

AFC

AFFINITY CHROMATOGRAPHY

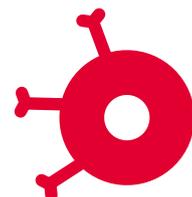
AFC PRODUCTS

- TSKgel ABA-5PW
- 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 TSK-GEL AFFINITY CHROMATOGRAPHY COLUMNS

The Tosoh Bioscience TSK-GEL Affinity Chromatography (AFC) column line consists of two group-specific stationary phases: **TSK-GEL BORONATE-5PW AND TSK-GEL CHELATE-5PW** as well as one activated packing material called **TSK-GEL 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.

TSK-GEL affinity chromatography columns are based on the well-known G5000PW porous resin, which is the basis for high performance size exclusion chromatography columns. The TSK-GEL 5PW-type resin is a hydrophilic media with 1,000 Å pores and an estimated protein exclusion limit of 5×10^6 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.

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

Column Selection

TSK-GEL affinity chromatography columns have been developed for purifying peptides, proteins, and nucleic acids. In addition, some columns have been successfully applied to the selective separation of small biomolecules such as nucleosides and catecholamines.

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 two formats: 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.

➤ FEATURES

BioAssist columns

- High size exclusion limit ($>5 \times 10^6$ Da)
- Small particle size
- Rigid polymeric base resin
- Stable affinity ligands
- Choice of four affinity ligands
- TSKgel BioAssist Chelate offered in PEEK hardware

➤ BENEFITS

- Enhanced access of large proteins to affinity ligands
- High efficiency for analytical (10 µm) and semi-preparative (13 µm) affinity applications.
- Wide pH range (2-12) of the base resin, enabling robust cleaning options
- Long lifetime, solvent compatibility, autoclavable
- Application flexibility, scalability from lab to commercial production.
- Eliminates undesirable interactions with column hardware.

AFC

TABLE 1

Characteristics of TSK-GEL AFC columns

Column packing	Ligand type	Ligand concentration	Adsorption capacity	Sample
Boronate-5PW	<i>m</i> -aminophenyl-boronate	not available	40 $\mu\text{mol/mL}$ resin	sorbitol
Chelate-5PW	iminodiacetic acid	20 $\mu\text{mol/mL}$ resin	not available	not available
Tresyl-5PW	tresyl	ca. 20 $\mu\text{mol/mL}$ resin	>60 mg/g dry resin (coupling capacity)	soybean trypsin inhibitor

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. At the recommended flow rates (see Ordering Information) the pressure drop across a TSK-GEL AFC glass or stainless steel column is less than 20 kg/cm².

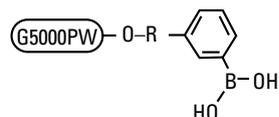
Separation columns should be protected with a guard column. Tosoh Bioscience offers a unique Guardgel kit consisting of guard column hardware and gel packing, allowing the user to repack the guard column as required. Guardgel kits are available for most affinity columns, both glass and stainless steel.

As with all columns used in gradient elution chromatography, affinity columns should be washed with final elution buffer prior to re-equilibration with initial (binding) buffer.

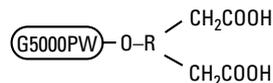
FIGURE 1

TSK-GEL affinity chromatography column packings

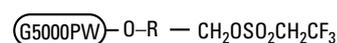
TSKgel Boronate-5PW



TSKgel Chelate-5PW



TSKgel Tresyl-5PW





APPLICATIONS OF TSK-GEL AFFINITY CHROMATOGRAPHY COLUMNS

TSK-GEL BORONATE-5PW

Coupling of m-aminophenyl boronate to the TSK-GEL 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.

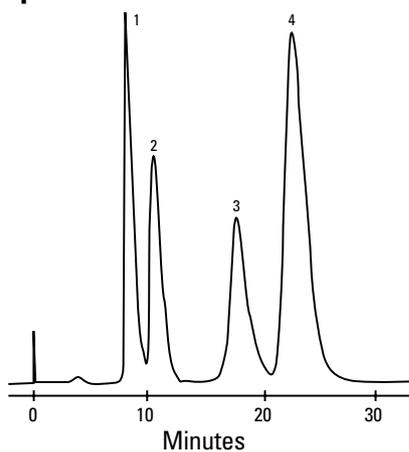
Applications for TSKgel Boronate-5PW include: nucleic acids, nucleotides and nucleosides. This affinity column has also been used to isolate catecholamines and other biomolecules containing the 1,2 cis-diol functionality. **FIGURES 3 & 4** demonstrate the applicability of the TSKgel Boronate-5PW affinity chromatography column for the separation of nucleosides and catecholamines.

TSK-GEL 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

≡ **FIGURE 2**

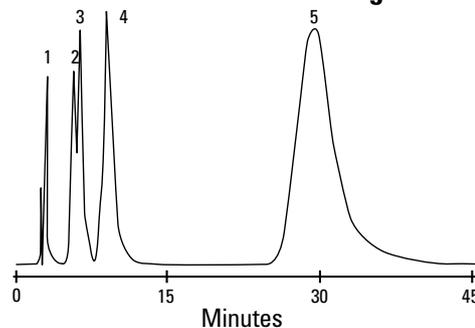
Isocratic separation of nucleosides



Column: TSKgel Boronate-5PW, 7.5mm ID x 7.5cm
 Sample: 1. cytidine, 2. uridine, 3. guanosine, 4. adenosine
 Elution: 0.1mol/L phosphate buffer, pH 8.0
 Flow Rate: 1.0mL/min
 Detection: UV @ 280nm

≡ **FIGURE 3**

Separation of catecholamines on TSKgel Boronate-5PW



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

AFC

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

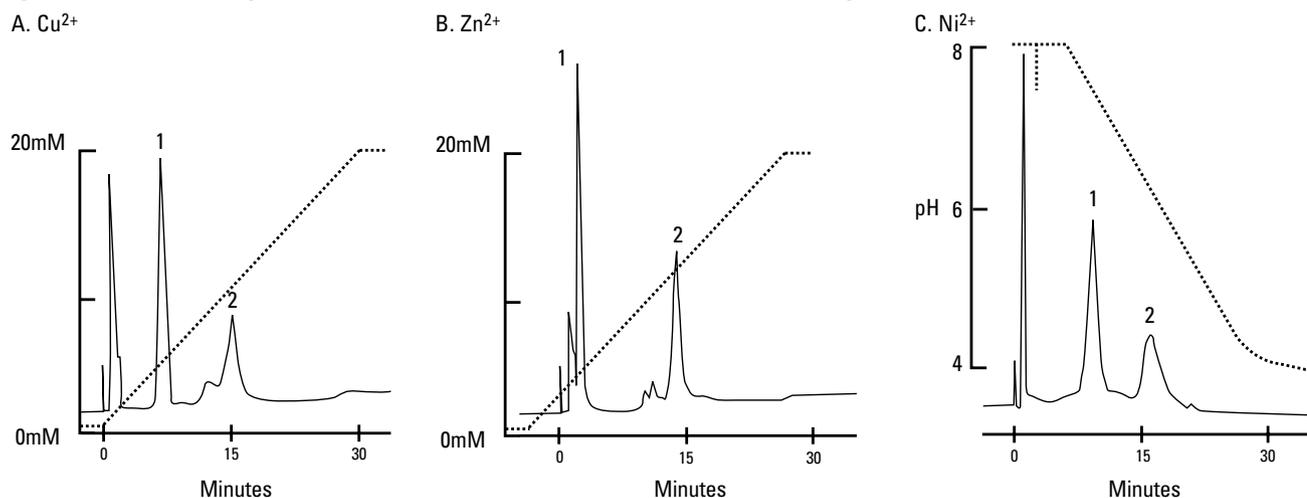
regeneration is necessary. Conversely, imidazole in phosphate buffer will extract the metal ions very slowly, avoiding frequent column regeneration.

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

In **FIGURE 5**, 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.

➤ **FIGURE 4**

Separation of standard proteins by immobilized metal ion affinity chromatography



Column: TSKgel Chelate-5PW, 5mm ID x 5cm

Metal Ion: A. Cu^{2+} , B. Zn^{2+} , and C. Ni^{2+}

Sample: 1. ribonuclease A (bovine), 2. transferrin (human)

Elution: A. and B.: 30min linear gradient from 1mmol/L to 20mmol/L imidazole in 20mmol/L HEPES-NaOH buffer, pH 8.0, containing 0.5mol/L NaCl
C. 30min linear pH gradient from 20mmol/L HEPES-MES-acetic acid, pH 8.0, to 20mmol/L HEPES-MES-acetic acid, pH 4.0, both in 0.5mol/L NaCl

Flow Rate: 0.8mL/min

Detection: UV @ 280nm

TSK-GEL TRESYL-5PW

Unlike other TSK-GEL 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.

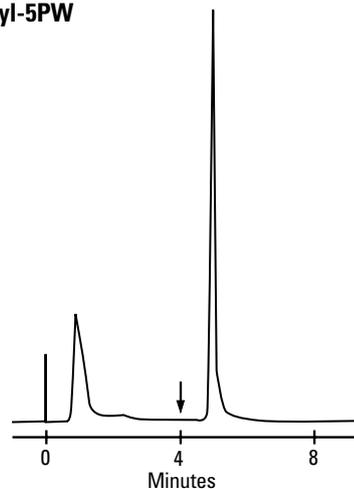
Principal applications for TSKgel Tresyl-5PW include the selective purification of antigens after coupling the appropriate antibody to the solid support. The antibody coupling yield at pH >7.5 is more than 90 %, with the maximum binding occurring at pH 7.5. Antigen adsorption to the antibody ligand is 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 6** shows the purification of peroxidase by the concanavalin A ligand coupled to the TSKgel Tresyl-5PW affinity support resin.

≡ **FIGURE 5**

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



- Washing step: Wash TSKgel Tresyl-5PW, 6mmID x 4cm, with DI water
- Ligand solution: Dissolve 40mg of concanavalin A in 10mL of 0.1mol/L NaHCO₃, pH 8.0, containing 0.5mol/L NaCl
- Coupling step: Recycle the ligand solution overnight through the column at 0.2mL/min at 25°C
- Blocking step: Block residual tresyl groups with 0.1mol/L Tris-HCl, pH 8.0, at 1.0mL/min for 1hr at 25°C
- Column: TSKgel Tresyl-5PW modified with concanavalin A
- Sample: Crude peroxidase, 0.5mg
- Binding: 0.05mol/L acetate buffer, pH 5.0, containing 0.5mol/L NaCl and 1mmol/L each of CaCl₂, MnCl₂, and MgCl₂
- Elution: Step gradient at 4min (see arrow on diagram) to 25mmol/L α-methyl-D-glucoside in binding buffer
- Flow Rate: 1.0mL/min
- Detection: UV @ 403nm

AFC

➤ ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min)		Maximum pressure drop (kg/cm ²)
						Range	Max.	
Glass columns								
14449	Boronate-5PW Glass, 1000 Å	5.0	5.0	10	≥ 500	0.5 - 1.0	1.2	20
14440	Chelate-5PW Glass, 1000 Å	5.0	5.0	10	≥ 500	0.5 - 0.8	1.0	20
Stainless steel columns								
13066	Boronate-5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	10
08645	Chelate-5PW, 1000 Å	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.2	10
14455	Tresyl-5PW, 1000 Å	6.0	4.0	10		0.2 - 0.5	1.0	10
14456	Tresyl-5PW, 1000 Å	7.5	7.5	10		0.5 - 1.0	1.2	10
PEEK columns								
20022	BioAssist Chelate, 1000 Å	7.8	5.0	10	≥ 800	0.5 - 1.0	1.2	10
Guard column products								
14451	Boronate-5PW Glass Guardgel Kit			10		For P/N 14450 and 14449		
13125	Boronate-5PW Guardgel Kit					For P/N 13066		
08647	Chelate-5PW Guardgel Kit					For P/N 08645		
Bulk packing								
16208	Tresyl-5PW, 2 g dry gel*							

* 1 g is approximately 3.5 mL