WP055

Rapid enzymatic digest of antibodies and proteins using Capturem technology

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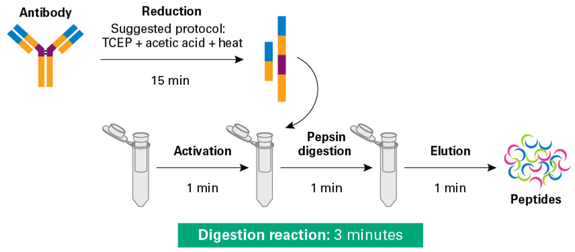
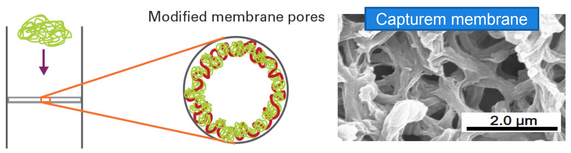
Introduction

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. These functionalized membranes are assembled into spin columns or 96-well plates, allowing for a rapid and controlled spin digest due to high membrane surface area and convective flow. This allows the fast digestion and analysis of protein and antibody samples in a high-throughput fashion. Here, we show that our Capturem Trypsin and Capturem Pepsin columns enable the digestion of proteins and antibodies within 2–3 minutes with no loss in sequence coverage and minimal over-digestion.

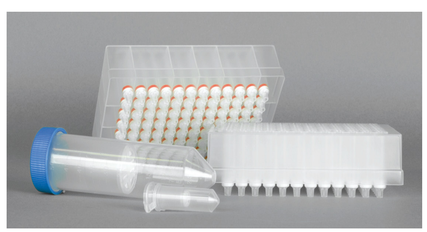
1 Schematic of the Capturem spin digest workflow





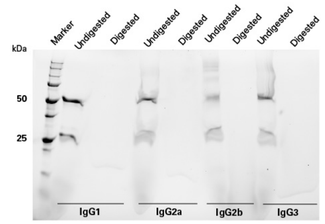
- Resin-free
- High capacity due to large internal surface
- Rapid flow-induced mass transfer
- No incubation time
- Room-temperature workflow

2 Capturem formats



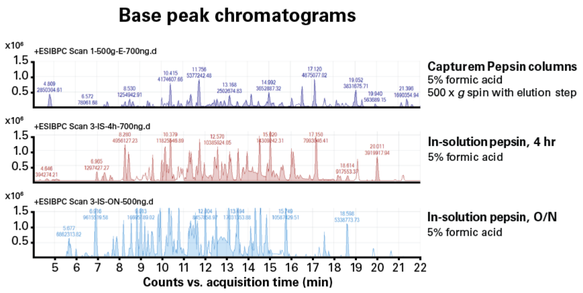
- Available in a variety of formats including mini-prep, maxi-prep, and 24- and 96-well plates

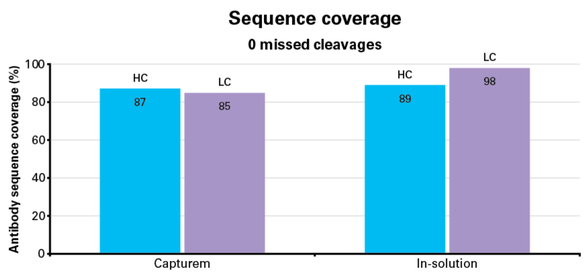
3 Capturem Pepsin spin digest of antibodies

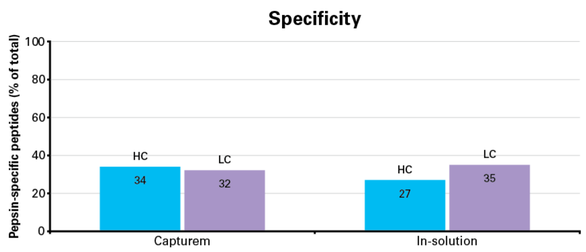


- In-solution pepsin digests are difficult to control due to high proteolytic reactivity, resulting in over-digestion of sample
- Spin digestion allows finer control over pepsin digests
- SDS-PAGE analysis of spin-digested antibodies shows no intact antibody after spin digest

4 Capturem Pepsin spin digest of anti-Her2 mAb

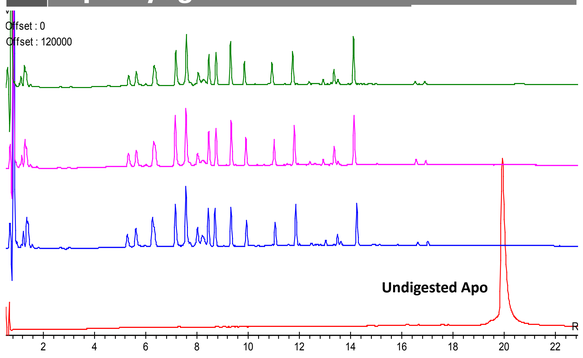






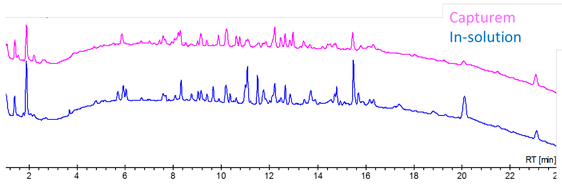
- The base peak chromatogram for in-solution digests shows a higher number of left-shifted peaks (indicative of over-digestion), while spin digestion allows finer control over pepsin digests
- Peptic peptides from spin digest cover antibody sequences in MS analysis similar to in-solution digest (4 hrs, 37°C)
- Capturem columns yield more pepsin-specific peptides

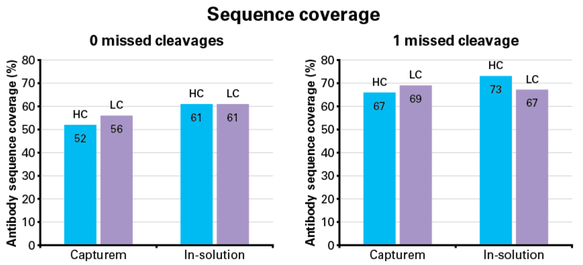
5 Capturem Trypsin spin digest of apomyoglobin

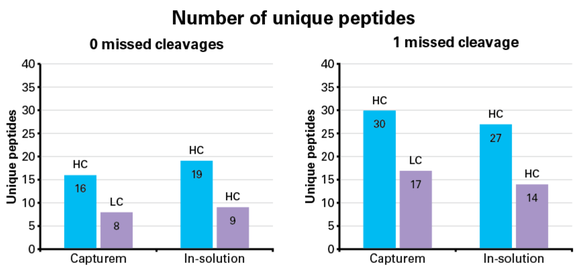


- Complete tryptic spin digest achieved in 2–3 minutes
- High well-to-well reproducibility
- Tryptic spin digest of 80 µg apomyoglobin under native conditions gives reproducible HPLC profiles (n=3)

6 Capturem Trypsin spin digest of standard mAb

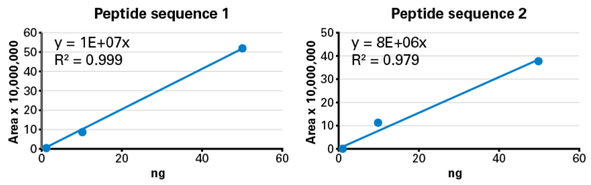






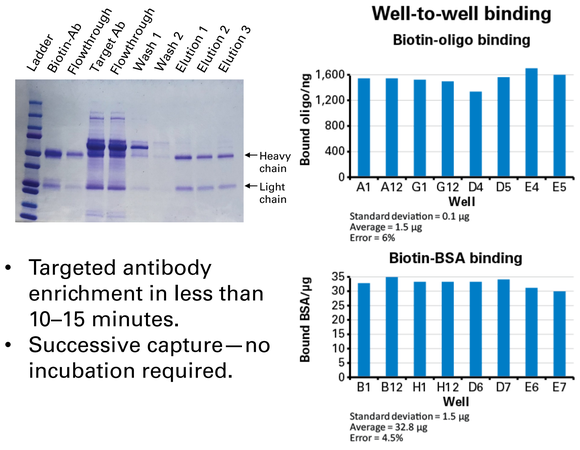
- Mass spectroscopy analysis of tryptic peptides from Capturem spin digestion (2 min, RT) of 20 µg of human IgG1 (NIST) reveals similar antibody sequence coverage to in-solution digestion (16 hrs, 37°C)
- Capturem spin digestion (2 min, RT) of 20 µg of human IgG1 (NIST) generates a similar number of unique peptides as in-solution digestion (16 hrs, 37°C)

7 Quantitation using Capturem Trypsin



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

8 Capturem Streptavidin pulldown



- Targeted antibody enrichment in less than 10–15 minutes.
- Successive capture—no incubation required.

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 Streptavidin in combination with Capturem Trypsin provides a complete solution for high-throughput workflows

Acknowledgments

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