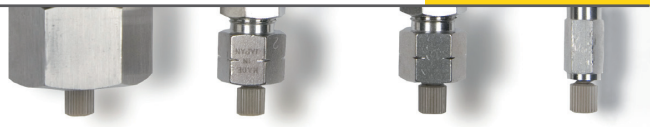

TOSOH

TSKgel® PROTEIN A-5PW AFFINITY HPLC COLUMN REPRODUCIBLE AND ROBUST ANTIBODY TITER ANALYSIS

TSKgel Protein A-5PW expands the line of TSKgel columns for antibody analysis with a high performance affinity chromatography column specifically designed for fast and accurate determination of monoclonal antibody (mAb) concentration during cell line selection or upstream bioprocess optimization and control.

INTRODUCTION

In many stages of mAb development, harvest cell culture samples must be screened for IgG titer. Antibody titer determination by Protein A affinity HPLC is much more robust, reliable and reproducible than enzyme-linked immunosorbent assays (ELISAs). Protein A affinity columns can be employed to determine the concentration of monoclonal antibody for the optimal time for harvest or to identify clones that express the most antibodies. If necessary, a partial purification can be accomplished using a Protein A affinity 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 can be captured and accurately quantitated in less than 2 minutes per injection.

The column can be used for more than 2,000 injections without regeneration or cleaning. Packed with hydroxylated methacrylic polymer beads with a high degree of crosslinking, it allows a high flow rate while still maintaining chromatographic efficiency, peak width and resolution. The recombinant Protein A ligand is a code-modified hexamer of the C domain. This 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 mAb development: from initial screening in R&D to process control in upstream development. Its reproducibility of injection-after-injection allows the users to accurately monitor the titer of mAb with high confidence. In addition, the low level of Protein A leaching makes this column a good candidate for small scale purification of mAbs for initial characterization.

HIGHLIGHTS

- Wide dynamic range
- Fast analysis: 1-2 min/analysis
- High sensitivity for mAb titer determination
- Long lifetime: > 2,000 injections per column

RAPID SEPARATION OF IgG FROM IMPURITIES

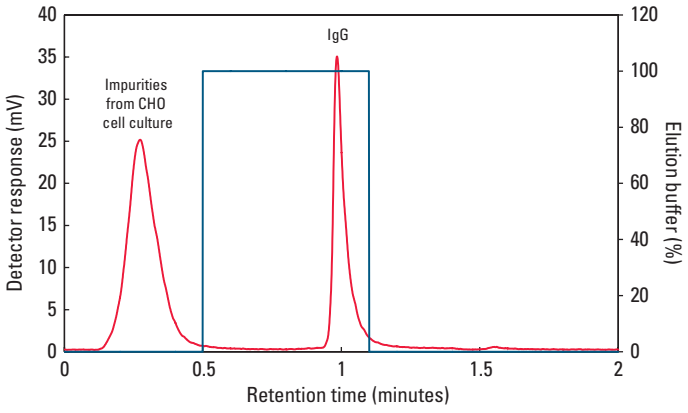

Figure 1

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 CHO cell culture supernatant containing polyclonal IgG (0.5 mg/mL)

DURABILITY AND DYNAMIC RANGE OF TSKgel PROTEIN A-5PW

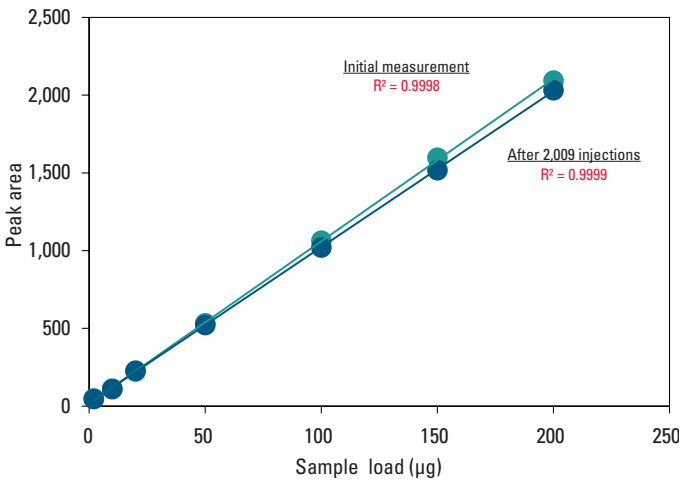

Figure 2

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).

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 colmn 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).

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	+++++	++++

Table 1

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. Figure 3 shows the results for rabbit antibody.

SEPARATION OF RABBIT mAb FROM IMPURITIES USING TSKgel PROTEIN A-5PW

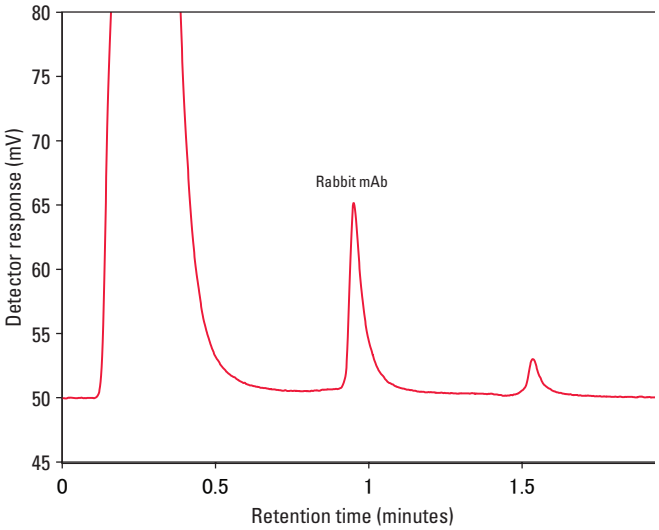

Figure 3

Ordering information

Part-No	Description	Matrix	Housing	Dimensions
0023483	TSKgel Protein A-5PW, 20 µm	Polymer	PEEK	4.6 mm ID x 3.5 cm L

MZ ANALYSENTECHNIK

AUTHORIZED DISTRIBUTOR
MZ-Analysentechnik GmbH, Barcelona-Allee 17• D-55129 Mainz
Tel +49 6131 880 96-0, Fax +49 6131 880 96-20
e-mail: info@mz-at.de, www.mz-at.de