Oct 27, 2025

In solution digestion (acetone precipitation)

DOI
https://dx.doi.org/10.17504/protocols.io.14egn6436l5d/v1

Cristina CARDENAL PERALTA[1]

[1]University of Edinburgh

Cristina CARDENAL PERALTA
University of Edinburgh

DOI: https://dx.doi.org/10.17504/protocols.io.14egn6436l5d/v1

Protocol Citation: Cristina CARDENAL PERALTA 2025. In solution digestion (acetone precipitation). **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn6436l5d/v1

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

Created: June 19, 2024

Last Modified: October 27, 2025

Protocol Integer ID: 102103

Keywords: acetone precipitation, in solution digestion, proteomics strategy for comprehensive protein analysis, ms analysis for global proteome profiling, global proteome profiling, used proteomics strategy, comprehensive protein analysis, efficient enzymatic cleavage of protein, protein, enabling efficient enzymatic cleavage, protein complexity, solution digestion, peptide, acetone precipitation, digestion, facilitating downstream lc

Abstract

In-solution digestion is a widely used proteomics strategy for comprehensive protein analysis, enabling efficient enzymatic cleavage of proteins into peptides in a liquid-phase environment. This method preserves protein complexity while facilitating downstream LC-MS/MS analysis for global proteome profiling.

Guidelines

After acetone precipitation O/N, it might be difficult to resuspend the peptide pellet just by vortexing. Gentle pipetting is advised at this point to break apart the pellet more efficiently.

Materials

Buffers
TEAB (Triethylammonium bicarbonate)

Enzymes
Trypsin: ThermoFisher 90057 20 µg per vial

Reagents
200 mM STOCK: Dithiothreitol (**DTT**) or 200 mM STOCK **TCEP** (tris(2-carboxyethyl)phosphine)
500 mM STOCK: Iodoacetamide (**IAA**)
Acetone 100%

Safety warnings

IAA incubation needs to happen in the dark

Sample reduction and alkylation (DAY 1)

1h 10m

1 Resuspend 100 µg of protein per condition with 100 µL with [M] 100 millimolar (mM) TEAB.

2 Add 5 µL of the [M] 200 millimolar (mM) TCEP/DTT and incubate sample at 55 °C for 40 min. Shake at 600-700 rpm. 40m

3 Add 5 µL [M] 500 millimolar (mM) IAA (making it a final concentration of [M] 25 millimolar (mM)). Incubate at Room temperature in the dark for 30 min. 30m

4 Add six volumes (roughly 600 µL) of pre-chilled (-20 °C) acetone and freeze at -20 °C . Allow precipitation O/N.

Protein digestion (DAY 2)

10m

5 Centrifuge the samples at 8000 x g for 10 minutes. 10m

6 Resuspend 100 µg of acetone-precipitated protein pellets with 100 µL of [M] 50 millimolar (mM) TEAB.
The pellet might not completely dissolve; however, after proteolysis at 37 °C all the peptides will be solubilised.

7 Immediately before use, add 20 µL of the Trypsin Storage Solution to the bottom of the trypsin glass vial and incubate for 5 minutes.

8 Add 2.5 µL of trypsin per 100 µg of protein (initial protein). Digest sample O/N at 37 °C