TSKgel® Protein A-5PW Column

Providing reproducible and robust analysis with high loading capacity

Introduction

Early in monoclonal antibody (mAb) development, there are many harvest cell culture samples that must be screened for IgG titer. Affinity protein A columns are often employed to determine the concentration of monoclonal antibody for the optimal time for harvest. This screening process is also used to identify clones that express the most antibodies. If necessary, a partial purification can be accomplished using an affinity protein A column for further analysis.

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.

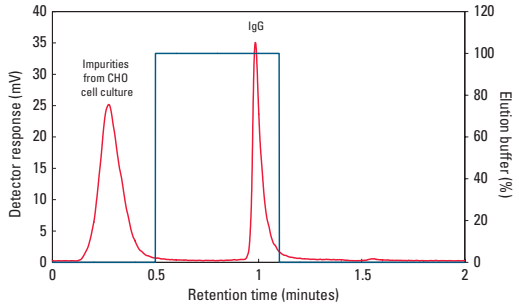
Highlights

- Wide dynamic range with high sample concentration load for mAb quantitation
- Fast analysis: 1-2 min/analysis
- High sensitivity for mAb titer determination
- Long lifetime: >2,000 injections per column with no sign of deterioration

Fast Capture of Monoclonal Antibody

As shown in *Figure 1*, IgG was separated well from impurities in CHO cell culture supernatant by stepwise pH gradient within 2 minutes. All host cell proteins from the supernatant are eluted in a flow-through peak and only IgG is captured and eluted by the column at approximately 1 minute. The IgG peak fraction was collected and subjected to size exclusion chromatography for further testing of its purity and aggregate analysis. The result of the analysis indicated that only IgG was present in this fraction (data not shown).

Figure 1. Rapid separation of IgG from impurities using TSKgel Protein A-5PW column

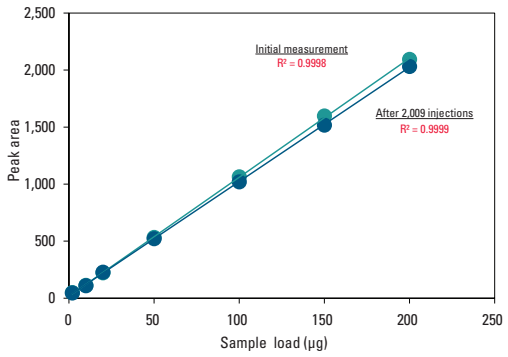


Column:	TSKgel Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm
Binding buffer:	20 mmol/L sodium phosphate buffer, pH 7.4
Elution buffer:	20 mmol/L sodium phosphate buffer, pH 2.5
Stepwise gradient:	0 – 0.5 min: binding buffer
	0.5 – 1.1 min: elution buffer
	1.1 – 2.0 min: binding buffer
Flow rate:	2 mL/min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

Robustness of the Column Design

Figure 2 demonstrates the high durability and the wide dynamic range of the TSKgel Protein A-5PW column. 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 2. Durability and dynamic range of TSKgel Protein A-5PW column



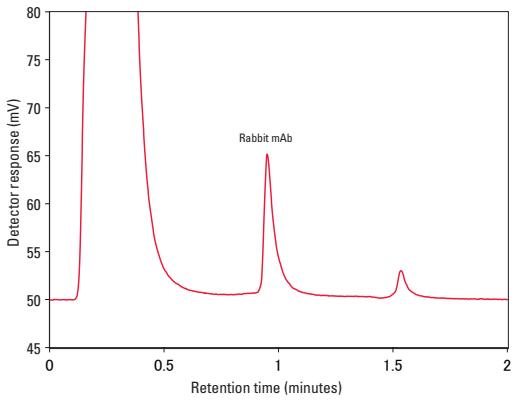
Affinity for Various Antibodies

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 1* and *Figure 3* below.

Table 1. 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	+++++	++++

Figure 3. Separation of rabbit mAb from impurities using TSKgel Protein A-5PW column



Leaching Analysis

With the 4.6 mm ID × 3.5 cm length, the TSKgel Protein-5PW column can also be used for small scale purification. The interference of protein A leaching in a collected IgG sample for characterization purposes makes this analysis cumbersome.

An analysis of an IgG sample was run on both a TSKgel Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm column and a similar competitive 20 µm protein A, 4.6 mm ID × 5 cm column. *Table 2* shows that the protein A ligand from the TSKgel Protein A-5PW column has 2.3 ppm of protein A leaching, which is many folds lower than the competitor's protein A column. This data suggests that the coupling process of protein A onto the TSKgel 5PW base bead is better than its competitor's coupling process.

Table 2. Comparison of protein A leaching with use of TSKgel Protein A-5PW and competitor column

Resin	Concentration (mg/mL)	Protein A (ppm)
TSKgel Protein A-5PW	0.538	2.3
Competitor protein A	0.508	10.8

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Ordering Information

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

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