Rapid purification and characterization of recombinant proteins and antibodies: Capturem high-capacity membranes

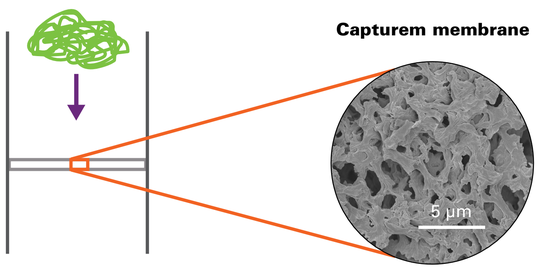
Gia Jokhadze*, Christian Hoppmann, Michael T. Vierra, Boris Levitan, Mandy Li & Andrew Farmer

Takara Bio USA, Inc., 1290 Terra Bella Avenue, Mountain View, CA 94043, USA *Corresponding author: gia_jokhadze@takarabio.com

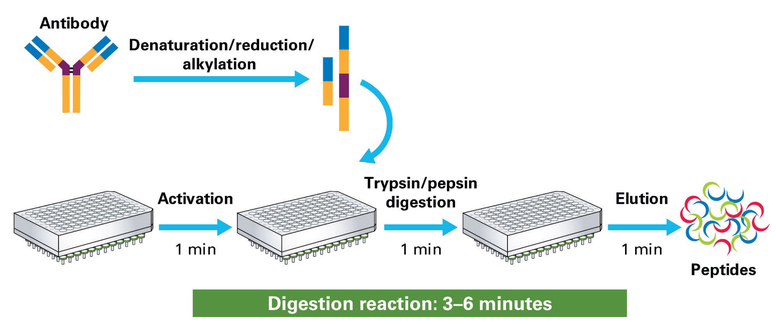
Abstract

Recombinant protein and antibody production is immensely important in many research settings, including academic research institutions, biopharmaceutical organizations, and enzyme and agricultural industries. Typical purification methods using immobilized metal affinity chromatography (IMAC) or immunoaffinity chromatography (protein A, G, A/G, L, etc.) columns take several hours to complete due to long column equilibration/binding times and slow diffusion of large macromolecules through the resin bed. These extended times increase the risk of proteolytic degradation and activity loss due to unfolding or denaturation. Membrane-based affinity systems employ rapid flow-induced mass transport and short residence times; however, they have been plagued with low capacity due to small internal surface areas. To overcome this limitation, we developed Capturem™ membrane technology. Capturem membranes consist of high-capacity nylon membranes with chemically enhanced surface areas. The pores of these modified membranes can be functionalized with ligands such as nickel, proteins A or G, streptavidin, lectins, various proteases (e.g., trypsin or pepsin), etc. We assembled these functionalized membranes into different spin column formats and high-throughput 24-well and 96-well filtration plates to allow for rapid purification, enrichment, labeling, or proteolytic spin digest of proteins and antibodies. For example, Capturem Protein A and G membranes enable extremely fast purification of antibodies from various matrices based on the affinity of these proteins for the fragment crystallizable (Fc) region of antibodies. Antibody purification can be accomplished in less than 15 minutes, with capacities far exceeding those of resin-based columns. We have also developed a 15-minute protocol for antibody conjugation and purification using Protein G Miniprep Columns without the need to purify before or after labeling. In addition, Capturem Trypsin and Pepsin membrane spin devices facilitate fast proteolysis of proteins and antibodies for their characterization, identification, and quantitation through mass spectrometry analysis. In contrast to the long incubation periods (6–24 hours) of conventional in-solution digestions, these proteolytic membranes generate peptides suitable for downstream analysis, with similar sequence coverage, within only 2–3 minutes. These novel, membrane-based spinnable affinity columns and high-throughput filtration devices will be useful for purifying a variety of recombinant proteins and antibodies as well as their proteomics characterization, in both academic and industrial settings.

Capturem membrane technology overview



- Resin-free
- High capacity due to large internal surface area
- Rapid flow-induced mass transfer
- No incubation time
- Room-temperature workflow



Digestion workflow using Capturem Pepsin or Capturem Trypsin spin columns. The digestion of the prepared sample is complete in just 3 min.

Materials and Methods

Experimental conditions for Capturem his-tagged minipreps: Spin columns were equilibrated with the addition of 400 µl xTractor™ Buffer to the column followed by centrifugation at 11,000*g* for 1 min. Equilibrated spin columns were then loaded with 400–800 µl clarified lysate from cells expressing a 6xhis-tagged fusion protein (GFPuv) and centrifuged at 11,000*g* for 1 min at room temperature. The bound protein was then washed with 300 µl Wash Buffer, followed by centrifugation. Elution was performed by the addition of 300 µl Elution Buffer containing 500 mM imidazole, followed by centrifugation. The eluted fraction was analyzed by electrophoresis on a 4–20% SDS polyacrylamide gel to verify the purity of the protein. Gels were stained with Coomassie blue and destained according to standard protocol. An expected band was observed at a molecular weight of ~29 kilodaltons (kDa), corresponding to 6xhis-tagged GFPuv (unless otherwise specified in the figure caption).

Experimental conditions for the antibody purification experiment are described in Figure 3. Details for trypsin digestion are provided in Figure 4. Antibody labeling and purification using Capturem Protein G is discussed in Figure 5. Antibody enrichment using Capturem Streptavidin for successive antibody capture is described in Figure 6.

1 High capacity with novel, membrane-based, spinnable, metal ion affinity columns

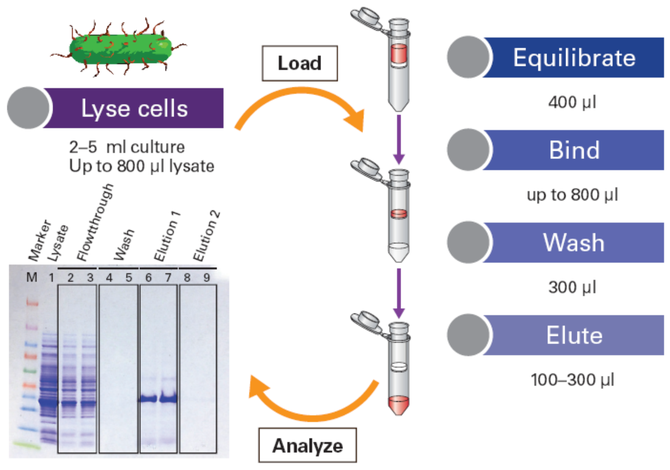


Figure 1. Capturem miniprep workflow for protein purification, as described in the Materials and Methods section. Briefly, the his-tagged protein is first bound to the membrane, then washed and eluted with the appropriate buffers. Over 90% of the bound protein can be eluted with as little as 100 µl of Elution Buffer. Each step is followed by spinning the tube for 1 min at 11,000*g*. The working bed volume of the membrane is ~3 µl. This entire purification is complete in <5 min.

2 Volume and yields of Capturem purification formats

	Bed volume	Sample volume	Time	Approximate yield
Nano	0.24 ± 0.01 µl	400 µl	5 min	5–25 µg
Mini	3.0 ± 0.2 µl	800 µl	5 min	40–100 µg
96 wells	3.0 ± 0.2 µl	1 ml	15 min	40–100 µg
24 wells	30 ± 2 µl	4.5 ml	15 min	0.4–1 mg
Maxi	64 ± 2 µl	25 ml	15 min	1–2.5 mg
Large volume	480 ± 20 µl	500 ml	30 min	10–25 mg

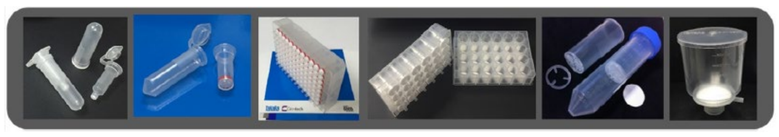
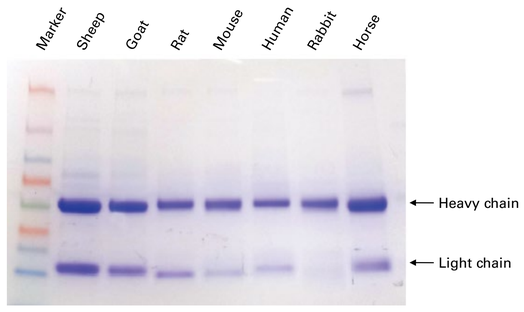


Figure 2. Loading volumes, protocol times, and approximate yields for different column formats. Yields are based on representative samples and will vary based on loading concentration and sample details (e.g., sample type, species, antibody isotype, protein size, etc.). Pictured from left to right: nanoprep, miniprep, 96-well, 24-well, maxiprep, and large-volume Capturem formats.

3 Animal sera antibody binding test



Animal sera	Purified antibody (µg)
Sheep	256
Goat	184
Rat	170
Mouse	140
Human	129
Rabbit	102
Horse	215

Figure 3. Capturem Protein A columns were tested for their ability to purify antibodies from various animal sera. Columns were equilibrated with 800 µl of a Protein A binding buffer, then centrifuged at 1,000*g* for 1 min. Serum samples (250 µl) were diluted in 1 ml of binding buffer, and 2 x 600 µl of each sample was loaded onto the column, followed by centrifugation each time at 1,000*g* for 1 min. The columns were then washed with 800 µl of binding buffer and centrifuged at 1,000*g* for 1 min. The bound antibody was eluted with 300 µl of Elution Buffer (0.1 M glycine, pH 2.5) directly into 30 µl of Neutralization Buffer (1 M Tris, pH 8.5). The eluates were resolved by gel electrophoresis, and absorbance at 280 nm was measured to quantify antibody.

4 Capturem Trypsin digest of a standard monoclonal antibody

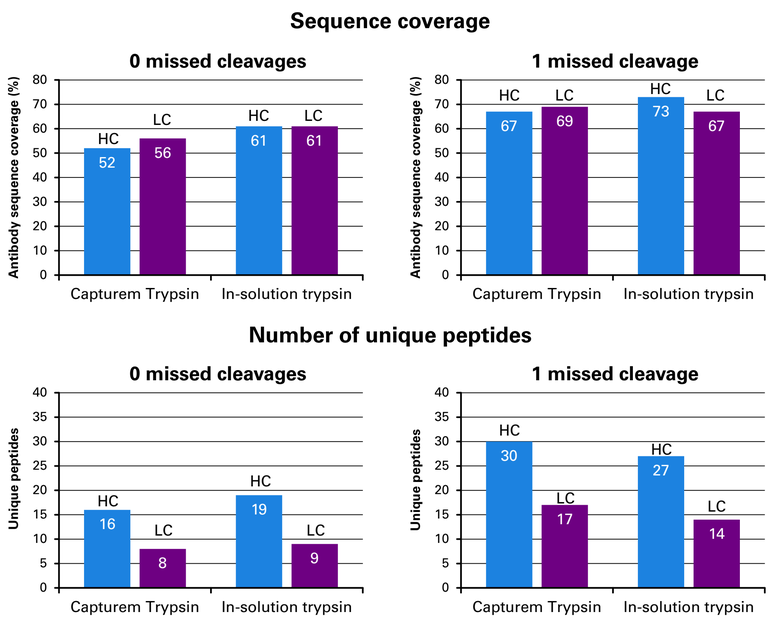


Figure 4. Analysis of monoclonal antibody digest using Capturem Trypsin spin columns. 20 µg of human IgG1 (NIST) was digested with Capturem columns (1 min, RT) or in-solution trypsin (16 hr, 37°C). Mass spectroscopy analysis of tryptic peptides reveals similar sequence coverage and unique number of peptides for both methods. HC = heavy chain (blue bars); LC = light chain (purple bars). Mass spectroscopy analyses of NIST-mAb were performed by Jadebio, Inc.

5 Schematic of antibody labeling and purification using Capturem Protein G

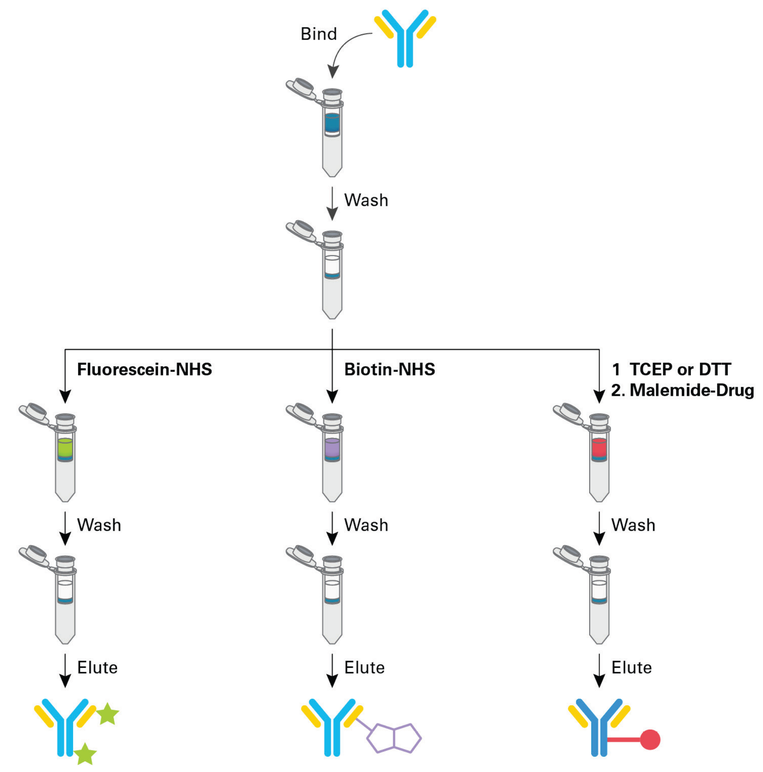


Figure 5. Workflow for labeling and purifying antibodies using Capturem Protein G. Antibodies are labeled directly from any starting solution, in just 15 min through the following process. The diluted antibody is bound to an equilibrated Capturem Protein G column. Labeling reagent is then spun through the column to complete the labeling process. The labeled antibody is recovered by elution with an appropriate elution buffer.

6 Capturem Streptavidin pulldown

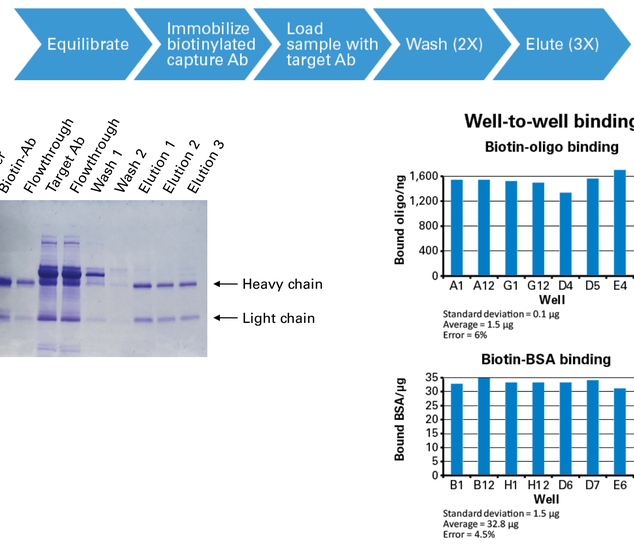


Figure 6. Binding capacity and reproducibility of Capturem Streptavidin pulldown experiments. 48 µg of biotinylated rabbit IgG in 200 µl Binding Buffer was passed through an equilibrated Capturem Streptavidin spin column, and 32.0 ± 1.4 µg was immobilized. After a single wash step, a sample containing the spiked-in target antibody (~100 µg of anti-rabbit IgG from goat) in hybridoma medium with 20% mouse serum was diluted with Binding Buffer and applied to the column. After two washing steps with Binding Buffer and then PBS, the target antibody was eluted with 1.0 M glycine in three steps to yield 42 ± 5 µg of highly pure target antibody. Technical replicates of biotinylated oligo or biotinylated BSA were loaded into a 96-well plate to test reproducibility. Absorbance measurements were used to determine the amount of biomolecules bound to the membranes. For protein binding experiments, 100 µg biotinylated BSA was diluted in 200 µl Binding Buffer and applied to each well. For oligonucleotide binding, 3.8 µg of oligo in 200 µl Binding Buffer was used.

Conclusions

- **No-waiting purification workflows**—Antibody and his-tagged purification protocols are complete in <5 min for the miniprep format and 15 min for the maxiprep format, protecting the protein from possible degradation or loss of activity.
- **High yield**—His-tagged miniprep columns yield up to ~0.3–1 mg/ml of protein and his-tagged maxiprep columns yield up to 0.5–4.5 mg/ml of protein. Protein A miniprep columns yield up to ~0.1–1 mg/ml of antibody and Protein A maxiprep columns yield up to 0.1–2 mg/ml of antibody.
- **Improved characterization**—Capturem Trypsin columns fully digest antibodies in 2–3 minutes and yield sequence coverage comparable to a 16-hour in-solution digest with minimal over-digestion.
- **Streamlined antibody labeling**—Capturem Protein G (or A) allows antibodies to be rapidly labeled without the need to purify before and after conjugation.
- **Rapid enrichment**—Streptavidin-functionalized membranes enable a variety of enrichment protocols (e.g., oligo capture, successive antibody capture, etc.) for the capture of biotinylated products or pulldown of binding partners for a captured biotinylated ligand.

Takara Bio USA, Inc.
United States/Canada: +1.800.662.2566 • Asia Pacific: +1.650.919.7300 • Europe: +33.(0)1.3904.6880 • Japan: +81.(0)77.565.6999