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How to optimize SMART Digestion

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Introduction

All proteins and protein mixtures vary with regards to digestion time. Digestion time must be optimized for your protein or protein mixture by determining the appropriate incubation time by performing a time course digestion for your platform. A strategy for screening digestion incubation time is outlined below.

Important notes

- Thermo Scientific™ SMART Digest™ is not compatible with samples containing urea or guanidine chloride as these will decrease the efficiency of SMART Digest enzymes.
- Samples should not be introduced in Ammonium Bicarbonate and Phosphate buffer solutions as these will form a precipitate with calcium in the SMART Digest buffer[1].
- If digestion is followed by TMT labeling and/or nano-LC, Low Salt SMART Digest buffer should be used in place of standard SMART Digest buffer. Following digestion and prior to labeling, excess ammonium ions from the Low Salt Digest Buffer should be removed by evaporation (e.g., SpeedVac).

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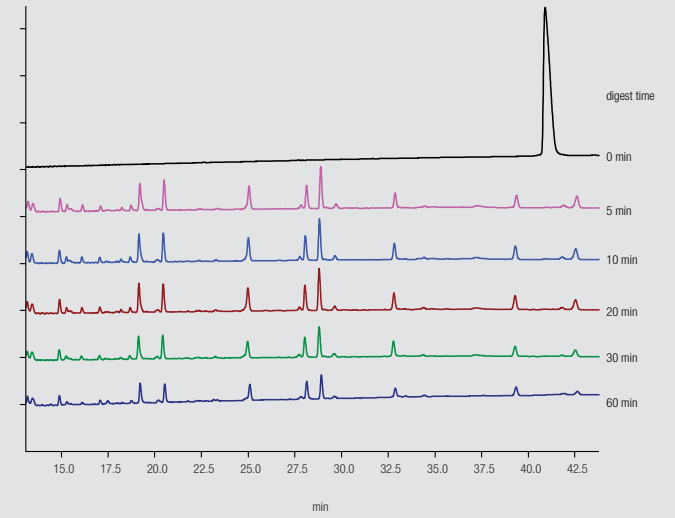
Carbonic Anhydrase, 29 KDa

Time course experiment for digestion optimization



Typical Digestion Times

Protein	Digest Time (min)
Insulin	4
BSA	< 5
Carbonic anhydrase	< 5
Lysozyme	< 5
Apo-B	30
IgG	45
IgG in 50 µL plasma*	75
Ribonuclease A	150
Thyroglobulin	240
C-reactive protein	240

200 µL protein solution (100 µg/mL) at 70 °C
*IgG in plasma (17.5 mg/mL total protein) at 70 °C

Digestion protocol

1. **Add 150 µL of buffer**—Add 150 µL of SMART Digest buffer to the SMART Digest tube
2. **Add 50 µL of sample**—Add 50 µL of sample to the SMART Digest tube (final volume of 200 µL per sample)
3. **Digestion**—Set your heater/shaker to 70 °C/1400 RPM, allow to equilibrate for 5 minutes. Then add your samples for the required time for digestion

Time course protocol

1. Prepare eight identical samples, using a known concentration of native analyte
2. Digest according to the Protein Digestion Procedure removing one sample from heater/shaker at a fixed interval (every 5–15 minutes)
3. Remove beads (centrifugation, filtration, magnetism) and perform sample clean-up of choice
4. Analyze samples to determine the extent of digestion (see chromatogram above) monitoring a known peptide, total peptide intensity and/or depletion of intact protein material
5. Disappearance of intact protein peak and stabilization of peptide peak intensities and peak ratios indicate a completion of digestion

References

1. Selecting buffers to remove uncertainty in tryptic digestion, AN 21179. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/AN-21179-SP-SMART-Digest-Buffers-AN21179-EN.pdf

Related products

Description	Part Number
Thermo Scientific™ SOLAµ™ SPE Plate, 2 mg, 1 mL 96-well plate	60209-001
Thermo Scientific™ SOLA™ HRP, 10mg, 2m: 96-well plate	60309-001

Current versions of product instructions are available at **separatedbyexperience.com/chromexpert**

Learn more about our SMART Digest products at
thermofisher.com/SMARTdigest

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