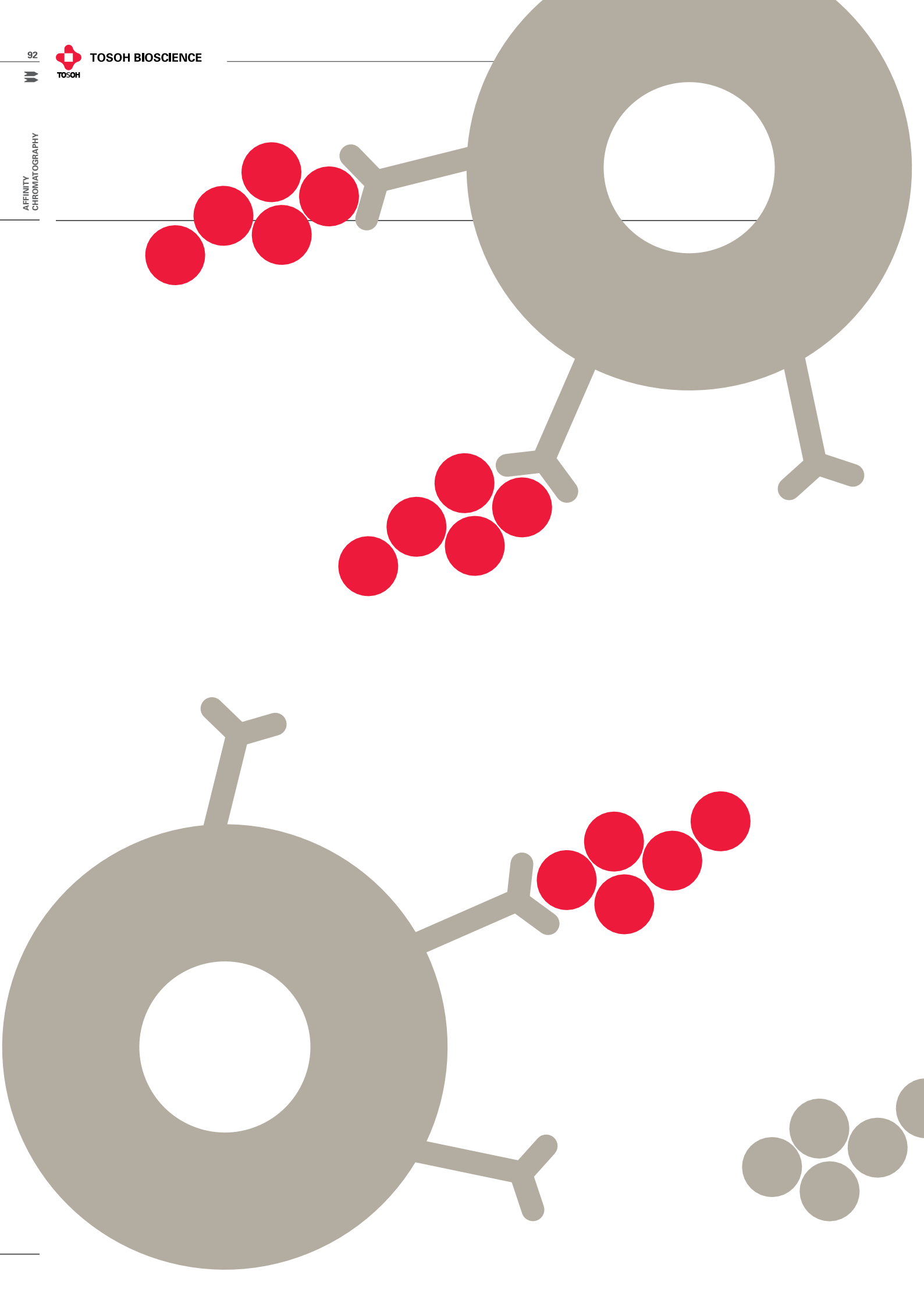


AFC
AFFINITY CHROMATOGRAPHY

AFC PRODUCTS

- TSKgel BORONATE-5PW
- TSKgel CHELATE-5PW
- TSKgel TRESYL-5PW

TOSOH FACT

The Tosoh logo symbolizes the corporate philosophy of Tosoh's vision of the ideal .

The curved lines represent the realization of happiness, reflecting Tosoh's management philosophy of putting people first. The square in the center expresses the advanced nature of Tosoh's technology and also represents the outstanding quality of Tosoh's products. The right-angle cut at the top portrays an image of contributing to society, Tosoh's stance towards the outside world. The red corporate color symbolizes the Tosoh spirit, which guides the ceaseless efforts to realize the ideal.

INTRODUCTION TO TSKgel AFFINITY CHROMATOGRAPHY COLUMNS

The Tosoh Bioscience TSKgel Affinity Chromatography (AFC) column line consists of two group-specific stationary phases: TSKgel BORONATE-5PW and TSKgel CHELATE-5PW as well as one activated packing material called TSKgel TRESYL-5PW. Affinity chromatography offers the highest level of specificity and selectivity in biomolecular separations and purifications. Tosoh Bioscience supplies a full range of products for analytical, preparative and process scale affinity chromatography.

TSKgel affinity chromatography columns are based on the well-known G5000PW porous resin, which is the basis for high performance size exclusion chromatography columns. The TSKgel 5PW-type resin is a hydrophilic media with 100 nm pores and an estimated protein exclusion limit of 5 x 10^6 Da. Tosoh Bioscience's process scale affinity media are based on the 65 µm particle size, semi-rigid TOYOPEARL HW-65 resin. Since analytical and semi-preparative columns are made from the same polymer chemistry as the process scale media, seamless scale-up from lab to process scale is achievable. Consult the chapter on bulk media for more information about resins for packing columns to purify medium to large volume samples.

COLUMN SELECTION

TABLE I lists the ligand concentration, adsorption capacity and the test analyte used to determine the capacity of each column type.

The structures of the functional ligands available from Tosoh Bioscience are shown in FIGURE 1. The choice of a specific ligand is dictated by the expected interaction between the sample and column bonded phase. For example, the TSKgel Chelate-5PW column will bind high concentrations of Zn^{2+} ions. If a given protein is known to bind to Zn^{2+} ions, the Chelate-5PW would be a candidate column for the isolation of that target compound.

Tosoh Bioscience offers AFC columns in both glass and stainless steel formats. Glass columns are available in 5 mm ID x 5 cm L and 8 mm ID x 7.5 cm L. Stainless steel columns are available as 7.5 mm ID x 7.5 cm L and 6 mm ID x 4 cm L (Tresyl-5PW only). TSKgel BioAssist Chelate is packed in 7.8 mm ID x 5 cm L PEEK hardware. The shipping solvent is distilled water for Boronate-5PW. The Chelate-5PW is shipped in 10 mmol/L acetate buffer, pH 4.5, and the Tresyl-5PW column shipping solvent is acetone.

Stainless steel or Pyrex frits are employed in the body of the column end-fittings for the metal and glass columns, respectively. The nominal frit size for stainless steel columns is engraved in the end-fittings and all Pyrex® frits are 10 µm nominal pore size.

TABLE I

Characteristics of TSKgel AFC columns

Column packing	Ligand type	Ligand concentration	Adsorption capacity	Sample
Boronate-5PW	*m*-aminophenyl-boronate	not available	40 µmol/mL resin	sorbitol
Chelate-5PW	iminodiacetic acid	20 µmol/mL resin	not available	not available
Tresyl-5PW	tresyl	ca. 20 µmol/mL resin	>60 mg/g dry resin (coupling capacity)	soybean trypsin inhibitor

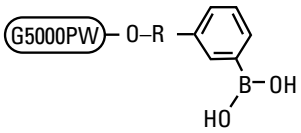
FEATURES	BENEFITS
High size exclusion limit (> 5 x 10^6 Da)	Enhanced access of large proteins to affinity ligands
Small particle size	High efficiency for analytical (10 µm) and semi-preparative (13 µm) affinity applications.
Rigid polymeric base resin	Wide pH range (2-12) of the base resin, enabling robust cleaning options
Stable affinity ligands	Long lifetime, solvent compatibility, autoclavable
Choice of four affinity ligands	Application flexibility, scalability from lab to commercial production.
TSKgel BioAssist Chelate offered in PEEK hardware	Eliminates undesirable interactions with column hardware.

AFC

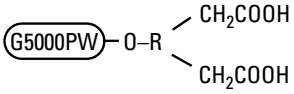
APPLICATIONS OF TSKgel AFFINITY CHROMATOGRAPHY COLUMNS

FIGURE 1
TSKgel affinity chromatography column packings

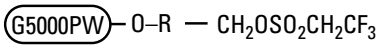
TSKgel Boronate-5PW



TSKgel Chelate-5PW



TSKgel Tresyl-5PW



Separation columns should be protected with a guard column. Tosoh Bioscience offers a unique Guardgel kit consisting of guard column hardware and gel packing, allowing the user to repack the guard column as required. Guardgel kits are available for most affinity columns, both glass and stainless steel.

TSKgel BORONATE-5PW

Coupling of m-aminophenyl boronate to the TSKgel 5PW-type polymeric support results in a ligand capable of forming a tetrahedral boronate anion under alkaline pH conditions. This anionic structure can bind with 1,2 cis-diol groups such as those found in carbohydrates, carbohydrate-containing compounds, and catecholamines. Interaction between the boronate anion and the 1,2 cis-diol groups is enhanced in the presence of Mg^{2+} ions and is inhibited by amine-containing buffers. Adsorption onto the TSKgel Boronate-5PW takes place in basic buffers such as HEPES and morpholine, while desorption takes place in carbohydrate or amine-containing mobile phases like sorbitol or Tris.

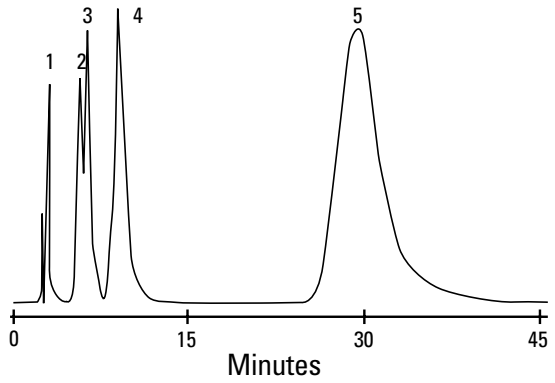
Applications for TSKgel Boronate-5PW include: nucleic acids, nucleotides and nucleosides. This affinity column has also been used to isolate catecholamines and other biomolecules containing the 1,2 cis-diol functionality (FIGURE 2).

TSKgel CHELATE-5PW

TSKgel Chelate-5PW utilizes the ability of iminodiacetic acid (IDA) to chelate ions such as Zn^{2+}, Ni^{2+} and Cu^{2+}. The column is pre-loaded with divalent metal ions by chelation. Peptides and proteins containing histidine residues will normally adsorb to these chelated ions at neutral pH. The retained compounds are then eluted with buffer containing imidazole or glycine.

The key to making successful use of this retention mechanism is the proper selection of metal ions for chelation and the elution buffer to desorb the analytes. In general, Cu^{2+} interacts better with protein; however, resolution is usually enhanced with Zn^{2+} ions. A gradient mobile phase containing increasing imidazole or glycine concentrations is used to elute the retained compounds. A decreasing pH gradient can also be used. Glycine, as well as HEPES buffers, will also elute the metallic ion so column regeneration is necessary. Conversely, imidazole in phosphate buffer will extract the metal ions very slowly, avoiding frequent column regeneration.

FIGURE 2
Separation of catecholamines on TSKgel Boronate-5PW



Column: TSKgel Boronate-5PW, 7.5 mm ID x 7.5 cm L; Sample: 1. tyrosine, 2. normetanephrine, 3. metanephrine, 4. DOPA, 5. epinephrine;
Elution: 0.1 mol/L phosphate buffer, pH 6.5; Flow rate: 1.0 mL/min;
Detection: UV @ 280 nm

APPLICATIONS OF TSKgel AFFINITY CHROMATOGRAPHY COLUMNS

Applications for TSKgel Chelate-5PW include: immunoglobulins, transferrin, lectins, milk proteins, membrane proteins, and peptides.

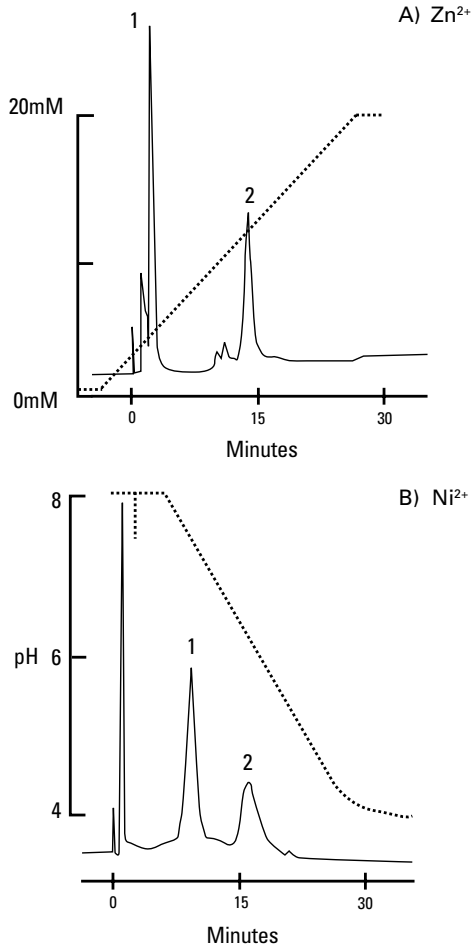
In FIGURE 3, the separation of ribonuclease A (bovine) and transferrin (human) are compared on TSKgel Chelate-5PW columns (glass, 5 mm ID x 5 cm L) containing different metal ions.

TSKgel TRESYL-5PW

Unlike other TSKgel affinity columns, the TSKgel Tresyl-5PW (tresyl; 2,2,2-trifluoroethanesulfonyl) requires activation with a user-selected ligand containing amino, thiol, phenol, or imidazole groups. The resulting structure is literally a custom affinity ligand with excellent pH stability and minimal ligand loss due to leaching. TSKgel Tresyl-5PW readily reacts with amino or thiol groups to form stable covalent alkylamines or thioethers.

FIGURE 3

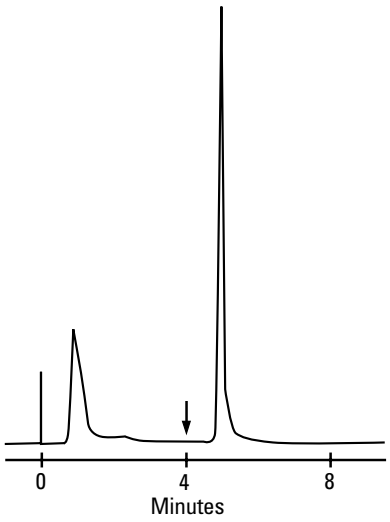
Separation of standard proteins by immobilized metal ion affinity chromatography



Column: TSKgel Chelate-5PW, 5 mm ID x 5 cm L; Metal Ion: A) Zn^{2+} and B) Ni^{2+}
Sample: 1. ribonuclease A (bovine), 2. transferrin (human)
Elution: A): 30 min linear gradient from 1 mmol/L to 20 mmol/L imidazole in 20 mmol/L HEPES-NaOH buffer, pH 8.0, containing 0.5 mol/L NaCl
B) 30 min linear pH gradient from 20 mmol/L HEPES-MES-acetic acid, pH 8.0, to 20 mmol/L HEPES-MES-acetic acid, pH 4.0, both in 0.5 mol/L NaCl; Flow rate: 0.8 mL/min; Detection: UV @ 280 nm

FIGURE 4

Purification of peroxidase on concanavalin A coupled to TSKgel Tresyl-5PW



Washing step: Wash TSKgel Tresyl-5PW, 6 mm ID x 4 cm L, with DI water; Ligand solution: Dissolve 40 mg of concanavalin A in 10 mL of 0.1 mol/L $NaHCO_3$, pH 8.0, containing 0.5 mol/L NaCl; Coupling step: Recycle the ligand solution overnight through the column at 0.2 mL/min at 25°C; Blocking step: Block residual tresyl groups with 0.1 mol/L Tris-HCl, pH 8.0, at 1.0 mL/min for 1 h at 25°C; Column: TSKgel Tresyl-5PW modified with concanavalin A; Sample: Crude peroxidase, 0.5 mg; Binding: 0.05 mol/L acetate buffer, pH 5.0, containing 0.5 mol/L NaCl and 1 mmol/L each of $CaCl_2$, $MnCl_2$, and $MgCl_2$; Elution: Step gradient at 4 min (see arrow on diagram) to 25 mmol/L -methyl-D-glucoside in binding buffer; Flow rate: 1.0mL/min; Detection: UV @ 403 nm

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Principal applications for TSKgel Tresyl-5PW include the selective purification of antigens after coupling the appropriate antibody to the solid support. The antibody coupling yield at pH >7.5 is more than 90 %, with the maximum binding occurring at pH 7.5. Antigen adsorption to the antibody ligand is most effective when the antibody concentration is < 2-3 mg/mL of affinity resin. To increase binding capacity, more antibody should be added to the coupling reaction.

However, higher concentrations of antibody can result in steric hindrance, thus lowering the binding capacity of the column. As a general rule, the time required for antibody attachment to the TSKgel Tresyl-5PW column is directly proportional to the antibody concentration. Small amounts of antibody require about 2 hours to complete the cross-linking reaction, whereas it may take 6-7 hours to fully attach an antibody at the concentration of 10 mg/mL-resin.

Examples of the wide range of applications using TSKgel Tresyl-5PW include the binding of such ligands as concanavalin A (a lipoprotein lectin that binds to glycoproteins), numerous antibodies and enzymes. The chromatogram in FIGURE 4 shows the purification of peroxidase by the concanvalin A ligand coupled to the TSKgel Tresyl-5PW affinity support resin.

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	*Number theoretical plates*	*Flow rate (mL/min) range*	*Maximum pressure drop (MPa)*
Glass columns							
0014449	Boronate-5PW Glass, 100 nm	5.0	5.0	10	≥ 500	0.5 - 1.0	2.0
0014440	Chelate-5PW Glass, 100 nm	5.0	5.0	10	≥ 500	0.5 - 0.8	2.0
TSKgel Stainless Steel Columns							
0013066	Boronate-5PW, 100 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.0
0008645	Chelate-5PW, 100 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.0
0014455	Tresyl-5PW, 100 nm	6.0	4.0	10		0.2 - 0.5	1.0
0014456	Tresyl-5PW, 100 nm	7.5	7.5	10		0.5 - 1.0	1.0
TSKgel PEEK columns							
0020022	BioAssist Chelate, 100 nm	7.8	5.0	10	≥ 800	0.5 - 1.0	1.0
Guard column products							
0014451	Boronate-5PW Glass Guardgel Kit			20	For P/N 0014449		
0013125	Boronate-5PW Guardgel Kit				For P/N 0013066		
0008647	Chelate-5PW Guardgel Kit				For P/N 0008645		
Bulk packing							
0016208	Tresyl-5PW, 2 g dry gel*			10			

** 1 g is approximately 3.5 mL*