TOSOH

APPLICATION NOTE

FAST MONOCLONAL ANTIBODY TITER DETERMINATION WITH TSKgel® PROTEIN A-5PW

INTRODUCTION

The antibody therapeutics market is enjoying high growth rates, the major areas of therapeutic application being cancer and immune/inflammation-related disorders. Six of the top ten best-selling global drug brands are monoclonal antibody-based. This market is predicated to show continued growth for many years to come, with more monoclonal antibodies (mAbs) designed and produced for treatments of specific diseases.

Early in mAb development many harvested CHO cell supernatant samples must be screened for their mAb titers. Antibody titer determination by Protein A affinity HPLC is much more robust, reliable and reproducible than enzyme-linked immunosorbent assays (ELISAs). During upstream processing the optimal time for harvesting mAbs from cell culture supernatant can also be detected by using Protein A HPLC. In addition, partial purification of mAb can be accomplished using an Protein A affinity column initially to establish the right cell lines and to partially characterize a newly produced mAb. With many samples to be screened for different purposes, a reliable and high throughput column is needed for this workflow.

FAST CAPTURE OF IgG FROM CHO CELL SUPERNATANT

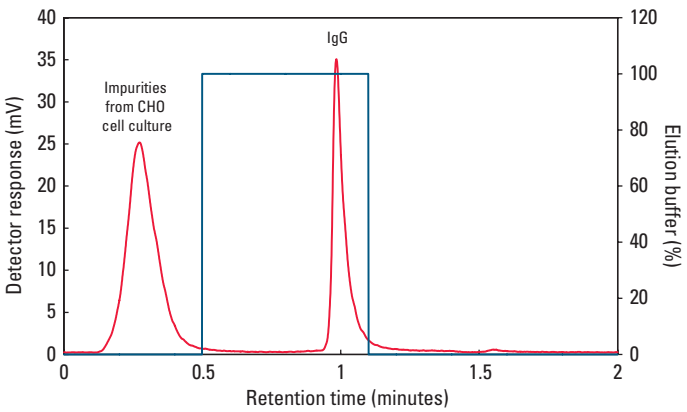


Figure 1

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 of CHO cell culture supernatant spiked with polyclonal IgG (0.5 mg/mL)

In this application note, the quick capture and accurate titer analysis over a wide concentration range of mAb is demonstrated using a TSKgel Protein A-5PW analytical column. Packed with 20 µm hydroxylated methacrylic polymer beads coupled with a recombinant Protein A ligand (a code-modified hexamer of the C domain), this 4.6 mm ID × 3.5 cm PEEK column can be used with high flow rates for high throughput analysis and still maintains chromatographic efficiency, peak width and resolution. In addition, the TSKgel Protein A-5PW column can perform for more than 2,000 injections with no sign of deterioration and without cleaning.

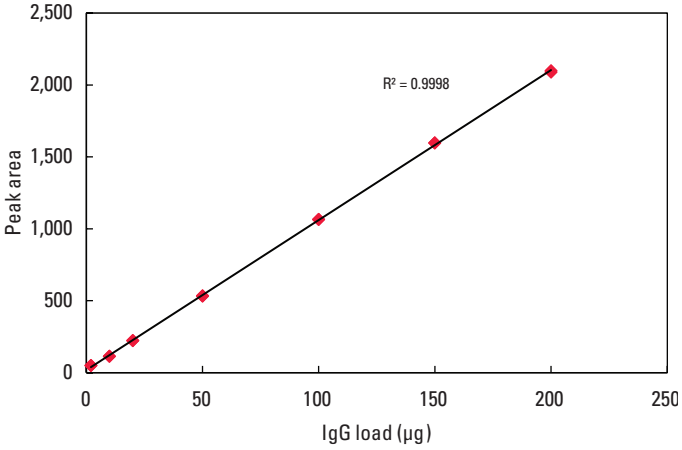
EXPERIMENTAL HPLC CONDITIONS

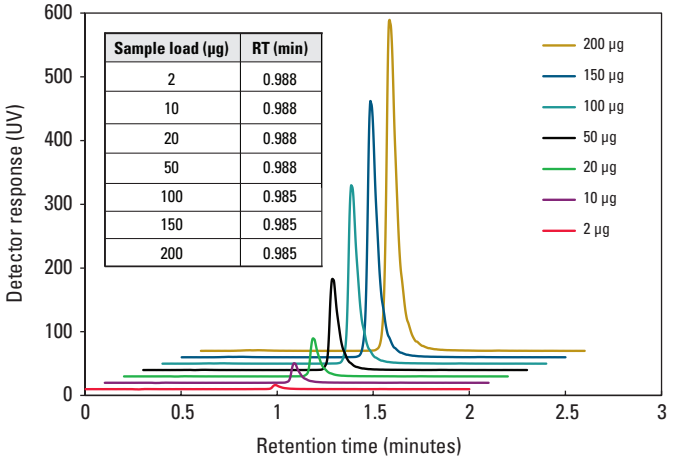
Columns:	TSKgel Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm (PEEK), P/N 0023483
Binding and washing buffer:	20 mmol/L sodium phosphate buffer, pH 7.4
Elution buffer:	20 mmol/L sodium phosphate buffer, pH 2.5 Note: IgG can also be eluted with 12 mmol/L HCl, 20-100 mmol/L citric acid, pH 2.5-3.5, 20-100 mmol/L glycine, pH 2.5-3.5, 5-10% acetic acid
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 as shown in the chromatograms

RESULTS AND DISCUSSION

Figure 1 shows the fast capture of mAb (human IgG) using a TSKgel Protein A-5PW column. 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).

WIDE DYNAMIC RANGE OF TSKgel PROTEIN A-5PW

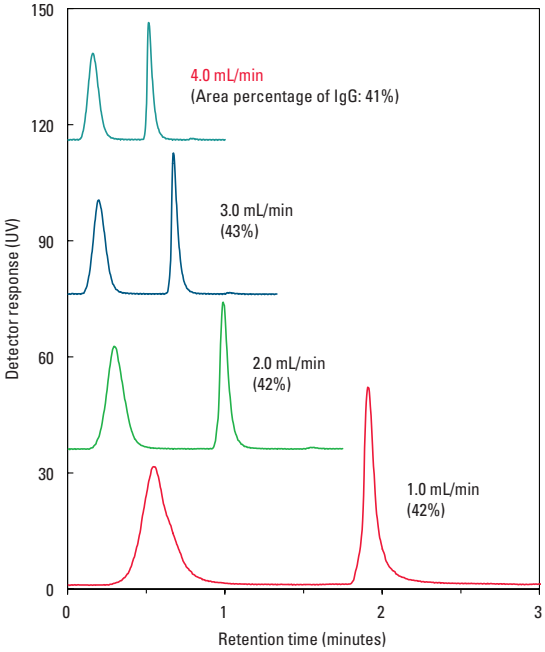




Sample load (µg)	RT (min)
2	0.988
10	0.988
20	0.988
50	0.988
100	0.985
150	0.985
200	0.985

Figure 2 & 3

VARYING FLOW RATES USED ON TSKgel PROTEIN A-5PW



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 5

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. Figure 2 is a calibration curve with good linearity (R^2 >0.999) showing the wide dynamic loading range of the TSKgel Protein A-5PW column for a polyclonal IgG (0.1 - 10 g/L). Similar chromatograms from 2 to 200 µg without any change of peak profile or retention are produced by this column (Figure 3).

DURABILITY AND DYNAMIC RANGE OF TSKgel PROTEIN A-5PW

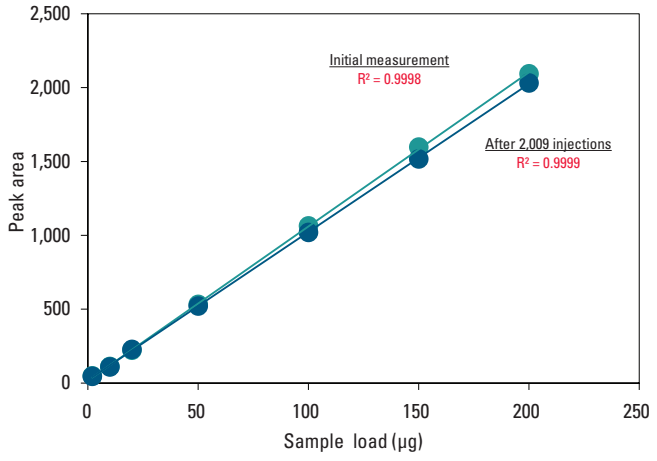


Figure 4

Figure 4 demonstrates the high durability and again the wide dynamic load 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).

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.

CONCLUSION

The TSKgel Protein A-5PW column can capture and accurately quantitate monoclonal antibody from harvested cell culture media in less than 2 minutes. The wide range loading capacity of this column allows the titer of mAb to be determined at various stages of development. Because of the high flow rate tolerance and durability of the TSKgel Protein A-5PW column, high throughput analysis can be accomplished.