

# Hydrophobic Interaction Chromatography

## TOYOPEARL Resins for HIC

TOYOPEARL Hexyl-650  
TOYOPEARL Butyl-650  
TOYOPEARL Phenyl-650  
TOYOPEARL Ether-650

## TSK-GEL Bulk Resins for HIC

TSKgel Phenyl-5PW  
TSKgel Ether-5PW

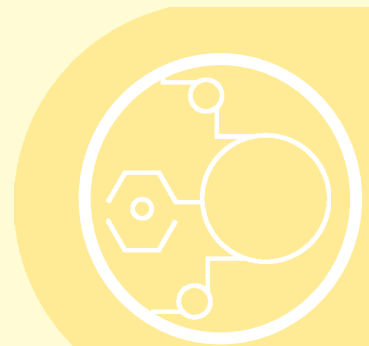
## Method Development Glass Columns (MD-G)

TOYOPEARL MD-G Butyl-650S  
TOYOPEARL MD-G Phenyl-650S  
TOYOPEARL MD-G Ether-650S  
TSKgel MD-G Phenyl-5PW (20)  
TSKgel MD-G Ether-5PW (20)



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## Hydrophobic Interaction Chromatography

Hydrophobic interaction chromatography (HIC) is a powerful technique for both analytical and preparative separations of biomolecules. The technique takes advantage of the hydrophobic areas located on the surface of proteins. HIC is an excellent complement to size exclusion and ion exchange chromatography in difficult separations, particularly those in which the impurities are of similar isoelectric point or molecular weight. It is preferred to reversed phase chromatography when retention of the biological activity of the protein is of importance. The selectivity differences exploited by HIC can also be used after affinity separations in which closely related proteins with similar recognition sites are not distinguishable by the affinity ligand.

### How does HIC work?

Proteins and other molecules with hydrophobic surface properties are attracted to the relatively mildly hydrophobic surface of a HIC resin (compared to the much stronger hydrophobic surface of reversed phase resins) under high salt aqueous conditions, but are released with decreasing salt concentration. Under zero salt conditions most components will be released. The matching of sample component hydrophobicity with resin hydrophobicity is critical to the

selection of the most appropriate resin. A wide range of resin hydrophobicities is highly desirable and is provided by Tosoh Bioscience for resin scouting and methods development.

### Applications:

- ◆ Separation of Proteins and Peptides
- ◆ Separation of DNA plasmids
- ◆ Ideal step after IEC

### Monoclonal antibodies

Hydrophobic interaction is one of the chromatographic modes used for the purification of MABs. The purification of mouse anti-chicken 14 kDa lectin MAB from ascites fluid was performed with 10µm TSKgel Ether-5PW columns. Highly purified MAB fractions were obtained after only one purification step (Figure 1). Identical selectivity was found with 65µm TOYOPEARL Ether-650M.

### Glycoproteins

TOYOPEARL HIC resins can purify glycoproteins with high carbon content, which often bind irreversibly to saccharide-based chromatographic media.

Features		Benefits
◆ hydrophilic polymer resin matrix	⇒	<ul style="list-style-type: none"> <li>• good chemical stability, pH 1-13 can be regulated with acid or base</li> <li>• temperature range 4°-60°C</li> <li>• autoclavable at 121°C</li> <li>• compatible with organic solvents</li> <li>• constant bed volume over a wide range of salt concentrations</li> <li>• low non specific protein binding</li> <li>• high protein recovery</li> </ul>
◆ good mechanical stability	⇒	<ul style="list-style-type: none"> <li>• excellent flow characteristics in large industrial size columns</li> <li>• direct scale-up from TSK-GEL HIC HPLC columns</li> </ul>



Examples of production scale column packing with TOYOPEARL

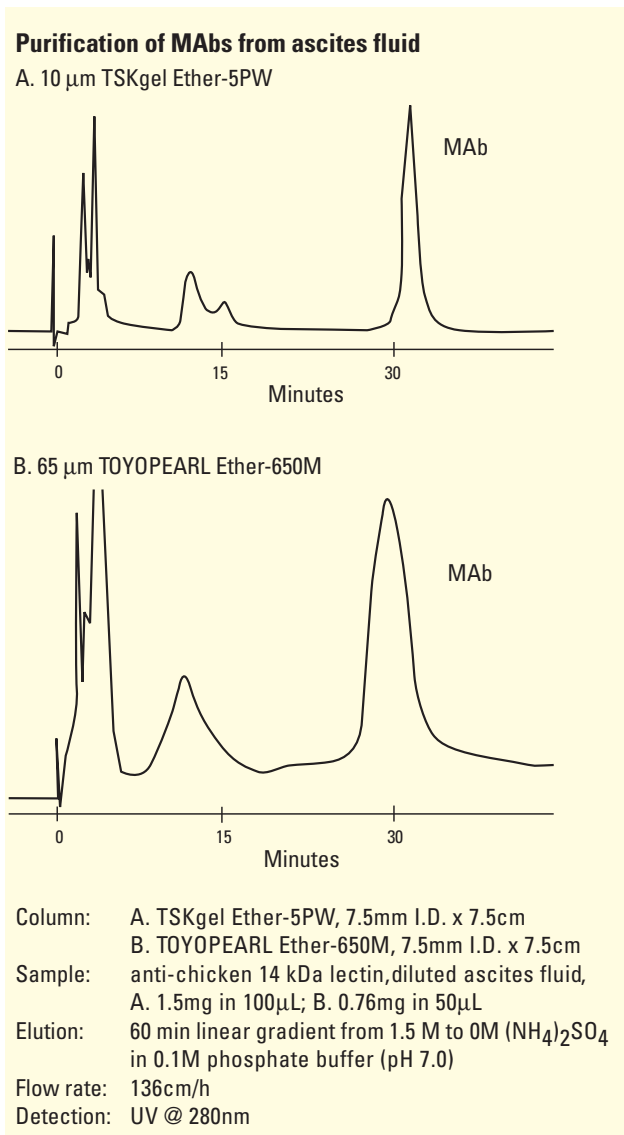
## Growth Factors

TOYOPEARL Butyl-650M as an intermediate purification step separates the correctly folded form of a growth factor (e.g. IGF-1) from a misfolded, yet stable form, which might be generated in a refolding process.

## DNA plasmids

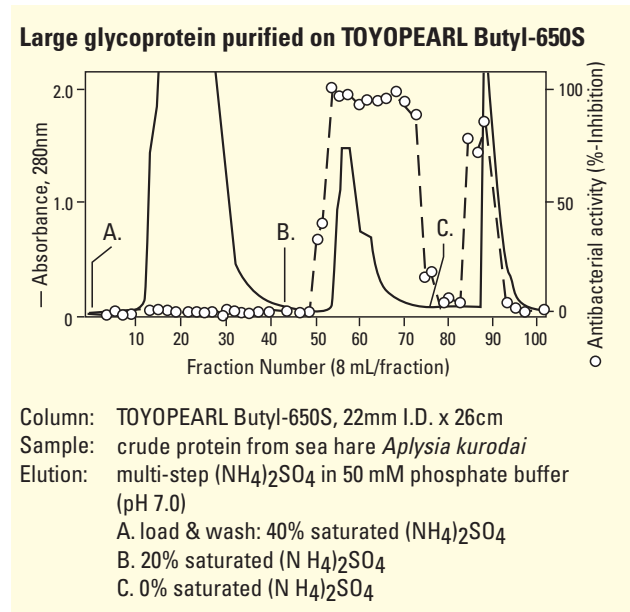
TOYOPEARL Hexyl-650C resin has been successfully used for plasmid DNA purification by CAMBREX, Baltimore, MD (Patent pending). Hexyl-650C has been shown to be the most effective among HIC resins for endotoxin removal with capacities exceeding 2 million EU/ml of resin. Additionally, RNA and protein contaminants were effectively eliminated. Hexyl-650C was also effective in separating of the supercoiled and open circular forms of plasmid DNA.

Figure 1



Please check the database on our website for additional applications

Figure 2



## Summary of HIC column range

Tosoh Bioscience provides prepacked analytical (TSK-GEL 5PW) and MD-G (method development) columns (TOYOPEARL and TSK-GEL resins) with ether, phenyl, butyl, or hexyl functional groups. Both TOYOPEARL and TSK-GEL HIC resins use 1000 Å pore size base resin.

## Summary of HIC resin range

- + **TSK-GEL** bulk polymeric media in 20 and 30 $\mu\text{m}$  particle size:
  - Ether-5PW
  - Phenyl-5PW
- + **TOYOPEARL** bulk polymeric media in 35, 65 and 100 $\mu\text{m}$  particle size:
  - Ether-650
  - Phenyl-650
  - Butyl-650
  - Hexyl-650 (only "C" grade)

# Hydrophobic Interaction Chromatography

## Four alternative hydrophobic surfaces/selectivities

Tosoh Bioscience offers four HIC resins featuring different surface chemistry and levels of hydrophobicity. The hydrophobicity of TOYOPEARL HIC resins increases through the series: Ether, Phenyl, Butyl, Hexyl. The structures are shown in Figure 3, with either TOYOPEARL HW-65 (1000 Å pores) and TSK-GEL 5PW as base resin.

For any particular protein, adsorption occurs at decreasing salt concentrations as the hydrophobicity of the resin increases. Strength of interaction is also directly related to strength of resin and salt concentration. Therefore by choosing the appropriate TOYOPEARL HIC resin, the use of inorganic salt can be minimized, and recovered bioactivity can be maximized. The data in Figure 4 shows the relative hydrophobicity of each resin for several test proteins.

This range of alternative resins enables chromatographic optimization of separations at extremes of the hydrophobic spectrum. Highly

retentive TOYOPEARL Hexyl or TOYOPEARL Butyl can be used to separate mildly hydrophobic proteins. This is of particular use when ion exchange has not resulted in achieving the required level of purity. TOYOPEARL Ether is recommended for the purification of very hydrophobic proteins such as monoclonal antibodies or membrane proteins. Such proteins may be too tightly bound to other HIC resins, resulting in unacceptably low recovery or loss of bioactivity. Retention and selectivity of protein standards on TOYOPEARL HIC resins are shown in Figure 5. The selection of salts that can be used to control selectivity form a series, called the lyotropic or "Hofmeister" series, with a range of effective elution strengths as indicated in Figure 6. Every salt will result in a different adsorption and desorption selectivity with each resin. This feature of HIC provides a wide number of variables for optimizing a process step.

Figure 3

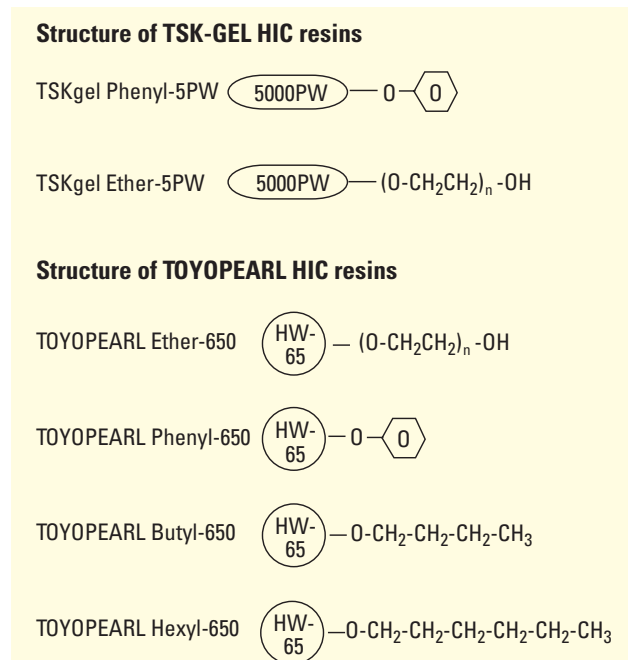


Figure 4

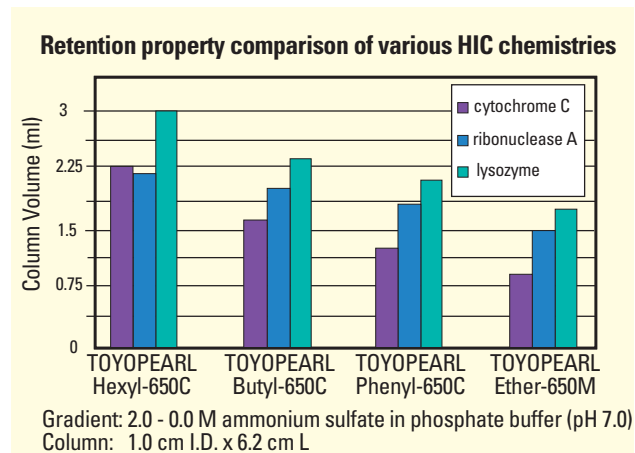


Figure 5

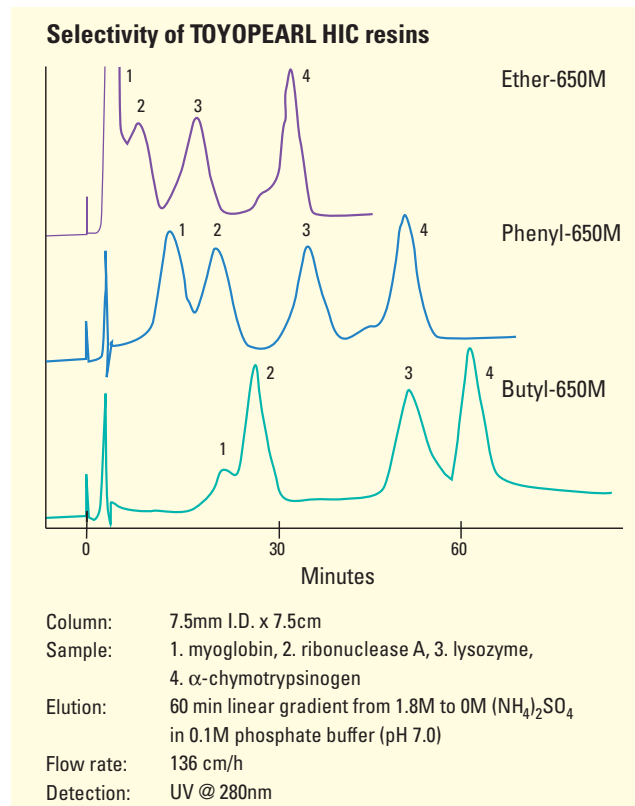
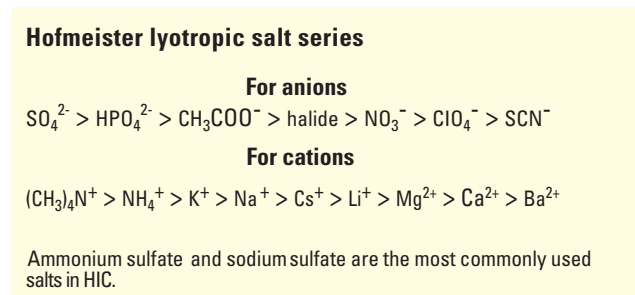


Figure 6



## Resins for research and manufacturing

TOYOPEARL hydrophobic interaction resins provide the performance characteristics vital to both successful laboratory and process scale chromatography at pressures up to 3 bar. A wide choice of resins and particle sizes enables optimization of selectivity, resolution and step productivity. The macroporous structure provides excellent adsorption capacities, and provides high mass and activity recovery for proteins and plasmids.

In HIC mode, the influence of particle size on resolution is more pronounced than in other adsorption modes (Table I). With each surface type, resolution improves from C to M to S grade as is clearly demonstrated in Figure 8.

## High flow rates for optimal throughput

The semi-rigid polymeric backbone of TOYOPEARL HIC resins permits high flow rates for maximum productivity. TOYOPEARL HIC resins are based on the rigid TOYOPEARL HW-65 polymer, which is stable to wide changes in pH and ionic strength. TOYOPEARL HIC resins show excellent performance in both fixed bed and axial compression columns.

The pressure/flow characteristics for each particle size grade of TOYOPEARL Phenyl-650 resins are shown in Figure 7. In these experiments the mobile phase was 2M ammonium sulfate.

## High performance HIC resins for process manufacture

The range of HIC resins includes the highly rigid TSK-GEL 5PW resins when high performance separations are required. Both 20µm and 30µm versions are available, when a smaller particle is required for higher resolution separations. These resins may be used at flow rates generating up to 20 bar pressure.

Figure 7

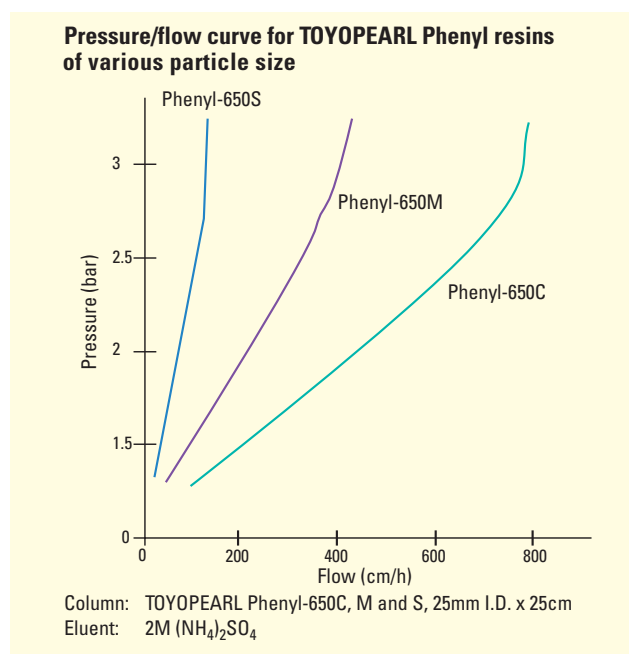
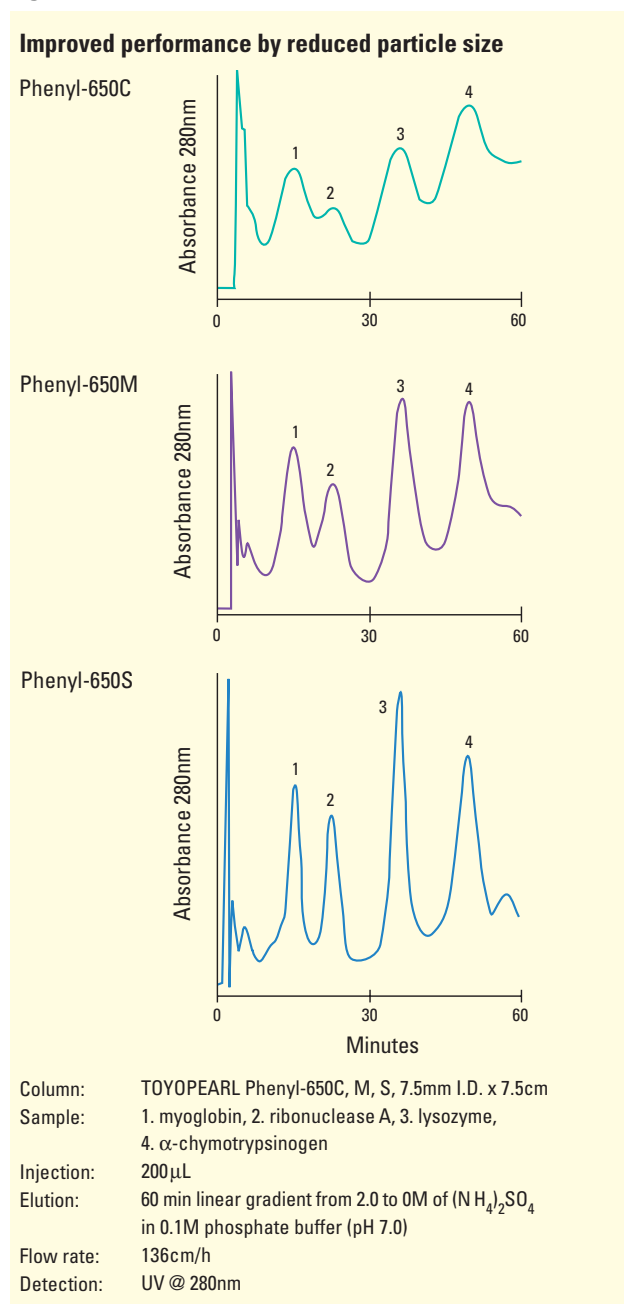


Table I

Resins	Particle size grade		
	C	M	S
Phenyl-650	1.12	1.52	2.19
Butyl-650	0.91	1.37	2.20

Figure 8



## High recovery of mass with activity

Achieving high mass and activity recovery are principal goals in process chromatography. The hydrophilic polymer used in TOYOPEARL and TSK-GEL-5PW minimizes nonspecific binding to enhance product recovery and assists with rapid cleaning and column regeneration. Protein mass recoveries are shown in Table I and activity recoveries in Table II.

## Scale-up with TOYOPEARL MD-G series columns

TOYOPEARL MD-G hydrophobic interaction columns are convenient, cost effective tools for scouting various resins and for methods development.

MD-G columns may be used in research as well as in process development where they provide an ideal starting point for a seamless scale up strategy.

Figure 9 shows an example purification using a TOYOPEARL MD-P column, an earlier "PEEK" version of the newer MD-G methods development glass columns which are now commercially available.

Table I

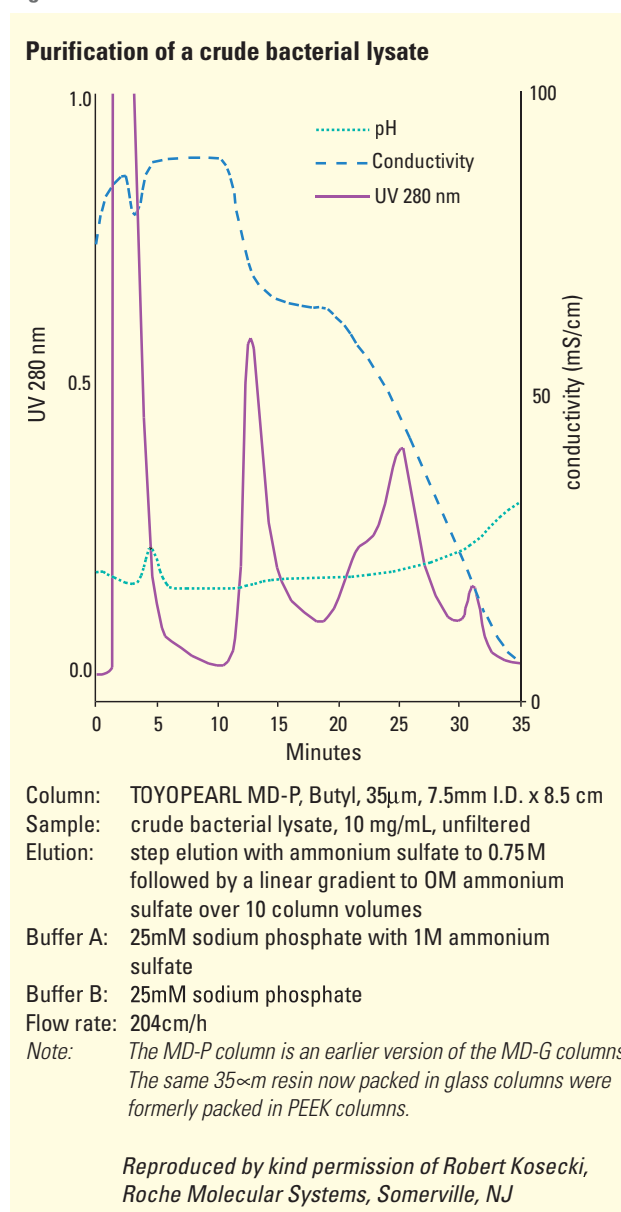
	High mass recovery (%) of proteins		
	TOYOPEARL HIC resin		
	<i>Ether</i> <i>-650M</i>	<i>Phenyl</i> <i>-650M</i>	<i>Butyl</i> <i>-650M</i>
Bovine serum albumin	84	62	76 *
$\alpha$ -Chymotrypsinogen	96	88 *	90
Cytochrome C	—	81 *	87 *
IgG	91	—	—
$\alpha$ -Lactalbumin	90	—	—
Lysozyme	94	92	85
Ovalbumin	83	88	73
Ribonuclease A	—	72 *	82 *

*Procedure: A 200 mL sample containing 200mg of protein was loaded onto a 7.5mm I.D. x 75cm column and eluted with a 60 minute gradient of 1.8M (\*1.5 M) to 0.0M ammonium sulfate in 0.1M sodium phosphate (pH 7.0). The mass recovery was determined spectrophotometrically, UV 280nm at 25°C.*

Table II

Recovery of enzymatic activity of proteins		
TOYOPEARL HIC resin	Protein	% Activity recovery
Phenyl	Phytochrome	79
Butyl	Halophilic protease	85
Butyl	Poly (3-hydroxybutyrate) depolymerase	88
Butyl	Aculeacin-A acylase	82
Butyl	Opine dehydrogenase	81

Figure 9



## Ordering Information

### TSK-GEL LABPAK:

Part #	Product description	Container size (ml)	Particle size ( $\mu\text{m}$ )
43278	HICPAK PW (20) (Ether-5PW, Phenyl-5PW)	2 x 25mL	10-30
43175	HICPAK PW (30) (Ether-5PW, Phenyl-5PW)	2 x 25mL	20-40

Conditions: Exclusion limits are  $\pm 30\%$  and are inferred from the base material, HW-65. Limits are determined using polyethylene glycol and polyethylene oxide standards.

### TOYOPEARL HIC resins:

Part #	Product description	Container size (mL)	Particle size ( $\mu\text{m}$ )	Adsorption capacity (mg lysozyme/mL resin)	Exclusion limit (Da)
43151	Ether-650S	25	20-50	10-30	$1 \times 10^6$
16172		100			
16174		1,000			
16176		5,000			
19805	Ether-650M	25	40-90	10-30	$1 \times 10^6$
16173		100			
16175		1,000			
16177		5,000			
43152	Phenyl-650S	25	20-50	30-50	$1 \times 10^6$
14477		100			
14784		1,000			
14935		5,000			
19818	Phenyl-650M	25	40-90	30-50	$1 \times 10^6$
14478		100			
14783		1,000			
14943		5,000			
18364		50,000			
43126	Phenyl-650C	25	50-150	30-50	$6 \times 10^5$
14479		100			
14785		1,000			
14944		5,000			
43153	Butyl-650S	25	20-50	30-50	$1 \times 10^6$
07476		100			
14701		1,000			
07975		5,000			
18826		50,000			
19802	Butyl-650M	25	40 - 90	30 - 50	$1 \times 10^6$
07477		100			
14702		1,000			
07976		5,000			
18355		50,000			
43127	Butyl-650C	25	50-150	30-50	$6 \times 10^5$
07478		100			
14703		1,000			
07977		5,000			
44465	Hexyl-650C	25	50-150	30-50	$6 \times 10^5$
19026		100			
19027		1,000			
19028		5,000			

## TSK-GEL MD-G series columns:

Part #	Product description	Dimensions (ID x L)	Particle size (µm)
22247	TSKgel MD-G Ether-5PW (20)	10mm x 6.8cm	20
22246	TSKgel MD-G Phenyl-5PW (20)	10mm x 6.8cm	20

## TOYOPEARL MD-G series columns:

Part #	Product description	Dimensions (ID x L)	Particle size (µm)
22237	TOYOPEARL MD-G Ether-650S	10mm x 6.8cm	35
22236	TOYOPEARL MD-G Phenyl-650S	10mm x 6.8cm	35
22235	TOYOPEARL MD-G Butyl-650S	10mm x 6.8cm	35

## TSK-GEL 5PW HIC resins for high resolution:

Part #	Product description	Container size (µm)	Particle size (µm)	Adsorption capacity (mg lysozyme/mL resin)
43276	Ether-5PW (20)	25	10-30	10-30
16052		250		
16053		1,000		
18437		5,000		
43176	Ether-5PW (30)	25	20-40	10-30
16050		250		
16051		1,000		
18439		5,000		
43277	Phenyl-5PW (20)	25	10-30	10-30
14718		250		
14719		1,000		
18438		5,000		
43177	Phenyl-5PW (30)	25	20-40	10-30
14720		250		
14721		1000		
17210		5000		

\*All TSK-GEL hydrophobic interaction chromatography resins have an exclusion limit of approximately  $9 \times 10^5$ Da.