Rapid and high-throughput purification of antibodies with Capturem Protein G

Capturem Protein G Miniprep, Capturem Protein G Maxiprep, Capturem Protein G 96, and Capturem Protein G 24-Well Plate

- Broad IgG affinity in a rapid purification workflow
- Rapid isolation of concentrated antibodies in a low elution volume
- 1–2 mg of purified antibody per column using Capturem Protein G Maxiprep
- High well-to-well reproducibility for high-throughput applications using Capturem Protein G 96-well plates

Introduction

Protein G is a cell wall protein from group G streptococcal bacteria with a high affinity for the fragment crystallizable (Fc) region of monoclonal and polyclonal IgG-type antibodies from several different species, e.g., mouse, rat, human, etc. Capturem Protein G products use a recombinant Protein G engineered to remove the albumin binding domain as well as the cell wall and cell membrane-binding domains in order to improve specificity towards the Fc region of antibodies.

Capturem membranes have been engineered to have a very large surface area with low nonspecific background binding. The large surface area results in a high binding capacity (approximately 75 mg/ml of bed volume), about threefold higher than similar resins. In addition to providing a high binding capacity, the 3D structure of the membrane causes the loaded sample to flow through narrow pores with very short diffusion distances. This allows the membranes to rapidly bind to the target protein, yielding purified product in 5 minutes using Capturem miniprep columns and 15 minutes using Capturem maxiprep columns and high-throughput plate formats.

Results

Comparison of Capturem Protein G and Capturem Protein A kits

The membranes in our Capturem Protein G minipreps, maxipreps, 24-well plates, and 96-well plates function in the same way as our Capturem Protein A membranes, except that they use Protein G to bind antibodies. Protein G has a slightly different affinity for antibodies than Protein A, and depending on the particular antibody and source species, Protein G might bind at a higher capacity than Protein A or vice versa. Typical binding capacities of Capturem Protein G Miniprep columns for serum from various source species are shown in Table I. For human IgGs, we expect that Protein G will be better for some isotypes. Even within a given isotype, there can be significant variability between IgG subclasses (Table II).

Typical binding capacities of Capturem Protein G Miniprep columns	
Serum source	**Eluted antibody (µg)**
Mouse	210
Rat	310
Rabbit	190
Human	430
Sheep	190
Goat	250

Table I. Typical binding capacities of Capturem Protein G Miniprep columns for different animal sera. Columns were loaded with 800 µl of serum from

each animal diluted 1:3 with Protein G binding buffer. The samples were then washed and eluted as described in the Methods section below.

Relative binding affinities of Capturem Protein A and Protein G Miniprep columns		
Human IgG subclass	**Capturem Protein A**	**Capturem Protein G**
IgG1	+++	++++
IgG2	++++	+++
IgG3	+	++++
IgG4	+++	++++

Table II. Relative binding affinities of Capturem Protein A and Protein G Miniprep columns for human IgGs 1–4. Columns were loaded with 800 µl serum samples diluted 1:3 with Protein A or Protein G binding buffer then washed and eluted as described in the Methods section below. The relative affinities of the various IgG subtypes were determined by ELISA.

Binding capacity of Capturem Protein G Maxiprep columns

We used Capturem Protein G Maxiprep columns to purify mouse antibody produced from a hybridoma in a bioreactor (Figure 1). This test demonstrated the high binding capacity of the Protein G membrane when using optimized conditions. Typical antibody purifications yielded 1–2 mg per column (Table III).

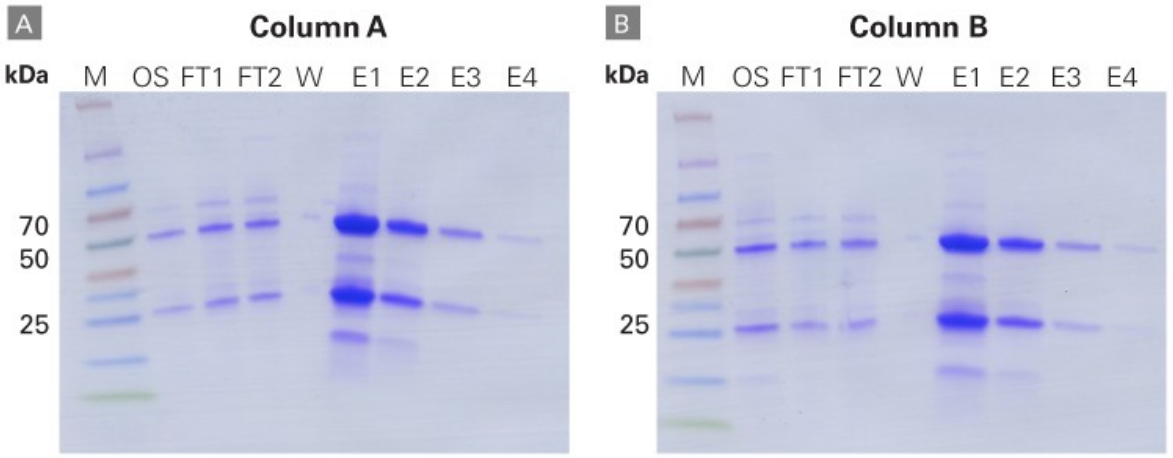

Figure 1. Purification of a hybridoma bioreactor supernatant using Capturem Protein G Maxiprep columns. SDS-PAGE analysis was performed on fractions purified from two separate Capturem Protein G Maxiprep columns (**Panels A & B**) as described in the Methods section below. The gels show a typical degree of purity for eluted antibody fractions and demonstrate that most of the antibody is eluted in the first two fractions (2.37 mg from Column A and 2.07 mg from Column B, as seen in Table III). Lane M: Marker. Lane OS: Original sample. Lane FT1: Flowthrough 1. Lane FT2: Flowthrough 2. Lane W: Wash. Lane E1: Elution 1. Lane E2: Elution 2. Lane E3: Elution 3. Lane E4: Elution 4.

Antibody yield in Capturem Protein G Maxiprep eluates			
	E1 (mg)	**E2 (mg)**	**Total (mg)**
Column A	1.89	0.48	2.37
Column B	1.56	0.51	2.07

Table III. Antibody yield in eluate fractions from a hybridoma bioreactor supernatant purified using Capturem Protein G Maxiprep columns.

Comparison of Capturem Protein G 96-well plates with resin spin plates

We tested the binding capacity of a Capturem Protein G 96-well plate side-by-side with a commercially available 96-well Protein G resin spin plate to purify dilute amounts of antibody from hybridoma supernatants (Figure 2). Antibodies were eluted from the Capturem membrane in a smaller volume using a shorter workflow (15 minutes instead of 1 hour), yielding a more concentrated antibody in less time.

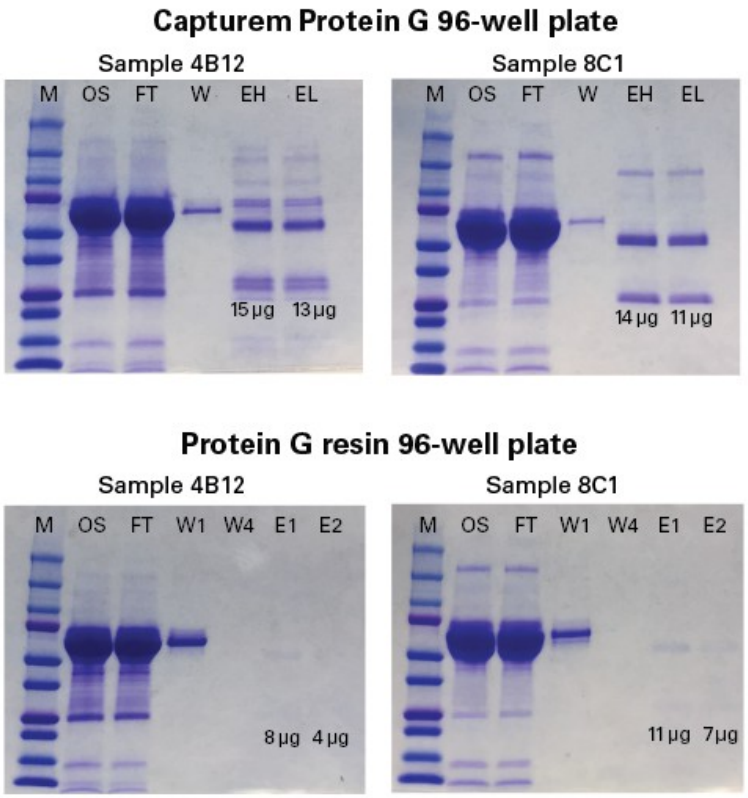

Figure 2. The Capturem Protein G 96-well plate outperforms a Protein G resin 96-well plate when used to purify a monoclonal antibody from dilute hybridoma supernatants. SDS-PAGE analysis was performed on fractions purified as described in the Methods section below from dilute hybridoma supernatants using either the Capturem Protein G 96-well plate (top row) or a commercially available Protein G resin 96-well plate (bottom row). The Capturem plate had a significantly faster workflow (15 min instead of one hour), higher recovery, and a significantly more concentrated eluate due to the small volumes required. Lane M: Marker. Lane OS: Original sample. Lane FT: Flowthrough. Lane W: Wash. Lane W1: Wash 1. Lane W4: Wash 4. Lane EH: Highest-yield elution. Lane EL: Lowest-yield elution. Lane E1: Elution 1. Lane E2: Elution 2.

Capturem Protein G 96-well plates have low variability for high-throughput applications

In addition to high-throughput screening, Capturem Protein G 96-well plates can also be used for high-throughput affinity purification upstream of applications such as mass spectrometry-based antibody quantification. We loaded eight replicates of two different hybridoma clone supernatants and a serum sample into individual plate wells to demonstrate the reproducibility and capacity of the Capturem Protein G 96-well plate (Figure 3).

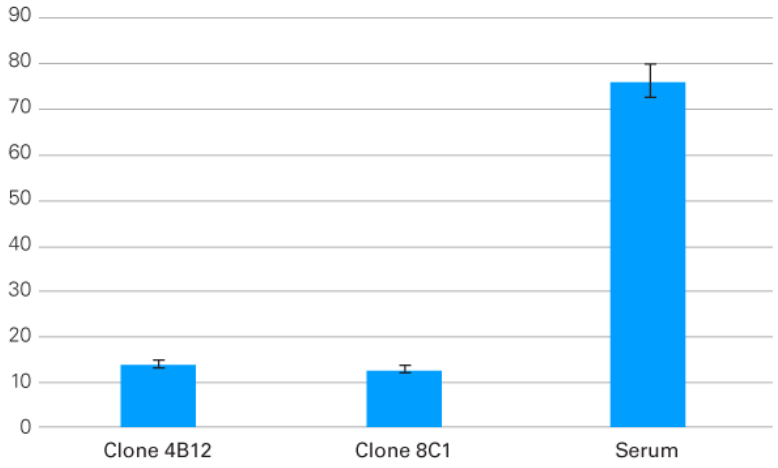

Figure 3. Reproducibility and capacity of the Capturem Protein G 96-well plate for high-throughput applications. We loaded eight wells with each sample type and then measured the amount of antibody in the eluted fraction. The standard deviation measured here was 6.0%, 6.5%, and 4.9% for the Clone 4B12, Clone 8C1, and serum samples, respectively.

Conclusions

Capturem Protein G products enable rapid IgG purification across a range of isotypes with a rapid workflow. Capturem Protein G 96-well plates offer consistent results for high-throughput applications, including affinity purification upstream of mass spectrometry-based antibody analysis, while Capturem Protein G Maxiprep columns can be used for projects requiring larger amounts of IgG. Capturem Protein G kits are appropriate for use with antibodies expressed in mammalian cells and whole serum, are compatible with various lysis buffers, and are available in miniprep, maxiprep, 24-well plate, and 96-well formats.

Methods

Capturem Protein G Miniprep columns were used according to the Capturem Protein G Miniprep protocol. First, the columns were equilibrated with 300 µl of Pierce Protein G Binding Buffer and then centrifuged at 1,000*g* for 1 min. Serum samples from sheep, goat, rat, mouse, human, rabbit, and horse (250 µl each) were diluted in 1 ml of Protein G binding buffer, and 600 µl of sample was loaded on an equilibrated column, followed by centrifugation at 1,000*g* for 1 min. The loading process was then repeated with another 600 µl of sample. The columns were then washed with 800 µl of Protein G binding buffer at 1,000*g* for 1 min. The bound antibody was then eluted with 300 µl of elution buffer (0.1 M glycine, pH 2.5) into a tube containing 30 µl of neutralization buffer (1 M Tris, pH 8.5) to neutralize the eluted antibody.

Capturem Protein G Maxiprep columns were used according to the Capturem Protein G Maxiprep protocol. First, the columns were equilibrated by loading 6 ml of Pierce Protein G binding buffer and centrifuging at 2,000*g* for 3 min. Next, 6.3-ml samples of mouse hybridoma supernatant from a bioreactor were diluted to 25 ml in Protein G binding buffer and then loaded onto the equilibrated columns by centrifugation at 2,000*g* for 3 min. We collected samples of the flowthrough for SDS-PAGE (Lane FT1 in Figure 1), then centrifuged the 25-ml samples through the columns a second time and collected another set of samples for SDS-PAGE (Lane FT2 in Figure 1). The columns were then washed once with 20 ml of Protein G binding buffer at 2,000*g* for 3 min. Lastly, four consecutive elutions were performed, each with 1 ml of elution buffer. The eluted fractions were analyzed using SDS-PAGE and quantified by measuring the absorbance at 280 nm with a NanoDrop 2000 spectrophotometer.

For the comparison of the Capturem 96-well plate and the Protein G resin 96-well plate, hybridoma supernatants from each clone were loaded onto each plate following the protocol for each product. The plates were first equilibrated, and then the supernatants were loaded and washed according to the protocols. Finally, the monoclonal antibody clones were eluted with 600 µl of elution buffer plus 60 µl of neutralization buffer in a collection tube.

To test the reproducibility of our 96-well plates, we loaded eight identical samples of two different hybridoma supernatants and animal sera into individual wells. Again, we equilibrated, loaded, washed, and eluted according to the protocol using Pierce Protein G Binding Buffer for all binding and washing steps. Eluates from various samples were quantified by measuring the absorbance at 280 nm with a NanoDrop 2000 spectrophotometer.

Related Products

Cat. #	Product	Size	License	Quantity	Details
635732	Capturem™ Protein G 24-Well Plate	1 x 24-well plate		*	
	Capturem Protein G 24-Well Plates are 24-well plates that provide rapid, high-quality, small-scale purification of monoclonal and polyclonal antibodies. It combines the specific antibody-binding properties of Protein G with novel Capturem technology to provide high-capacity, membrane-based affinity purification. Typical yields for monoclonal antibodies are up to 600 µg with a 4.5-ml sample capacity. Documents Components				
635726	Capturem™ Protein G 96-Well Plate	1 x 96-well plate		*	
635727	Capturem™ Protein G Maxiprep Columns	6 Columns		*	
635725	Capturem™ Protein G Miniprep Columns	10 Columns		*	

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