TECH NOTE

Rapid Screening of Hybridoma Clones for the Best Monoclonal Antibody Using Capturem Protein A Technology

Capturem Protein A Miniprep, Capturem Protein A Maxiprep, and Capturem Protein A 96

Technology overview: Capturem Protein A high-capacity membranes >>

Five-minute, no-incubation protocol for high-quality antibody purification >>

Improved animal sera antibody binding compared to traditional protein A resin >>

Fast screening for the best Cas9 monoclonal antibody from hybridoma clones >>

Introduction

Antibody therapeutics is ever increasing in complexity and demand. Consequently, those supporting research in this area find a constant need for more efficient tools and methods to help with the work of antibody engineering, production, and purification. Capturem Protein A Minipreps, Maxipreps, and 96-well plates—part of the ever-growing Capturem family, which also includes products for his-tagged protein purification—provide unique solutions for antibody purification without resins or the need for incubation steps. Consisting of spinnable affinity columns or plates containing novel high-capacity Protein A nylon membranes (Figure 1), this system enables researchers to bring speed, ease-of-use, flexibility, and high yield to antibody purification and screening.

Our unique, high-capacity membranes have a high surface area, leading to protein binding capacity better than that of resins at 75 mg or more per cm^3 of membrane, and yielding 250 µg of protein per column or well in just five minutes. Where resin-based purification requires a significant amount of work and may take up to a few hours to complete, the entire Capturem purification process can be completed in less than five minutes for minipreps and less than 15 minutes for maxipreps and 96-well plates, without any incubation steps required. Combined with the specific antibody-binding properties of Protein A, Capturem technology provides rapid, high-quality purification and screening of monoclonal and polyclonal antibodies, as well as utility in immunoprecipitation and co-immunoprecipitation experiments.

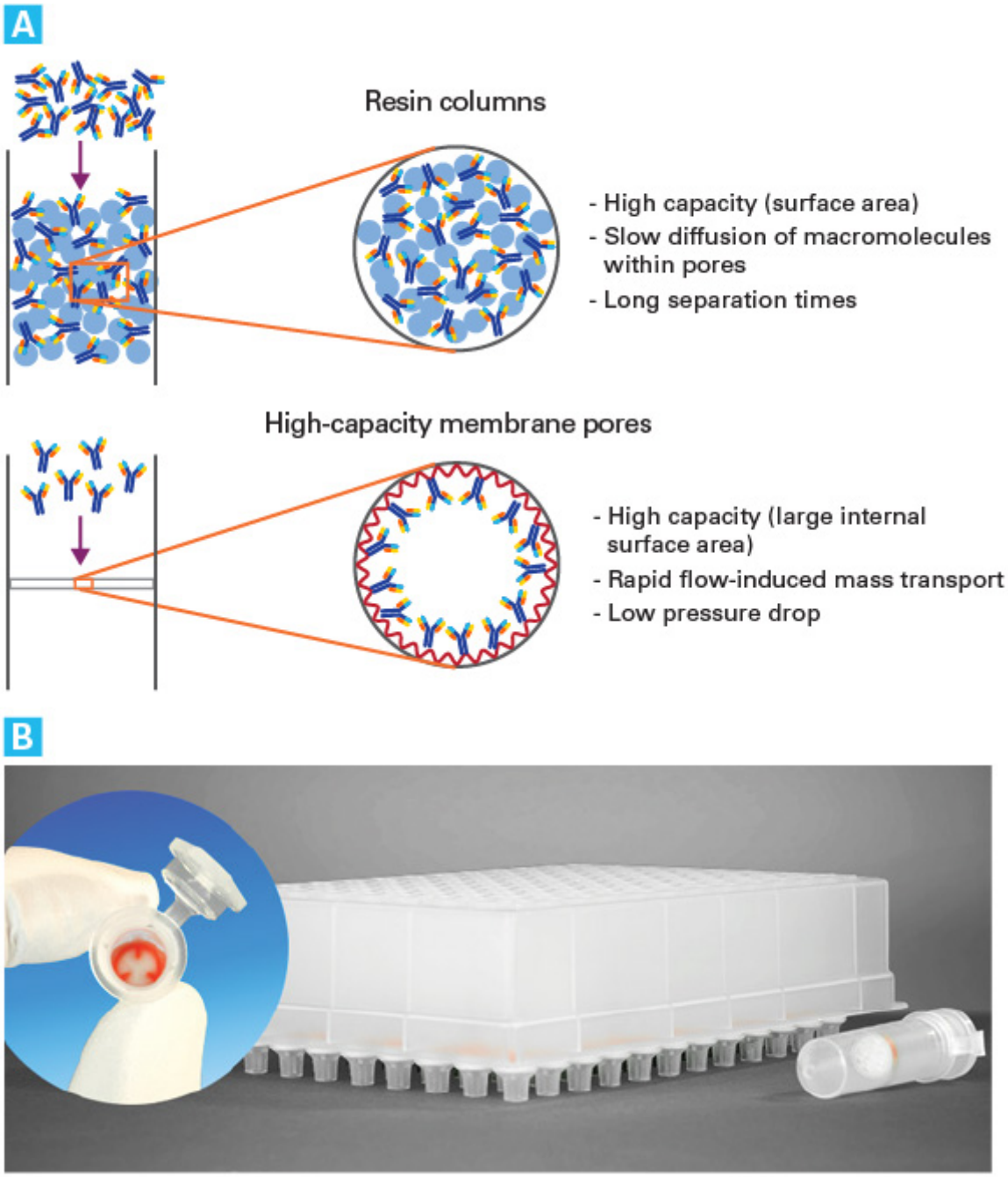


Figure 1. Capturem Protein A technology. Panel A. Each Capturem Protein A spin column contains a high-capacity Protein A membrane with increased surface area, providing much higher protein binding capabilities per ml of membrane than per ml of resin. **Panel B.** Capturem Protein A miniprep spin columns and plates accommodate up to 800 µl of antibody sample and a capacity of up to 250 µg antibody per tube or well, respectively. Capturem Protein A maxiprep spin columns (not shown) accommodate up to ~25 ml of antibody sample and have a capacity of up to 2.5 mg antibody per tube.

Results

Fast, high-capacity membrane-based antibody purification

Capturem Protein A technology removes the effort from antibody purification and screening with a no-waiting workflow that produces high-quality antibodies quickly and easily. With a protocol as simple as that of DNA minipreps, each column or well is equilibrated and then loaded with diluted antibody sample. Antibodies are bound to the membrane, washed, and eluted. Each step is followed by a one-minute spin for a total purification time of just five minutes (Figure 2). Over 80% of the antibody can be eluted with as little as 100 µl elution buffer.

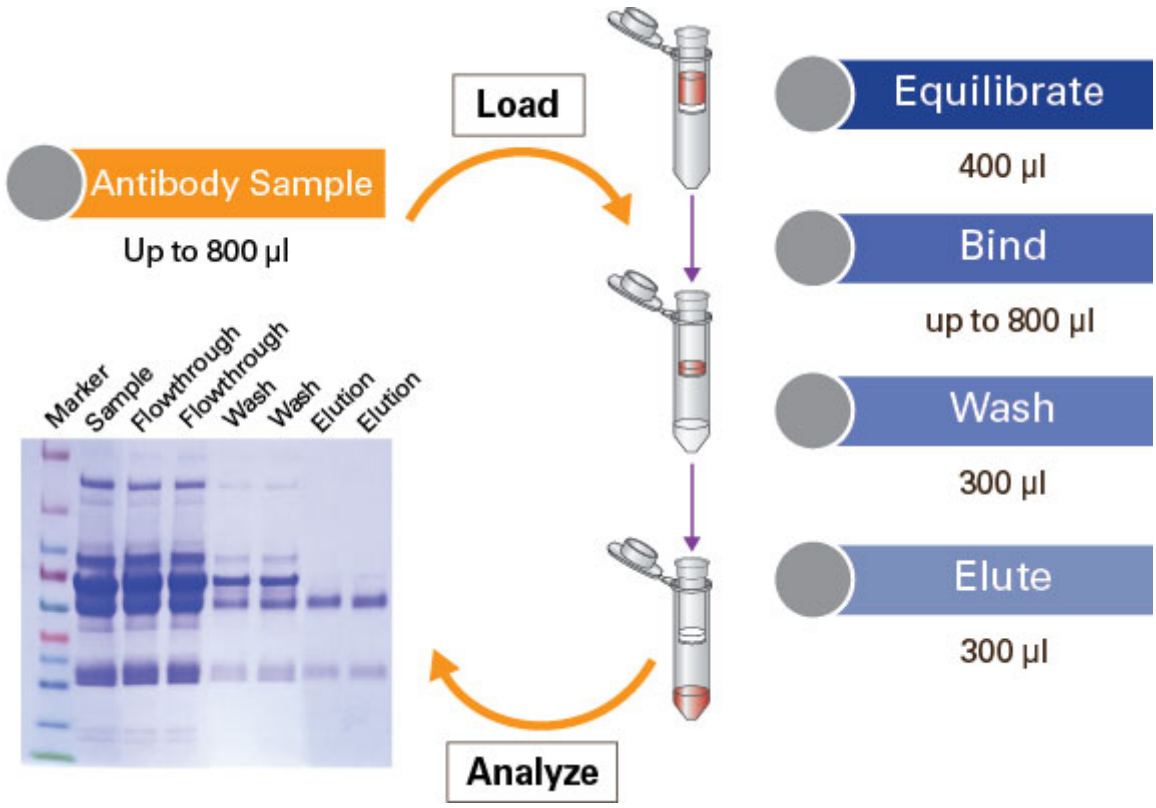
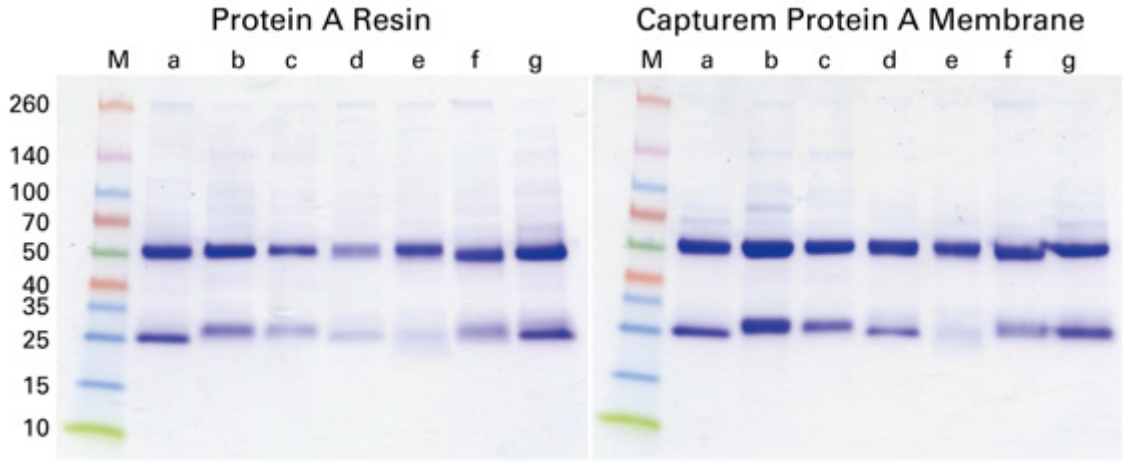


Figure 2. Capturem Protein A Miniprep workflow for antibody purification. Each mini spin column can be loaded with up to 800 µl of a diluted sample (antibody sample diluted from 1:1 to 1:20 with buffer). Antibodies are first bound to the membrane, followed by washing with 300 µl of wash buffer, and elution with 300 µl of elution buffer. Each step is followed by spinning the tube for 1 min at 1,000*g*. The working bed volume of the membrane is <3 µl. This entire miniprep purification is complete in ~5 min.

Animal sera antibody binding with Capturem Protein A vs. resins

The Capturem Protein A purification system allows for flexibility in sample type, handily accommodating animal sera, cell culture media, and other sources. In order to directly test the performance of Capturem Protein A Minipreps versus traditional Protein A resin, seven different animal sera were used as samples for antibody purification with both methods (Figure 3). Standard manufacturers' conditions were used for both, entailing a five-minute Capturem protocol and a 45–minute resin protocol. Samples purified with Capturem Protein A Minipreps showed excellent purity with Coomassie blue staining, and even showed higher yields than those obtained with the Protein A resin.



	Amount in Elution Samples (µg)	
Sample	Protein A Resin	Capturem Protein A Membrane
a) Mouse	90	122
b) Sheep	94	207
c) Goat	55	104
d) Rat	42	191
e) Rabbit	70	94
f) Horse	80	251
g) Human	114	180

Figure 3. Antibodies purified from various animal sera with Protein A resin and Capturem Protein A Minipreps. Capturem Protein A Minipreps and Protein A resin were both used to purify antibodies from 250 µl each of the following animal sera: sheep, goat, rat, mouse, human, rabbit, and horse. Manufacturers' protocols were followed for each purification method. Purities of the final elution samples from both sets were analyzed by gel electrophoresis, followed by staining with Coomassie blue. The amount of antibody eluted was determined by measuring absorbance at 280 nm.

Screening for the best monoclonal Cas9 antibody

In addition to high-quality antibody purification, the Capturem Protein A protocol enables quick and easy antibody screening. With this method, screening hybridomas for the best monoclonal antibody becomes a much simpler process, taking advantage of speed and the ability to directly apply antibody supernatant to the Capturem Protein A columns. In the data below (Figure 4), this technology was used to screen different hybridoma clones for the best monoclonal antibody against Cas9. Crude antibody supernatant was purified with Capturem Protein A, elution was done in a low volume in order to yield a concentrated antibody, and the resulting purified anti-Cas9 antibodies were resolved on an SDS-PAGE gel (Panel A). Cell lysates expressing low and high amounts of Cas9 (Lanes 1 and 2, respectively) were resolved by electrophoresis and transferred to nitrocellulose membranes. These membranes were then blotted using the anti-Cas9 antibodies purified above (Panel B). From these results, two clones were selected for blotting against dilutions of the Cas9 protein (Panel C). With just five minutes of hands-on time, Capturem Protein A maintained excellent results, providing pure, active antibodies for streamlined screening of hybridoma clones.

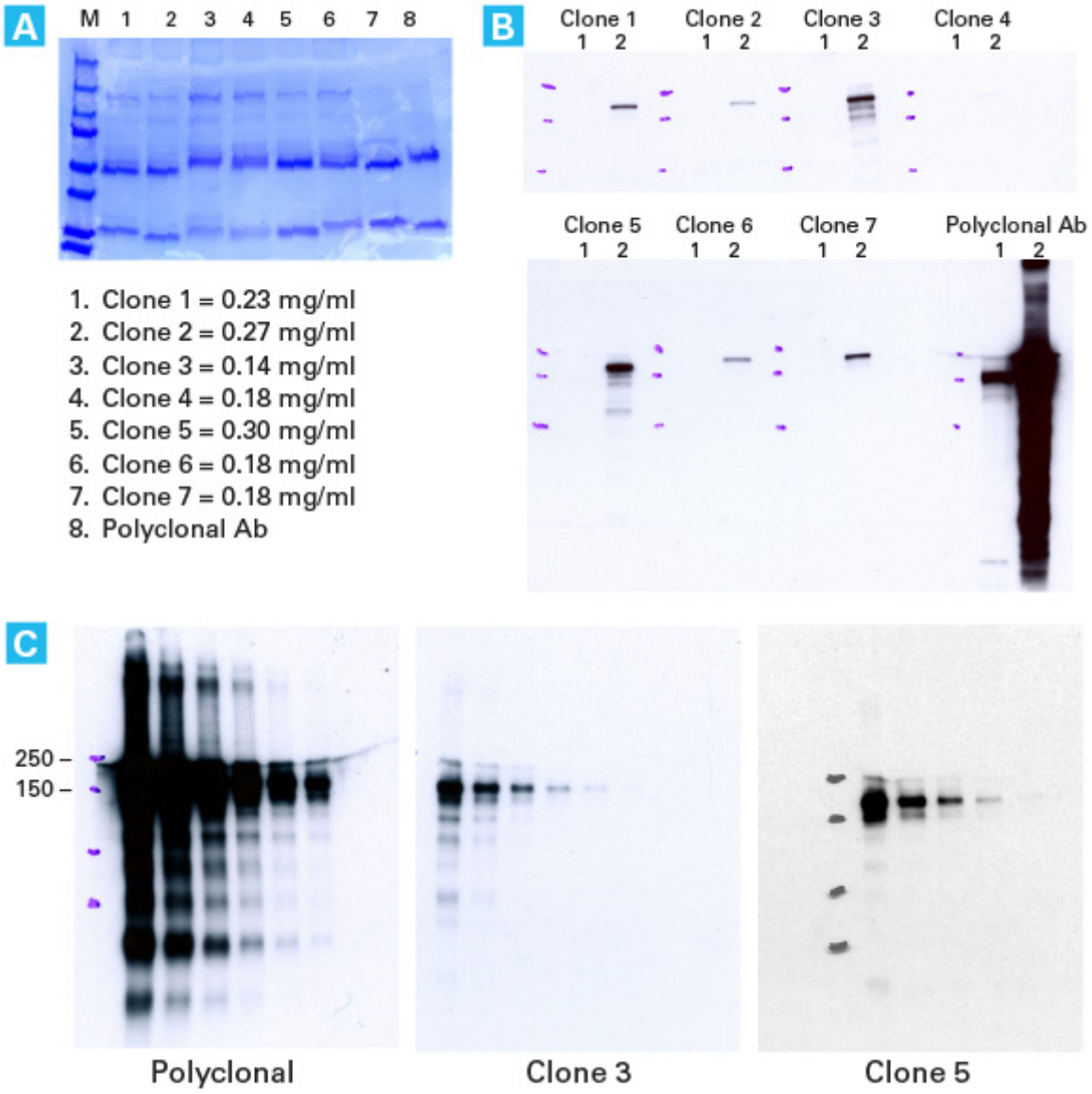


Figure 4. Screening for the best monoclonal antibody against Cas9 from hybridoma supernatants. All clones were of the mouse IgG1 isotype, and thus purified using a high-salt method. Capturem Protein A maxi columns were equilibrated with buffer and centrifuged. Antibody supernatant was then loaded on a Capturem Protein A maxi column, followed by centrifugation. The loading process was repeated with the flowthrough to maximize antibody binding. The column was then washed with wash buffer, centrifuged, and eluted with 0.5 ml of elution buffer into the collection tube, and neutralized. **Panel A.** Antibodies purified from cell culture supernatants using Capturem columns were resolved on an SDS-PAGE gel. **Panel B.** Cell lysates expressing low (Lane 1) or high (Lane 2) amounts of Cas9 were resolved by gel electrophoresis and transferred to nitrocellulose membranes. The membranes were blotted using the antibodies purified with the maxi columns.**Panel C.** Cas9 protein dilutions (from left to right: 20, 10, 5, 2.5, 1.25, and 0.625 ng), blotted with the selected antibodies.

Conclusions

Capturem Protein A technology provides researchers with a unique solution to save time and hassle in antibody research. With an incubation- and resin-free workflow, antibody purification is complete in record time while still yielding high-quality, concentrated antibodies. Consisting of single-use spin columns containing high-capacity membranes with mobilized Protein A, this novel system is easy to use and reduces the risk of contamination and carryover. Hybridoma screening also benefits from this fast protocol, with minimal hands-on time and the ability to apply supernatants directly

to the columns, thus streamlining the whole process. Capturem Protein A is appropriate for use with antibodies expressed in mammalian cells and whole serum, is compatible with various lysis buffers, and miniprep, maxiprep, and 96-well formats available.

Methods

Animal sera antibody binding test

Capturem Protein A Miniprep columns were first equilibrated with 800 µl of Protein A binding buffer (0.5 M phosphate, pH 8.0 containing 2 M NaCl), 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 A binding buffer, and 600 µl of sample was loaded on an equilibrated column, followed by centrifugation at at 1,000*g* for 1 min. The loading process was then repeated with another 600 µl sample. The columns were then washed with 800 µl of Protein A 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.

For the resin (Thermo Fisher Scientific), 20 µl of slurry was used for each purification, following the vendor's protocol for antibody purification. Storage buffer was first removed, followed by equilibration of the beads with Protein A binding buffer (Thermo Fisher Scientific; 600 µl x 2). The equilibration buffer was then removed and the beads were incubated with diluted serum samples (1 ml of serum in 3 ml of buffer, 1.2 ml of diluted sample) for 4 min with end-over-end rotation. Following loading, the resin was washed twice with 600 µl of Protein A binding buffer, drained, and eluted with 600 µl of elution buffer plus 60 µl of neutralization buffer in a collection tube.

The eluates from various sera were resolved by gel electrophoresis and stained with Coomassie blue. Absorbance at 280 nm was measured with a NanoDrop 2000 spectrophotometer, and used to quantify the amount of antibody in eluted fractions.

Screening for the best monoclonal antibody against Cas9 from hybridoma supernatants.

All clones were of the mouse IgG1 isotype, and thus purified using the high-salt method as detailed below. Capturem Protein A maxi columns were equilibrated with 6 ml of buffer (10 mM sodium borate, pH 8.9, 3 M NaCl), and centrifuged at 2,000*g* for 3 min. The NaCl concentration of the crude antibody supernatant was adjusted to 3.3 M, and to this, 1/10 of its volume of 1.0 M sodium borate (pH 8.9) was added. The supernatant (20 ml) was then loaded on a Capturem Protein A maxi column, followed by centrifugation at 2,000*g* for 3 min. The loading process was repeated with the flowthrough to maximize antibody binding. The column was then washed with 10 ml of wash buffer (3 M NaCl, 10 mM sodium borate, pH 8.9), centrifuged, and eluted with 0.5 ml of elution buffer (100 mM glycine, pH 3.0). The eluate was neutralized by adding 50 µl of 1 M Tris, pH 8.5, into the collection tube. Antibodies purified from cell culture supernatants using Capturem columns were resolved on an SDS-PAGE gel. Cell lysates expressing low or high amounts of Cas9 were resolved by gel electrophoresis and transferred to nitrocellulose membranes. The membranes were blotted using the antibodies purified with the maxi columns. Cas9 protein dilutions (20, 10, 5, 2.5, 1.25, and 0.625 ng), were blotted with the selected antibodies.

View web page >>

http://www.clontech.com/US/Support/Applications/Antibody_Purification/Purification_and_Screening_with_Capturem_Protein_A