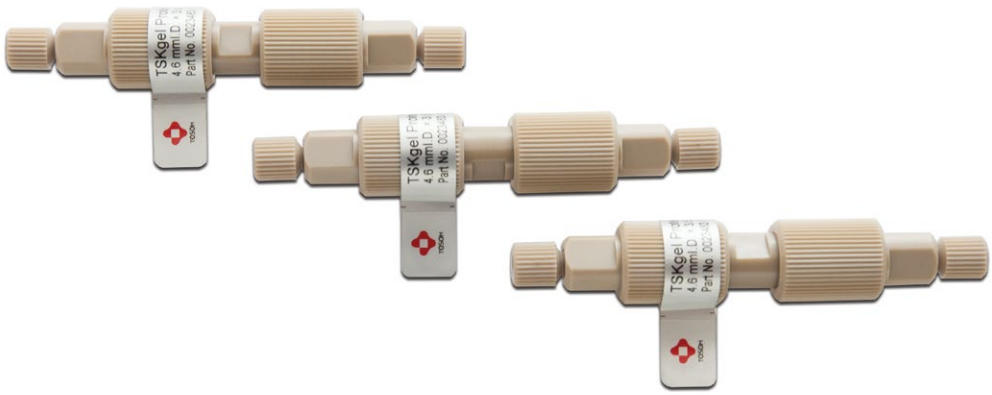
TSKgel Protein A-5PW

Protein A Tips:

- The TSKgel Protein A-5PW column is offered in PEEK hardware.
- 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.
- Column shipping solvent is an aqueous solution containing 20% ethanol.
- The TSKgel Protein A-5PW column is supplied with an Inspection Data Sheet, which includes a QC chromatogram 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).

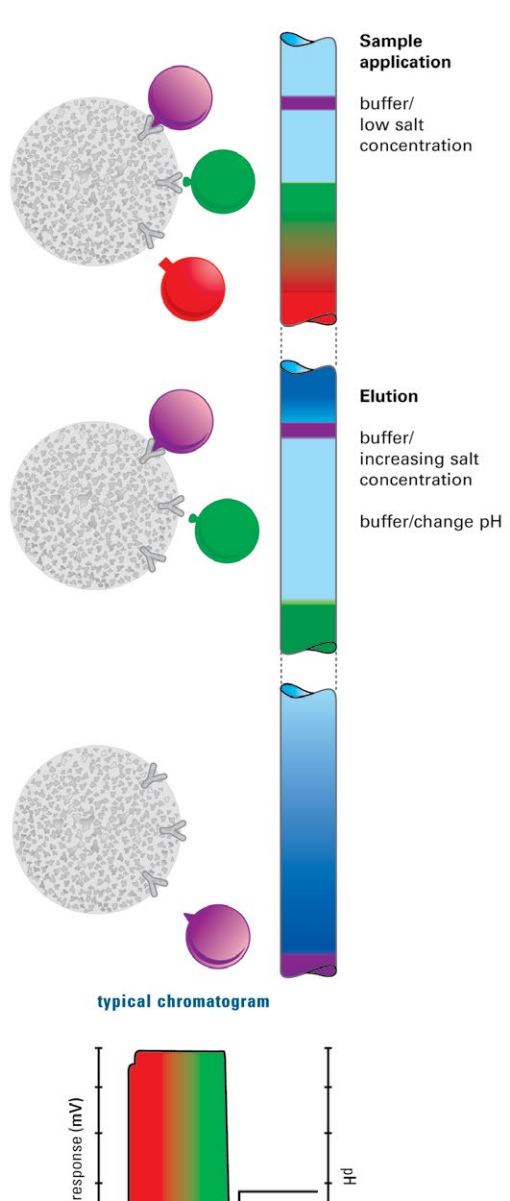


About: Protein A Chromatography

Protein A chromatography, the most widely used type of affinity chromatography, relies on the specific and reversible binding of antibodies to an immobilized ligand; in this case protein A. Protein A is a 56 kDa surface protein native to the cell wall of the bacterium *Staphylococcus aureus*. It is composed of five immunoglobulin-binding domains, each of which are able to bind proteins from many mammalian species, most notably Immunoglobulin G (IgG) through the heavy chain within the Fc region. While the native form of Protein A was used as the ligand for first generation Protein A resins, the recombinant form (rProtein A) produced in *E. coli* is the most prevalent today. The protein A ligand can either bind directly to the Fc region of an antibody or to an Fc tag that has been fused to the target of interest.

In protein A chromatography, crude feed stock is passed through a column under conditions that promote binding. After loading is complete, the column is washed under conditions that do not interrupt the specific interaction between the target and ligand, but that will disrupt any non-specific interactions between process impurities (host cell proteins, etc.) and the stationary phase. The bound protein is then eluted with mobile phase conditions that disrupt the target/ligand interactions. Elution of the target molecule from protein A resin is most commonly accomplished by lowering the pH of the mobile phase, creating an environment whereby the structure of the target molecule is altered in such a way as to inhibit binding. Low pH elution can have a negative effect on protein stability and it is advised that the eluted protein solution be neutralized to minimize aggregation and denaturation.

Figure 1: Protein A Chromatography



About: TSKgel Protein A-5PW Affinity Chromatography Column

TSKgel Protein A-5PW is a 20 µm, 4.6 mm ID × 3.5 cm column for high performance affinity chromatography. Made of PEEK hardware, this column has been designed for the rapid separation and robust quantification of a variety of antibodies. Monoclonal antibodies from harvested cell culture media can be captured and accurately quantitated in less than 2 minutes per injection.

The TSKgel Protein A-5PW column can be used for more than 2,000 injections without regeneration or cleaning. Packed with hydroxylated methacrylic polymer beads, this column is designed with a high degree of crosslinking, which allows a high flow rate for chromatography while still maintaining chromatographic efficiency, peak width and resolution. The recombinant protein A ligand is a code-modified hexamer of the C domain. An enhanced rProtein A ligand is bound to the TSKgel 5PW base bead via multipoint attachment resulting in excellent base stability in 0.1 mol/L NaOH.

The wide range loading capacity of the TSKgel Protein A-5PW column can accurately determine the titer of mAb at various stages of cell culture media processing. The low level of protein A leaching makes this column a good candidate for small scale purification of mAbs for initial characterization. Its reproducibility of injection-after-injection allows the users to accurately monitor the titer of mAb with high confidence.

Attributes

Products attributes of the TSKgel Protein A-5PW column is listed in Table 1.

Table 1: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Particle size	20 µm
pH stability	2.5-7.5
Exclusion limit	1,000 kDa
Ligand	Recombinant protein A, hexamer of C domain

Affinity for Various Antibodies

Because the recombinant protein A ligand of the TSKgel Protein A-5PW column is a code-modified hexamer of the C domain, this column has an affinity for various antibodies that the native protein A and some other recombinant protein A ligands do not possess. For example, it has high affinity for different subclasses of antibodies from rat and goat which native protein A does not have any affinity for, as demonstrated in Table 2.

Table 2: Affinity of protein A to various antibodies

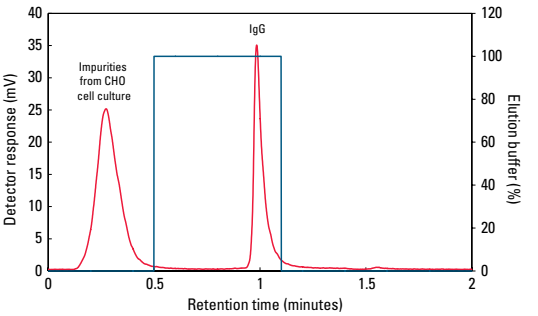
Species	Subclass	Protein A ligand of Protein A-5PW	Native Protein A
Human	IgG_1 IgG_2 IgG_3 IgG_4	+++++ +++++ - +++++	++++ ++++ - ++++
Mouse	IgG_1 IgG_{2a} IgG_{2b} IgG_3	++++ +++++ +++++ ++++	+ ++++ +++ ++
Rat	IgG_1 IgG_{2a} IgG_{2b} IgG_{2c}	++++ - +++ ++++	- - - -
Goat	IgG_s	++++	-
Chicken	IgY	-	-
Rabbit	IgG	+++++	++++

Titer Analysis

Figure 2 shows the fast capture of mAb (human IgG) using a TSKgel Protein A-5PW column. After harvesting, the supernatant from a CHO cell culture is briefly spun to remove cell debris and to concentrate the sample. It is then injected onto a protein A column for titer analysis.

The run was completed within 2 minutes, including bind, wash, elution, and re-equilibration steps. Host cell proteins from the supernatant were not absorbed by the column and so eluted as a flow-through peak. Only IgG was captured and then eluted from the column at approximately a 1 minute retention time. The IgG peak fraction was subjected to size exclusion chromatography using a TSKgel UP-SW3000 column for aggregate and monomer analysis. The result of that analysis indicated that the collected IgG consisted of more than 98% monomer (data not shown).

Figure 2: Fast capture of IgG in the mixture of CHO cell supernatant spiked with IgG

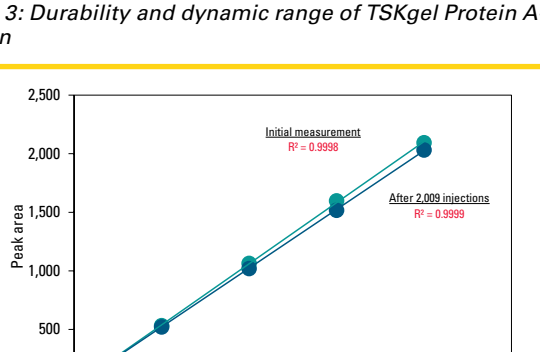


Column:	**TSKgel Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm**
Binding buffer:	20 mmol/L sodium phosphate buffer, pH 7.4
Elution buffer:	20 mmol/L sodium phosphate buffer, pH 2.5
Stepwise gradient:	0 – 0.5 min: binding buffer 0.5 – 1.1 min: elution buffer 1.1 – 2.0 min: binding buffer
Flow rate:	2 mL/min
Detection:	UV @ 280 nm
Sample:	20 µL of CHO cell culture supernatant spiked with polyclonal IgG (0.5 mg/mL)

Durability and Wide Dynamic Range

The high durability and wide dynamic range of the TSKgel Protein A-5PW column is demonstrated in Figure 3. The column was subjected to a linearity analysis test. Purified IgG was initially injected onto the column with subsequent injections of IgG made at different volumes. The column was then used up to 2,009 injections without being cleaned. A linearity analysis test was then repeated. No significant change in the calibration curve for IgG was seen. The column still maintained its high loading capacity with an excellent linearity (R^2 = 0.9999).

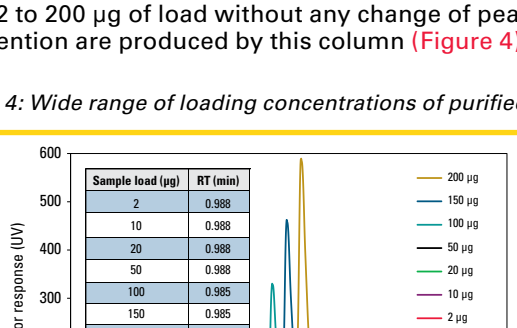
Figure 3: Durability and dynamic range of TSKgel Protein A-5PW column



Wide Dynamic Range and Sensitivity of Detection

Determination of mAb concentration from harvested cell culture supernatant requires a column with good linearity over a wide dynamic range so that the concentrations of mAb can be accurately determined. Similar chromatograms from 2 to 200 µg of load without any change of peak profile or retention are produced by this column (Figure 4).

Figure 4: Wide range of loading concentrations of purified IgG

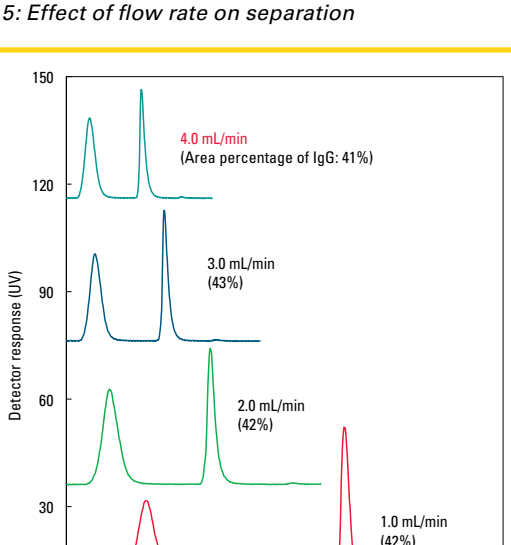


Column:	**TSKgel Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm**
Binding and washing buffer:	20 mmol/L sodium phosphate buffer, pH 7.4
Elution buffer:	20 mmol/L sodium phosphate buffer, pH 2.5
Stepwise gradient:	0 - 0.5 min: binding buffer 0.5 - 1.1 min: elution buffer 1.1 - 2.0 min: binding buffer
Flow rate:	2 mL/min
Detection:	UV @ 280 nm
Sample:	CHO supernatant and IgG

High Flow Rate Tolerance for High Throughput

Four different flow rates (1, 2, 3 and 4 mL/min) were used to demonstrate the high flow rate performance of the TSKgel Protein A-5PW column. Figure 5 shows there is a minimal effect of flow rate on IgG binding or absorbing onto the column. The relative peak area percentages of the unbound (flow-through) protein peak and the bound IgG remained unchanged at different flow rates. Less than 1 minute analysis was available at 4.0 mL/min with a similar peak profile. At 4.0 mL/min, the TSKgel Protein A-5PW column showed a wide dynamic range (2~200 µg) with good linearity (R^2 = 1.0000) for IgG (Figure 6).

Figure 5: Effect of flow rate on separation

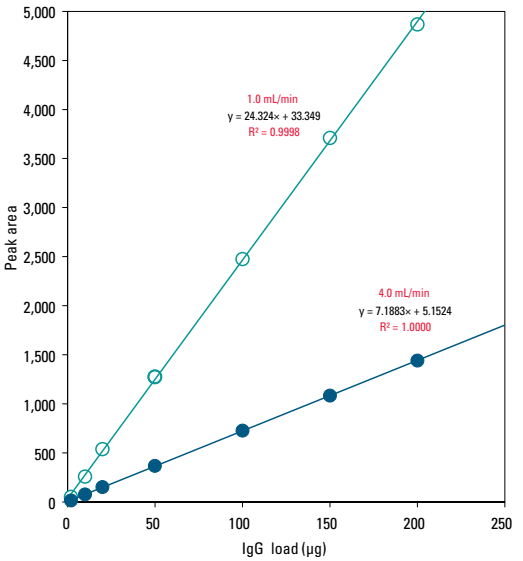


Gradient conditions

Flow rate (mL/min)	Binding buffer (min)	Elution buffer (min)	Binding buffer (min)
4.0	0-0.25	0.25-0.55	0.55-1.00
3.0	0-0.33	0.33-0.73	0.73-1.33
2.0	0-0.50	0.50-1.10	1.10-2.00
1.0	0-1.00	1.00-2.20	2.20-4.00

20 µL of CHO cell supernatant spiked with polyclonal antibody (0.5 mg/mL)

Figure 6: Effect of flow rate on calibration curve

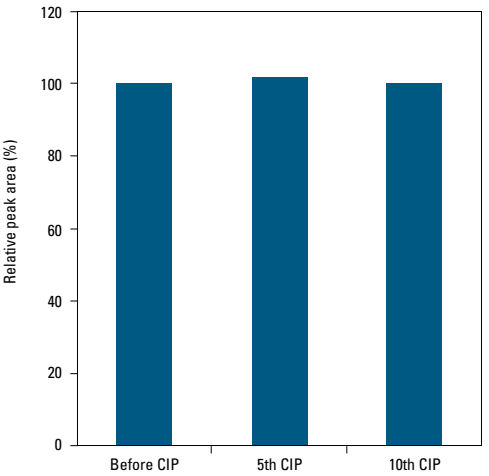


Column:	**TSKgel Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm**
Binding and washing buffer:	20 mmol/L sodium phosphate buffer, pH 7.4
Elution buffer:	20 mmol/L sodium phosphate buffer, pH 2.5
Stepwise gradient:	0 - 0.5 min: binding buffer 0.5 - 1.1 min: elution buffer 1.1 - 2.0 min: binding buffer
Flow rate:	1.0, 2.0, 3.0, 4.0 mL/min
Detection:	UV @ 280 nm
Sample:	CHO supernatant containing 0.5 g/L IgG

Alkaline Stability

A clean-in-place (CIP) study using a polyclonal IgG sample (10 g/L, dissolved in binding buffer) was conducted to test the alkaline stability of the TSKgel Protein A-5PW column. Prior to CIP, IgG was injected onto the column to establish the efficacy of the column for IgG capture. Following this step, 500 µL of 0.1 mol/L NaOH solution was injected onto the column. After the 5th and 10th CIP cycle, the column was injected with polyclonal IgG and the peak area of IgG was integrated and compared to the data prior to the CIP being performed. As demonstrated in Figure 7, the TSKgel Protein A-5PW column shows alkaline stability up to 10 cycles of CIP.

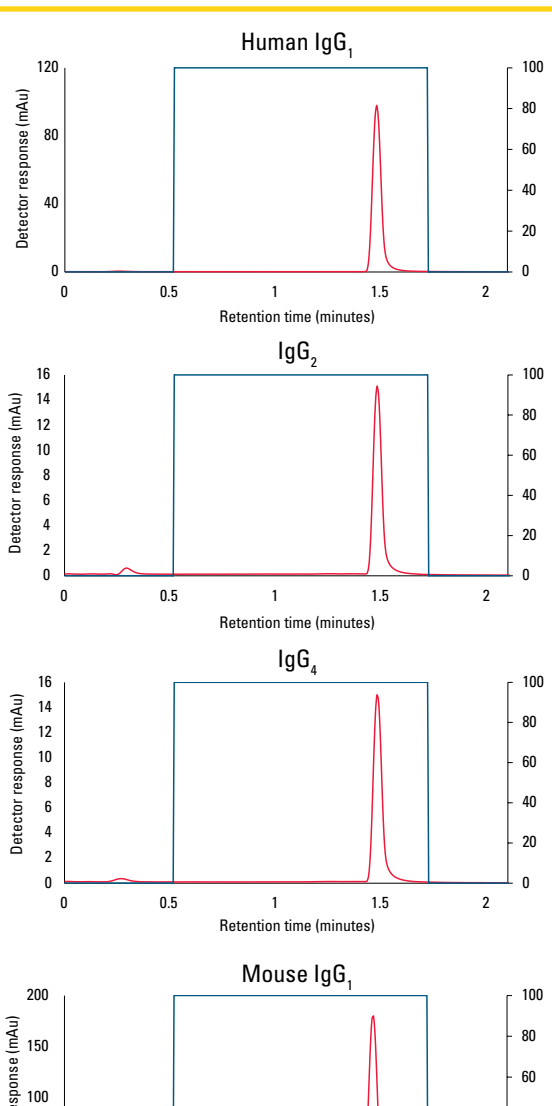
Figure 7: Alkaline stability



Fast Capture of Various IgG Subclasses and Species

Human IgG_1, IgG_2, and IgG_4 and Mouse IgG_1 were subsequently injected onto a TSKgel Protein A-5PW column for titer analysis. Figure 8 shows the fast capture of the various IgG subclasses using this column. The run was completed in less than 2.2 minutes, including bind, wash and re-equilibration steps. The peaks were eluted within the elution step of the chromatographic conditions as shown in the figures.

Figure 8. Fast capture of various IgG species and subclasses using TSKgel Protein A-5PW column

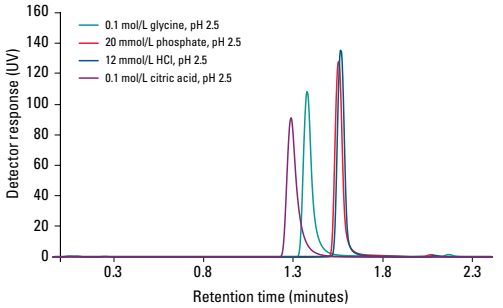


Column:	**TSKgel Protein A-5PW, 4.6 mm ID × 3.5 cm, 20 µm**
Mobile phase:	A. 20 mmol/L sodium phosphate, pH 7.4 B. 12 mmol/L HCl, pH 2.5
Gradient:	equilibration: mobile phase A: 0.0-0.5 min A elution: mobile phase B: 0.51-1.7 min re-equilibration: mobile phase A 1.71-2.2 min
Flow rate:	2.0 mL/min
Detection:	UV @280nm
Temperature:	ambient
Injection vol.:	5 µL
Sample:	as listed

Compatibility with Various Mobile Phases

The TSKgel Protein A-5PW column can be successfully used to analyze mAbs within 2.2 minutes under a variety of mobile phase conditions. A number of elution conditions, specifically 12 mmol/L HCl, pH 2.5; 100 mmol/L citric acid, pH 2.5; 100 mmol/L glycine, pH 2.5; 20 mmol/L phosphate, pH 2.5 were used for eluting the IgGs bound to the TSKgel Protein A-5PW column. The peaks were eluted within the elution step of the chromatographic conditions as shown in Figure 9.

Figure 9. Compatibility with multiple elution buffers



Column:	**TSKgel Protein A-5PW, 4.6 mm ID × 3.5 cm, 20 µm**
Mobile phase:	A. 20 mmol/L sodium phosphate; pH 7.4 B. 12 mmol/L HCl, pH 2.5 100 mmol/L citric acid, pH 2.5 100 mmol/L glycine, pH 2.5 20 mmol/L phosphate, pH 2.5
Gradient:	equilibration: mobile phase A: 0.0-0.5 min A elution: mobile phase B: 0.51-1.7 min re-equilibration: mobile phase A 1.71-2.2 min
Flow rate:	2.0 mL/min
Detection:	UV @280nm
Temperature:	ambient
Injection vol.:	5 µL
Sample:	IgG_1 (5mg/mL)

Ordering Information

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
23483	TSKgel Protein A-5PW, 20 µm, 100 nm	Polymer	PEEK	4.6	3.5