-Amplified 130 bp target

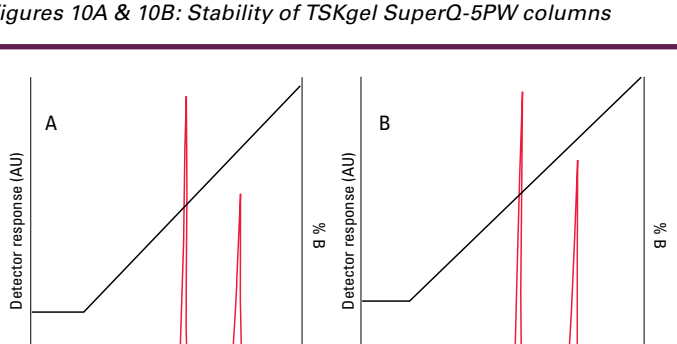


Column:	**TSKgel DEAE-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	A: 25 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0 B: 25 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0, with 1 mol/L NaCl
Gradient:	25-60%B in 30 minutes
Flow rate:	1 mL/min
Detection:	UV @ 260 nm
Temperature:	25 °C
Sample:	pBR322 plasmid
Sample load:	2.5 mg in 1 mL

Column Stability

Figures 10A & 10B demonstrate the stability of the TSKgel SuperQ-5PW columns. Ovalbumin and trypsin inhibitor were initially loaded onto a TSKgel SuperQ-5PW, 7.5 mm ID × 7.5 cm column (Figure 10A). The column was then cleaned in place (CIP) using a solution of 0.5 mol/L NaOH. This cleaning procedure was repeated once each day for a total of 15 days. The resolution after this cleaning protocol was equivalent to the resolution of the initial injection of the compounds on the column (Figure 10B).

Figures 10A & 10B: Stability of TSKgel SuperQ-5PW columns

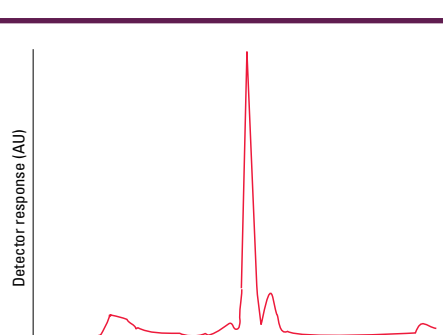


Column:	**TSKgel SuperQ-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	A: 50 mmol/L Tris-HCl, pH 8.6
	B: 0.5 mmol/L sodium cloride in 50 mmol/L Tris-HCl, pH 8.6
Gradient:	A-B (60min)
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	100 µL
Sample load:	each of 1 mg
Samples:	1. ovalbumin
	2. trypsin inhibitor
Note:	A: before CIP
	B: after 15 times (15 days)

Oligonucleotides

Figure 11 shows the analysis of a 16-mer morpholine oligonucleotide on a TSKgel SuperQ-5PW column using a NaCl gradient in a 10 mmol/L sodium hydroxide mobile phase.

Figure 11: Analysis of 16-mer oligonucleotide

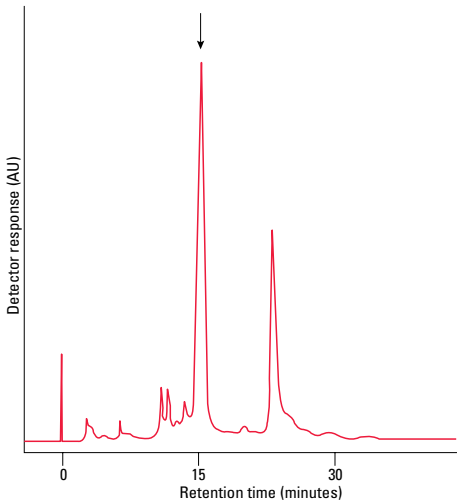


Column:	**TSKgel SuperQ-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	A: 10 mmol/L NaOH
	B: 10 mmol/L NaOH with 1 mol/L NaCl
Gradient	0 min (0% B) 40 min (50% B)
	41 min (100%B) 46 min (100%B)
Flow Rate:	1 mL/min
Detection:	UV @ 254 nm
Sample:	16-mer morpholine oligonucleotide, AAG AAG AAG AGG GGA G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

Monoclonal Antibody

The separation of a monoclonal antibody (IgG_1) from mouse ascites fluid using a TSKgel DEAE-5PW column is shown in Figure 12. IgG_1 elutes in about 15 minutes, well separated from the impurities that elute before and after IgG_1, such as transferrin (about 11 minutes) and albumin (about 22 minutes).

Figure 12: Separation of monoclonal antibody

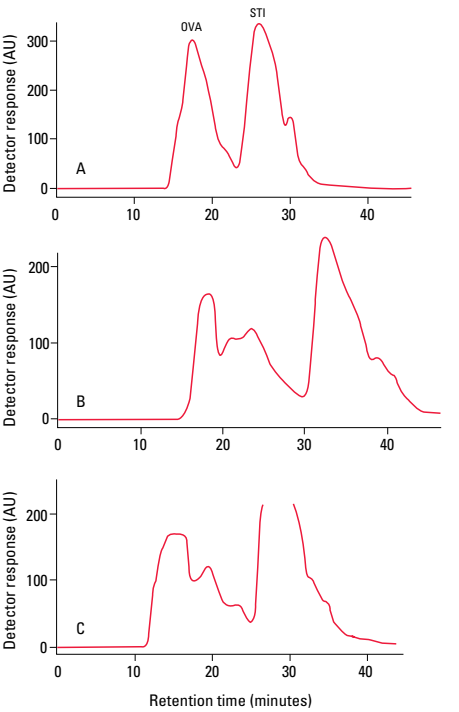


Column:	**TSKgel DEAE-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	A: 20 mmol/L Tris-HCl, pH 8.5 B: A + 0.5 mol/L NaCl A → B linear gradient (60 min)
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Sample:	anti-human albumin (IgG_1), diluted solution of mouse ascites (168 µg in 40 µL)

Performance Data

In Figure 13, a separation performed under high sample load conditions was compared on various anion exchange columns. When a 20 mg protein sample is loaded on a 1 mL column volume, only the TSKgel SuperQ-5PW column shows a chromatogram with "normal" looking peaks. Other anion exchange columns show multiple artifact peaks from sample overloading. A 5 mm ID × 5 cm TSKgel SuperQ-5PW column provides sufficient retention and resolution. Thus, isolation of proteins at semi-preparative scale is possible on TSKgel SuperQ-5PW when using an analytical column.

Figure 13: Comparison of various anion exchange columns under large sample load



Columns:	**A: TSKgel SuperQ-5PW, 10 µm, 5 mm ID × 5 cm** B: Company A, Q type, 5 mm ID × 5 cm C: Company A, perfusion Q type, 6.4 mm ID × 3 cm All column volumes were 1.0 mL
Mobile phase:	A: 50 mmol/L Tris-HCl buffer, pH 8.3 B: A + 0.5 mol/L NaCl A → B linear gradient (60 min)
Flow rate:	0.8 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	2 mL
Samples:	ovalbumin, 20 mg trypsin inhibitor, 20 mg

About: TSKgel DEAE-NPR and DNA-NPR Anion Exchange Columns

Methacrylate is the backbone of nonporous resin (NPR) columns such as TSKgel DEAE-NPR and DNA-NPR, which 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. Due to the absence of pores, protein recovery is generally very high on TSKgel DEAE-NPR and DNA-NPR columns.

TSKgel DNA-NPR columns are packed with hydrophilic polymer beads which are surface modified with a weak anion exchange group. Because TSKgel DNA-NPR columns contain nonporous particles, binding capacity is low compared to porous columns with the same ligand functionality. Column dimensions are optimized for the high efficiency separation of DNA fragments, PCR products, or plasmids.

The hydrophilic polymer beads used to pack the TSKgel DEAE-NPR columns are also surface modified with a weak anion exchange group. These columns are used for the high speed separation and analysis of proteins and poly- and oligonucleotides. TSKgel DEAE-NPR columns are particularly useful for high resolution separation of DNA digests or fragments.

Attributes and Applications:

Table 6 lists the product attributes of TSKgel DNA-NPR and DEAE-NPR columns. These columns are stable in a pH range from 2.0 – 12.0 and are packed with spherical 2.5 µm, nonporous particles.

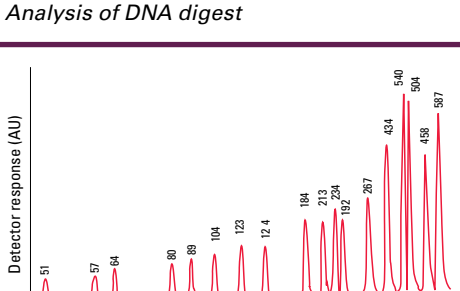
Table 6: Product attributes

TSKgel column	DEAE-NPR	DNA-NPR
Matrix	polymethacrylate	
Particle size (mean)	2.5 µm	
Pore size (mean)	nonporous	
Functional group	$CH_2CH_2N^+(C_2H_5)_3$	proprietary
Counter ion	Cl^-	ClO_4^-
pH stability	2.0-12.0	
Capacity (mg BSA/mL)	5	
Small ion capacity	>0.1 eq/L	
pKa	11.2	

DNA Digests

Because of their small (2.5 µm) particle size, TSKgel DEAE-NPR nonporous columns excel in rapid separations of large polynucleotides in DNA digests. A chromatogram of a standard Hae III digest of pBR322 plasmid DNA is shown in Figure 14.

Figure 14: Analysis of DNA digest

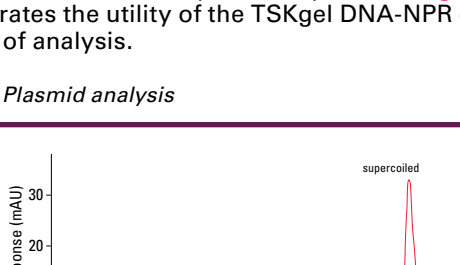


Column:	**TSKgel DEAE-NPR, 2.5 µm, 4. 6 mm ID × 3.5 cm, with guard column, 4.6 mm ID × 0.5 cm**
Mobile phase:	A: 0.02 mol/L Tris-HCl, pH 9.0 B: mobile phase A plus 1.0 mol/L NaCl
Gradient:	15 min linear gradient from 48% to 65% mobile phase B
Flow rate:	1.5 mL/min
Detection:	UV @ 260 nm
Pressure:	14 MPa
Temperature:	40 °C
Sample:	Hae III digest of pBR322 DNA, (base pair number for each peak is indicated)

Plasmid

One of the purity checks used for plasmids in gene therapy assays is the measure of the relative amount of open circular plasmid versus supercoiled plasmid. Figure 15 demonstrates the utility of the TSKgel DNA-NPR column for this type of analysis.

Figure 15: Plasmid analysis



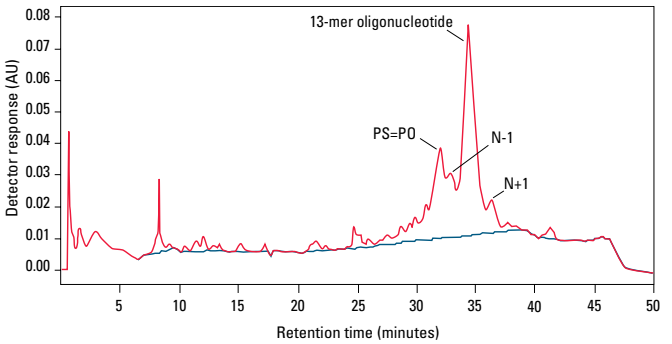
Column:	**TSKgel DNA-NPR, 2.5 µm, 4. 6 mm ID × 7.5 cm**
Mobile phase:	A. 20 mmol/L Tris, pH 9.0 B. 20 mmol/L Tris, pH 9.0 with 1 mol/L NaCl
Gradient:	linear gradient from 50% to 65%B in 10 column volumes
Flow rate:	1 mL/min
Detection:	UV @ 260 nm
Sample:	PUC19 plasmid

Oligonucleotides

Figure 16 contains the chromatographic trace of the crude deprotected 13-mer oligonucleotide using a TSKgel DNA-NPR column. The early eluting peaks from 0–5 minute exhibit a lambda max range of 220–230 nm, indicating the presence of protecting groups used in the synthesis. The N-1 peak as confirmed by mass spectrometry elutes just before the main substance peak. The PS=PO peak elutes before N-1. Structurally, the N-1 analog is completely thioated but is missing one nucleotide. As a result, the N-1 compound is more thioated and hydrophobic than the PS=PO analog. The backside peak is an N+1 impurity verified by mass spectrometry.

The method conditions are designed to optimize resolution of all impurity peaks and inhibit any aggregation, secondary structure formation, and PS=PO conversion. Specifically, sodium bromide acts as the eluting agent and diethylamine provides the buffering capacity while contributing mild chaotropic effects. The step gradient is designed to remove all the protecting groups from the column before elution of the impurity analogs.

Figure 16: Oligonucleotide analysis

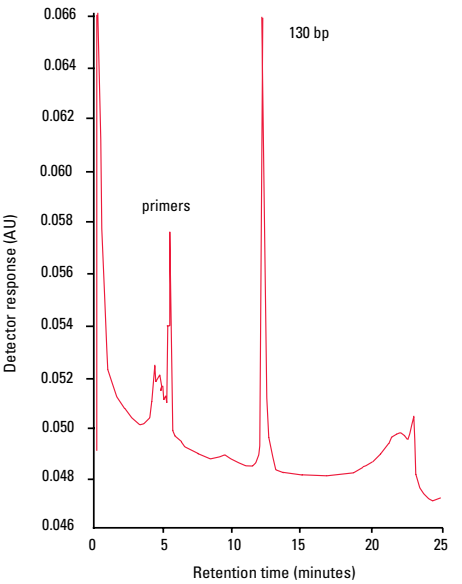


Column:	**TSKgel DNA-NPR, 2.5 µm, 4.6 mm ID × 7.5 cm**
Mobile phase:	A: 10 mmol/L sodium bromide, 20 mmol/L NaOH, pH 12, 1% diethylamine
	B: 1 mol/L sodium bromide, 20 mmol/L NaOH, pH 12, 1% diethylamine
Gradient	3.5 min (20%B) 12 min (20%B) 45 min (55%B)
Flow rate:	1.0 mL/min
Temperatures:	60 °C (column), 4 °C (sample chamber)
Sample:	crude deprotected 13-mer oligonucleotide

HIV-1 PCR-amplified 130 bp Target

Figure 17 shows the detection of a 130 bp target derived from HIV using a nonporous TSKgel DEAE-NPR column.

Figure 17: Detection of HIV-1 PCR-Amplified 130 bp target



Column:	**TSKgel DEAE-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase:	A: 20 mmol/L Tris-HCl with 0.25 mol/L NaCl, pH 7.7
	B: 20 mmol/L Tris-HCl with 1 mol/L NaCl, pH 7.7
Flow rate:	1 mL/min
Detection:	UV @ 260 nm
Temperature:	ambient
Sample:	HIV-1 PCR-amplified 130 bp target
Sample load:	20 µL

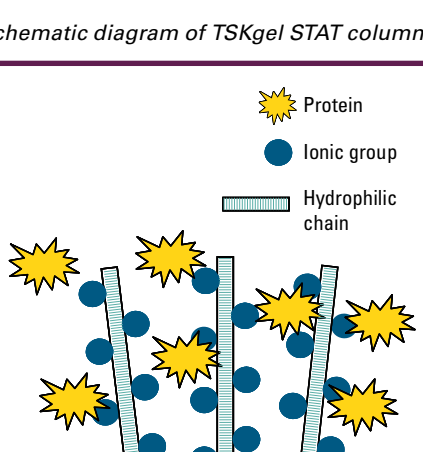
About: TSKgel Q-STAT and DNA-STAT Anion Exchange Columns

TSKgel Q-STAT and DNA-STAT columns are packed with hydrophilic nonporous resin particles of which the surface consists of an open access network of multi-layered anion exchange groups (see Figure 18). The innovative bonding chemistry, combined with a relatively large particle size of these nonporous columns, results in a respectable loading capacity and a low operating pressure.

TSKgel Q-STAT columns are packed with 7 or 10 µm nonporous particles. Applications for these columns include the separation of proteins, peptides, low molar mass nucleic acids, aggregates and charge isomers of monoclonal antibodies, PEGylated proteins, oligo DNA, and siRNA.

Applications for the 5 µm TSKgel DNA-STAT columns include the separation of DNA fragments, nucleic acids and nucleotides.

Figure 18: Schematic diagram of TSKgel STAT columns



Attributes and Applications:

Table 7 lists the product attributes of the TSKgel Q-STAT and DNA-STAT columns. These columns are an excellent choice for high resolution protein and DNA separations. TSKgel Q-STAT and DNA-STAT nonporous columns are supplied in stainless steel (SS) housing with SS fittings and PEEK frits and are stable in a pH range from 3.0 – 10.0.

Table 7: Product attributes

TSKgel column	Q-STAT	DNA-STAT
Matrix	hydrophilic polymer	
Particle size (mean)	7 µm and 10 µm	5 µm
Pore size (mean)	nonporous	
Functional group	quaternary ammonium	
Counter ion	Cl^-	
pH stability	3.0-10.0	
Static binding capacity (mg BSA/g dry gel)	ca. 25 (7 µm) ca. 20 (10 µm)	ca. 35 (5 µm)
Small ion capacity	270 µeq/g dry gel	
pKa	10.5	

Binding Capacities

Table 8 illustrates that despite the fact that surface area decreases with increasing particle size, the larger TSKgel Q-STAT and TSKgel DNA-STAT particles have higher binding capacities than the smaller particles used in TSKgel NPR columns. The novel bonding chemistry used in the preparation of the TSKgel STAT resin resulted in a dramatic increase in static binding capacity, more than compensating for the lower external surface area of the larger particles.

Table 8: Binding capacities of TSKgel STAT anion exchange columns

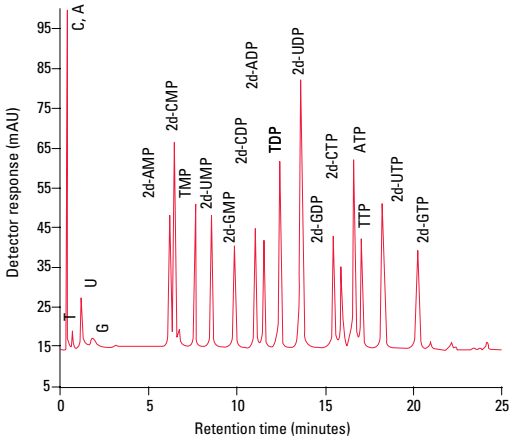
Property	TSKgel NPR column	TSKgel DNA-STAT	TSKgel Q-STAT	
Particle size	2.5 µm	5 µm	7 µm	10 µm
Capacity*	9.1	38.6	27.0	20.9

*Static binding capacity in mg BSA/mg dry gel.

DNA Fragments

Mono-, di-, and tri-nucleotides were separated with excellent peak shape on a TSKgel DNA-STAT column packed with 5 µm particles. The narrow, symmetrical peaks, as shown in Figure 19, demonstrate the absence of micro-pores on this new generation of nonporous resin columns. TSKgel DNA-STAT columns are, as the name implies, first choice for large nucleic acid fragments.

Figure 19: Separation of large DNA fragments

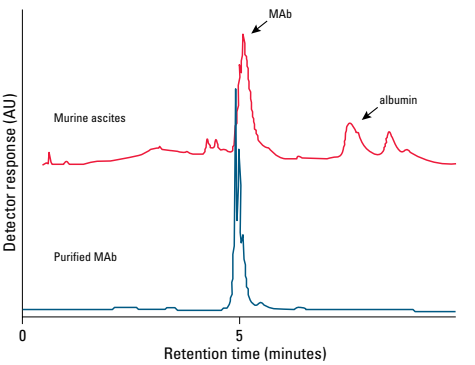


Column:	**TSKgel DNA-STAT, 5 µm, 4.6 mm ID × 10 cm**
Mobile phase:	A: 20 mmol/L Tris-HCl, pH 8.5
	B: 0.75 mol/L NaCl in buffer A
Gradient:	0 min (50% B) 25 min (75% B)
Flow rate:	0.8 mL/min
Detection:	UV @ 260 nm

Mouse Ascites Fluid

Figure 20 shows the separation of mouse ascites fluid containing a monoclonal antibody (top) and a partially purified monoclonal antibody (bottom) on a TSKgel Q-STAT column. The top chromatogram clearly shows that the antibody and albumin components are well separated. The bottom chromatogram shows that multiple peaks are present in the partially purified monoclonal sample.

Figure 20: Separation of mouse ascites fluid containing monoclonal antibodies and purified monoclonal antibodies

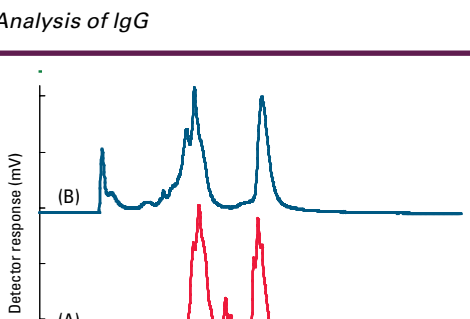


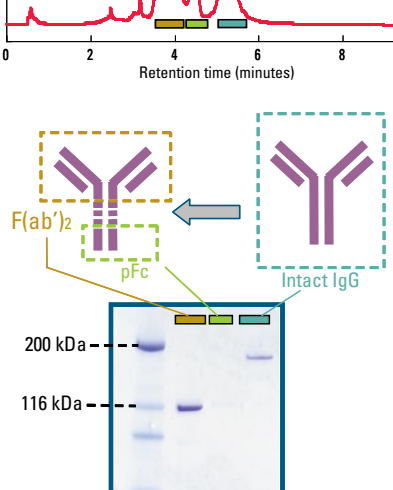
Column:	**TSKgel Q-STAT, 7 µm, 4.6 mm ID × 10 cm**
Mobile phase:	A:20 mmol/L Tris-HCl buffer, pH 8.5
	B:0.5 mol/L NaCl in 20 mmol/L Tris-HCl buffer, pH 8.5
Gradient:	A → B linear gradient (10 min)
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	Top: 1/10 dilution of mouse ascites containing mAb
	Bottom: purified mouse mAb
	Sample diluted 10-fold with eluent A

Immunoglobulin G (IgG)

Immunoglobulin G (IgG) is a monomeric immunoglobulin, built of two heavy chains and two light chains. Each IgG has two antigen binding sites. It is the most abundant immunoglobulin and is approximately equally distributed in blood and in tissue liquids, constituting 75% of serum immunoglobulins in humans. IgG was digested using pepsin and separated on a TSKgel Q-STAT column and a competitive nonporous WAX-10 column. As shown in Figure 21, three peaks were isolated from the TSKgel Q-STAT column and assigned as F(ab')2, pFc, and intact IgG by SDS-PAGE. No correlation could be established between the peaks obtained on the WAX-10 column and SDS-PAGE results.

Figure 21: Analysis of IgG





Non-reduced SDS - PAGE

Columns:	**A: TSKgel Q-STAT, 7 µm, 4.6 mm ID × 10 cm**
	B: ProPac® WAX-10, 10 µm, 4 mm ID × 25 cm
Mobile phase:	A: 20 mmol/L Tris-HCl, pH 8.5
	B: 0.5 mol/L NaCl in buffer A
Gradient:	0 min (0% B) 10 min (100% B)
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Sample:	pepsin digested mAb

About: TSKgel Sugar AXG, Sugar AXI and SAX Anion Exchange Columns

TSKgel Sugar AXG and Sugar AXI columns are specialty columns for the analysis of mono- and disaccharides, as well as sugar alcohols. Both columns are packed with porous spherical polymer beads which are surface modified with a strong anion exchange group.

The TSKgel Sugar AXG column contains 10 µm particles for the gradient separation and analysis of monosaccharides, disaccharides, and sugar alcohols, whereas the TSKgel Sugar AXI column is packed with 8 µm particles for the isocratic separation of carbohydrates where lower and constant back pressures may be generated.

TSKgel SAX columns are packed with 5 µm porous spherical polymer beads which are surface modified with a strong anion exchange group. They are used for the separation of isomerized sugars, alcohols, and low molar mass organic acids.

Attributes and Applications:

Table 9 lists the product attributes of the TSKgel Sugar AXG, Sugar AXI, and SAX columns. These columns are packed with silica particles and are stable in a pH range from 1.0 – 14.0.

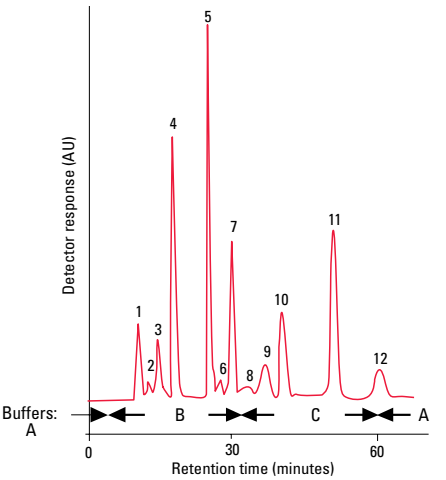
Table 9: Product attributes

TSKgel column	Sugar AXG	Sugar AXI	SAX
Matrix	PS-DVB polymer		
Particle size (mean)	10 µm	8 µm	5 µm
Pore size (mean)	6 nm		
Functional group	trimethylamino		
Counter ion	HBO_3^-	HBO_3^-	Cl^-
pH stability	1.0-14.0		
Small ion capacity	>1.2 eq/L	>1.2 eq/L	>1.0 eq/L
pKa	12.5		

Saccharide Mixture

Saccharides are retained on TSKgel Sugar AX columns following the formation of negatively charged complexes with boric acid at alkaline pH. Figure 22 shows the separation of twelve mono- and disaccharides using a TSKgel Sugar AXG column.

Figure 22: Separation of saccharide mixture

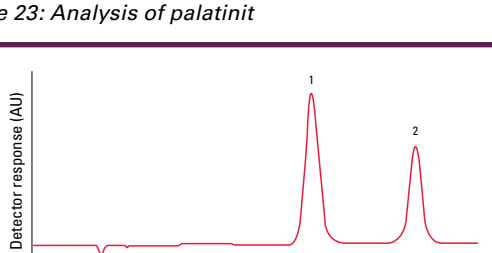


Column:	**TSKgel Sugar AXG, 10 µm, 4. 6 mm ID × 15 cm**
Mobile phase:	step gradient: 6 min buffer A, 0.6 mol/L boric acid, pH 7.7; then 27 min buffer B, 0.7 mol/L boric acid, pH 7.25; then 30 min buffer C, 0.7 mol/L boric acid, pH 8.7
Flow rate:	0.4 mL/min (column and post column reagent solution)
Detection:	fluorescence; Ex: 331 nm, Em: 383 nm
Pressure:	16 kg/cm²
Temperature:	70 °C (column), 100 °C (post column reactor)
PC reagent:	2.5% 2-cyanoacetamide solution
Samples:	disaccharides, 25 nm; monosaccharides, 50 nm: 1. cellobiose 2. maltose 3. lactose 4. rhamnose 5. lyxose 6. ribose 7. mannose 8. fructose 9. arabinose 10. galactose 11. xylose 12. glucose

Sugar Alcohol

Palatinit is a sugar alcohol used as a low-calorie and anti-decay food additive. It can be obtained by reducing palatinose and is composed of two isomers, 6-O-alpha-D-Glucopyranosyl-D-glucitol and 1-O-alpha-D-glucopyranosyl-D-mannitol. As shown in Figure 23, a TSKgel Sugar AXG column can separate the isomers.

Figure 23: Analysis of palatinit

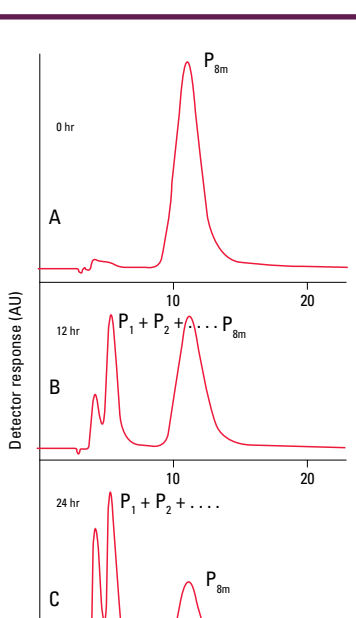


Column:	**TSKgel Sugar AXG, 10 µm, 4.6 mm ID × 15 cm**
Mobile phase:	0.7 mol/L borate buffer, pH 8.6
Flow rate:	0.8 mL/min
Detection:	RI
Temperature:	65 °C
Injection vol.:	10 µL
Samples:	1. alpha-D-glucopyranosyl-1,6-soribitol (GPS) 2. alpha-D-glucopyranosyl-1,6-mannitol (GPM)

Polyphosphates

The stability of the TSKgel SAX column allows a wide pH range for separations of polyphosphates. Figure 24 shows the monitoring of cyclooctaphosphate hydrolysis products over the course of 24 hours with a pH 10.2 mobile phase.

Figure 24: Hydrolysis products of cyclooctaphosphate



Column:	**TSKgel SAX, 5 µm, 4 mm ID × 25 cm**
Mobile phase:	0.4 mol/L KCl, 0.1% EDTA, pH 10.2
Sample:	cyclooctaphosphate hydrolysis products A. 0 hours B. 12 hours C. 24 hours