Protein digestion with Capturem Trypsin

Capturem membranes are a revolutionary technology that consist of porous, high-capacity membranes that can be functionalized with not only proteases (trypsin or pepsin) for digestion but also ligands such as Protein A or G, nickel, and streptavidin for purification. We've assembled these functionalized membranes into a number of different spin-column, multiwell-plate, and large-volume formats to bring you effortless, high-quality results across your protein research workflow.

Digestion workflow

The high local trypsin concentration in the column plus the large internal surface area across a short distance ensures almost instant binding and cleavage of proteins and antibodies at room temperature. Complete antibody digestion takes 3–6 minutes as compared to traditional methods that may require overnight digestion.

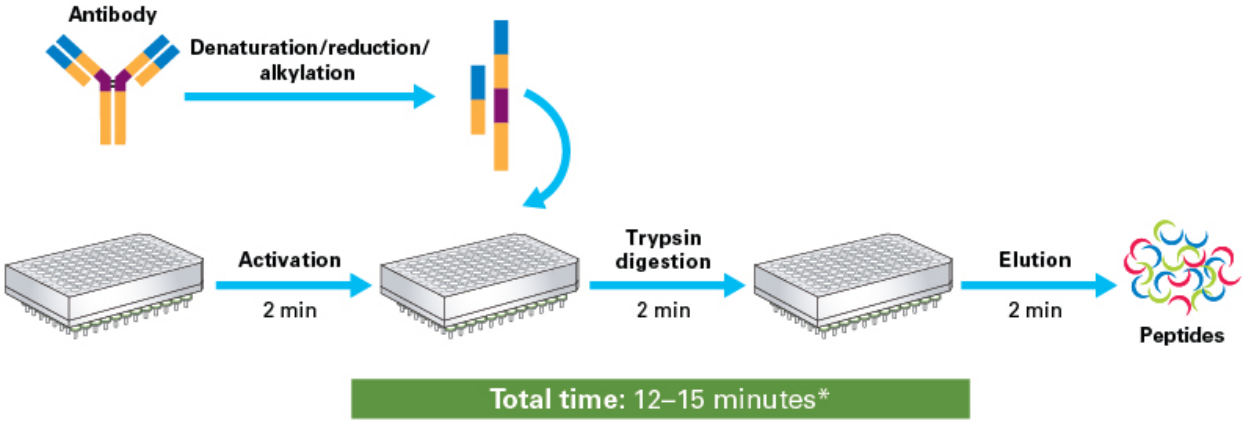


Figure 1. Capturem Trypsin digestion workflow. After initial activation of the columns with the included Capturem Trypsin Activation Buffer, the sample solution (50–800 µl) containing the native or denatured sample is passed through the spin column via two-minute centrifugation. Digestion by trypsin occurs on the column, and subsequent peptide fragments are eluted in a second two-minute centrifugation. *Denaturation/reduction/alkylation time is not included.

Data

High well-to-well reproducibility

A well-characterized protein commonly used as a standard in peptide studies, apomyoglobin (Apo), was used to test the reproducibility of Capturem Trypsin plates. Apo was digested under native conditions using three different wells of Capturem Trypsin 96-well plates. All three digests yielded nearly identical HPLC profiles (Figure 2), thus demonstrating the reliability of the Capturem Trypsin protocol. Here we show the digestion of proteins within ~2–3 minutes using Capturem Trypsin 96-well plates.

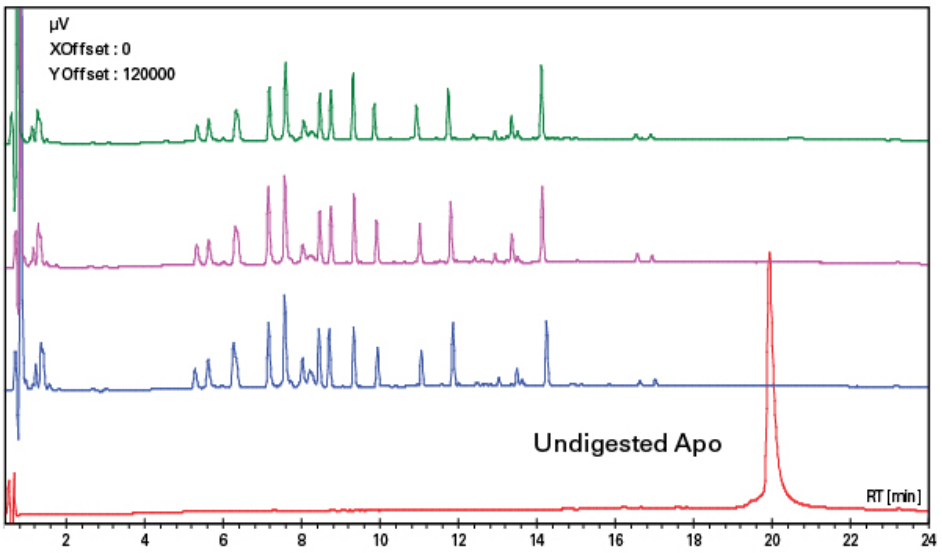


Figure 2. HPLC profiles well-to-well reproducibility of apomyoglobin digests using Capturem Trypsin. Tryptic spin digests of 80 µg apomyoglobin under native conditions give reproducible HPLC profiles.

Quantitation using Capturem Trypsin

Capturem 96-well trypsin plates allow specific peptide fragments to be detected and quantified in a high-throughput manner (Figure 3) with excellent well-to-well reproducibility. We performed and analyzed the tryptic spin digest of SILuLite. The quantitative analysis showed that Capturem Trypsin digestion yielded surrogate peptides detectable at ng-input amounts (1, 10, and 50 ng).

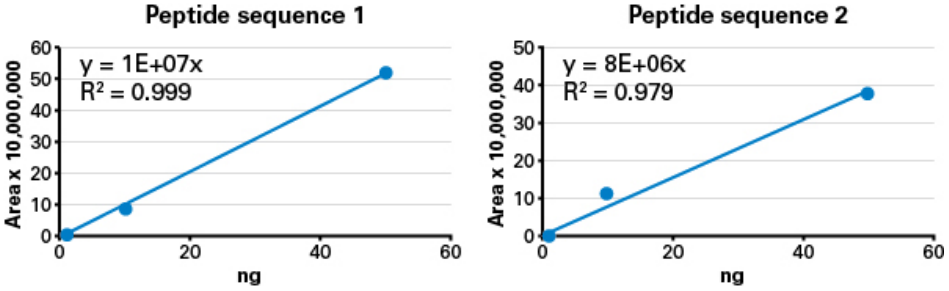
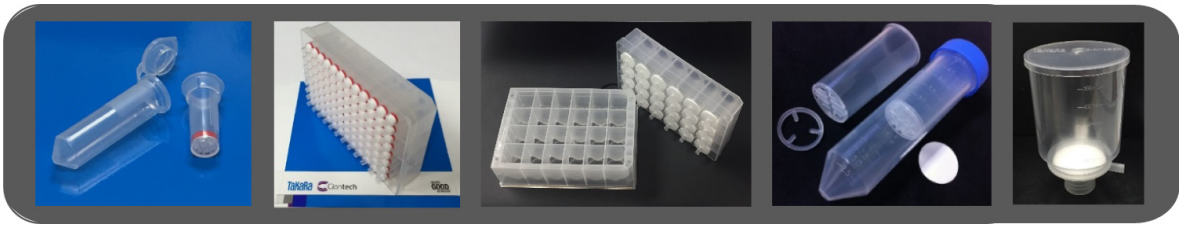


Figure 3. Reliable quantitation. Tryptic spin digest of SILuLite yields surrogate peptides detectable at ng-input levels (1, 10, 50 ng). AUCs of specific peptides give a linear correlation.

Chemistries & formats

Technology	Format	Typical yield	Time	Maximum single load volume
Capturem Trypsin/Pepsin	Miniprep	25 µg IgG‡	5 min§	850 µl
	96-well plate†	25 µg IgG‡	15 min§	1 ml
Capturem Protein A	Miniprep	40 µg	5 min	850 µl
	96-well plate	40 µg	15 min	1 ml
	24-well plate	400 µg	15 min	4 ml
	Maxiprep	1 mg	15 min	23 ml
Capturem Protein G	Miniprep	60 µg	5 min	850 µl
	96-well plate	600 µg	15 min	1 ml
	Maxiprep	600 µg	15 min	4 ml
	24-well plate	1.2 mg	15 min	23 ml
Capturem Streptavidin	Miniprep	25 µg IgG#	5 min	850 µl
	96-well plate	25 µg IgG#	15 min	1 ml
Capturem His	Miniprep	80 µg	5 min	850 µl
	96-well plate	80 µg	15 min	1 ml
	24-well plate	800 µg	15 min	4 ml
	Maxiprep	1.5 mg	15 min	23 ml
	Large volume	8–20 mg	30 min	500 ml

†Not available for Capturem Pepsin
‡80 µg protein
§Excludes denaturation/reduction/alkylation time
#4,000 pmol free biotin



Related Products

Cat. #	Product	Size	License	Quantity	Details
635736	Capturem™ Trypsin 96-Well Plate (Mass Spectrometry Grade)	4 x 96-Well Plates		*	

Capturem Trypsin 96-Well Plate (Mass Spectrometry Grade) enables rapid and efficient digestion of proteins, including antibodies, at room temperature. This product uses our Capturem technology with membrane-immobilized trypsin from porcine pancreas that has been modified by reductive methylation to increase its stability and resistance to autolytic digestion. Capturem Trypsin-mediated digestion of protein samples takes less than 4 minutes. This kit does not include Capturem Trypsin Activation Buffer. The buffer can be purchased using Cat. No. 635739.

Documents	Components	Image Data

635737	Capturem™ Trypsin 96-Well Plate (Mass Spectrometry Grade)	1 x 96-Well Plate		*	
635738	Capturem™ Trypsin 96-Well Plate (Mass Spectrometry Grade)	1 x 96-Well Plate		*	
635740	Capturem™ Trypsin Miniprep Kit (Mass Spectrometry Grade)	20 Rxns		*	

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