

Hydrophobic Interaction Chromatography

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Hydrophobic Interaction Chromatography

In HIC, a weakly non-polar stationary phase is used with an aqueous mobile phase containing a high concentration of a chaotropic salt. The technique is mainly applied to the separation of proteins, which are eluted by gradually reducing the salt concentration.

As in reversed phase LC, proteins are retained by interaction with alkyl or aryl functional groups on the packing material. Unlike RPLC, in HIC the density of these functional groups is low and protein molecules are adsorbed on only one or a few sites.

Sorption takes place at high salt concentration, and desorption is accomplished by decreasing the salt concentration or by adding a low percentage of organic solvent.

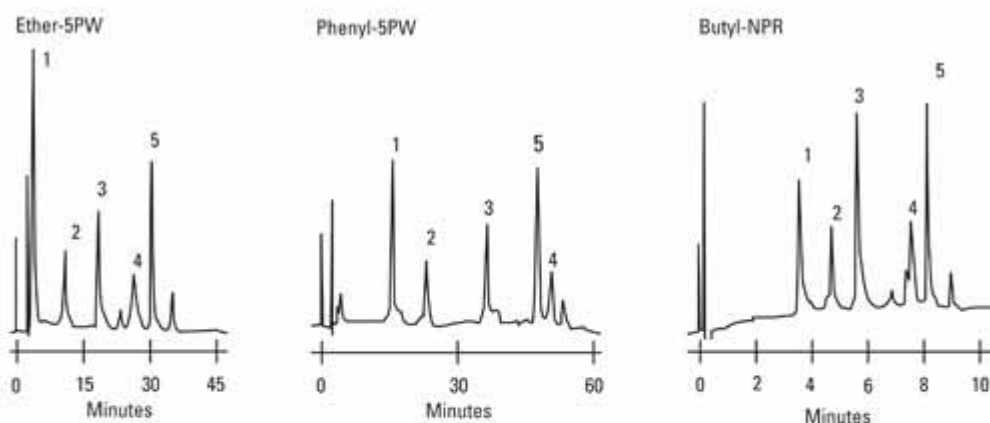
Although also based on hydrophobic interactions, selectivity in HIC separations is distinctly different from that in reversed phase LC.

Despite the lower peak capacity in HIC compared to RPC, HIC has the advantage that the mobile phase conditions (primarily aqueous) do not usually disrupt higher-order protein structures.

TSK-GEL HIC packings consist of polymethacrylate base material and a choice of three ligands (butyl, ether and phenyl) with varied hydrophobicities from low to high, respectively.

The polymethacrylate base resin for the ether and phenyl columns is a porous material with a 1000Å pore size and 1,000,000 Da exclusion limit, offering high capacity and high sample loads. The TSKgel Butyl-NPR features a non-porous support in which only the surface of the bead is available for adsorption. Shorter run times and excellent recovery result, making the butyl columns an optimal choice for high throughput or QC analysis. See the figure below for a comparison between the three column types.

Comparing conventional and nonporous HIC columns



Column: TSKgel Ether-5PW & TSKgel Phenyl-5PW, 7.5mm x 7.5cm
TSKgel Butyl-NPR, 4.6mm x 3.5cm

Sample: 1. myoglobin, 2. ribonuclease A, 3. lysozyme, 4. α -chymotrypsin, 5. α -chymotrypsinogen

Injection: 5PW columns: 100 μ L (50-100 μ g); NPR: 20 μ L (1.5-40 μ g)

Elution: 60min linear gradient from 1.8M to 0M $(\text{NH}_4)_2\text{SO}_4$ in 0.1M phosphate buffer, pH 7.0 for 5PW columns; 12min linear gradient from 2.3M to 0M $(\text{NH}_4)_2\text{SO}_4$ in 0.1M phosphate buffer, pH 7.0 for TSKgel Butyl-NPR

Flow Rate: 1.0mL/min

Detection: UV @ 280nm

Hydrophobic Interaction Chromatography

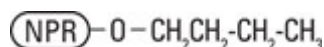
	<i>Features</i>	<i>Benefits</i>
<i>TSKgel Phenyl-5PW</i>	Most hydrophobic	Requires modest salt concentration to retain proteins. Most popular column applicable for the widest range of hydrophobicities
<i>TSKgel Ether-5PW</i>	Less hydrophobic	Excellent choice for hydrophobic proteins such as membrane proteins or monoclonal antibodies.
<i>TSKgel Butyl-NPR</i>	Least hydrophobic	Excellent choice for high speed applications Usually high recovery due to absence of pores.

Hydrophobic Interaction Chromatography

TSKgel Butyl-NPR

The least hydrophobic of the HIC column offerings, **TSKgel Butyl-NPR** is the best choice for high speed separations with excellent recovery even for more hydrophobicity samples. The non-porous resin requires lower sample loading and leads to faster analysis time because the binding kinetics occur only on the bead's surface. The ultra efficient 2.5 micron bead allows for fast, high efficiency separations using a 3.5cm x 4.6mm ID column format. Thus, TSKgel Butyl-NPR is ideal for time critical QC analysis or sample limited applications. Featuring polymethacrylate base material, the TSKgel Butyl-NPR column is stable in either acid or caustic cleaning regimes up to 1N.

Structure:

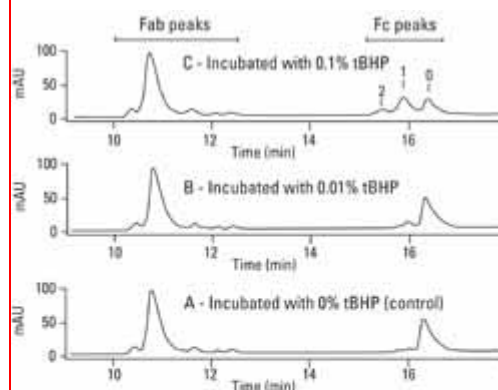


Product Attributes:

Pore size (mean):	Non-porous
Particle size (mean):	2.5µm
pH stability:	2.0 - 12.0
Functional group:	Butyl

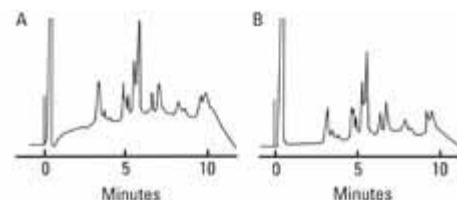
Applications

Separation of Fab and Fc fragments on TSKgel Butyl NPR



Column: TSKgel Butyl-NPR, 4.6mm ID x 3.5cm
 Elution: Buffer A: 2M (NH₄)₂SO₄, 20mM Tris, pH7
 Buffer B: 20mM Tris, pH7
 Gradient: linear from 10%B to 100%B in 34 minutes
 Flow rate: 1mL/min
 Temperature: 30°C

Effect of sample load on the separation of phosphoglucose isomerase



Column: TSKgel Butyl-NPR, 4.6mm x 3.5cm
 Sample: crude sample of phosphoglucose isomerase
 Loads: A. 25µg; B. 100µg
 Elution: 10min linear gradient of (NH₄)₂SO₄ from 1.8M to 0M in 0.1M phosphate buffer, pH7.0
 Flow Rate: 1.0mL/min
 Temperature: 25°C
 Detection: UV @ 280nm

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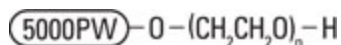
P/N	Description	Particle size	Housing material	ID (mm)	Length (cm)	Price (\$)
14947	TSKgel Butyl-NPR	2.5 μ m	Stainless Steel	4.6	3.5	908
42168	TSKgel Butyl-NPR	2.5 μ m	Stainless Steel	4.6	10	1,134

Hydrophobic Interaction Chromatography

TSKgel Ether-5PW

Of the three TSK-GEL HIC columns, **TSKgel Ether-5PW** has intermediate hydrophobicity. It is an excellent choice for separating hydrophobic molecules such as membrane proteins or monoclonal antibodies such as IgG or IgM. Featuring a 1000Å pore size in a polymethacrylate base material, the Ether-5PW column is stable in either acid or caustic cleaning regimens and provides excellent access to larger molecules with low diffusion coefficients.

Structure:

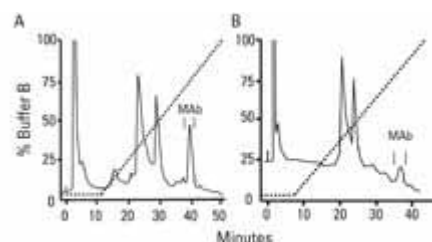


Product Attributes:

Pore size (mean):	1,000Å
Particle size (mean):	10, 13 and 20µm
pH stability:	2.0 - 12.0
Functional group:	Ether

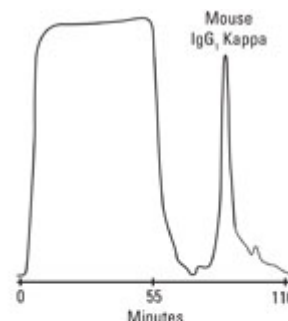
Applications

Screening of mouse monoclonal antibodies



Column: TSKgel Ether-5PW, 8.0mm x 7.5cm, glass
Sample: A. 20µL unequilibrated mouse IgG_{2b} ascites
B. 20µL unequilibrated mouse IgM_k ascites
Elution: linear gradient from buffers A to B as shown above
Buffer A: 0.05M sodium phosphate (pH7.0),
2.0M ammonium sulfate, 1.0M glycine
Buffer B: 0.05M sodium phosphate (pH7.0),
1.0M glycine
Flow rate: 1.0mL/min
Detection: UV @ 280nm

Monoclonal antibody purified on TSKgel Ether-5PW



Column: TSKgel Ether-5PW, 8.0mm x 7.5cm glass
Sample: 25mL raw cell culture supernatant,
~ 200mg total protein,
~ 15mg total antibody diluted to 50mL
with initial elution buffer
Injection: 50mL
Elution: 67.5min isocratic load and wash with
1M (NH₄)₂SO₄ in 0.1M glycine, 0.5M phosphate
buffer pH 7.0, followed by a 37.5 min linear
gradient from 1.0M to 0M (NH₄)₂SO₄ in
1.0M glycine, 0.05M phosphate, pH 7.0
Flow rate: 1.0mL/min
Detection: UV @ 280nm, 3.0 AUFS

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P/N	Description	Particle size	Housing material	ID (mm)	Length (cm)	Price (\$)
14013	TSKgel Ether-5PW	10µm	Glass	5	5	926
14014	TSKgel Ether-5PW	10µm	Glass	8	7.5	1,193
14025	Guardgel Kit for P/Ns 14013 and 14014		Glass			580
18760	TSKgel Ether 5PW	10µm	Stainless Steel	2	7.5	900
42156	Guard Cartridge for P/N 18760, pk 3		Stainless Steel	2	1	319
19308	Guard Cartridge Holder for P/N 42156		Stainless Steel	2	1	292
08641	TSKgel Ether-5PW	10µm	Stainless Steel	7.5	7.5	1,193
08643	Guardgel Kit for P/N 08641		Stainless Steel			543

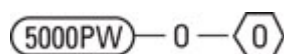
Hydrophobic Interaction Chromatography

TSKgel Phenyl-5PW

The most hydrophobic among the three TSK-GEL HIC columns, **TSKgel Phenyl-5PW** is an excellent choice to screen for the selectivity, retention and recovery of most biomolecules. Featuring a 1000Å pore size in a polymethacrylate base material, the Phenyl-5PW column is stable in either acid or caustic cleaning regimens and provides excellent access to larger molecules with low diffusion coefficients.

Stainless steel is the standard column format for TSKgel Phenyl-5PW. Glass and PEEK column housings are available for samples that may adsorb to stainless steel. The alternative column formats are also suitable for general applications.

Structure:

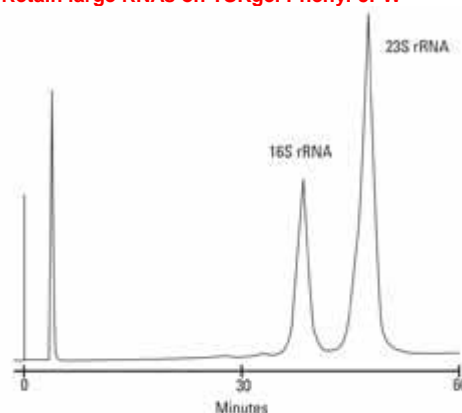


Product Attributes:

Pore size (mean):	1,000Å
Particle size (mean):	10, 13 and 20µm
pH stability:	2.0 - 12.0
Functional group:	Phenyl

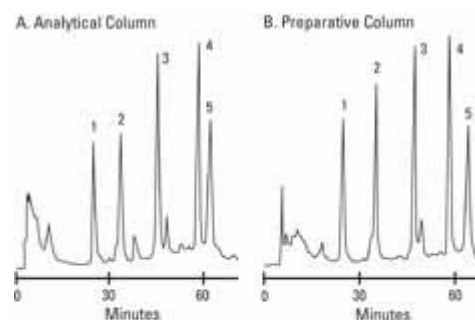
Applications

Retain large RNAs on TSKgel Phenyl-5PW



Column: TSKgel Phenyl-5PW, 7.5mm x 7.5cm
Sample: 16S and 23S rRNA from *E.coli*, 0.05mg in 0.1mL
Elution: 60min linear gradient from 2M to 0M (NH₄)₂SO₄ in 0.1M phosphate buffer, pH7.0
Flow Rate: 0.5mL/min
Detection: UV @ 280nm

Scale up to preparative separations



Column: TSKgel Phenyl-5PW, A.) 7.5mm x 7.5cm and B.) 21.5mm x 15cm
Sample: 1. myoglobin, 2. ribonuclease A, 3. lysozyme, 4. a-chymotrypsinogen, 5. a-chymotrypsin
Elution: 60min linear gradient from 1.8M to 0M (NH₄)₂SO₄ in 0.1M phosphate buffer, pH7.0
Flow rate: 0.5mL/min (7.5mm ID) or 4mL/min (21.5mm ID)
Detection: UV @ 280nm

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P/N	Description	Particle size	Housing material	ID (mm)	Length (cm)	Price (\$)
13063	TSKgel Phenyl-5PW	10µm	Glass	5	5	926
08804	TSKgel Phenyl-5PW	10µm	Glass	8	7.5	1,193
18759	TSKgel Phenyl-5PW	10µm	Stainless Steel	2	7.5	900
42155	Guard Cartridge for P/N 18759		Stainless Steel	2	1	319
19308	Guard Cartridge Holder for P/N 42155		Stainless Steel	2	1	292
07573	TSKgel Phenyl-5PW	10µm	Stainless Steel	7.5	7.5	1,193
07652	Guardgel Kit for P/N 07573		Stainless Steel			543
07656	TSKgel Phenyl-5PW	13µm	Stainless Steel	21.5	15	4,059
16095	Guardgel Kit for P/N 07656		Stainless Steel			838
07938	TSKgel Phenyl-5PW	20µm	Stainless Steel	55	20	13,259
07936	Guard Column for P/N 07938		Stainless Steel	45	5	3,747
20023	TSKgel BioAssist Phenyl	10µm	PEEK	7.8	5	1,193