Introduction

Capture™ 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.

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

Capture Trypsin digest of a standard monoclonal antibody

- Complete trypsic 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)

Capture Trypsin digest of apomyoglobin

- Digestion of 20 µg of human lgG1 (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)

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

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

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