WP035

Rapid conjugation, proteolysis, and purification of antibodies using high-capacity Capturem membranes

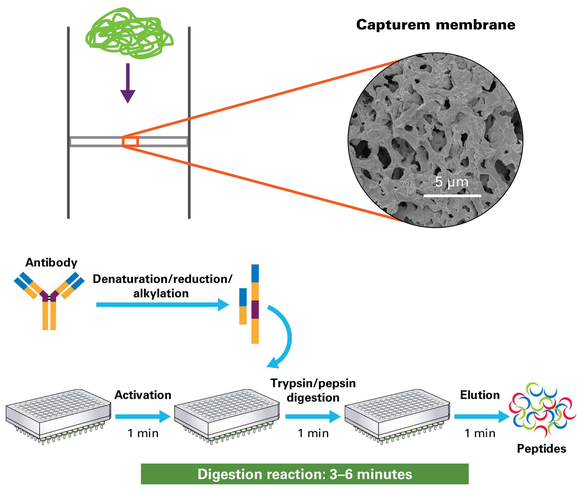
Christian Hoppmann*, **Mandy Li, Michael Vierra, Boris Levitan, Gia Jokhadze, Andrew Farmer**

Takara Bio USA, Inc., Mountain View, CA 94043, USA *Corresponding author: christian_hoppmann@takarabio.com

Introduction

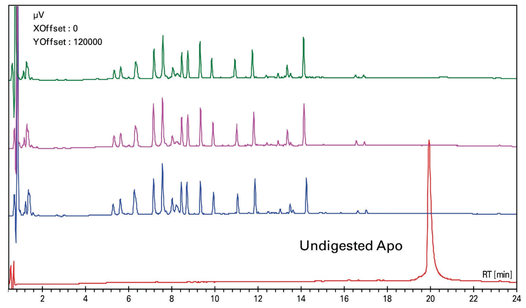
Capturem™ is a new technology that consists of a porous, high-capacity membrane that can be functionalized with not only proteases (e.g., trypsin or pepsin) but also with other ligands such as Protein A and G, nickel, streptavidin, etc. The functionalized membranes are assembled in different spin column formats and multiwell plates to allow for rapid purification, enrichment, labeling, or spin digest. Here we show the digestion of antibodies within ~2–3 minutes using Capturem Trypsin 96-well plates, and a 15-minute protocol for antibody conjugation and purification using Protein G Miniprep Columns without the need to purify before and after labeling. This allows the fast digestion and analysis of protein and antibody samples in a high-throughput fashion.

1 Schematic of the Capturem spin digest workflow



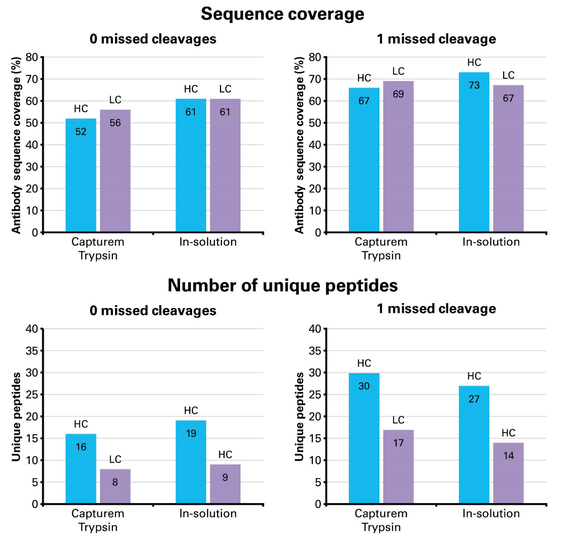
- Resin-free
- High capacity due to large internal surface
- Rapid flow-induced mass transfer
- No incubation time
- Room-temperature workflow
- Available in a variety of formats including miniprep and maxiprep spin columns, and 24- and 96-well plates

2 Capturem Trypsin digest of apomyoglobin



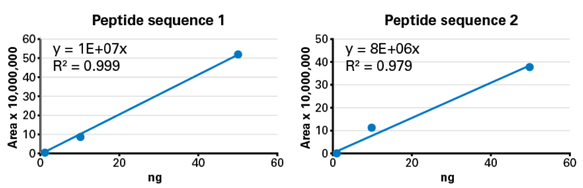
- Complete tryptic digest of 80 µg of apomyoglobin achieved in 2–3 minutes under native conditions
- High well-to-well reproducibility as demonstrated by consistent HPLC profiles (n = 3)

3 Capturem Trypsin digest of a standard monoclonal antibody



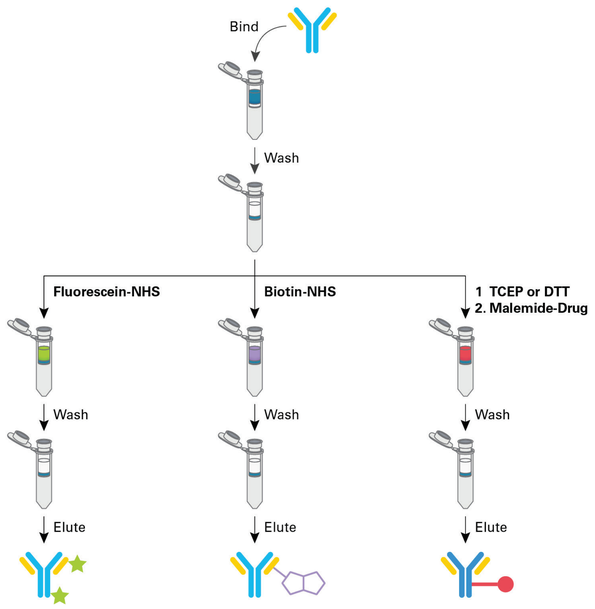
- Digestion of 20 µg of human IgG1 (NIST) with Capturem columns (2 min, RT) or in-solution trypsin (16 hr, 37°C)
- Mass spectroscopy analysis of tryptic peptides reveals similar sequence coverage and unique number of peptides for both methods
- HC = heavy chain (blue bars); LC = light chain (purple bars)

4 Quantitation using Capturem Trypsin



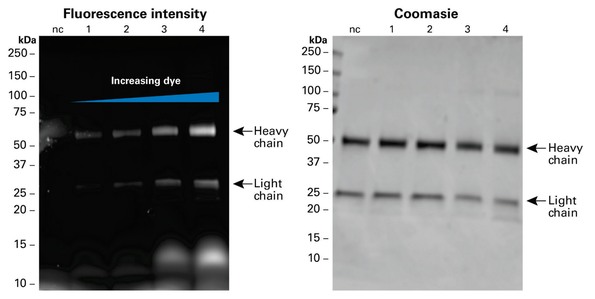
- Tryptic spin digest of SILuLite yields surrogate peptides detectable at ng-input amounts (1, 10, and 50 ng)
- AUCs of specific peptides give linear correlation

5 Schematic of antibody labeling and purification using Capturem Protein G



- Label antibodies (Abs) directly from any starting solution in just 15 minutes
- Diluted antibody is bound to an equilibrated column
- Labeling reagent is then spun through the column to complete the antibody labeling process
- Labeled antibody is retrieved by elution with an appropriate elution buffer

6 Fluorescein labeling of a Cas9 antibody using Capturem Protein G



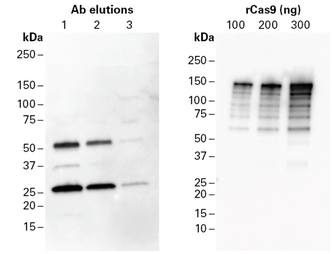
- Fluorescence signal rises with the ratio of dye molecules per antibody in the labeling reaction
- Each well has an equal mass of antibody as seen in the Coomassie-stained gel

Acknowledgments

We thank and acknowledge Weijing Liu (Merlin Bruening lab at University of Notre Dame) for help with low-input LC-MS quantitation.

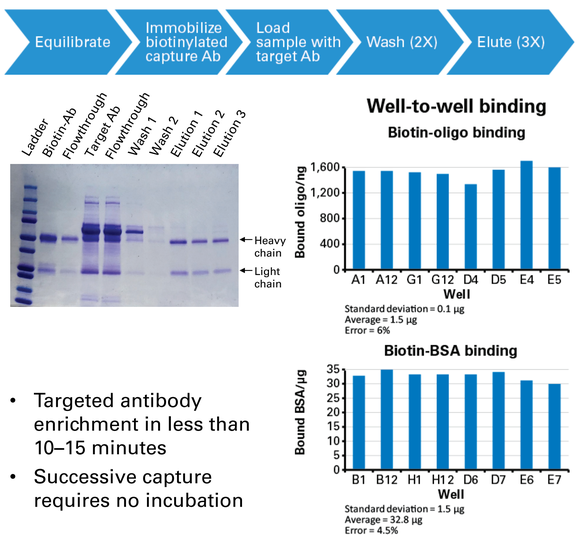
Mass spectroscopy analyses of NIST-mAb were performed by Jadebio, Inc.

7 Biotin labeling of a Cas9 antibody using Capturem Protein G



- >90% of biotinylated Ab captured in first two elutions
- Recognition of Cas9 by the biotinylated Cas9 Ab demonstrates that the epitope remains active after labeling and eluting

8 Capturem Streptavidin pulldown



- Targeted antibody enrichment in less than 10–15 minutes
- Successive capture requires no incubation

Conclusions

- Capturem spin membranes enable complete protein digestion in 2–3 minutes at room temperature with high temporal control and reproducibility
- Antibodies are fully digested in 2–3 minutes and yield sequence coverage comparable to a 16-hour in-solution digest with minimal over-digestion
- Capturem 96-well trypsin plates allow specific peptide fragments to be detected and quantified in a high-throughput manner with excellent well-to-well reproducibility
- Capturem Protein G (or A) allows antibodies to be rapidly labeled without the need to purify before and after conjugation
- Capturem Streptavidin in combination with Capturem Trypsin provides a complete solution for high-throughput workflows

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