Oct 27, 2025

In solution digestion for protein standards (Urea)

DOI
https://dx.doi.org/10.17504/protocols.io.ewov19db7lr2/v1

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Protocol Citation: Cristina CARDENAL PERALTA 2025. In solution digestion for protein standards (Urea). **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov19db7lr2/v1

Protocol status: Working
We use this protocol and it's working

Created: June 14, 2024

Last Modified: October 27, 2025

Protocol Integer ID: 101821

Keywords: Protein Standards, trypsin, Lys-C, Mass Spectrometry, solution digestion for protein standard, digestion of protein standard, protein standard, different protein standard, spectrometry instrument, solution digestion, digestion, house quality standards for technical calibration, preparation for injection

Abstract

Digestion of protein standards, such as albumin, is a common practice to keep in-house quality standards for technical calibration of Mass-Spectrometry instruments. Here we describe a protocol to digest three different protein standards and their preparation for injection in a Mass-Spectrometry instrument.

Guidelines

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Materials

Protein Standards

- 1 mg Albumin from bovine serum
- 1 mg Creatine kinase (rabbit)
- 1 mg Beta-lactoglbulin (bovine)

Buffers

- [M] 8 Molarity (M) Urea in ABC
- [M] 50 millimolar (mM) Ammonium bicarbonate (ABC))
- [M] 0.1 % volume TFA (trifluoro acetic acid)

Reagents

- [M] 1 Molarity (M) DTT (Dithiothreitol)
- [M] 1 Molarity (M) IAA (Iodoacetamide)

Enzymes

Lys-C proteinase
Trypsin proteinase

Equilibration buffers for SEP-PAK C18

Solution A: 0.1 % TFA in Milli Q water
Solution B: 0.1 % TFA, 80 % ACN (acetonitrile)

C18 Cartridge Vac 1cc (100 mg)

Sep-Pak C-18 (Waters) WAT023590

Safety warnings

Incubation with IAA needs to be performed in the dark.

Before start

Make the Urea and ABC buffers fresh on the same morning of the experiment.

Steps (Day 1) 5h 20m

1 Weight 1 mg of each standard and resuspend in 100 µL of 8 M Urea in ABC. 10m

2 Add DTT to a final concentration of 25 mM, incubate for 30 min at Room temperature in a shaker (1200 rpm) 30m

3 Add IAA to a final concentration of 55 mM, incubate for 30 min Room temperature in the **dark**. 30m

4 Add 5 µL of Lys-C (stock at [M] 1 µg/µL), for 3-4 hours at 37 °C
(This is adding 1 µg of Lys-C per 20 µg of protein). 4h

5 Dilute sample 5X times (add 400 µL) with ABC buffer 5m
This step is performed because trypsin does not work in high urea concentrations. Be careful to use the ABC buffer and NOT the one with Urea and ABC when diluting.

6 Add 5 µL of trypsin (stock at [M] 1 µg/µL, resuspend in 20 µL of 0.1 % TFA) to each sample. Incubate O/N at 37 °C 5m
(This is adding 1 µg of trypsin per 20 µg of protein).

Steps (Day 2) Sep-Pak C-18 cartridge loading 20m

7 Equilibrate the Sep-Pak C-18 column: 5m
Flush the column with 5 ml of solution B or Acetonitrile

8 Add 10 ml of solution A. Add the digested sample into the 10 ml of solution A and let it into the Sep-Pak column slowly. 5m

9 Wash the sample with 10 ml of Buffer A 5m

10 Elute with 2 × 600 µl of solution B (collect your purified sample in a 2 mL tube) 5m

11 Dry down purified peptides completely in a speed-vac. Resuspend in Buffer A (up to 20 µM)