TSKgel Boronate-5PW

TSKgel Chelate-5PW

TSKgel Heparin-5PW

TSKgel Tresyl-5PW

Affinity Tips:

- TSKgel Affinity columns are offered in glass and stainless steel. Stainless steel (SS) or Pyrex frits are embedded in the body of the column end-fittings of metal and glass columns, respectively. The nominal frit size for SS columns is engraved in the end-fittings; Pyrex frits in the glass columns have a 10 µm nominal pore size.
- Halide salts corrode stainless steel tubing, fitting, and frits. Do not store SS columns in a mobile phase containing NaCl and, where possible, use another salt in the operating buffer. Chlorotrifluorethylene and tetrafluorethylene are the materials in the glass column fittings that come into contact with the mobile phase and sample.
- Good laboratory procedures demand that the analytical column be protected by a guard column. TSKgel guardgel kits, containing column hardware and gel packing, are available to pack your own guard column for use with TSKgel affinity columns.
- As with all columns used with gradient elution, affinity columns should be washed with final elution buffer prior to their re-equilibration with initial (binding) buffer. Always wash the column and the LC system with halide-free buffer at the end of the day.
- The recommended pH range for some TSKgel affinity columns is not as large as that for the base gel. Solvents outside of this pH range can be utilized for clean-up by injecting small volumes (100 µL) of the cleaning solution as part of a standard mobile phase. For the most appropriate clean-up procedure, consult the Operating Conditions and Specifications (OCS) Sheet that is shipped with each column.
- Column shipping solvents are: distilled water (TSKgel Boronate-5PW and TSKgel Heparin-5PW); 10 mmol/L acetate buffer, pH 4.5 (TSKgel Chelate-5PW); and acetone (TSKgel Tresyl-5PW).
- TSKgel affinity columns (except TSKgel Tresyl-5PW columns, since the ligand is activated) are supplied with an Inspection Data Sheet, which includes a QC chromatogram and test data, and an OCS Sheet summarizing the recommended operating conditions for optimum column performance.
- A separate TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems, can be downloaded from the Tosoh Bioscience LLC website (www.tosohbioscience.com).

About

Affinity Chromatography (AFC) offers the greatest potential specificity and selectivity for the isolation or purification of biomolecules. Almost all biological molecules can be purified on the basis of a specific interaction between their chemical or biological structure and a suitable affinity ligand.

In AFC, the target molecule is specifically and reversibly adsorbed by a complementary ligand and immobilized on a matrix. Examples of a complementary ligand include an inhibitor, substrate analog or cofactor, or an antibody which specifically recognizes the target molecule. The selectivity is often based on spatial recognition, a 'lock-and-key' mechanism.

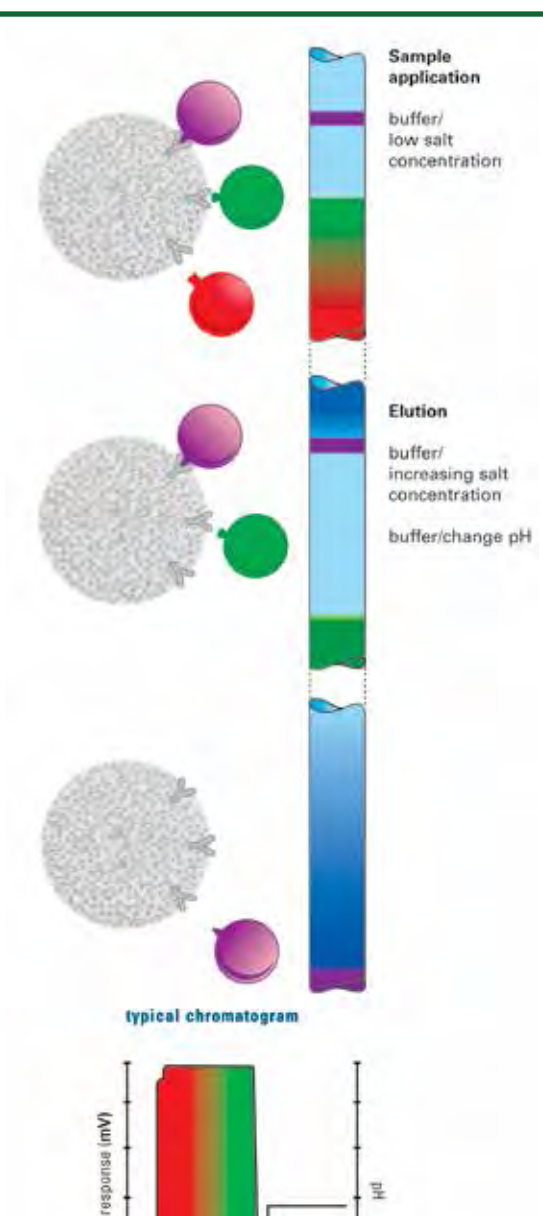
The adsorbed molecule is subsequently eluted either by competitive displacement or a conformation change through a shift in pH or ionic strength. Typical molecular pairs are antigens and antibodies, enzymes and coenzymes, and sugars with lectins.

Purification of several thousand-fold may be obtained due to the high selectivity of the affinity interactions. Although affinity chromatography is not specific, in that no enzyme interacts with only one substrate, it is the most selective method for separating proteins. The features and benefits of AFC are detailed in Table 1 below.

Table 1: Features and benefits of Affinity Chromatography

Features	Benefits
High size exclusion limit (>5 × 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 polymer 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 columns offered in PEEK hardware	Eliminates undesirable interactions with column hardware

Figure 1: Affinity Chromatography



TSKgel Affinity Chromatography Columns

The TSKgel affinity chromatography column line consists of three group-specific stationary phases: Boronate-5PW, Chelate-5PW, and Heparin-5PW, as well as one with a chemically-activated functionality, Tresyl-5PW. All analytical TSKgel AFC columns are based on the well-established 10 µm rigid TSKgel G5000PW resin. This resin features 100 nm pores that have an estimated exclusion limit of 1 million Dalton, along with excellent stability from pH 2 to 9.

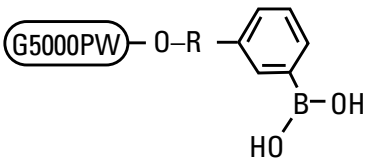
The structures of the available functional ligands are shown in Figure 2. The choice of a specific ligand is dictated by the expected interaction between the sample and the bonded phase. Table 2 lists well known applications for each type of TSKgel affinity column.

Table 2: Applications of TSKgel Affinity Chromatography columns

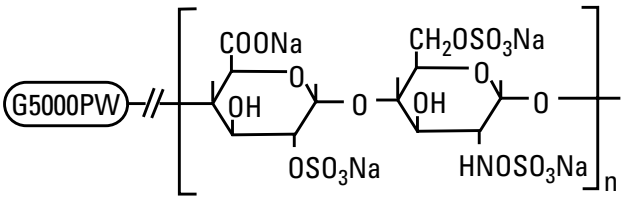
Group Specific Ligands	Application
TSKgel Boronate-5PW	carbohydrates, nucleic acids, nucleosides, nucleotides, catecholamines
TSKgel Chelate-5PW	immunoglobulins, transferrin lectins, milk proteins, membrane proteins, peptides
TSKgel Heparin-5PW	blood clotting factors: antithrombin III, Factor VII, Factor IX, etc., anti-heparin proteins: glycoproteins, endoglycosidase, hyaluronidase, lipases, growth factors, RNA polymerases, other nucleic acid-binding proteins
Activated Ligands	**Application**
TSKgel Tresyl-5PW	glycoproteins, antigens

Figure 2: Structure of TSKgel Affinity ligands

TSKgel Boronate-5PW

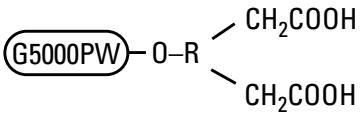


TSKgel Heparin-5PW

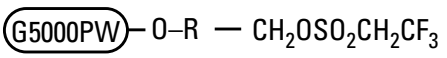


Approximate Ligand Density: 5 g/L

TSKgel Chelate-5PW



TSKgel Tresyl-5PW



About: TSKgel Boronate-5PW Affinity Chromatography Columns

The coupling of *m*-aminophenyl boronate to TSKgel G5000PW polymeric support results in the TSKgel Boronate-5PW column. This coupling makes 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 matrix 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.

Attributes and Applications

Table 3 lists the attributes of TSKgel Boronate-5PW columns. Applications for TSKgel Boronate-5PW columns include nucleic acids, nucleosides, nucleotides, catecholamines, and other biomolecules containing the 1,2 *cis*-diol functionality.

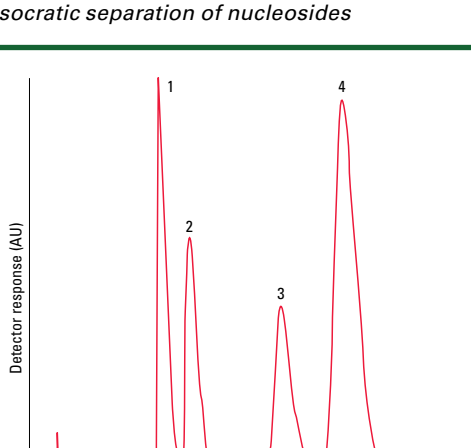
Table 3: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Exclusion limit (base resin, estimate)	<1.0 × 10^7 Da globular proteins
Adsorption capacity	40 mmol/L resin (sorbitol)
Particle size	10 µm
pH stability	2.0-9.0
Functional group	m-aminophenyl boronate

Nucleosides

Nucleosides are glycosylamines consisting of a nucleobase (often referred to as simply *base*) bound to a ribose or deoxyribose sugar via a beta-glycosidic linkage. Examples of nucleosides include cytidine, uridine, adenosine, guanosine, thymidine, and inosine. Figure 3 shows a TSKgel Boronate-5PW column for the selective separation of nucleosides using an isocratic mobile phase.

Figure 3: Isocratic separation of nucleosides

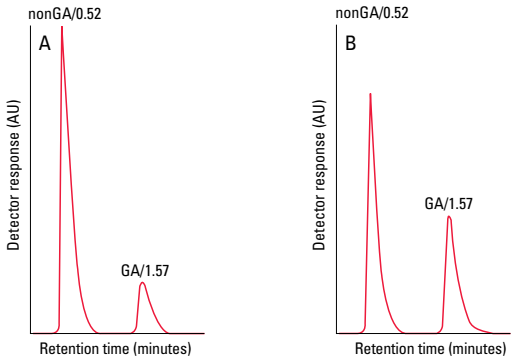


Column:	**TSKgel Boronate-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	0.1 mol/L phosphate buffer, pH 8.0
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Samples:	1. cytidine 2. uridine 3. guanosine 4. adenosine

Glycated Albumin in Human Serum

Glycated (glycosilated) and non-glycated (non-glycosilated) proteins in human serum were rapidly analyzed within 4 minutes using a TSKgel Boronate-5PW column. The amount of glycated human serum albumin provides useful information on short term blood glucose control in diabetic patients. Figure 4 shows chromatograms of pooled serum samples from normal adults and diabetic patients, respectively. Non-glycated and glycated albumin were eluted at 0.52 and 1.57 minutes as sharp peaks, and were well separated.

Figure 4: Glycated human serum albumin analysis

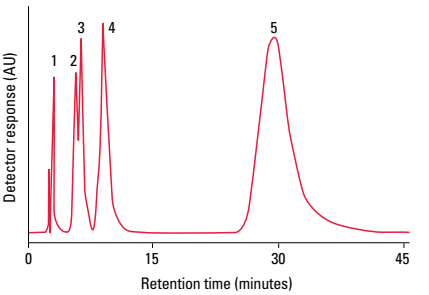


Column:	**TSKgel Boronate-5PW, 10 µm, 4.6 mm ID × 10 cm**
Mobile phase:	50 mmol/L glycine-NaOH buffer, pH 7.5, 200 mmol/L magnesium chloride and 0.05% sodium azide with a step gradient elution of sorbitol from 0 to 200 mmol/L
Flow rate:	0.8 mL/min
Temperature:	37 °C
Injection vol.:	1 µL after a 2 minute equilibration of the column with the starting eluent. The starting eluent was delivered for 1 minute to elute unbound non-glycated proteins. The eluent containing sorbitol was then delivered for 1 minute to elute glycated proteins bound on the column.
Samples:	pooled serum; A. normal adults B. diabetic patients

Catecholamines

Catecholamines are "fight-or-flight" hormones that are released by the adrenal glands in response to stress. They are called catecholamines because they contain a catechol group and are derived from the amino acid tyrosine. Figure 5 demonstrates the analysis of catecholamines on a TSKgel Boronate-5PW column.

Figure 5: Analysis of catecholamines



Column:	**TSKgel Boronate-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	0.1 mol/L phosphate buffer, pH 6.5
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Samples:	1. tyrosine 2. normetanephrine 3. metanephrine 4. DOPA 5. epinephrine

About: TSKgel Chelate-5PW Affinity Chromatography Columns

TSKgel Chelate-5PW columns contain iminodiacetic acid (IDA) groups that are covalently bonded to the TSKgel G5000PW polymeric support. Prior to chromatography, a metal ion, such as Zn^{2+}, Ni^{2+} or Cu^{2+}, is chelated to the IDA group. The selected metal ion is fixed at three coordinating sites on the IDA group. Therefore the target molecule can be tightly bound at three free binding sites at the metal ion. Because of the high concentration of the fixed metal ion (20 µmol metal ion per mL gel), the TSKgel Chelate-5PW column has high binding capacity for the target molecules.

Peptides and proteins containing histidine residues will normally adsorb to these chelated ions at neutral pH. A buffer containing imidazole or glycine is used for protein desorption. The key to making successful use of this retention mechanism is selecting the proper metal ion and elution buffer. Cu^{2+} interacts more strongly with proteins, while Zn^{2+} usually enhances resolution. With Zn^{2+} the column is loaded to saturation, while stronger binding Cu^{2+} is loaded to about half the total capacity. Gradients of increasing imidazole or glycine concentrations or decreasing pH are often used for protein elution. Glycine (in HEPES buffer) is a strong eluent that also desorbs the metal ion, making it necessary to regenerate the column after each run. Imidazole (in phosphate buffer) is a weak eluent, allowing several runs before column regeneration is necessary.

Attributes and Applications

Table 4 lists the attributes of TSKgel Chelate-5PW columns. Applications for TSKgel Chelate-5PW columns include: the analysis of lectins, serum proteins such as immunoglobulins and transferrin, milk proteins, membrane proteins, and peptides.

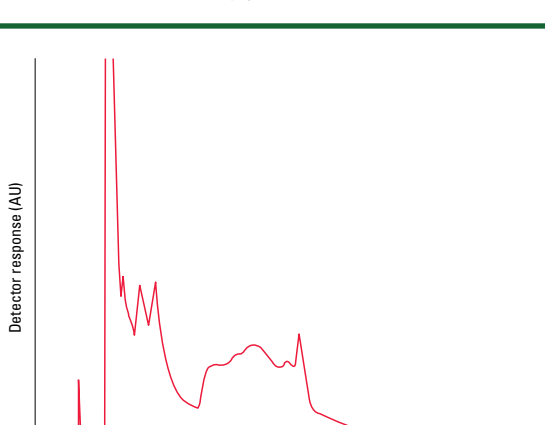
Table 4: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Exclusion limit (base resin, estimate)	$<1.0 \times 10^7$ Da globular proteins
Ligand concentration	20 mmol/L resin
Particle size	10 µm and 13 µm
pH stability	2.0-12.0
Functional group	iminodiacetic acid

Monoclonal Antibody

Figure 6 demonstrates monoclonal antibody (mAb) purification from culture supernatant on a TSKgel Chelate-5PW column loaded with Zn^{2+} ion. This figure shows that the mAb (IgG_1) is eluted in about 32 minutes by pH gradient elution and is well separated from other impurities.

Figure 6: Monoclonal antibody purification

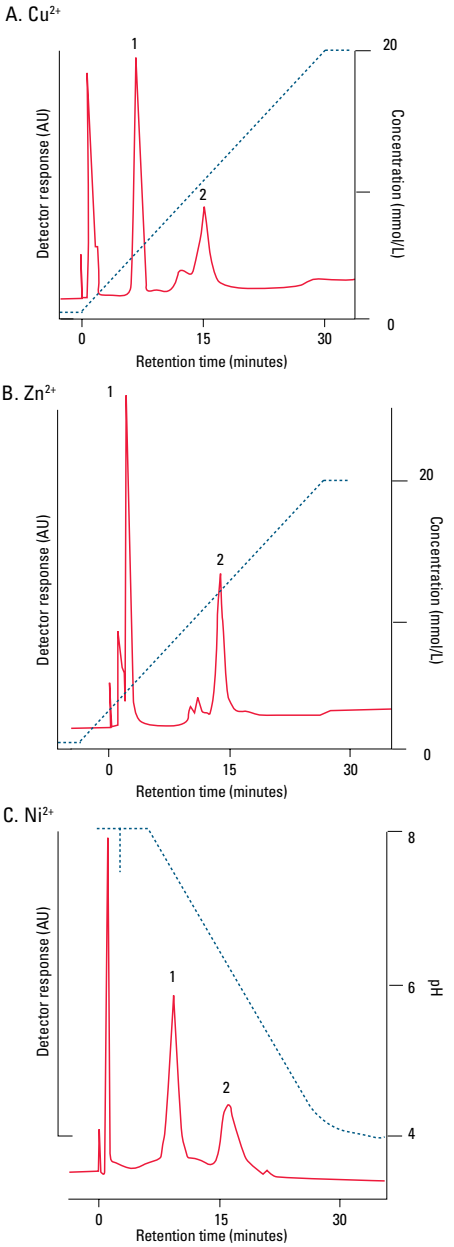


Column:	**TSKgel Chelate-5PW (Zn^{2+}), 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	A: 20 mmol/L Tris-HCl, pH 8.0, + 0.5 mol/L NaCl B: A + 200 mmol/L glycine
Gradient:	30 min (A→B), linear
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Sample:	Anti-HLA-A, B, C (IgG_1), NS-1 cultured supernatant

Standard Proteins

Retention and peak shape of two globular proteins on Zn-, Cu-, and Ni-loaded TSKgel Chelate-5PW columns are compared in Figure 7.

Figure 7: Analyses of ribonuclease A and transferrin

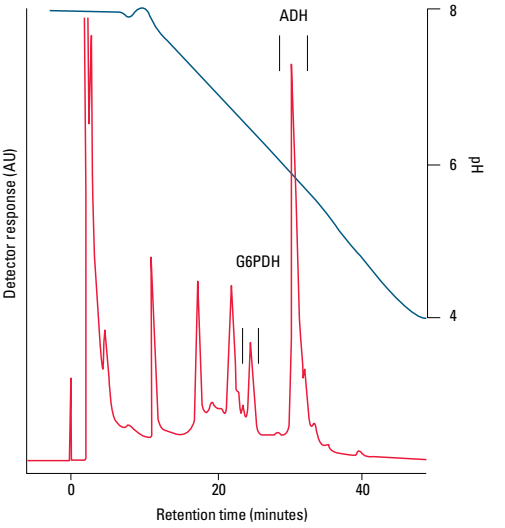


Column:	**TSKgel Chelate-5PW, 10 µm, 5 mm ID × 5 cm, glass**
Metal ions:	A: Cu^{2+} B: Zn^{2+} C: Ni^{2+}, all saturated
Mobile phase:	A and B: 30 min linear gradient from 1 mmol/L to 20 mmol/L imidazole in 20 mmol/L HEPES-NaOH buffer, pH 8.0, containing 0.5 mol/L NaCl C: 30 min linear pH gradient from 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
Samples:	1. ribonuclease A (bovine) 2. transferrin (human)

Yeast Enzymes

A TSKgel Chelate-5PW column was used in Figure 8 to recover yeast enzymes with high activity and yield.

Figure 8: Analysis of yeast enzymes



Column:	**TSKgel Chelate-5PW, 10 µm, 8 mm ID × 7.5 cm, glass**
Metal ions:	Zn^{2+}, saturated
Mobile phase:	40 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:	1.0 mL/min
Detection:	UV @ 280 nm
Recovery:	G6PDH*: 90%, and ADH*: 97% of enzymatic activity
Sample:	yeast enzyme concentrate
Purification:	G6PDH: 8.7-fold, and ADH: 3.9-fold

*G6PDH: glucose-6-phosphate dehydrogenase
*ADH: alcohol dehydrogenase

About: TSKgel Heparin-5PW Affinity Chromatography Columns

TSKgel Heparin-5PW columns are made by immobilizing heparin from porcine intestinal mucosa on a TSKgel G5000PW polymeric support. Heparin is a 1.6×10^4 Da polysaccharide consisting of D-glucosamine and D-glucuronic acid, linked through α-1, 4-glycoside linkage. Immobilized heparin interacts with proteins via two modes:

- On one site, the acidic character of O-sulfate and N-sulfate groups make TSKgel Heparin-5PW a cation exchange resin with elution strength similar to that of the strong cation exchange resin TSKgel SP-5PW, but with a different selectivity for basic proteins. The heparin ligand reduces the effective pH range for this column to between 5.5 and 9.0.
- On the other site, heparin interacts as a specific affinity ligand as follows: as an anti-blood clotting agent in tissue, heparin prevents the formation of thrombin and fibrin by interacting with prothrombin and fibrinogen.

Attributes and Applications

Table 5 lists the attributes of TSKgel Heparin-5PW columns. Typical applications using TSKgel Heparin-5PW columns include the separation of blood clotting factors such as antithrombin III, Factor VII, Factor IX, etc., and anti-heparin proteins such as glycoproteins, endoglycosidase, hyaluronidase. TSKgel Heparin-5PW columns are also used to separate lipases, growth factors, RNA polymerases, and other nucleic acid-binding proteins.

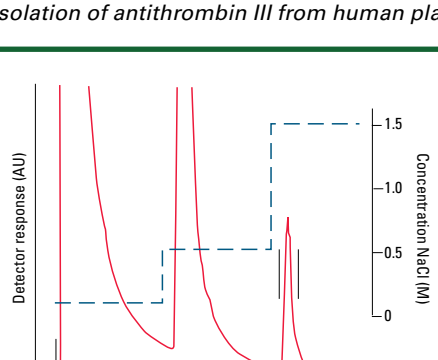
Table 5: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Exclusion limit (base resin, estimate)	$<1.0 \times 10^7$ Da globular proteins
Adsorption capacity for antithrombin III	>1.8 g/L resin
Particle size	10 µm
pH stability	5.5-9.0
Functional group	heparin

Antithrombin III from Human Plasma

Heparin binds to the enzyme inhibitor antithrombin causing a conformational change, resulting in its activation through an increase in the flexibility of its reactive site loop. The activated antithrombin then inactivates thrombin and other proteases involved in blood clotting, most notably factor Xa. The step gradient purification of antithrombin from plasma is illustrated in Figure 9, demonstrating the affinity properties of a TSKgel Heparin-5PW column.

Figure 9: Isolation of antithrombin III from human plasma



Column:	**TSKgel Heparin-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	0.15 mol/L NaCl in 0.02 mol/L Tris-HCl, pH 7.5, followed by a step gradient to 0.5 mol/L NaCl at 30 min, followed by a step gradient to 1.5 mol/L NaCl at 60 min
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Recovery:	Antithrombin III activity was quantitatively recovered from the fraction collected between the vertical lines.
Sample:	human plasma, 0.5 mL

About: TSKgel Tresyl-5PW Affinity Chromatography Columns

Unlike other TSKgel affinity columns, the TSKgel Tresyl-5PW columns which are derivatized with the 2,2,2-trifluroethanesulfonyl ligand, require 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 columns 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 g/L 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 g/L resin.

Attributes and Applications

Table 6 lists the attributes of TSKgel Tresyl-5PW columns. Examples of the wide range of applications using TSKgel Tresyl-5PW columns include the binding of such ligands as concanavalin A (a lipoprotein lectin that binds to glycoproteins), numerous antibodies, and enzymes.

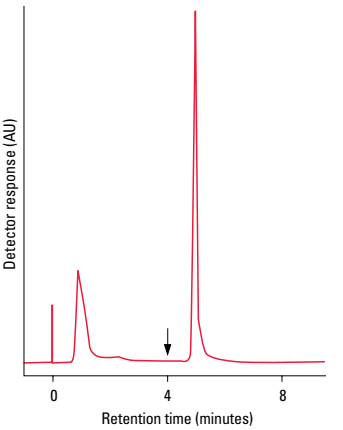
Table 6: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Particle size (mean)	10 µm
pH stability	2.0-12.0
Ligand concentration	ca. 20 mmol/L resin
Adsorption capacity	>60 mg/g dry resin (coupling capacity with soybean tryspin inhibitor)
Exclusion limit (base resin, estimate)	$<1.0 \times 10^7$ Da globular proteins
Active group	tresyl

Peroxidase on Concanavalin A

Concanavalin A is a lipoprotein lectin that binds to glycoproteins such as peroxidase. TSKgel Tresyl-5PW is activated by binding concanavalin A to the resin. The chromatogram in Figure 10 shows the purification of peroxidase by the concanavalin A ligand coupled to the TSKgel Tresyl-5PW affinity support resin.

Figure 10: Purification of peroxidase

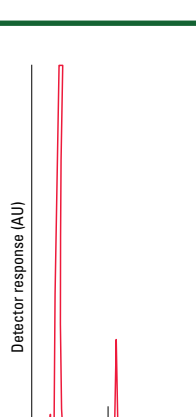


Column:	**TSKgel Tresyl-5PW, 10 µm, 6 mm ID × 4 cm, modified with concanavalin A**
Binding:	0.05 mol/L acetate buffer, pH 5.0, containing 0.5 mol/L NaCl, and 1 mmol/L each of $CaCl_2$, $MnCl_2$ and $MgCl_2$
Mobile phase:	Step gradient at 4 min (see arrow on diagram) to 25 mmol/L α-methyl-D-glucoside in binding buffer
Flow rate:	1.0 mL/min
Detection:	UV @ 403 nm
Sample:	crude peroxidase, 0.5 mg
Washing step:	Wash TSKgel Tresyl-5PW, 6 mm ID × 4 cm, with dissolved H_2O
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 the residual tresyl groups with 0.1 mol/L Tris-HCl, pH 8.0, at 1.0 mL/min for 1 hr at 25 °C

Human Transferrin

Human transferrin is a plasma protein for iron ion delivery. When human transferrin loaded with iron encounters a transferrin receptor on the surface of a cell, it binds to it and is consequently transported into the cell in a vesicle. The cell will acidify the vesicle, causing human transferrin (TF) to release its iron ions. The purification of human transferrin using a TSKgel Tresyl-5PW column immobilized with an anti-human transferrin antibody is shown in Figure 11.

Figure 11: Purification of human transferrin



Column:	**TSKgel Tresyl-5PW, 10 µm, 6 mm ID × 10 cm**
Mobile phase:	0.1 mol/L phosphate buffer, pH 7.4 eluted by pulse method with 0.1 mol/L citrate-hydrochloride buffer, pH 1.6
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Injection vol.:	20 µL
Sample:	human transferrin in serum