Application Note

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Robust, Reliable, Recombinant Protein A Monolith Column for Antibody Titer Determination

Authors

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Abstract

Rapid screening of crude cell culture supernatant allows decisions to be made on the optimum time for harvest during the manufacture of biotherapeutic antibodies. There are several advantages to using a Bio-Monolith column with recombinant Protein A affinity ligand. First, the Bio-Monolith structure has wide through-pores that minimize the risk of clogging. Second, the use of recombinant Protein A provides the selectivity towards IgG that is associated with native Protein A, but with a higher ligand purity and a more robust structure. Finally, the column can also be used for small-scale purification so that other analytical techniques can be applied, particularly in the determination of critical quality attributes (CQAs).

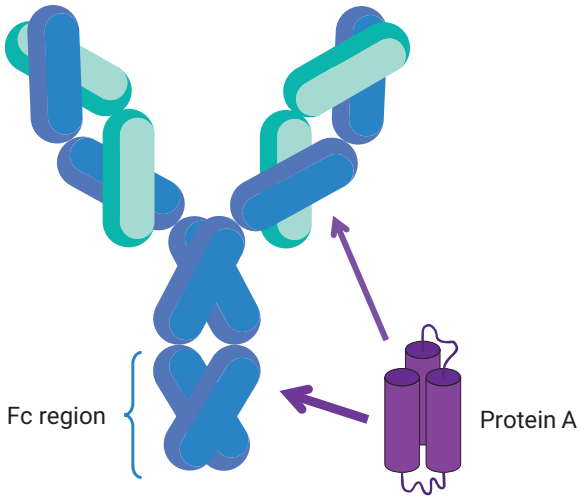

Figure 1. Protein A interaction with immunoglobulin G (IgG).

Introduction

Native Protein A is a surface protein isolated from Staphylococcus aureus, which has a high binding affinity for the Fc region of many different types of immunoglobulin from different species. Native Protein A affinity chromatography has become the method of choice for the purification of monoclonal antibodies from crude cell culture supernatant. Recombinant Protein A can provide some extra benefits since it can be produced in a purer form and can be engineered to ensure that its immobilization onto a stationary phase creates the ideal orientation for optimum binding.

It is also helpful for improving column lifetime, which can otherwise be compromised due to the crude nature of cell culture supernatant. This is because it can withstand the harsh conditions required for column cleanup better than native Protein A columns.

This application note tests the lifetime of a new Agilent recombinant Protein A Bio-Monolith column.

Experimental

Reagents and chemicals

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 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:

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

Method conditions

HPLC Conditions	
Column	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
Cleanup Buffer	1) 1 M NaCl in 100 mM sodium phosphate, pH 7.4 2) 20% isopropanol in 50 mM sodium phosphate, pH 7.4
Gradient Profile	Time %B 0.0 to 0.5 0 (binding) 0.6 to 1.8 100 (elution) 1.9 to 4.0 0 (reconditioning)
Flow Rate	1 mL/min
Column Temperature	24 °C
Detection	UV, 280 nm
Injection Volume	As required (1 to 20 µL)

Results and discussion

A crude cell culture supernatant solution containing much higher levels of host cell proteins than in the previous work[1] was chosen to investigate the robustness of Bio Monolith rProtein A columns.

A repetitive sequence involving step gradients for binding, elution, and column reconditioning was used and the results from every 250th injection are shown in Figures 2, 3, and 4. After 1,500 injections, a column regeneration step (see Method conditions) was introduced using a cleanup buffer, which was performed every 500 injections thereafter. As expected from such a challenging crude sample matrix, a gradual build-up of pressure was observed (Figure 5).

However, with regular cleanup, the column continued to provide consistent, reliable peak area and titer analysis during the 3,000 injections, as shown in Figures 6 and 7.

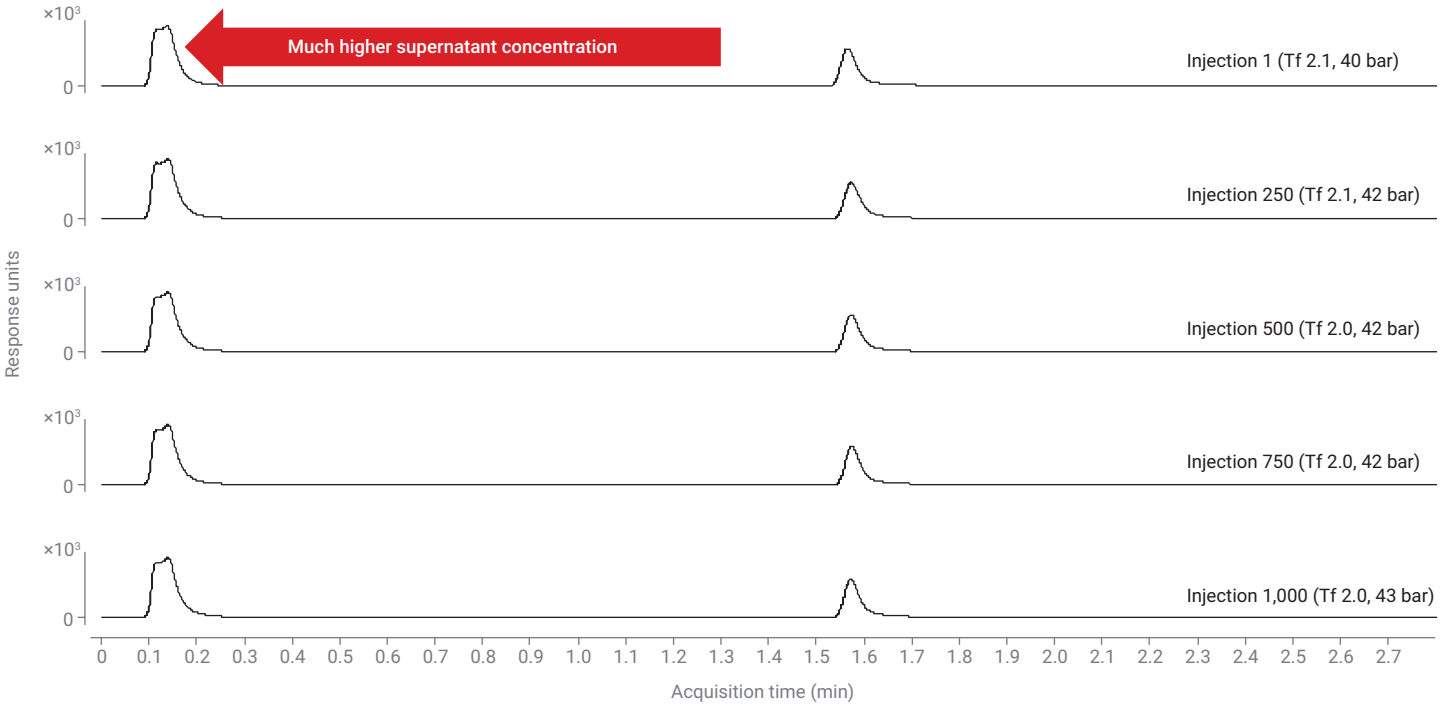

Figure 2. Agilent Bio-Monolith rProtein A column lifetime: Injections 1 to 1,000.

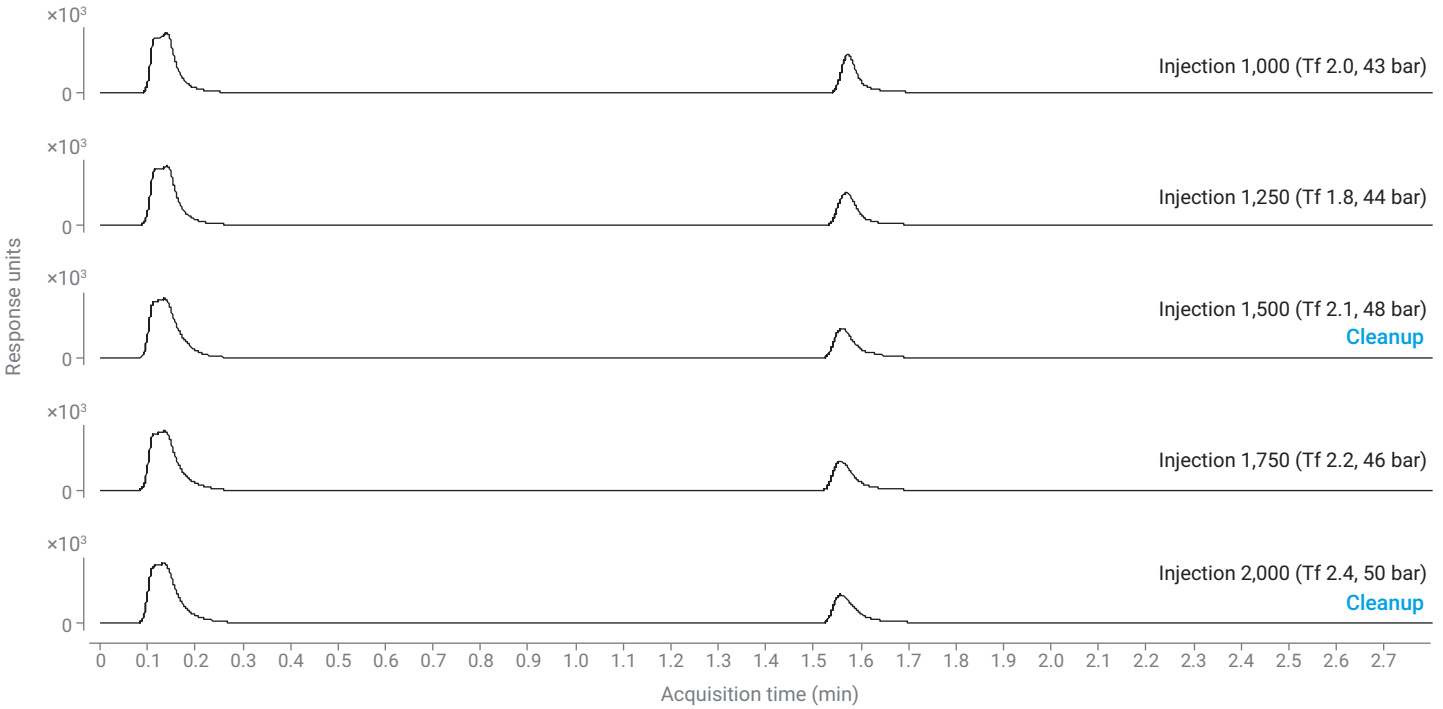

Figure 3. Agilent Bio-Monolith rProtein A column lifetime: Injections 1,000 to 2,000.

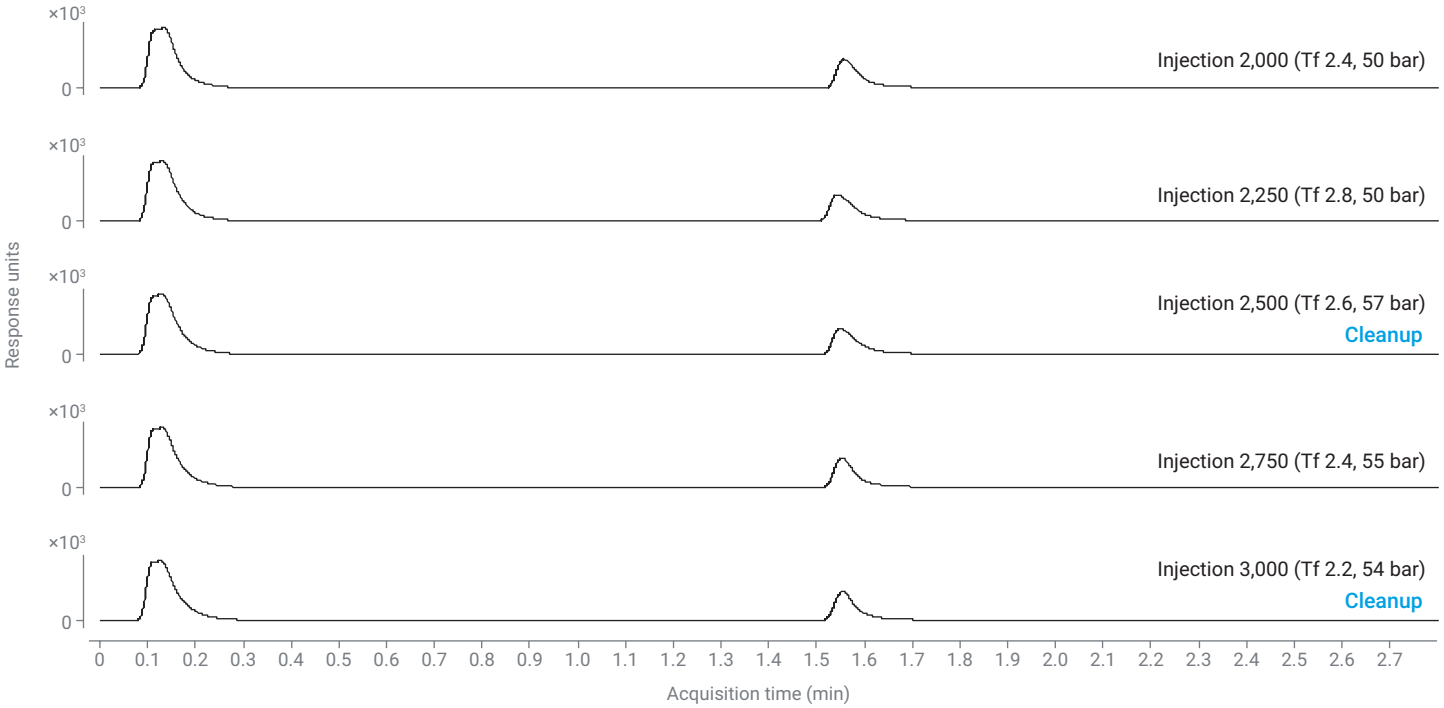

Figure 4. Agilent Bio-Monolith rProtein A column lifetime: Injections 2,000 to 3,000.

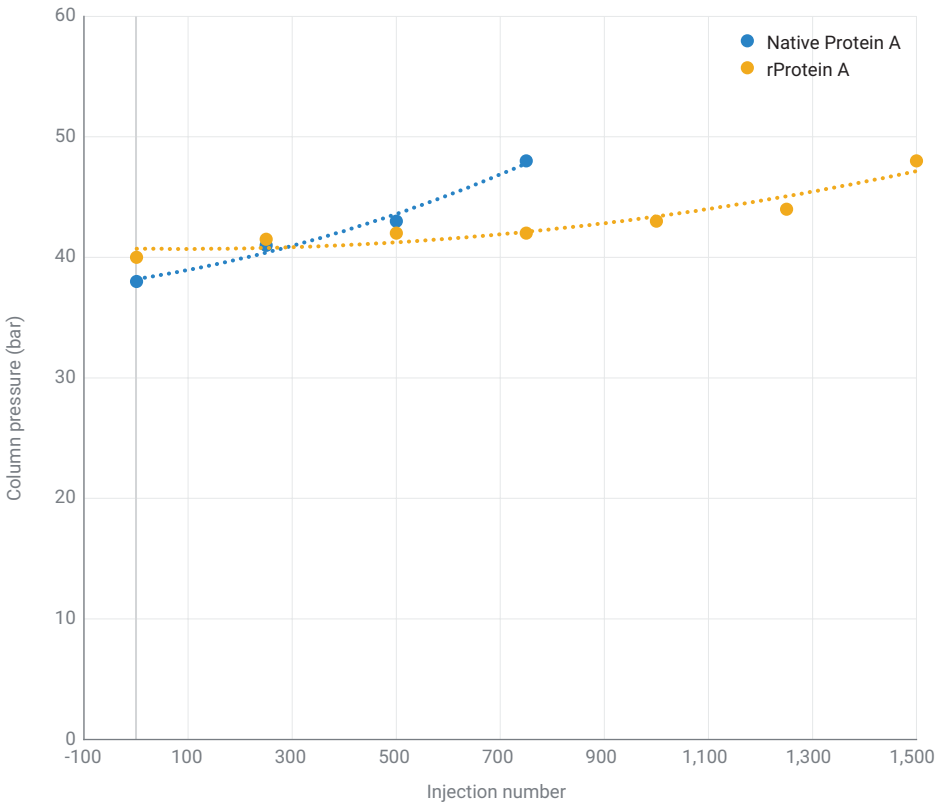

Table 1. Column pressure versus injection number during lifetime.

Injection	Native Protein A	rProtein A
1	38.0	40.0
250	41.0	41.5
500	43.0	42.0
750	48.0	42.0
1,000		43.0
1,250		44.0
1,500		48.0

Figure 5. Column pressure versus injection number during lifetime.

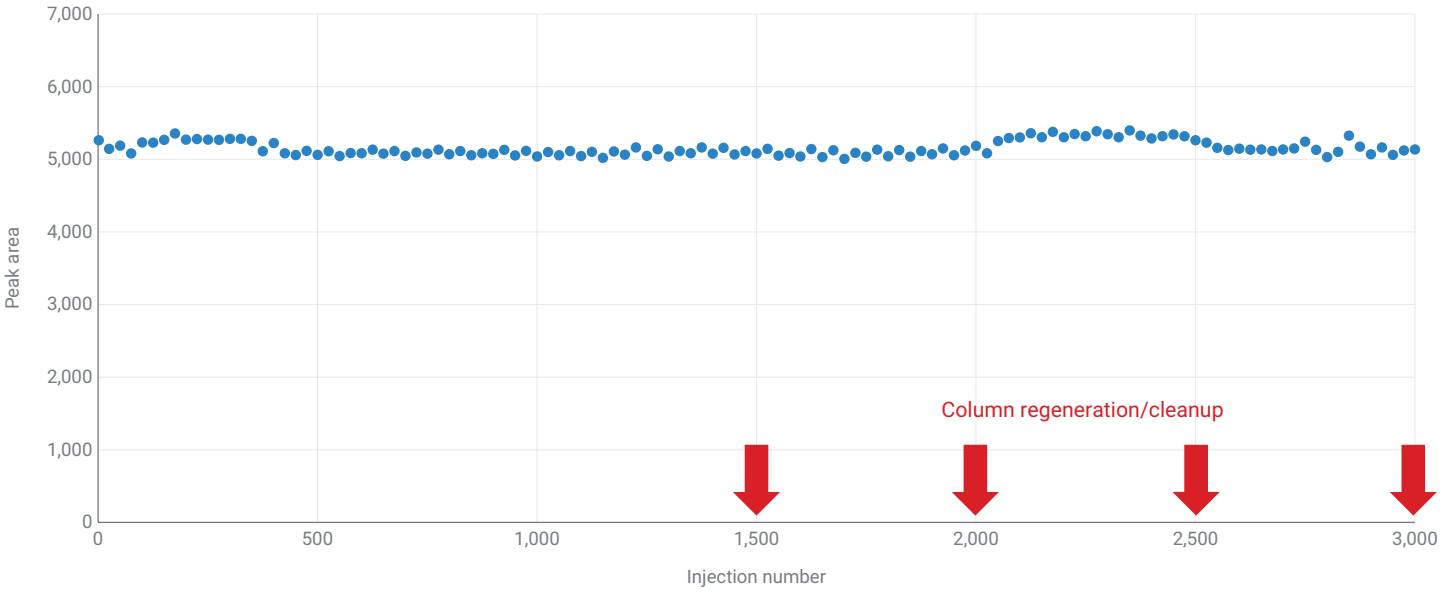

Figure 6. Peak area versus injection number during column lifetime.

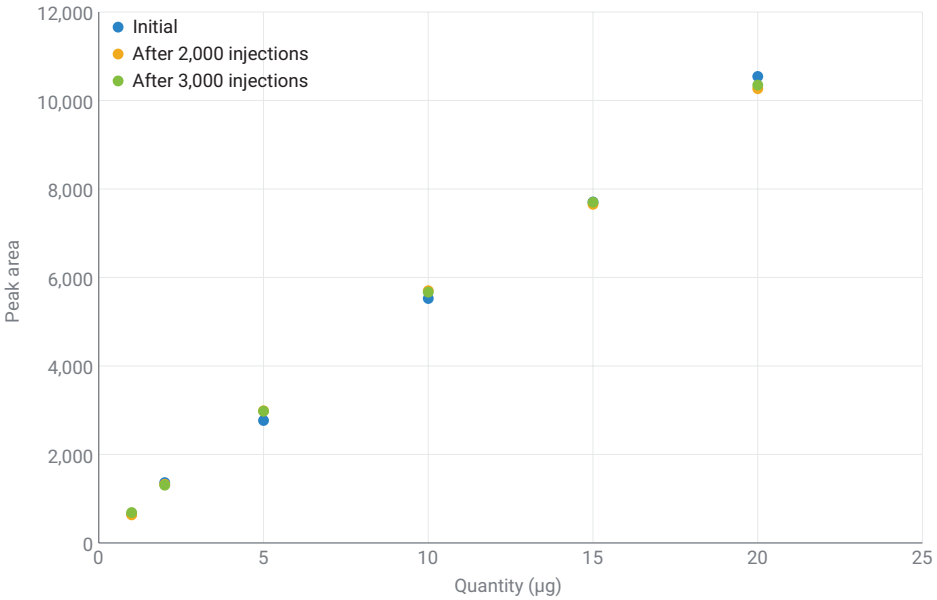

Figure 7. Peak area versus injection quantity (µg) during column lifetime.

Table 2. Peak area versus injection quantity (µg) during column lifetime.

Quantity (µg)	Initial	After 2,000 Injections	After 3,000 Injections
1	654	633	688
2	1,363	1,323	1,308
5	2,766	2,984	2,979
10	5,526	5,699	5,666
15	7,706	7,653	7,699
20	10,541	10,268	10,347

Conclusion

This application note has shown that the Bio-Monolith rProtein A column is capable of consistent and reliable performance in titer analysis beyond what we have observed with equivalent native Protein A columns.

Reference

1. Coffey, A.; Kondaveeti. Improved Lifetime of Bio-Monolith Protein A Columns for Titer Determination. *Agilent Technologies application note*, publication number 5994-2168EN, **2020**.

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