Application Note

Biopharma

Agilent

Trusted Answers

mAb Titer Determination in 60 Seconds Using the Agilent Bio-Monolith rProtein A Column

High-throughput clonal selection for process development and process optimization

Authors

Dr. Te-Wei Chu and
Ivan Huang
Agilent Technologies, Inc.

Introduction

The Agilent Bio-Monolith rProtein A (recombinant protein A) analytical column is the latest addition to the Bio-Monolith and affinity chromatography family. The column enables high-speed analysis of monoclonal antibody (mAb) titer and small-scale purification, and can easily be integrated into other analytical workflows, such as 2D-LC. This application note tests the recombinant protein A column at the maximum flow rate and performs the bridging study against the native protein A column. A chromatography bind/elute method for mAb titering is demonstrated, which delivers an ultrafast run time (1 minute) suitable for high-throughput applications such as clonal selection, process development, and optimization. In the bridging study, performance characteristics such as retention time, linearity and deviation of the standard curve, sample carryover, and recovery showed no detectable difference between the native and the recombinant columns. This work serves to give confidence to those who are transitioning from the native protein A column to the rProtein A column.

Experimental

1. High-throughput mAb titer analysis

Chemicals and reagents

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).

Sample

The sample was a crude Chinese Hamster Ovary (CHO) cell culture supernatant collected from a bioreactor that contained 1 mg/mL of recombinant IgG monoclonal antibody.

Instrumentation

Agilent 1260 Infinity II bio-inert LC comprising:

- 1260 Infinity II bio-inert pump (G5654A)
- 1260 Infinity II bio-inert multisampler (G5668A) with sample cooler (option 100)
- 1260 Infinity II multicolumn thermostat (G7116A) with bio-inert heat exchanger (option 019)
- 1260 Infinity II variable wavelength detector (G7114A)

Method conditions

HPLC Conditions	
Column	Agilent Bio-Monolith rProtein A, 4.95 × 5.2 mm (p/n 5190-6903)
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 0.2 0 (binding) 0.3 to 0.65 100 (elution) 0.66 to 0.90 0 (reconditioning) (0.1 min postrun)
Flow Rate	3 mL/min
Column Temperature	25 °C
Detection	UV, 280 nm
Injection Volume	4 µL (10 µg loading)

2. Bridging study

Chemicals and reagents

The same chemicals and reagents as used in the high-throughput mAb titer analysis experiment.

Sample

The samples were crude Chinese Hamster Ovary (CHO) cell culture supernatant collected from a bioreactor that contained 1.5 mg/mL of recombinant IgG monoclonal antibody and purified recombinant IgG monoclonal antibody at the same concentration.

Instrumentation

The Agilent 1290 Infinity II Bio LC system consists of the following components:

- 1290 Infinity II bio high-speed pump (G7132A)
- 1290 Infinity II bio multisampler (G7137A)
- 1290 Infinity II multicolumn thermostat with bio heat exchanger (G7116B)
- 1290 Infinity II diode array detector (G7117B) and variable wavelength detector with respective bio flow cell

Method conditions

HPLC Conditions	
Column	Agilent Bio-Monolith rProtein A, 4.95 × 5.2 mm (p/n 5190-6903) Agilent Bio-Monolith Protein A, 4.95 × 5.2 mm (p/n 5069-3639)
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 2.6 100 (elution) 2.7 to 4.0 0 (reconditioning)
Flow Rate	1.5 mL/min
Column Temperature	25 °C
Detection	UV, 280 nm
Injection Volume	5 to 50 µL (25 µg loading)

Results and discussion

High-throughput mAb titer analysis

With the high-throughput method, high-speed mAb titering with a 1-minute chromatography run time was demonstrated (Figure 1). The retention time of the purified (bound/eluted) mAb was approximately 0.61 minutes, well separated from the impurities peak at ~0.05 minutes containing host cell proteins from the CHO cell culture supernatant. In Figure 1, repeated injection of crude supernatant spiked with mAb showed consistent and robust performance of 60 samples/hour throughput with backpressure leveling at 125 bar. Throughout the study, there was no noticeable change in peak shape, retention time, and backpressure. Figure 2 showed the chromatograms of different sample loading amounts. A calibration curve was then generated by plotting peak area versus injection quantity (Figure 3). Results indicated excellent linearity response (R^2 = 0.9993), as shown in the calibration curve, and accurate measurement of mAb quantity from two separate sets of samples. These data demonstrated the feasibility of accurate mAb titer measurement using this fast analysis method.

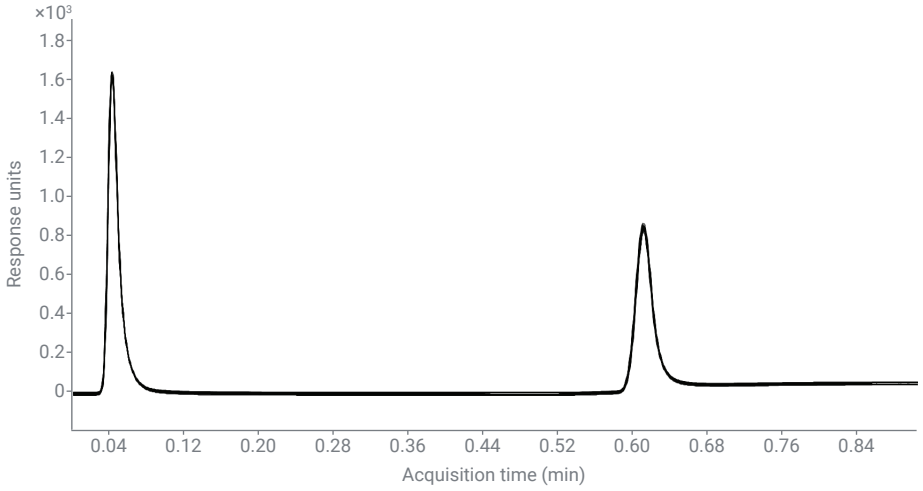
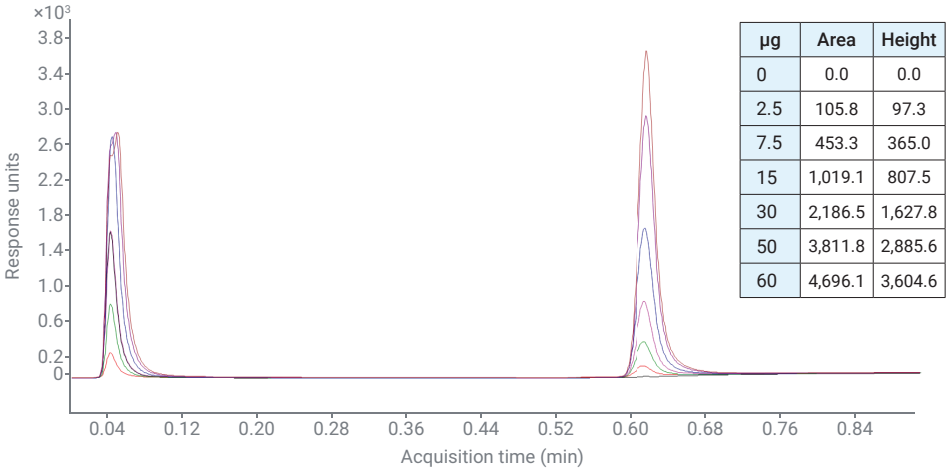
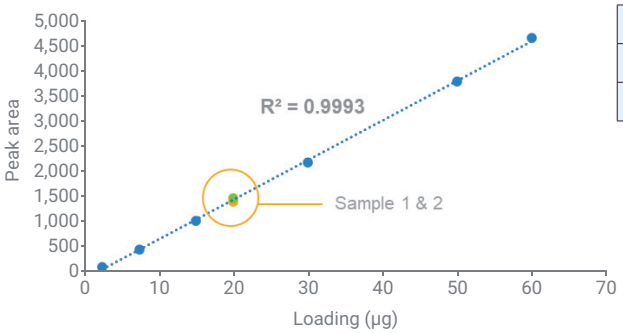


Figure 1. Agilent Bio-Monolith rProtein A column: Overlaid chromatograms of 60 consecutive injections. First peak indicates host cell protein impurities in culture supernatant; second peak is purified mAb.



µg	Area	Height
0	0.0	0.0
2.5	105.8	97.3
7.5	453.3	365.0
15	1,019.1	807.5
30	2,186.5	1,627.8
50	3,811.8	2,885.6
60	4,696.1	3,604.6

Figure 2. Agilent Bio-Monolith rProtein A column: Calibration curve. Overlay chromatograms of increasing sample loading amount for calibration curve generation.



Spiked Sample	Measured	% Deviation
20 µg (Sample 1)	19.53 µg	-2.35%
20 µg (Sample 2)	20.51 µg	+2.53%

Figure 3. Agilent Bio-Monolith rProtein A column: Standard curve linearity response and % deviation.

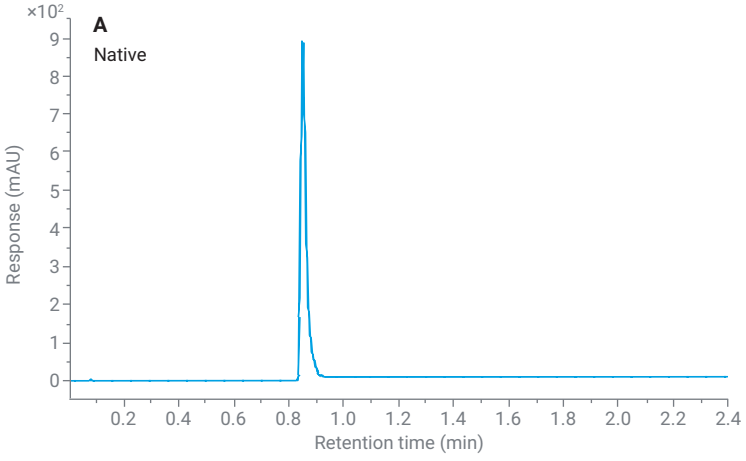
Bridging study

Performance of the two Bio-Monolith protein A columns were tested under the same conditions. All characteristics showed little or no difference between the native and the rProtein A columns, including retention time and peak shape of the purified mAb (Figure 4), linearity response of the standard curve and spiked sample recovery (Figures 5 and 6), and sample carryover (Figure 7).

Recovery analysis

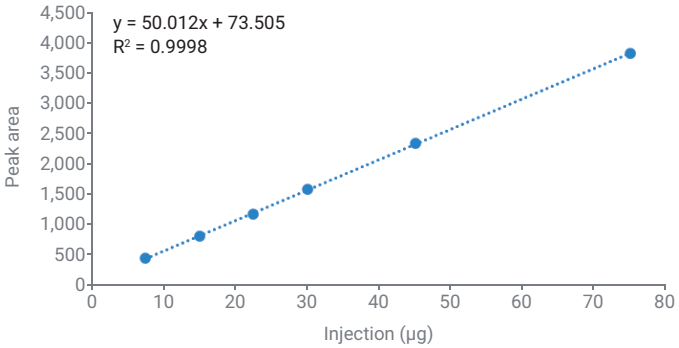
In addition to comparing the recovery between native and the rProtein A columns, two non-Agilent rProtein A columns were included in this study. Flow rate was adjusted to 2 mL/min to accommodate a non-Aglient column's operating flow rate. Additional mAb samples were included:

- Agilent-NISTmAb (part number 5191-5744)
- Sigma SiLu mAb from Sigma-Aldrich (SiLu Lite, part number MSQC4)



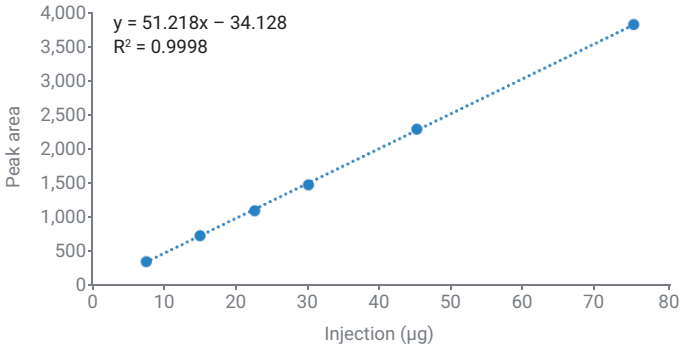
	Native	rProtein A
Retention Time (Min)	0.850 ±0.001	0.850 ±0.001
Peak Height	880.9 ±8.1	881.8 ±5.3

Figure 4. Chromatogram and mAb peak result comparison between Native and rProtein A columns.



Spiked Sample	Measured	% Deviation
25 µg (pure)	25.25 µg	0.99%
25 µg (in sup.)	26.09 µg	4.35%

Figure 5. rProtein A column: Linearity response.



Spiked Sample	Measured	% Deviation
25 µg (pure)	24.94 µg	–0.23%
25 µg (in sup.)	25.78 µg	3.12%

Figure 6. Native Protein A column: Linearity response.

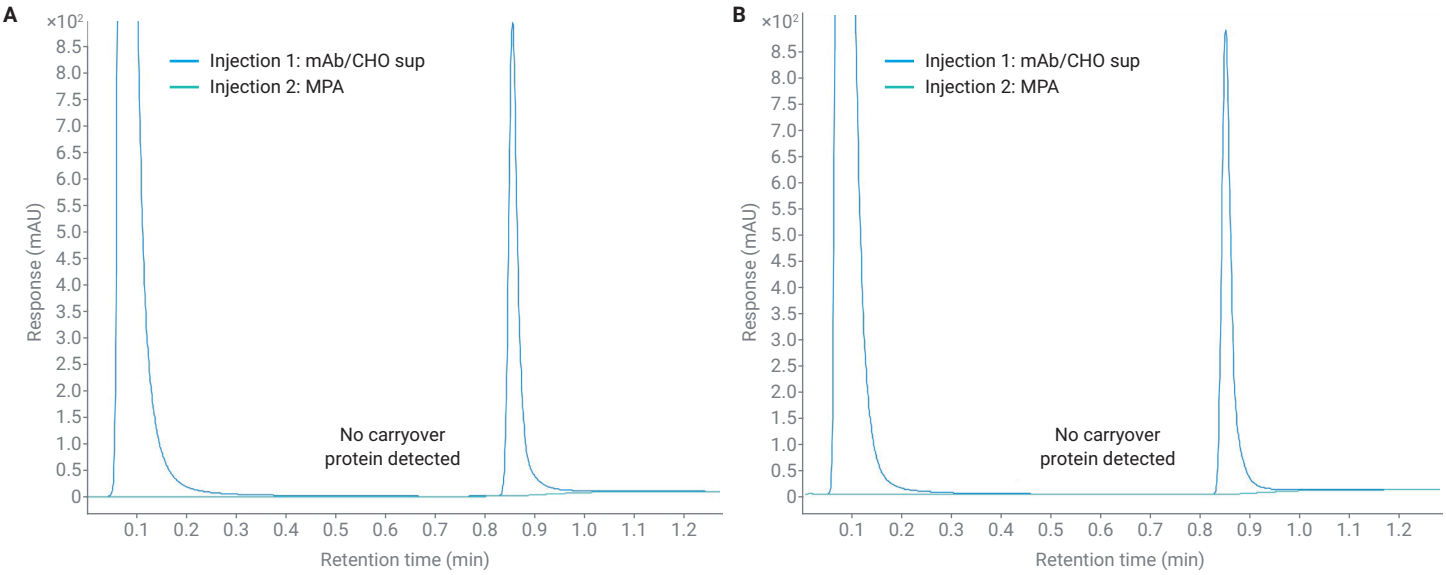


Figure 7. Carryover analysis. Subsequent injection of binding buffer (MPA) showed no detectable protein carryover. (A) rProtein A column. (B) Native Protein A column.

Baseline area under the curve (AUC) of the mAb peak was obtained by injecting purified mAb sample, which was diluted with mobile phase B, without a column (with a union). The column was applied and AUC of eluted mAb was obtained. The same amount of mAb sample as baseline AUC was used.

Recovery % = (AUC of eluted mAb/Baseline AUC) × 100

Result and analysis

The average recovery of the rProtein A column was 1% lower than the native protein A column but still showed better recovery than the two non-Agilent columns. While the nProtein A column took the slight lead in recovery, it was the rProtein A column that demonstrated the most robust recovery across the three mAb samples.

HPLC Conditions	
Column	Agilent Bio-Monolith rProtein A, 4.95 × 5.2 mm (p/n 5190-6903)
	Agilent Bio-Monolith Protein A, 4.95 × 5.2 mm (p/n 5069-3639) Vendor A & B rProtein A column
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 0.4 0 (binding) 0.5 to 1.3 100 (elution) 1.31 to 4.0 0 (reconditioning)
Flow Rate	2 mL/min
Column Temperature	25 °C
Detection	UV, 280 nm
Injection Volume	4 µL (10 µg loading)

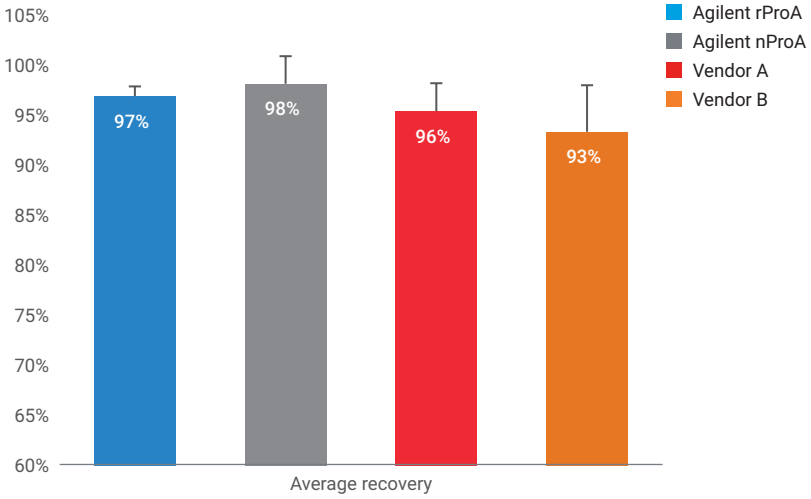


Figure 8. mAb recovery results comparison.

Conclusion

This application note has shown that, at the maximum flow rate of 3 mL/min, the Agilent Bio-Monolith rProtein A column delivers a robust performance. In the second part of this application note, the bridging study between native column and the rProtein A column has demonstrated that rProtein A delivers a similar or equivalent performance to the native protein A column.

www.agilent.com/chem

DE44389.5535300926

Printed in the USA, August 18, 2021
5994-3969EN

Agilent
Trusted Answers