High-capacity system for rapid purification of antibodies using Protein A and Protein G membranes

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Abstract

Antibody engineering, production, and purification are critical in a wide range of research settings such as academic research institutions and biopharmaceutical organizations. There is a constant need for better, faster, and more efficient processes for antibody purification at various scales. Protein A has historically been one of the most widely used methods for affinity purification of immunoglobulins (IgGs) and allows the opportunity for several-fold enrichment in fewer steps along with high recovery rates. Agarose resins immobilized with Protein A are typically used for this process, with capacities ranging from 18 to 35 mg/ml. Resin-based purification requires a significant amount of work and may take up to a few hours to complete due to long column equilibration/binding/loading/elution/diffusion of large macromolecules through the resin bed. These longer times, in turn, increase the possibility of antibody aggregation or precipitation, or loss of activity due to unfolding or denaturation. Membrane-based affinity systems have rapidly flow-induced mass transport, and short residence times; however, traditionally they have been plagued with low capacity due to small internal surface areas. Here we describe a novel, membrane-based system with Protein-A and Protein-G affinity chemistry in which the pore surface area has been chemically enhanced, leading to a protein binding capacity better than that of resins at 75 mg or more per gram of membrane. Unlike traditional resin-based systems, the entire purification process—from loading the sample to eluting pure antibody—can be completed at room temperature in less than five minutes. We have assembled these high-capacity membranes into spin columns and filtration devices such as 96-well plates, and demonstrate that they can purify antibodies from a variety of samples such as animal sera, cell culture supernatants, and hybridoma supernatants, and demonstrate their utility in immunoprecipitation (IP) and co-IP experiments. We have compared our Protein A and Protein-G membranes with commercially available resins, and show that Capturem membranes result in more concentrated antibodies in significantly less time. These novel membrane-based affinity columns are extremely useful for purification and characterization of various antibody isotypes for a variety of applications.

1. Fast, high-capacity membrane-based antibody purification

2. Animal sera antibody binding test

3. Comparison with resins

4. Volume and capacity for different Capturem column formats

5. Cas9 antibody screening

6. Protein A column IP

7. IP compatibility

8. Fast Co-IP of p53 and SV40 T antigen with Capturem Protein A

Conclusions

Capturem Protein A and Protein G membranes are unique solutions for antibody purification, consisting of amenable affinity columns and filtration devices containing novel, modified nylon membranes. The benefits of these systems make it a powerful tool for a wide range of research settings.

- No-waiting workflow — The protocol is complete in <5 min for the miniprep and 15 min for the maxiprep, from loading the antibody to elution, saving the antibody from possible degradation and/or loss of activity.
- High purity and yield — Typical elutions of 50–100 mg antibody/column, with a concentration of ~80–110 mg/ml for the mini column
- Appropriate for different samples — The purification protocol works for antibodies expressed in mammalian cells and whole serum
- Fast immunoprecipitation — Capturem Protein A enables IP in less than 5 minutes of hands-on time starting from antibody-antigen complex

Materials and methods

Spin columns were first equilibrated by addition of 400 μl of binding buffer to the column, followed by centrifugation at 1,000 g for 1 min. Equilibrated spin columns were then washed with 200 μl of wash buffer to the column, followed by centrifugation at 1,000 g for 1 min. The wash buffer was then removed, followed by elution of antibody beads with Protein-a binding buffer (Thermo Fisher Scientific). The elution buffer was incubated for 3 min. The bound immunocomplexes were then eluted with 30 μl of either low pH glycine buffer (3 M NaCl, 10 mM sodium borate, pH 8.9), centrifuged, and eluted with 0.5 ml of elution buffer (100 mM Tris, pH 9.4) in the collection tube, centrifuged at 1,000 g for 1 min. The elution buffer was then diluted to 500 μl with buffer, and applied to the spin column, followed by centrifugation at 1,000 g for 1 min. All elution samples were then diluted to 500 μl with buffer, and applied to the spin column, followed by centrifugation at 1,000 g for 1 min. All elution samples were then diluted to 500 μl with buffer, and applied to the spin column, followed by centrifugation at 1,000 g for 1 min.

Graphs and tables

- Graph A: Comparison of Capturem Protein A to commercially available Protein A resins
- Graph B: Comparison of Capturem Protein A to commercially available Protein A resins
- Table 1: Comparison of Capturem Protein A to commercially available Protein A resins

References


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