Purification of antibodies from cell culture supernatant using the Agilent AssayMAP Bravo platform

Application Note

BioPharma

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Abstract

The Agilent AssayMAP Bravo platform has been developed to automate a variety of operations used to prepare and analyze biomolecules. This Application Note describes purification of poly- and monoclonal antibodies from different media backgrounds constructed to mimic those encountered during antibody screening, production, and scale-up in commercial settings. Antibodies were added at different concentrations to phosphate buffered saline or supernatant media harvested from Chinese hamster ovary cell culture. Automated purifications were performed on the Agilent AssayMAP Bravo platform using specially formulated microchromatography cartridges containing immobilized protein A. Yields from each antibody source and dilution were not altered by the background media, or the presence of up to 10% fetal bovine serum in the media. By increasing sample throughput, the AssayMAP Bravo platform will shorten timelines for development of new antibodies for research and therapeutics.

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Introduction

Development of antibodies as biotherapeutic agents has become widespread and continues to increase. Antibodies with specific affinities may be selected from various organisms, or constructed synthetically and produced by cell culture. Throughout the selection, scale-up and production phases, it is important to monitor specific attributes for each antibody to ensure the highest degree of biological purity. Affinity chromatography using immobilized protein A, an antibody-specific binding protein derived from the bacterium *Staphylococcus aureus*, has been in use for many years and has become widely accepted as the method-of-choice for purifying antibodies. Standard liquid chromatography workflows use serial processing of samples on expensive dedicated instruments. However, as investigation and development of antibodies for therapeutic uses has increased, current methods of purification have become a bottleneck. Agilent has developed the AssayMAP Bravo platform to purify and process proteins from multiple samples quickly and in parallel. The system consists of a specially-developed 96-channel positive-displacement pipetting head designed to mate specifically with AssayMAP microchromatography cartridges. The head is capable of moving fluids at rates as low as 1 µL/minute to as high as 500 µL/second. Cartridges are available pre-filled with an assortment of different chromatography resins. Here we demonstrate the ability of the AssayMAP system to purify antibodies from cell culture supernatants using cartridges filled with immobilized protein A.

Materials and Methods

This Application Note used an Agilent AssayMAP Bravo configured with the 96-channel AssayMAP pipetting head, wash station, and pump module. Protocols were developed using VWorks automation control software developed specifically for the platform. Purifications were carried out using AssayMAP microchromatography cartridges packed with protein A (PA-W cartridges, p/n G5496-60000). Phosphate buffered saline, pH 7.4 (PBS, Sigma, p/n D5652) was used for washing and dilution steps. Samples were prepared by adding either human immunoglobulin, G (Hu IgG, Athens Research & Technology, Altanta, GA, p/n 16-16-090707) or a well-characterized anti-streptavidin monoclonal antibody to PBS, cell culture supernatant used to grow nonantibody-expressing Chinese hamster ovary cells (CCS, Expression Systems, Woodland, CA), or CCS supplemented with fetal bovine serum (FBS, Sigma, p/n F2442).

A 1.32-fold dilution series of Hu IgG from 4.6 to 0.33 mg/mL was made in in PBS or CCS, each with or without 10% FBS. To represent concentrations encountered during screening, scale-up and production, dilutions of the monoclonal antibody were prepared at 10, 50, 500, and 1,000 µg/mL in PBS or CCS augmented with 0, 5, or 10% FBS. Samples were purified with AssayMAP PA-W cartridges on the AssayMAP Bravo platform using a protocol which consisted of priming and equilibrating cartridges, loading samples, washing unbound material, then eluting with 12 mM HCl, followed by regenerating the column and re-equilibrating with PBS. The volumes, rates, and solutions used for each step are presented in Table 1. Purified products were eluted into half-area microtiter plates (Greiner Bio-One, p/n 675801) and read at 280 nm. The automation protocols are available on request. The mass of eluted protein was compared to that of unpurified PBS dilutions. The automation protocols are available on request.

Results and Discussion

To demonstrate performance of the AssayMAP Bravo platform, samples similar to those encountered during development of antibody-expressing cell lines were constructed by spiking different concentrations Hu IgG into CCS with and without FBS. The results presented in Figure 1 show that regardless of background, antibodies were purified at titers similar to those of the PBS controls. FBS, which is often used as a supplement for growing cells in culture, is known to contain antibodies, and thus would be expected to raise the apparent antibody concentration in eluates from those samples. However, others have shown that protein A has low affinity for bovine antibodies, which is further confirmed by these results as there was no apparent increase in titer from samples containing 10% FBS. Results were linear (R^2 values of 0.99) from approximately 10 µg to 130 µg. Previous work with this system has indicated that while the AssayMAP cartridges can bind as much as approximately 180 µg, the linear range peaks at approximately 100 µg, which is also seen in this data.

During different phases in antibody production, cell lines generally produce widely varying amounts of antibody. While primary cell lines used for selection may generate only about 10 mg/L, subsequent selection and optimization of growth conditions can increase yields to as much as 1 g/L. To simulate conditions encountered during various stages in antibody production, a monoclonal antibody was mixed with CCS containing different amounts of FBS. Replicates of each preparation were purified using a modified rapid protocol which combines equilibration, loading and washing into a single step.

Volumes and rates were kept the same as presented in Table 1, except column regeneration was eliminated.

Purification results for the monoclonal antibody were similar to those observed with Hu IgG and are shown in Figure 2. Similar to results shown in Figure 1, the presence of different levels of FBS in the CCS apparently did not alter yields. These results demonstrate the AssayMAP Bravo platform can be used to determine antibody titers produced by different cells lines over a range of initial antibody concentrations. To investigate the ability of the system to purify levels of antibody required for subsequent analytical procedures, 10 µg of antibody was loaded onto PA-W cartridges in volumes of 10, 20, or 100 µL. Recoveries from those different loads ranged from 9 to 10 µg (data not shown), demonstrating that the AssayMAP Bravo platform can be used to concentrate, purify and recover antibody from cell lines expressing low levels of antibody.

Conclusion

The results achieved confirm that the Agilent AssayMAP Bravo is a robust platform for purifying antibodies from samples similar to those presented during antibody selection, development, and production. In addition to titer determination, higher yields of antibody may be prepared from each cartridge by increasing the volume of sample loaded. In preliminary experiments, 25 µg of antibody was purified from solutions containing from 10–1,000 µg/mL by passing volumes from 2,500 to 250 µL, respectively, through the cartridges. When analyzed by mass spectroscopy, the products maintained the same peptide and glycoforms compared with samples not purified by AssayMAP Bravo.

Step	Fluid	Volume (µL)	Rate (µL/min)
Prime	PBS	125	1,980
Equilibrate	PBS	50	25
Load	(sample)	50	2
Wash	PBS	50	10
Elute	12 mm HCl	50	5
Regenerate	12 mm HCl	50	10
Re-equilibrate	PBS	50	25

Table 1
Main steps and conditions used to purify antibodies with the AssayMAP platform.

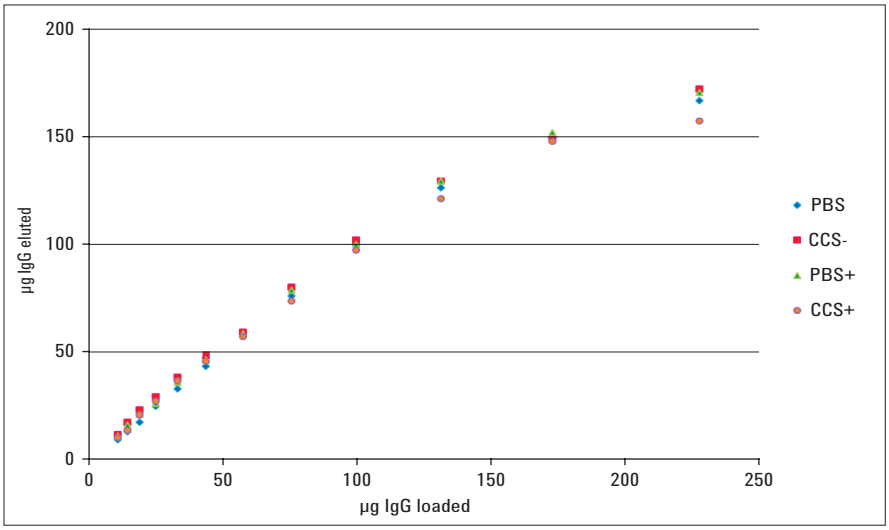

Figure 1
Human IgG was diluted in PBS and CCS, augmented with 10% FBS (PBS+ and CCS+) and purified with the AssayMAP Bravo platform as described. The graph shows comparative results between the amount of antibody loaded and the amount eluted from the cartridges.

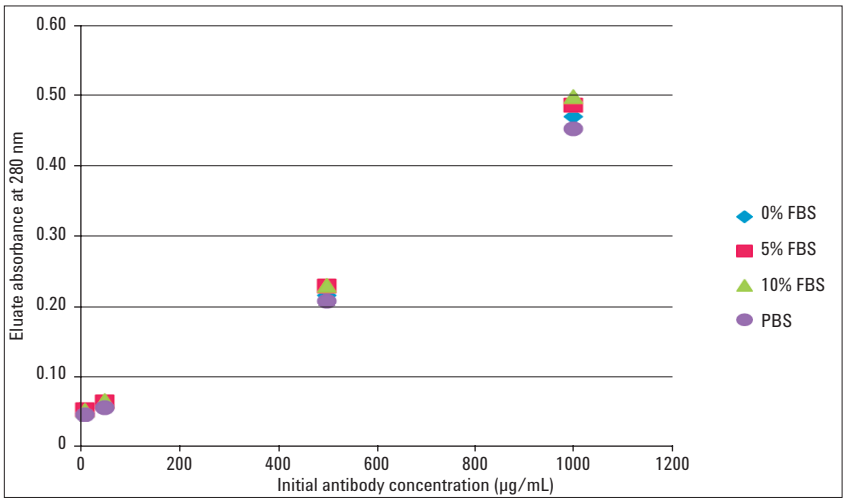

Figure 2
Purification of a monoclonal antibody at four different concentrations from four different media.

www.agilent.com/lifesciences/Assaymap-Bravo

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