Complete antibody digestion with pepsin in just three minutes

- Complete digestion of antibody isotypes
- High lot-to-lot reproducibility
- Improved performance over in-solution methods

Introduction

Capturem Pepsin was developed for rapid and efficient digestion of antibodies—with a room-temperature protocol (Figure 1)—designed for downstream proteomics analysis. Pepsin is an acidic protease with utility in a wide variety of applications, most notably in the preparation of antibody samples for characterization by mass spectrometry (MS). The specificity (Keil, B. et al. 1992) and activity of digestion are both pH-dependent, with an active range between pH 1–5. For the purpose of MS, digestions are typically carried out at pH 1–2 in order to cleave peptide bonds at both N- and C-terminal phenylalanine, leucine, tryptophan, and tyrosine residues. Altering the pH of the reaction will, in turn, affect the amount of digestion that occurs; 90% of maximum activity is achieved at pH 1.5, while only 35% activity is achieved at pH 4.5 (Bohak, Z. et al. 1969).

Conventional methods for sample preparation involve in-solution digestion with pepsin, in which incubation times can be as long as overnight. In addition to being a time-consuming process, such a prolonged digestion may lead to protein modifications, poor specificity, and over-digestion. On the other hand, shorter incubations can lead to incomplete digestion and thus compromise downstream efforts to characterize the protein. Capturem Pepsin is part of the ever-expanding Capturem family and takes advantage of this novel technology, modified here to provide membrane-immobilized pepsin in a mini spin column format. This new method offers high performance with an incredibly simple workflow, and maintains full digestion capabilities in the presence of common antibody sample prep reagents (e.g., TCEP, urea, formic acid, etc.). These single-use, disposable spin columns allow for complete digestion of antibody samples in just three minutes, providing reliable, consistent results that outperform those of lengthier in-solution methods.

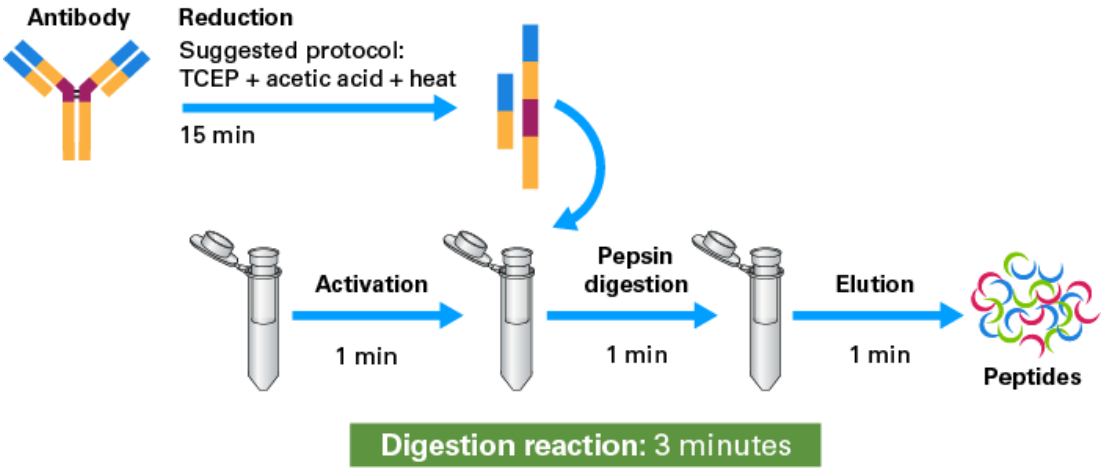

Figure 1. Capturem Pepsin workflow. Antibody samples are denatured and reduced before enzymatic digestion. After initial activation of the columns with the included Activation Buffer, the sample solution (50–800 µl) containing the reduced antibody is passed through the spin column via a one-minute centrifugation. Digestion by pepsin occurs on the column, and subsequent peptide fragments are eluted in a second one-minute centrifugation. Eluted peptides are neutralized with the addition of NaOH and ready for downstream analysis.

Results

Complete digestion of different antibody isotypes

To demonstrate a typical use of Capturem Pepsin, various immunoglobulin subtypes were processed using the kit. Mouse IgG1, IgG2a, IgG2b, and IgG3 were all denatured, diluted, and then digested according to the standard Capturem Pepsin protocol. SDS-PAGE analysis of digested

and undigested samples shows that bands for heavy and light chains were absent from only the digested lanes, demonstrating that each of the four different IgGs were fully digested after just a single pass through the Capturem Pepsin membrane (Figure 2). These columns have also shown complete digestion in the presence of urea, DTT, and IAA (data not shown).

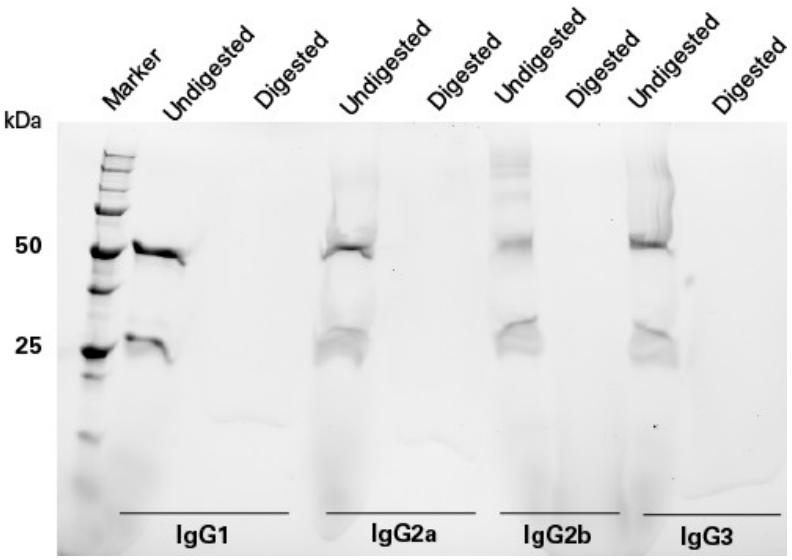

Figure 2. Digestion of different mouse antibody isotypes. 100 µg each of mouse IgGs (IgG1, IgG2a, IgG2b, and IgG3) was denatured in TCEP and acetic acid at 75°C for 15 min, and then diluted in 5% formic acid buffer. The diluted samples were digested using Capturem Pepsin spin columns. Both digested and undigested samples (4 µg each) were evaluated by SDS-PAGE.

High reproducibility between experiments

A well-characterized protein commonly used as a standard in peptide studies, apomyoglobin (Apo), was used to test the reproducibility of Capturem Pepsin experiments. Apo in a 5% formic acid solution was digested using three different lots of Capturem Pepsin. All three digests yielded nearly identical HPLC profiles (Figure 3), in which Apo was cleaved three times into the same fragments, thus demonstrating the reliability of the Capturem Pepsin protocol.

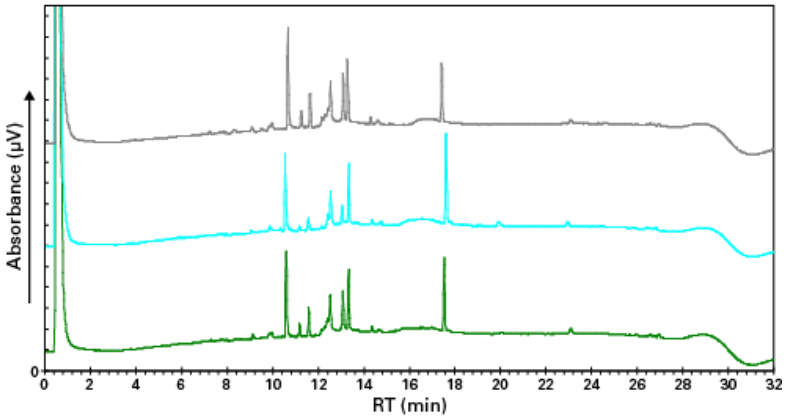

Figure 3. HPLC profiles of lot-to-lot comparisons of Capturem Pepsin digests. 50 µg of Apo was diluted in 5% formic acid and passed through Capturem Pepsin columns from three different lots. HPLC profiles show the same fragments for each sample digestion.

On-column digestion efficiency meets or exceeds that of in-solution methods

Digestion efficiency of Capturem Pepsin columns was compared to an in-solution approach using a monoclonal antibody (mAb) anti-HER2 in combination with MS analysis. Digestion was performed via one of three methods: Capturem Pepsin, a 4-hr in-solution incubation, and an overnight in-solution incubation. MS analysis was performed on the digested samples, and the resulting base peak chromatograms were compared (Figure 4). Increasing the incubation time for the in-solution protocol resulted in over-digestion of the sample, as indicated by the increasing number of peak signals shifted to smaller fragments (bottom trace). However, Capturem Pepsin yielded distinctive peaks and an overall profile not considered to be over-digested (top trace).

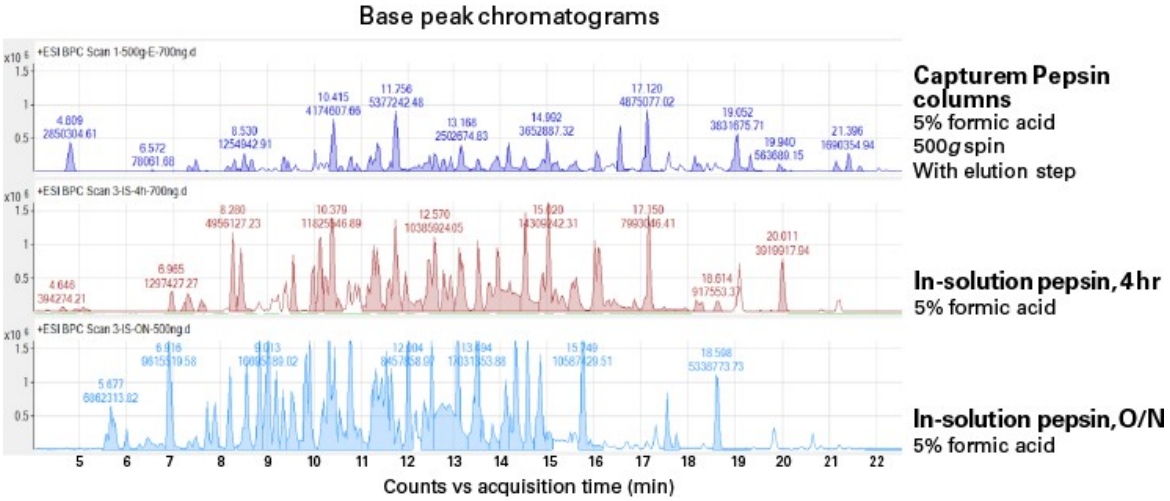

Figure 4. Comparison of digestion with Capturem Pepsin columns versus standard in-solution pepsin methods. 50 µg of anti-HER2 mAb was digested using three different methods: Capturem Pepsin, in-solution pepsin incubated for four hours, and in-solution pepsin incubated overnight. MS analysis was performed following digestion. The left shift of the peaks for the in-solution digests and the higher number of peaks overall is indicative of over-digestion. Neither of these results was observed for the Capturem Pepsin digest.

Conclusions

Capturem Pepsin completely digests antibody samples in just three minutes, with an easy, room-temperature protocol. While easily meeting and exceeding the results of standard in-solution methods, this membrane-based system offers consistent results without over-digestion and shows no ill effects from the presence of common sample preparation reagents. These single-use, disposable spin columns facilitate an uninterrupted MS workflow for antibody characterization.

Methods

Digestion of different mouse antibody isotypes

100 µg each of mouse immunoglobulins (IgG1, IgG2a, IgG2b, and IgG3) was denatured in TCEP and acetic acid at 75°C for 15 min and then diluted in 5% formic acid buffer. The diluted samples were digested using Capturem Pepsin spin columns as per the standard protocol. 100 µg of digested and undigested samples were evaluated by SDS-PAGE with a stain-free gel.

HPLC profiles from different Capturem Pepsin lots

50 µg of apomyoglobin was diluted in 5% formic acid and digested using three different lots of Capturem Pepsin as per the standard protocol. HPLC profiles were generated for each sample using an Aeris PEPTIDE XB-C18 column.

Comparison of Capturem Pepsin columns with in-solution pepsin

50 µg of anti-HER2 mAb (Trastuzumab, Herceptin, human IgG1, kappa chain) was digested using three different methods: Capturem Pepsin, in-solution pepsin incubated for four hours, and in-solution pepsin incubated overnight. In-solution digests were performed at 37°C with 50 µg of antibody in a reaction volume of 500 µl (weight ratio of 1:20 pepsin:antibody). In-solution digests are stopped by heating at 95°C for 10 min, followed by the addition of NaOH. The Capturem Pepsin method was performed as per the standard protocol. MS analysis was performed following digestion. 1D LC-MS/MS QTOF analysis was performed by JadeBio, Inc.

References

Keil, B., *et al.* Essential substrate residues for action of endopeptidases. *Specificity of Proteolysis* Springer-Verlag Berlin-Heidelberg-New York, pp.335 (1992).

Bohak, Z., *et al.* Purification and characterization of chicken pepsinogen and chicken pepsin. *J. Biol. Chem.* **244**:4368–4648 (1969).

Related Products

Cat. #	Product	Size	License	Quantity	Details
635728	Capturem™ Pepsin Miniprep Kit	20 Rxns		*	

Capturem Pepsin provides fast, efficient, and complete digestion of proteins at room temperature, allowing for an uninterrupted proteomic analysis workflow. This product is based on our novel Capturem technology and provides membrane-immobilized pepsin in a mini spin column format. Capturem Pepsin completely digests protein samples in just a few minutes while avoiding over-digestion by controlling the time the sample is exposed to the protease. This kit contains Capturem Pepsin mini spin columns and an activation buffer.

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