High throughput purification of human IgG using the Agilent Bravo for Protein Purification and AssayMAP[1] protein A cartridges

Application Note

Authors

David Knorr and Marc Beban
Agilent Technologies, Inc.
Santa Clara, CA USA

Zachary Van Den Heuvel
BioSystem Development
Madison, WI USA

Abstract

To address a growing desire for robust, high-throughput chromatography capability, Agilent Technologies has developed a 96-channel liquid handling head to enable microchromatography on the Bravo Automated Liquid Handling Platform using AssayMAP protein purification cartridges from BioSystem Development. This system is called the Agilent Bravo for Protein Purification. The system can operate using direct fluid displacement for chromatography applications, or air displacement for conventional liquid handling. Early results using the system to purify immunoglobulin from artificially constructed samples show the system can purify between 1 and 100 µg of target protein when presented in backgrounds as high as 10 mg/mL of non-specific protein. Recovered protein quantities accurately reflect input quantities, and these results are repeatable over a number of purifications using used or unused cartridges.

Introduction

The rise of biologics as important pharmaceutical agents has driven an ever-increasing demand for protein analysis methods that are precise, sensitive, and amenable to high throughput. These methods are used to analyze complex samples from throughout the biopharmaceutical discovery process, from research samples taken from protein expression systems to biological samples present in serum to production cell lysates and cell culture supernatants. In typical workflows, HPLC and immunoassays are used to purify specific proteins from complex samples and quantify using absorbance, fluorescence, or chemiluminescence. While these methods have been successfully used for years, a significant challenge has been adapting these methods to the demands of high throughput analysis where concerns of sample capacity and reagent use are prominent.

1. AssayMAP is a registered US trademark of BioSystem Development.

Agilent Technologies

The Agilent Bravo for Protein Purification is a simple, precise, and high throughput platform for micro-scale purification and preparation of polyclonal or monoclonal antibody (MAb) from bioprocess samples. The 96AM liquid handling head utilizes AssayMAP cartridges with the same protein A chemistry as traditional affinity HPLC and adds advanced liquid handling of the Agilent Bravo to enable protein purifications from complex matrices. This automated platform allows for a full range of high throughput, highly parallelized liquid handling operations, including precision flow rate control to enable true chromatographic separation of the target molecule in low sample volume. The result is a robust system for multiplexing up to 96 samples in a single run.

Here we present results showing system performance purifying human immunoglobulin G (hIgG) from samples with different concentrations of background protein.

Materials and Methods

Reagents: IgG from human plasma (#16-16-090707, Athens Research & Technology, Athens, GA, USA) was diluted to 4 mg/mL in PBS (Sigma). Fish gelatin from cold water fish (FGel, Sigma, #G7041) was reconstituted in PBS at a stock concentration of 20 mg/mL. Bound protein was eluted in 100 mM glycine (pH 2.5, Fisher Scientific, #G46). Phosphate buffered saline (PBS) was diluted from 10X stock (Sigma, #P5493). Protein A purification cartridges were obtained from BioSystem Development.

Instrumentation: Experiments were carried out using a standard Agilent Bravo Automated Liquid Handling Platform with a 96AM liquid handling head and an Agilent 96-channel wash station and pump module controlled by VWorks Automation Control software. Absorbance values were collected at 280 nm using a Varioskan Flash multi-mode reader (Thermo). Electrophoresis was performed using Agilent Protein 230 chips (Agilent Technologies # 5067-1518) run on an Agilent 2100 Bioanalyzer with 2100 Expert software.

Workflow: Cartridges were pre-conditioned by priming with 125 µL PBS at a flow rate of 33 µL/s before equilibrating by aspirating an additional 50 µL of PBS at 0.41 µL/s. Cartridges were loaded by aspirating 50 µL of sample at a rate of 0.1 µL/s before washing by aspirating 50 µL of PBS at a rate of 0.41µL/s. Cartridges were dismounted before washing probes three times with PBS. Human IgG was eluted by aspirating 50 µL of elution buffer into the probes, mounting the cartridges, and then dispensing the syringe contents at a rate of 0.1 µL/s into a 96-well microtiter plate (Greiner µv Star, # 675801). Following elution, the cartridges were flushed with additional PBS, dismounted, and the syringes washed.

Linearity was measured by creating three sets of 2-fold serial dilutions of hIgG prepared with final concentrations of FGel at 0, 5, and 10 mg/mL. HIgG concentrations for each set were 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.015625 mg/mL. Each dilution set was introduced to the system using a 12-well sample reservoir (Porvair Sciences, Ltd., # 390012) with each well corresponding to a column of eight syringes. A single concentration was placed into each of eight wells and 1X PBS was added to a ninth well as a control. Following purification as outlined above, absorbance values for each set of dilutions were averaged and plotted as shown in **Figure 1**.

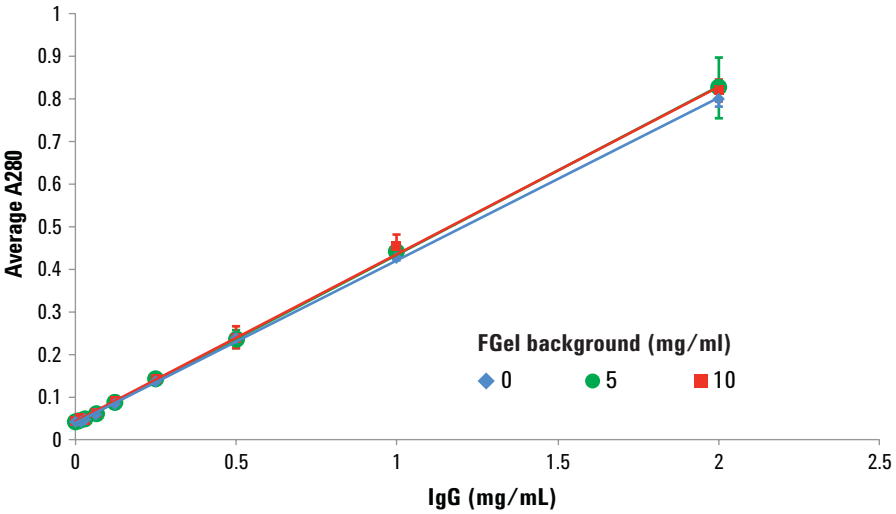

Figure 1. Absorbance values of eluates from Protein A cartridges loaded with hIgG and different concentrations of FGel. R2 values for the three different sample sets of 0, 5, and 10 mg/mL background protein were 0.9997, 0.9998, and 0.9992, respectively. Average values for each concentration were calculated from eight readings. Error bars indicate standard deviation.

Purity was determined using the Bioanalyzer to run sets of unpurified and purified products from individual cartridges. Aliquots of 4 µL of sample were mixed with 2 µL of non-reducing denaturing solution, then heated before dilution with 84 µL of de-ionized water and loading onto an Agilent Protein 230 chip, which was run as described in the Agilent Protein 230 Kit Guide (P/N G2938-90054). **Figure 2** shows the super-imposed output from four lanes.

To examine reproducibility and robustness of the assay, a set of samples was constructed with 0.1, 0.4, or 2 mg/mL hIgG in a background of 10 mg/mL FGel. Next, a sample plate was prepared such that each dilution filled four columns (i.e., A1-H4, A5-H8, or A9-H12). This plate was used during six successive purification runs. See **Figure 3**. Between each run, the sample plate was rotated 180° so that cartridges used to purify high or low amounts of hIgG in one run would be used for the most different concentration in the next run, and so on. After four runs, the cartridge box was replaced and two more runs were performed.

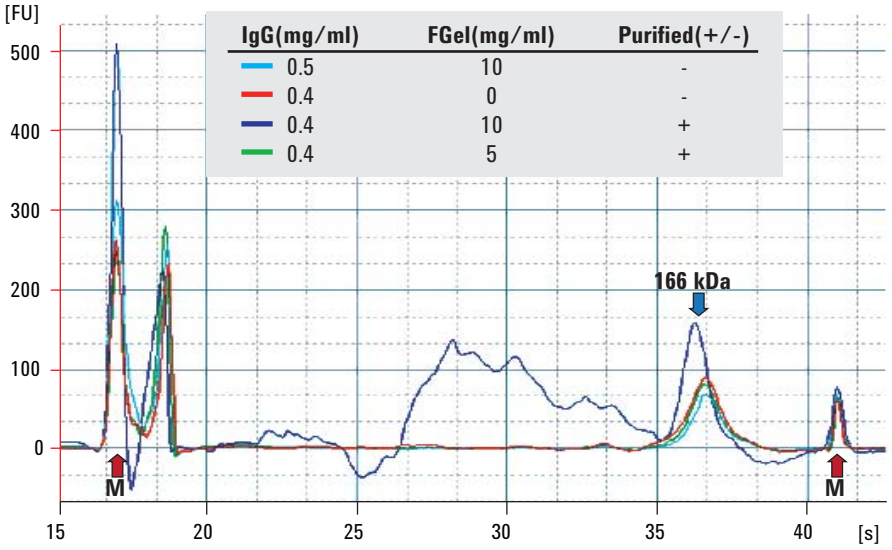

Figure 2. Superimposed electropherogram traces from Protein 230 chips. The 166 kDa peak indicates the expected position for hIgG. Arrows labeled M indicate upper and lower protein ladder markers.

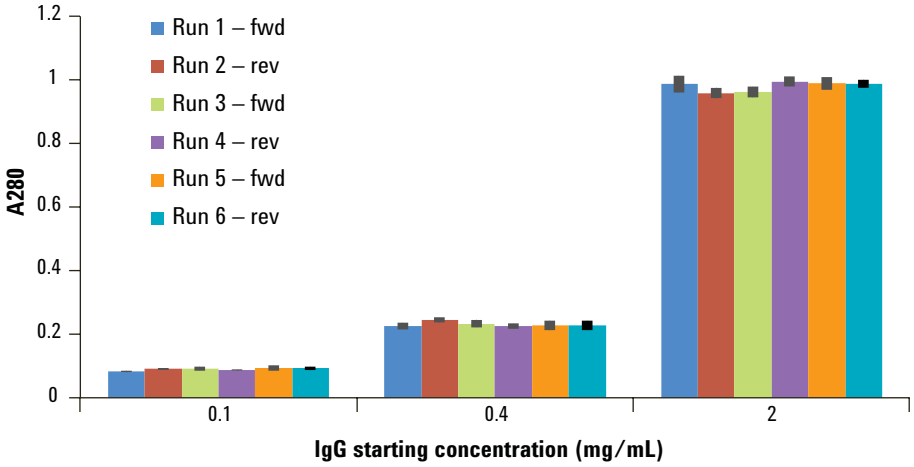

Figure 3. Run-to-run reproducibility of cartridge-based purification of hIgG. Six purifications were run using 96 cartridges and the same sample input plate containing three different concentrations of hIgG along with 10 mg/mL FGel. Between each run, the sample plate was rotated 180° (relative rotation is indicated next to each sample in the legend). Average absorbance values for each input concentration (24 samples) are given including standard deviation. Runs 5 and 6 used a separate set of cartridges.

Results and Discussion

Performance of the system was examined by purifying different concentrations of hIgG that were presented in different backgrounds of a contaminating protein. Linearity of the response was nearly perfect throughout the 1 to 100 µg binding capacity of the cartridges. Purified products co-migrated with hIgG during capillary electrophoresis. In addition, purification results were consistent over multiple assays, and for different cartridges.

Results in Figure 1 illustrate the ability of the 96AM platform to purify protein directly proportional to input of specific target presented. Yields of hIgG were linear throughout the range of target binding capacity for the cartridges. This performance was obtained even with non-specific backgrounds as high as 10 mg/mL, which approximates that seen with many biological samples. Purified samples examined using electrophoresis under native conditions maintained the expected major peak at approximately 166 kDa while non-specific material was minimized. See Figure 2. Sample-to-sample results throughout six purification runs showed excellent agreement in the amount of eluted product. See Figure 3. Results were similar for new and used cartridges, regardless of whether they had been used to purify widely different amounts of target in a previous run. This indicates robustness in both the protocol and the hardware, including cartridge production.

The Agilent Bravo for Protein Purification provides a robust system for hIgG titer in support of biopharmaceutical discovery and development. The 96AM head is designed to maintain syringe dead volumes at less than 500 nL, which is important in eliminating air bubbles and allows direct displacement of fluid to maintain constant flow rate regardless of column resistance. The direct displacement mode can be used with either AssayMAP cartridges or bare probes. By controlling whether cartridges are mounted during aspirate and dispense cycles, the system can dictate unidirectional flow through the cartridges. The head can also use pipette tips in air-displacement mode, delivering 2 µL at ±5% CV, which is identical to the Agilent Bravo 96LT head. While the normal velocity range of 1-500 µL/s is maintained for use with pipette tips, in direct-displacement mode flow rates can be controlled to as low as 1 µL/minute to address challenging separations. The combination of flow control with direct displacement fluid handling in a multichannel system enables high throughput microchromatography.

Conclusion

Two critical factors for developing a successful chromatography method are quantitative binding of desired targets and efficient removal of non-specific material. Flow rate control is of key importance for both factors to optimize interactions between the sample and column bed material. This is especially important for assays that rely on enzymatic reactions. Because Bravo for Protein Purification combines a wide range of flow rates with high-quality microscale media cartridges, it will be useful for developing additional affinity purification methods as well as enzyme-based applications such as immunoassays and glycan analyses.

www.agilent.com/lifesciences/automaton

Published in the U.S.A., January 21, 2011
5990-7203EN

Agilent Technologies