Application Note

BioPharma

Agilent

Trusted Answers

Improved Lifetime of Bio-Monolith Protein A Columns for Titer Determination

Authors

Andrew Coffey and
Sandeep Kondaveeti
Agilent Technologies, Inc.

Abstract

Bio-Monolith protein A columns have proven to be excellent tools for rapid screening of IgG concentration in cell culture supernatant. The wide dynamic range (from less than 1 µg to more than 150 µg on column injection) means that fast and accurate IgG concentrations can be obtained from crude cell culture supernatant for rapid clone screening or determination of optimal time to harvest.

Previously, crude samples like these have often led to limited column lifetime due to column fouling, issues with nonspecific binding, or carryover. Procedures such as clean-in-place (CIP) can aid recovery of column performance, but this application note demonstrates enhanced lifetime without the need for intermediate CIP steps.

Introduction

Titer determination is the measurement of protein concentration, an important feature of any cell culture procedure. For monoclonal antibodies, affinity chromatography using protein A provides the ideal tool for capture and release (and quantification) of the target immunoglobulin IgG. Rapid affinity chromatography is essential for determining the most productive clone, assessing the impact of varying process parameters, or monitoring the progress of a batch to determine the optimal time to harvest. However, crude cell culture supernatant is a complex sample matrix and can rapidly foul conventional protein A affinity columns. Alternatively, a wide pore monolithic stationary phase containing 1.2 to 1.5 µm channels provides excellent robustness with minimal carryover. The Bio-Monolith protein A column contains highly crosslinked poly(glycidylmethacrylate-co-ethylenedimethacrylate) polymer coated with native protein A. It has been shown to give good performance for µg quantities of IgG1 and IgG2.[1] This is enough for titer determination, for very small-scale purification and subsequent analysis, or even for combination with complementary techniques in 2D-LC configurations.

This application note demonstrates that extended column lifetime, even before performing CIP, benefits customers by allowing more runs to be made than previously possible.

Experimental

Reagents and chemicals

All reagents were HPLC grade or higher.

Equipment and materials

All chemicals and reagents were HPLC grade or higher and were obtained from Sigma-Aldrich (now Merck) or VWR Scientific. Water was purified using a Milli-Q A10 (Millipore).

Instrumentation

Agilent 1260 Infinity II Bio-inert LC instrument comprising:

- Agilent 1260 Infinity II Bio-inert pump (p/n G5654A)
- Agilent 1260 Infinity II Bio-inert multisampler (p/n G5668A) with sample cooler (option 100)
- Agilent 1260 Infinity II multicolumn thermostat (p/n G7116A) with bio-inert heat exchanger (option 019)
- Agilent 1260 Infinity II diode array detector WR (p/n G7115A) with bio-inert flow cell (option 028)

Software

OpenLab 2.3 CDS

Method conditions

HPLC Conditions	
Column	Bio-Monolith protein A
Binding Buffer (Eluent A)	50 mM sodium phosphate, pH 7.4
Eluting Buffer (Eluent B)	100 mM citric acid, pH 2.6
Gradient Profile	Time (min) %B 0.0 to 0.5 0 (binding) 0.6 to 1.8 100 (elution) 1.9 to 4.0 0 (regeneration)
Flow Rate	1 mL/min
Column Temperature	24 °C
Detection	UV, 280 nm
Injection Volume	As required (2 to 40 µL)

Sample

The sample solution consisted of CHO cell lysate supernatant diluted 1:3 v/v with phosphate buffer and spiked with human IgG to 5 mg/mL final concentration.

Blanks of CHO cell lysate supernatant, diluted 1:3 v/v with phosphate buffer and phosphate buffer alone, were also used to determine protein carryover.

Results and Discussion

Before performing a series of injections to determine the performance over a longer period, the Bio-Monolith protein A column was subjected to a series of injections to create a calibration curve. This was achieved by making a series of injections of a CHO cell supernatant solution spiked with 5 mg/mL human IgG, shown in Figure 1. Following flushing with binding buffer (100% mobile phase A, 50 mM sodium phosphate, pH 7.4) the spiked sample was applied with increasing injection volumes using blank injections between each injection of sample. The bound hIgG was then eluted by switching to the elution buffer (100% mobile phase B, 100 mM citric acid, pH 2.6). Under such elution conditions, it was observed that a 200 µg on column injection (shown in orange and excluded from the linear fit) resulted in a peak response >3,000 mAU, which is beyond the linear range of the detector. Plotting the peak area versus quantity of protein injected creates the calibration curve seen in Figure 2.

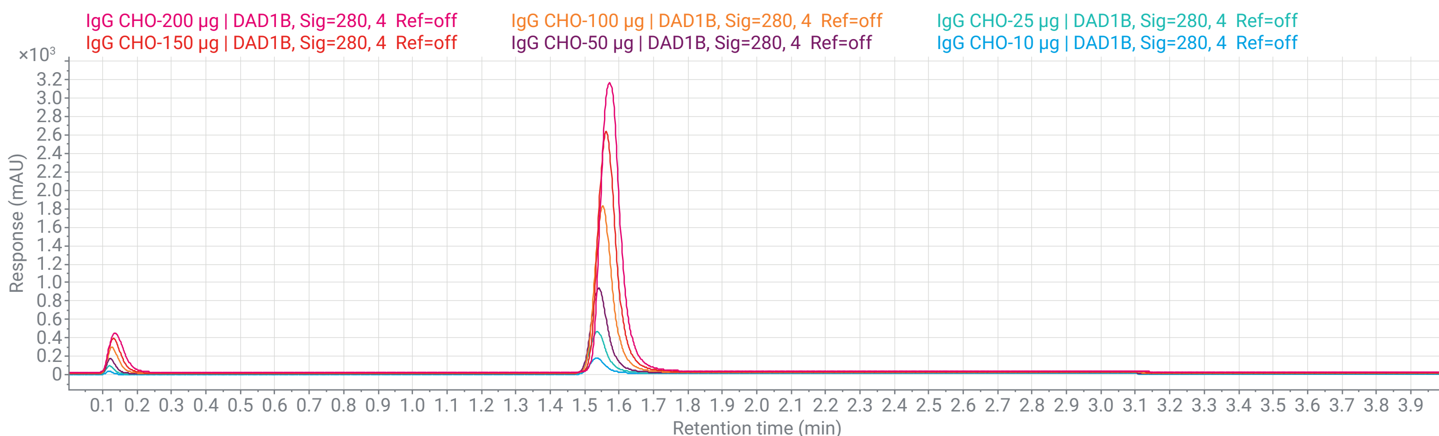
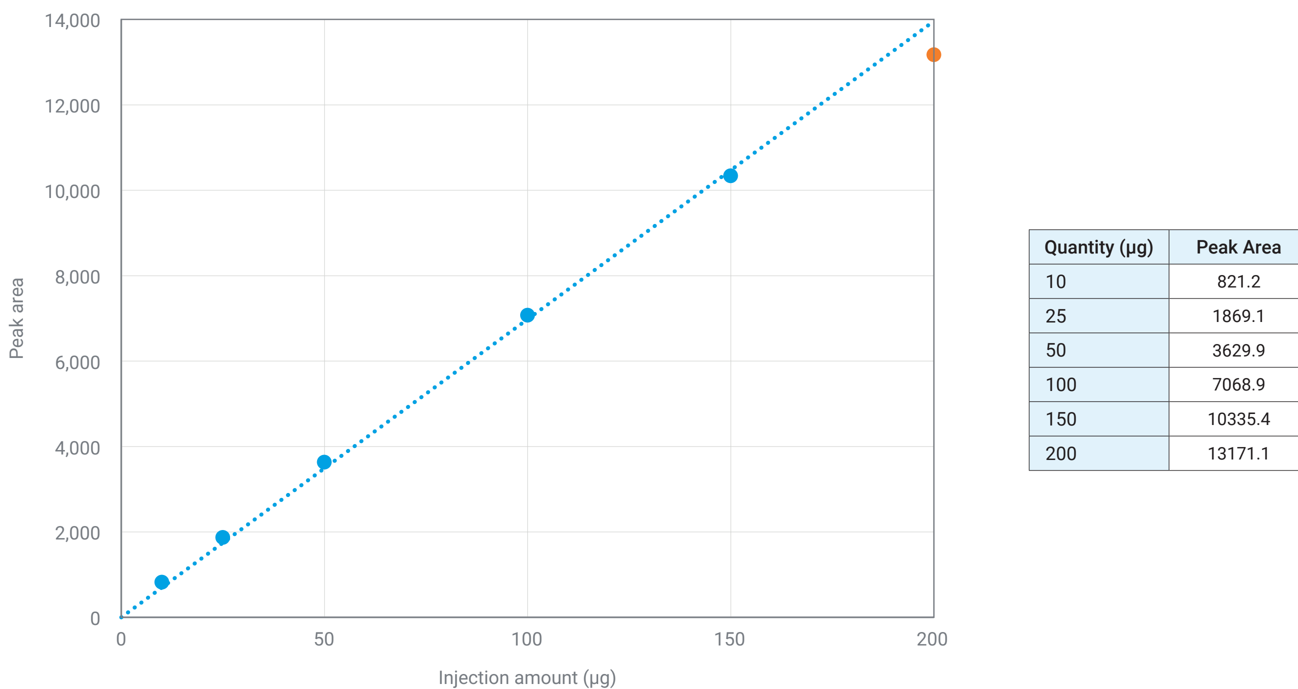


Figure 1. Chromatograms showing increasing injection volumes.



Quantity (µg)	Peak Area
10	821.2
25	1869.1
50	3629.9
100	7068.9
150	10335.4
200	13171.1

Figure 2. Calibration curve for increasing hIgG quantity.

Ensuring detector saturation does not occur may mean further dilution of the cell culture supernatant where protein concentrations are too high.

Following calibration, the Bio-Monolith protein A column was subjected to blank gradients of diluted cell culture supernatant to ensure there was no carryover of IgG or any nonspecific binding of host cell proteins that may coelute with the hIgG (Figure 3). Following that, a sequence was run comprising 50 injections of the diluted cell culture supernatant spiked with hIgG, then additional blank injections were carried out repeatedly to over 800 injections. An overlay of injections 1, 250, 500, and 750 is shown in Figure 4. During the experiment (carried out over the course of four days), there was no noticeable deterioration in peak shape. Peak height and tailing factors remained consistent without the need to perform column cleaning, due to the crude nature of the samples.

Figure 5 shows the first blank injection following 50 injections of the diluted cell culture supernatant spiked with hIgG. Even after 50 injections, there is very little carry over of IgG, <0.2%. This then disappears after a subsequent blank gradient.

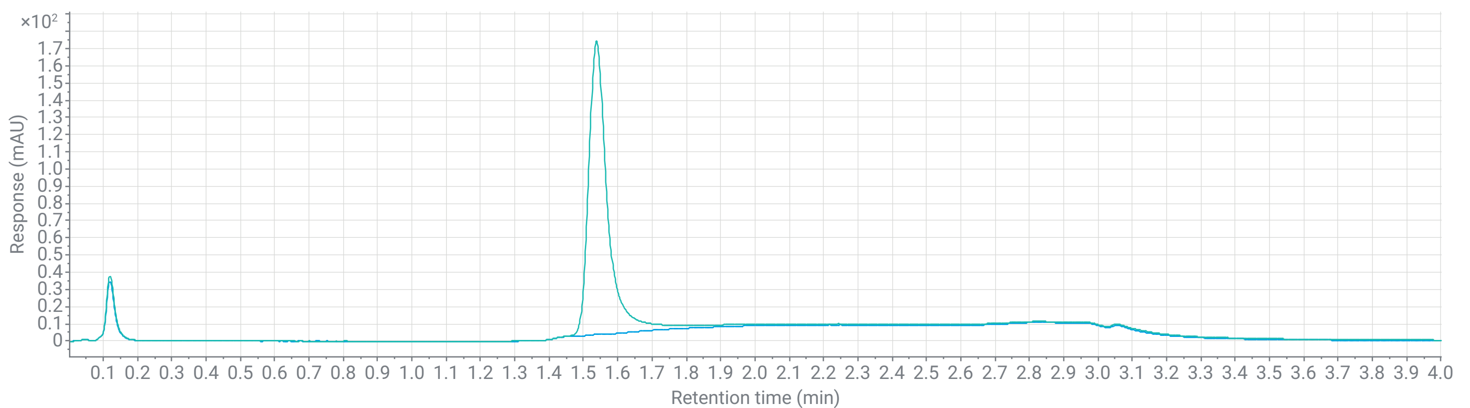


Figure 3. Initial blank injection (supernatant only) followed by first injection (10 µg hIgG).

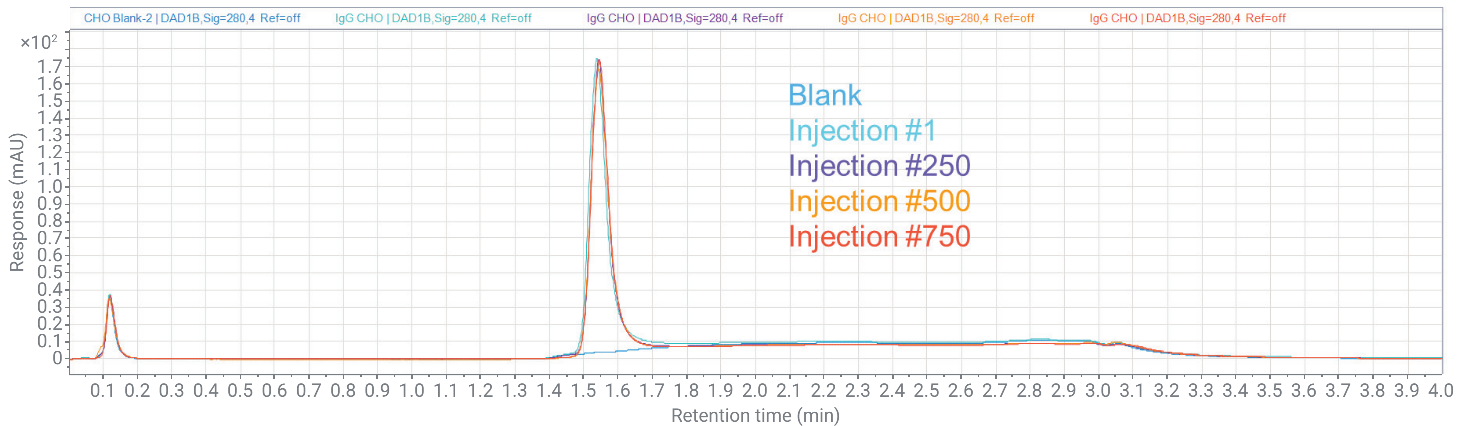


Figure 4. Overlay of injection 1, 250, 500 and 750 showing minimal change in peak shape or area.

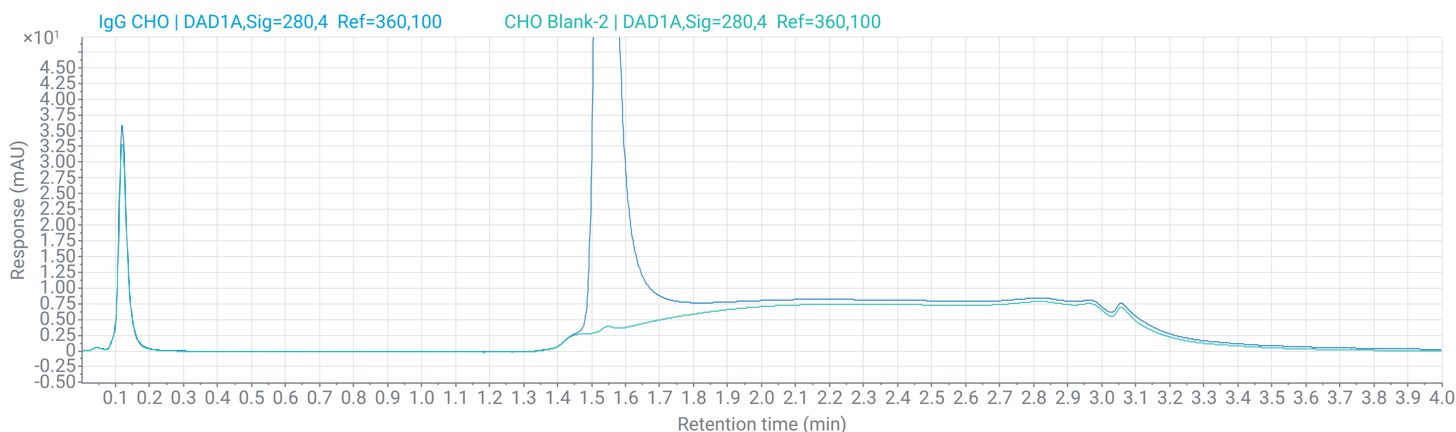


Figure 5. Fiftieth injection followed by blank supernatant injection showing minimal carry over (<0.2%).

Conclusions

Through minor modifications to reduce clogging, Bio-Monolith protein A columns have been shown to demonstrate little or no nonspecific binding of host cell proteins. They also exhibit very low carry over and exceptional column lifetime, with minimal deterioration in peak shape even after 750 injections of crude cell culture supernatant.

This provides an excellent tool for determining antibody titer levels in a rapid but accurate manner, suitable for screening many hundreds of samples at a time.

References

1. Dumont, E. *et al.* mAb Titer Analysis with the Agilent Bio-Monolith Protein A Column, *Agilent Technologies application note,* publication number 5991-5135EN, **2017**.

www.agilent.com/chem

For Research Use Only. Not for use in diagnostic procedures.

DE.3805555556

This information is subject to change without notice.

© Agilent Technologies, Inc. 2020
Printed in the USA, June 26, 2020
5994-2168EN