



HYDROPHOBIC INTERACTION CHROMATOGRAPHY

HIC
HYDROPHOBIC
INTERACTION
CHROMATO
GRAPHY

TOSOH BIOSCIENCE

HIC

HYDROPHOBIC INTERACTION

CHROMATOGRAPHY

HIC PRODUCTS

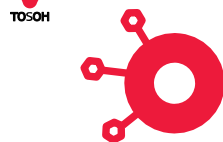
- TSKgel Ether-5PW
- TSKgel Phenyl-5PW
- TSKgel Butyl-NPR

≡ TOSOH FACT

Tosoh Bioscience provides solutions for today's biological purification needs. In fact, some of the first commercial HIC products were manufactured by Tosoh. We take pride in our ability to design new products based on existing chemistries to solve specific customer applications.

We encourage you to have a confidential discussion with us about your specific needs. Whether it is a surface modification of an existing product or the creation of a new one, we encourage you to call on us to meet your needs for a customized solution.





INTRODUCTION TO TSKgel HIC COLUMNS

Hydrophobic Interaction Chromatography (HIC) is based on the interaction between hydrophobic groups on a protein and a hydrophobic ligand on the solid support. HIC offers a distinct advantage for easily denatured proteins; it can be run using moderate concentrations of ammonium sulfate, which favors the stability of many proteins.

The binding of proteins to a hydrophobic matrix is affected by a number of factors including (1) the type of ligand, (2) the ligand density on the solid support, (3) the backbone material of the matrix, (4) the hydrophobic nature of the protein, and (5) the type of salt used. All of these factors help to make HIC a powerful technique for the separation of biomolecules.

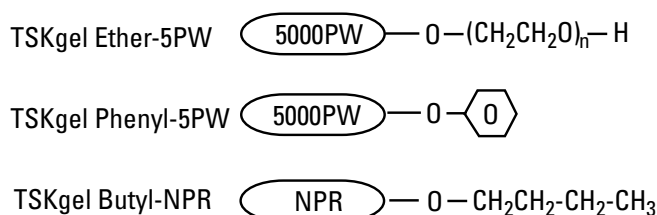
Tosoh Bioscience offers three different HIC column types in analytical format: TSKgel Phenyl-5PW, Ether-5PW and Butyl NPR. TSKgel Phenyl-5PW is also available in preparative column formats. See **FIGURE 1** for the structure of the HIC resins.

COLUMN SELECTION

TSKgel HIC stationary phases are polymethacrylate-based with a choice of three ligands with varied hydrophobicities from low to high. TSKgel Ether-5PW and Phenyl-5PW are based on a porous base matrix with 100 nm pores and available with various particle sizes depending on column dimensions, while TSKgel Butyl-NPR is based on a 2.5 µm nonporous base particle. Nonporous resins (NPR) are typically used for high-speed analytical applications.

TSKgel **ETHER-5PW** is less hydrophobic than TSKgel Phenyl-5PW. It displays weaker interaction and thus shorter retention times compared to Phenyl-5PW.

FIGURE 1
Structure of TSKgel HIC resins



FEATURES

- Choice of three hydrophobic ligands (ether, phenyl or butyl)
- Rigid polymeric base resin
- Similar chemistry to TOYOPEARL resins
- TSKgel Phenyl-5PW offered in PEEK hardware
- Ether and Phenyl available in 2 mm ID format

TSKgel Ether-5PW is the best choice for the separation of very hydrophobic proteins such as membrane proteins or monoclonal antibodies.

The **TSKgel PHENYL-5PW** columns were the first commercially available, polymer-based columns for high performance HIC. These columns have been instrumental to the increase in popularity of this technique for analytical, preparative, and process scale separations of biopolymers.

TSKgel **BUTYL-NPR** is the least hydrophobic among the three TSKgel HIC columns and requires a higher salt concentration for binding. TSKgel Butyl-NPR columns provide fast and quantitative HIC, because smaller particles provide higher efficiency. By packing the 2.5 µm nonporous resin particles into shorter columns, typical analysis times are reduced to less than 10 minutes. Pore diffusion is often the rate-limiting step in the overall mass transport of large biomolecules through a porous column. Eliminating the pores provides higher resolution at higher flow rates. Another benefit of NPR resins is excellent mass recovery, allowing quantitation down to nanogram levels. These properties make TSKgel Butyl-NPR the preferred choice for process monitoring and quality control. TSKgel Butyl-NPR is getting increasingly popular for the analysis of antibody drug conjugates (ADCs) and is available in two dimensions: 3.5 cm length for high throughput and 10 cm length for high resolution.

TSKgel HIC columns are compatible with water soluble organic solvents at concentration below 50 % (20 % for Butyl-NPR).

TABLE I
Column selection for the TSKgel HIC columns

Sample	MW range (Da)	TSKgel Column
Peptides	< 10,000	Butyl-NPR
Medium to large proteins	> 10,000	Phenyl-5PW Ether-5PW Butyl-NPR
DNA, RNA, and PCR products	> 500,000	Phenyl-5PW Butyl-NPR
Oligonucleotides	> 10,000	Phenyl-5PW Butyl-NPR

BENEFITS

- Added flexibility during method development
- Wide pH range (2-12) enabling robust cleaning options
- Seamless scalability from analytical to preparative scale
- Eliminates undesirable interactions with column hardware
- LC-MS applications

HIC

COMPARISON OF SELECTIVITY

FIGURE 2 compares the separation of standard proteins on the Ether, Phenyl, and Butyl supports under similar operating conditions.

SAMPLE CAPACITY

One definition of sample capacity is the amount of pure compound injected onto the column at which the peak width is 10% larger than the peak width under low loading conditions. Using this definition, the capacity of a 7.5 mm ID x 7.5 cm L TSKgel Phenyl-5PW column varies from 0.1 to 1 mg of protein. Resolution and peak width are dependent on sample loading, as shown in **FIGURE 3**. Therefore, sample loading should be kept within 0.1 - 0.5 mg in order to obtain the highest resolution.

Separations on TSKgel Ether-5PW columns usually take 30 - 60 minutes. 0.5 mg of pure protein can be purified from a 5 - 10 mg crude protein mixture using a 7.5 mm ID x 7.5 cm L column.

Since almost all of the surface area of a porous particle is inside the pores, the capacity of the 4.6 mm ID x 3.5 cm L TSKgel Butyl-NPR column is significantly less than that for the 7.5 mm ID x 7.5 cm L Phenyl-5PW column. Capacities for the Butyl-NPR column are 100 µg for crude sample and 2 µg for pure sample.

CHEMICAL STABILITY

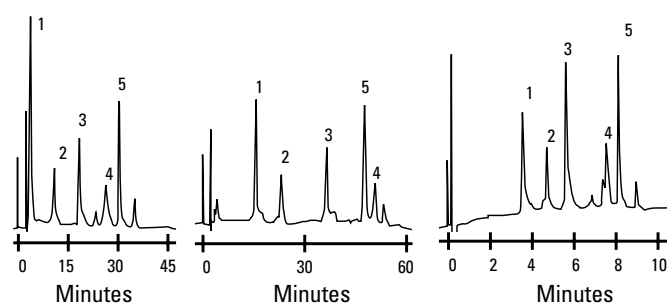
TSKgel 5PW-type HIC columns are physically and chemically stable in water soluble organic solvents (at < 50% methanol, ethanol, ACN, DMF, DMSO or < 30 % chloroform). Change the solvent gradually by reducing the flow rate (preferably with a gradient) because rapid change may cause degradation of column efficiency. Note: When changing to an organic solvent, reduce the salt concentration to prevent precipitation of the salt on the column. Also, chaotropic agents (urea, SDS, etc.) will reduce the adsorption of biomolecules; therefore, use low levels of these agents (<2 mol/L).

Polymer-based columns are stable when cleaning at alkaline pH. All TSKgel HIC columns can be routinely operated from pH 2-12. **TABLE II** shows that the phenyl groups on the TSKgel Phenyl-5PW are stable for more than 10 days upon exposure to 0.5 mol/L NaOH or 0.5 mol/L acetic acid.

TABLE II
Long-term exposure of TSKgel Phenyl-5PW to acid and base

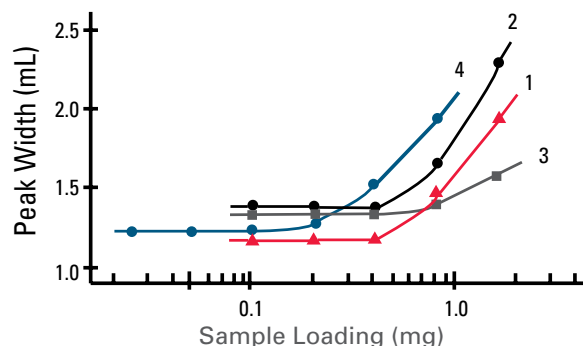
Acid/base	Phenyl content (mmol/mL - resin)	
	Before exposure	After 10 days exposure
0.5 mol/L CH ₃ COOH	0.105	0.106
0.5 mol/L NaOH	0.105	0.104

FIGURE 2
Comparing HIC columns



Column: TSKgel Ether-5PW & TSKgel Phenyl-5PW, 7.5 mm ID x 7.5 cm L; TSKgel Butyl-NPR, 4.6 mm ID x 3.5 cm L; Sample: 1. myoglobin, 2. ribonuclease A, 3. lysozyme, 4. α -chymotrypsin, 5. α -chymotrypsinogen; Injection: 5PW-type columns: 100 µL (50-100 µg), NPR-type column: 20 µL (1.5-40 µg); Elution: 60 min linear gradient from 1.8 mol/L to 0 mol/L (NH₄)₂SO₄ in 0.1 mol/L phosphate buffer, pH 7.0, for 5PW-type columns; 12 min linear gradient from 2.3 mol/L to 0 mol/L (NH₄)₂SO₄ in 0.1 mol/L phosphate buffer, pH 7.0 for TSKgel Butyl-NPR; Flow rate: 1.0 mL/min; Detection: UV @ 280 nm

FIGURE 3
Dependence of peak width on sample loading in the separation of proteins



Column: TSKgel Phenyl-5PW, 7.5 mm ID x 7.5 cm L; Sample: 1. myoglobin; 2. ribonuclease A; 3. ovalbumin; 4. α -chymotrypsin; concentration: 0.025 % to 1.6 %; Elution: 60 min linear gradient of (NH₄)₂SO₄ from 1.5 mol/L to 0 mol/L in 0.1 mol/L phosphate buffer (pH 7.0); Flow rate: 0.5 mL/min; Temperature: 25 °C; Detection: UV @ 280 nm

APPLICATIONS - TSKgel HIC COLUMNS

ANTIBODY FRAGMENTS

FIGURE 4 shows the separation of Fab and Fc fragments of an antibody on TSKgel Butyl-NPR. The appearance of additional Fc fragments is due to the oxidation of methionine residues by 0.10% t-butylhydroperoxide (tBHP). The numbers above the Fc peaks correspond to the number of oxidized residues in each fragment.

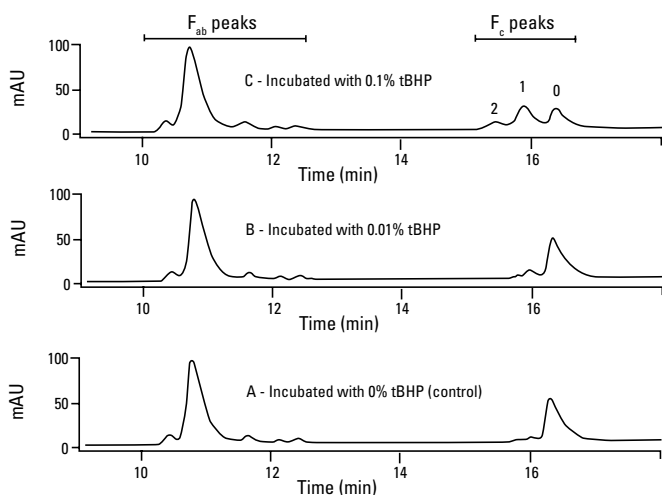
ANTIBODY AGGREGATES

The use of a short TSKgel Butyl-NPR column for the separation of a monoclonal antibody and its high molecular weight aggregates is shown in FIGURE 5. The total aggregate content of this sample is about 11 %, which was also confirmed by SEC on TSKgel G3000SWxl (5 micron, 7.8 x 300 mm) the current industrial standard for mAb aggregate analysis. Because of the high efficiency of the nonporous particles of TSKgel Butyl-NPR only low sample amounts are needed for aggregate analysis.

ANTIBODY DRUG CONJUGATES (ADCs)

ADCs are becoming an increasingly important class of therapeutic agents for treatment of cancer. One of the most important quality attributes of an ADC is the drug to antibody ratio (DAR), the average number of drugs that are conjugated. This determines the amount of "payload" that can be delivered to the tumor cell. * Aditya Wakankar and others described the analysis of an ADC on TSKgel Butyl NPR that yielded five predominant peaks that corresponding to mAb containing zero, two, four, six and eight drugs.

FIGURE 4
Separation of Fab and Fc fragments on TSKgel Butyl-NPR



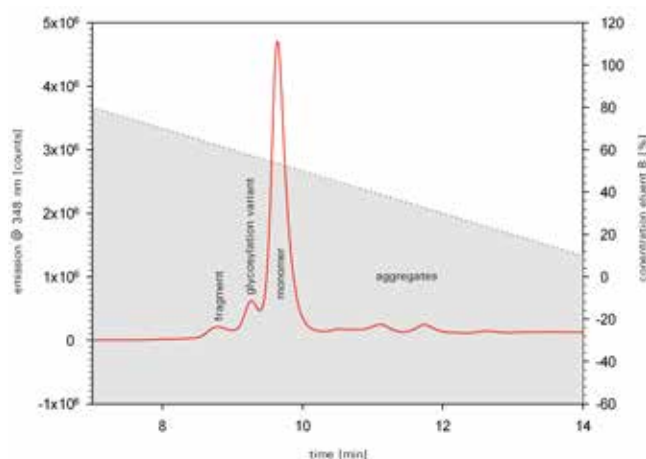
Column: TSKgel Butyl-NPR, 4.6 mm ID x 3.5 cm L; Elution: Buffer A: 2 mol/L $(\text{NH}_4)_2\text{SO}_4$, 20 mmol/L Tris, pH 7, Buffer B: 20 mmol/L Tris, pH 7; Gradient: linear from 10 % B to 100 % B in 34 minutes; Flow rate: 1 mL/min; Temperature: 30°C

TSKgel ETHER-5PW

ANTIBIOTICS

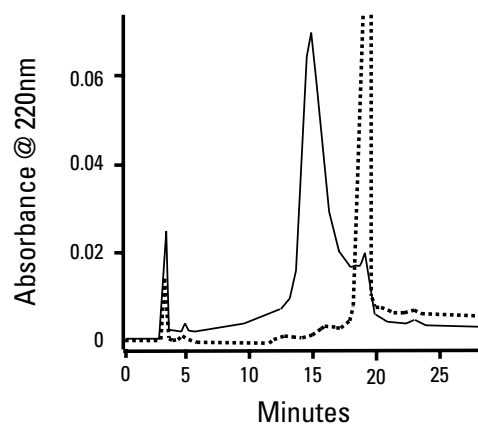
The TSKgel Ether-5PW column was used to determine the relative purity of the antibiotic components C-1027 and C-1027-AG (FIGURE 6). Antibiotic C-1027 is composed of a protein consisting of many hydrophobic and hydroxyamino acids with a non-protein chromophore. Antibiotic C-1027-AG is composed of the hydrophobic and hydroxyamino acids without the chromophore.

FIGURE 5
Analysis of monoclonal antibody and aggregates using a TSKgel Butyl-NPR column



Column: TSKgel Butyl-NPR, 2.5 μm , 4.6 mm ID x 3.5 cm L, Mobile phase: A: 3 mol/L NaCl, B: H_2O , Gradient: 0-100%B in 10 min Flow rate: 1.0 mL/min, Detection: fluorescence, Ex: 280 nm, Em: 348 nm Injection vol.: 5 μg , Sample: IgG₁

FIGURE 6
Purification of anti-tumor antibiotic



Column: TSKgel Ether-5PW, 7.5 mm ID x 7.5 cm L; Sample: C-1027, C-1027-AG concentration: 1 mg/mL; Injection: 20 μL ; Elution: linear gradient from 1.5 mol/L to 0 mol/L $(\text{NH}_4)_2\text{SO}_4$ in 0.1 mol/L phosphate buffer, pH 7.0; Flow rate: 0.8 mL/min; Detection: UV @ 220 nm

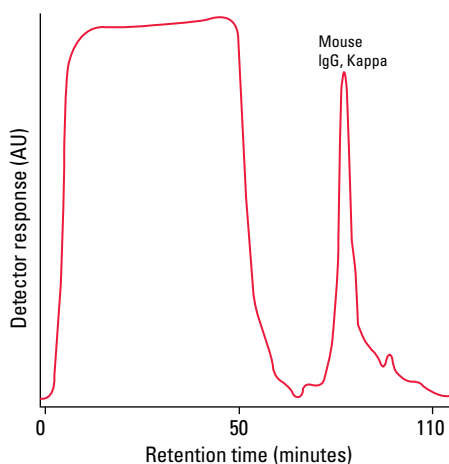
* Aditya Wakankar et al. 'Analytical methods for physicochemical characterization of antibody drug conjugates', mAbs 3:2, pages 161-172; March/April 2011.

APPLICATIONS - TSKgel HIC COLUMNS

MONOCLONAL ANTIBODIES

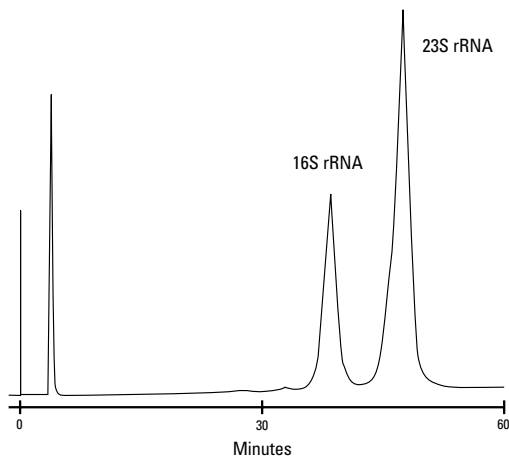
Monoclonal antibodies (mAbs) play a part in many research, diagnostic, and therapeutic applications. Monoclonal antibodies are generally the most hydrophobic proteins in ascites fluid and cell culture supernatant.

FIGURE 7
Monoclonal antibody purification



Column: TSKgel Ether-5PW, 10 μ m, 8.0 mm ID \times 7.5 cm, glass
Mobile phase: 67.5 min isocratic load and wash with 1 mol/L $(\text{NH}_4)_2\text{SO}_4$ in 1 mol/L glycine, 0.5 mol/L phosphate buffer, pH 7.0, followed by a 37.5 min linear gradient from 1.0 mol/L to 0 mol/L $(\text{NH}_4)_2\text{SO}_4$ in 1.0 mol/L glycine, 0.05 mol/L phosphate, pH 7.0; Flow rate: 1.0 mL/min; Detection: UV @ 280 nm, 3.0 AUFS; Injection vol.: 50 mL; Sample: 25 mL raw cell culture supernatant, 200 mg total protein, 15 mg total antibody diluted to 50 mL with initial elution buffer

FIGURE 8
Retain large RNAs on TSKgel Phenyl-5PW



Column: TSKgel Phenyl-5PW, 7.5 mm ID \times 7.5 cm L;
Sample: 16S and 23S rRNA from *E. coli*, 0.05 mg in 0.1 mL; Elution: 0 min linear gradient from 2 mol/L to 0 mol/L $(\text{NH}_4)_2\text{SO}_4$ in 0.1 mol/L phosphate buffer, pH 7.0; Flow rate: 60.5 mL/min; Detection: UV @ 280 nm

FIGURE 7 shows typical results from the screening of two mAbs using a TSKgel Ether-5PW column.

TSKgel PHENYL-5PW

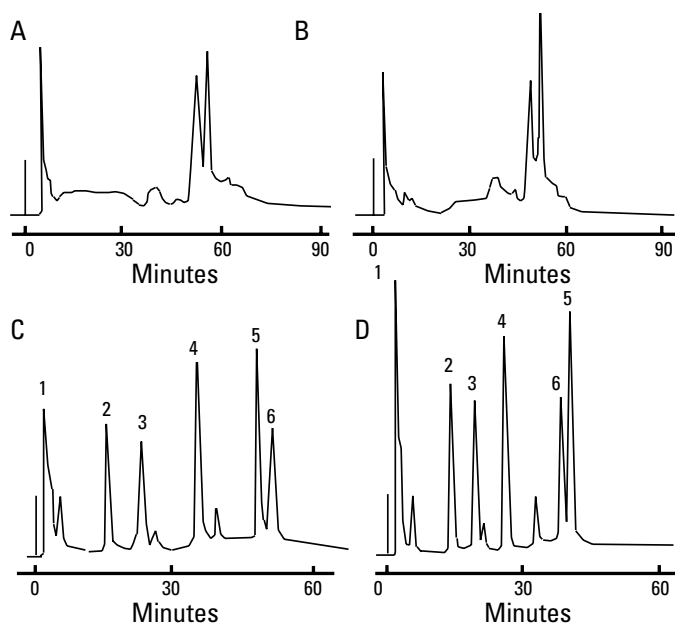
RNAs

FIGURE 8 illustrates the separation of 16S and 23S ribosomal RNA on a TSKgel Phenyl-5PW column. The approximate molecular weights of these RNAs are 560,000 and 1,100,000 Da, respectively.

MODULATION OF SELECTIVITY

The addition of organic solvents or chaotropic agents in the final buffer can improve separations. However, relative elution positions may change. Therefore, add chaotropic agent and organic solvent in small quantities. See **FIGURE 9** for the effect of chaotropic agents and organic solvents on the HIC separation of two different samples.

FIGURE 9
Effect of urea and isopropanol on the separation of commercial lipoxidase and a standard protein mixture



Column: TSKgel Phenyl-5PW, 7.5 mm ID \times 7.5 cm L;
Sample: A & B: commercial lipoxidase, C & D: protein mixture: 1. cytochrome C, 2. myoglobin, 3. ribonuclease A, 4. lysozyme, 5. α -chymotrypsinogen, 6. α -chymotrypsin; Elution: A: 60 min linear gradient from 0.1 mol/L phosphate buffer containing 1.5 mol/L $(\text{NH}_4)_2\text{SO}_4$ (pH 7.0) to 0.1 mol/L phosphate buffer (pH 7.0), B: 60 min linear gradient from 0.1 mol/L phosphate buffer containing 1.5 mol/L $(\text{NH}_4)_2\text{SO}_4$ (pH 7.0) to 0.1 mol/L phosphate buffer containing 2 mol/L urea (pH 7.0), C: 60 min linear gradient from 0.1 mol/L phosphate buffer containing 1.8 mol/L $(\text{NH}_4)_2\text{SO}_4$ (pH 7.0) to 0.1 mol/L phosphate buffer (pH 7.0), D: 60 min linear gradient from 0.1 mol/L phosphate buffer containing 1.8 mol/L $(\text{NH}_4)_2\text{SO}_4$ (pH 7.0) to 0.1 mol/L phosphate buffer (pH 7.0) containing 7% isopropanol; Flow rate: A & B: 0.5 mL/min; C & D: 1.0 mL/min; Temp.: 25°C; Detection: UV @ 280 nm



► ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min) range	Maximum pressure drop (MPa)
TSKgel Glass columns							
0014013	Ether-5PW Glass, 100 nm	5.0	5.0	10.0	≥ 600	0.5 - 0.8	2.0
0014014	Ether-5PW Glass, 100 nm	8.0	7.5	10.0	≥ 1,000	0.5 - 1.0	2.0
0013063	Phenyl-5PW Glass, 100 nm	5.0	5.0	10.0	≥ 600	0.5 - 0.8	2.0
0008804	Phenyl-5PW Glass, 100 nm	8.0	7.5	10.0	≥ 1,000	0.5 - 1.0	2.0

TSKgel Stainless Steel Columns

0018760	Ether-5PW, 100 nm	2.0	7.5	10.0	≥ 1,000	0.05 - 0.1	0.6
0008641	Ether-5PW, 100 nm	7.5	7.5	10.0	≥ 1,000	0.5 - 1.0	2.0
0018759	Phenyl-5PW, 100 nm	2.0	7.5	10.0	≥ 1,000	0.05 - 0.1	0.8
0007573	Phenyl-5PW, 100 nm	7.5	7.5	10.0	≥ 1,000	0.5 - 1.0	2.0
0007656	Phenyl-5PW, 100 nm	21.5	15.0	13.0	≥ 3,000	4.0 - 6.0	2.0
0014947	Butyl-NPR, nonporous	4.6	3.5	2.5		0.5 - 1.0	20.0
0042168	Butyl-NPR, nonporous	4.6	10.0	2.5	> 4,000	0.5 - 1.0	20.0

TSKgel PEEK columns

0020023	BioAssist Phenyl, 100 nm	7.8	5	10.0	≥ 1,000	0.5 - 1.0	2.0
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Guard column products

	ID (mm)	Length (cm)	Particle size (µm)	
0014025	Ether-5PW Guardgel Kit, Glass		20.0	For P/Ns 0014013 and 0014014
0008643	Ether-5PW Guardgel Kit		20.0	For P/N 0008641
0007652	Phenyl-5PW Guardgel Kit		20.0	For P/N 0007573
0016095	Phenyl-5PW Prep Guardgel Kit		20.0	For P/N 0007656

HIC





TSKgel® Butyl-NPR

INTRODUCTION

Hydrophobic Interaction chromatography (HIC) is based on the interaction between hydrophobic patches on the protein surface and a hydrophobic ligand on the solid support. HIC sorts biomolecules by degree of their surface hydrophobicity. Samples are adsorbed to the stationary phase at relatively high salt concentrations and eluted by applying a decreasing salt gradient. The mild conditions used in HIC separation of peptides and proteins typically maintain protein structure and biologic activity.

HIGHLIGHTS

- Small, non-porous 2.5 µm micron particles
- Dimensions optimized for high speed or high resolution analysis, respectively
- Hydrophilic polymer base matrix enables high recovery of proteins and trace analysis
- High chemical stability and wide pH range (pH 2 – pH 10)

FEATURES

Non-porous resins (NPR) are typically used for high-speed analytical applications. The base material of TSKgel Butyl-NPR consists of spherical 2.5 µm non-porous polymethacrylate particles. TSKgel Butyl-NPR columns provide fast and quantitative HIC, because smaller particles provide higher efficiency. By packing the 2.5 µm non-porous resin particles into short columns, typical analysis times are reduced to less than 10 minutes. TSKgel Butyl-NPR columns are available in 3.5 cm length for fast analysis and 10 cm length for high resolution.

Pore diffusion is often the rate-limiting step in the overall mass transport of large biomolecules through a porous stationary phase. Eliminating the pores provides higher resolution at higher flow rates.

Another benefit of NPR media is excellent mass recovery, allowing quantitation down to nanogram levels. Table 1 shows the recovery of some standard proteins with TSKgel Butyl-NPR. These properties make TSKgel Butyl-NPR the preferred choice for process monitoring and quality control.

RECOVERY OF PROTEINS

Proteins	Recovery (%)
Myoglobin	96
Ribonuclease	90
Lysozyme	102
Alpha-Chymotrypsin	95
Alpha-Chymotrypsinogen A	98
Trypsin inhibitor	83
Ovalbumin	92

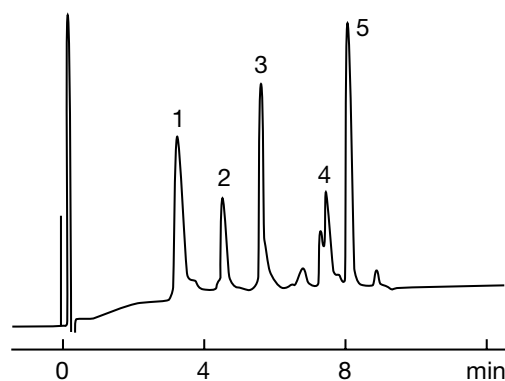
➤ **Table 1**

Column: TSKgel Butyl-NPR (4.6 mm ID x 3.5 cm);
 Mobile phase: A: 0.1 mol/L phosphate buffer (pH 7.0) + 2.3 mol/L ammonium sulfate, B: 0.1 mol/L phosphate buffer (pH 7.0); Gradient: 0 - 100% mobile phase B, 12 min, linear; Flow rate: 1.5 mL/min;
 Detection: UV @ 280 nm; Sample load: 5 µg

APPLICATIONS

TSKgel Butyl-NPR can be applied in a broad range of applications ranging from the separation of the open circular and supercoiled forms of plasmid DNA to the analysis of modified or aggregated peptides and proteins. Figure 1 shows the separation of five standard proteins on a short TSKgel Butyl-NPR column within ten minutes.

FAST SEPARATION OF PROTEINS



➤ **Figure 1**

Column: TSKgel Butyl-NPR (4.6 mm ID x 3.5 cm);
 Mobile phase: A: 0.1 mol/L phosphate buffer (pH 7.0) + 2.3 mol/L ammonium sulfate; B: 0.1 mol/L phosphate buffer (pH 7.0); Gradient: 0 - 100% mobile phase B, 12 min, linear; Flow rate: 1.0 mL/min; Detection: UV @ 280 nm; Samples: 1. myoglobin (4 µg) 2. ribonuclease (4 µg) 3. lysozyme (1.5 µg) 4. α-chymotrypsin (3 µg) 5. α-chymotrypsinogen A (2 µg)

ANALYSIS OF PROTEIN OXIDATION

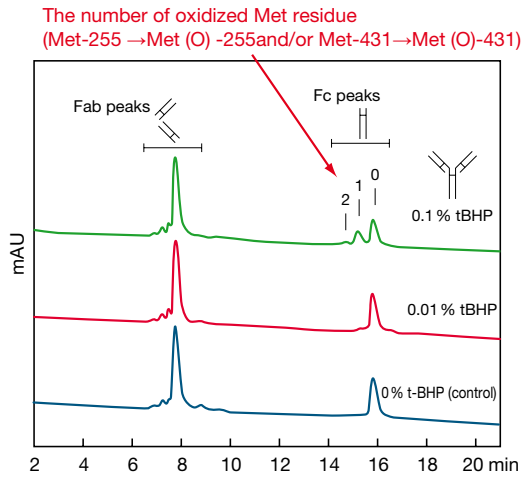


Figure 2

Column: TSKgel Butyl-NPR (4.6 mm ID × 10 cm)
 Mobile phase A: 20 mmol/L phosphate buffer + 2 mol/L ammonium sulfate (pH 7.0); B: 20 mmol/L phosphate buffer (pH 7.0); Gradient: 25 to 60 % mobile phase B, 20 min, linear; Flow rate: 1.0 mL/min;
 Detection: UV @ 215 nm; Temp.: 35 °C; Inj. volume: 2 µL;
 Sample conc.: 2 g/L; Sample: enzymatic digest of antibody therapeutics;
 Oxidation: 0.01 % / 0.1 % tert-butylhydroperoxide (tBHP) was added to antibody solution. After digestion by papain aliquots of reacted solution were subjected to HPLC.

Figure 2 shows the analysis of oxidation variants of monoclonal antibodies. The antibody was cleaved by papain and subjected to HPLC analysis. The number of oxidized methionine residues can be easily detected.

Figure 3 shows the analysis of structural differences of a therapeutic antibody. Charge variants were fractionated by cation-exchange chromatography (CEC), cleaved with papain and fragments were analyzed by HIC. The main CEC peak (Peak 2) represents the main form of the antibody without any modification. Deamidation of the light chain asparagine 30 to aspartate in one light chain is responsible for an acidic form (Peak 1). The variant collected in Peak 3 is based on isomerization of one heavy chain aspartate 102.

Figure 4 shows the application of the short TSKgel Butyl-NPR column for the separation of an aggregated mAb sample. As aggregates are more hydrophobic than the corresponding mAb monomers, aggregates, monomers and fragments can easily be identified. The total aggregate content of this sample is about 11 %, which was also confirmed by SEC on TSKgel G3000SW_{XL}, the current industrial standard for mAb aggregate analysis.

ANALYSIS OF PROTEIN DEAMIDATION

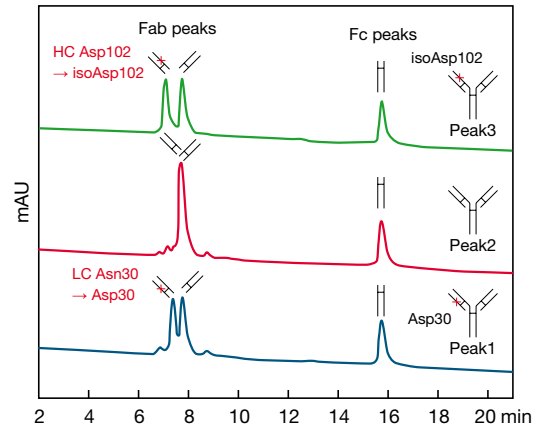


Figure 3

Column: TSKgel Butyl-NPR (4.6 mm ID × 10cm)
 Mobile phase A: 20 mmol/L phosphate buffer + 2 mol/L ammonium sulfate (pH 7.0); B: 20 mmol/L phosphate buffer (pH 7.0)
 Gradient: 25 to 60 % mobile phase B, 20 min, linear; Inj. volume: 2 µL
 Flow rate: 1.0 mL/min; Detection: UV @ 214 nm; Temp.: 35°C;
 Sample conc.: 2 g/L Sample: enzymatic digest of therapeutic antibody
 The antibody was fractionated by cation-exchange chromatography (TSKgel BioAssist® S). Each fraction was digested by papain and aliquots were subjected to HPLC.

SEPARATION OF mAb AGGREGATES AND FRAGMENTS

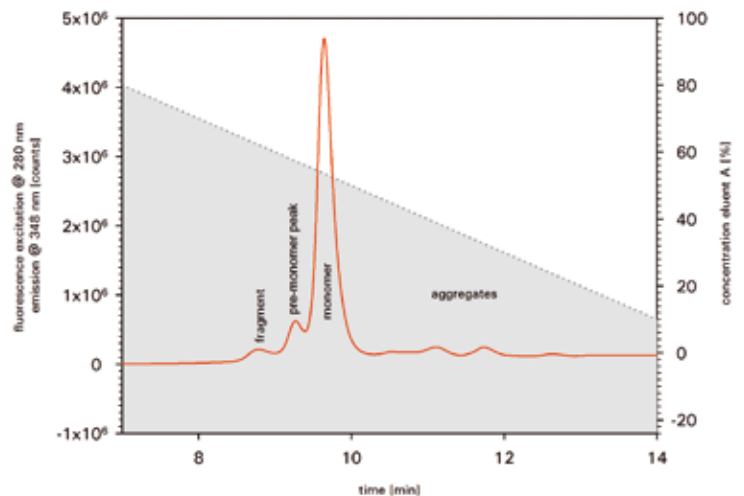


Figure 4

Column: TSKgel Butyl-NPR (4.6 mm ID × 3.5 cm)
 Mobile phase A: 10 mmol/L sodium phosphate buffer (pH 7.0) + 3 mol/L NaCl; B: 10 mmol/L sodium phosphate buffer (pH 7.0)
 Gradient: 0 - 100 % mobile phase B, 25 min, linear; Flow rate: 1.0 mL/min
 Detection: Fluorescence; Sample: aggregated monoclonal antibody

Ordering information

Part-No	Description	Particle Size (µm)	Dimensions
14947	TSKgel Butyl-NPR	2.5	4.6 mm ID x 3.5 cm L
42168	TSKgel Butyl-NPR	2.5	4.6 mm ID x 10.0 cm L