

Mar 12, 2024

BAF_Protocol_007 Solution Digest with Protein Precipitation

DOI

dx.doi.org/10.17504/protocols.io.bp2l6xjp5lqe/v1

Nicholas Sherman¹

¹University of Virginia Biomolecular Analysis Facility Core



Nicholas Sherman

University of Virginia Biomolecular Analysis Facility Core

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.bp2l6xjp5lqe/v1>

Protocol Citation: Nicholas Sherman 2024. BAF_Protocol_007 Solution Digest with Protein Precipitation. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bp2l6xjp5lqe/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: February 29, 2024

Last Modified: March 12, 2024

Protocol Integer ID: 95972

Keywords: Cells, Solution digest, mass spectrometry, protein precipitation, tissue, baf-protocol-007 solution digest with protein precipitation, baf-protocol-007 solution digest, protein precipitation, proteins before digestion, using protocol 003, protocol 003, protein mixture, protocol, protein, resulting peptide, digestion, precipitation step

Abstract

The protocol is a method to digest a protein mixture obtained from a cell lysis or tissue that is in solution. The protocol includes a precipitation step to clean up the proteins before digestion. The protein mixture can then be digested and the resulting peptides cleaned up using protocol 003 and/or 006.

Materials

Pre-cleaned microtubes 1.5 mL - SEAL-RITE® 1.5 ML MICROCENTRIFUGE TUBES color: natural, USA Scientific

Pipette tips - Fisher Brand, yellow, part number: 02-681-151.

Reinforced tubes with screw caps and O-rings - Fisher Brand bulk tubes opaque: 15-340-162.

Stainless steel balls - 2.4 mm Metal bead media 19-640-3

15 mL tubes - Nunc 15mL: 339650.

BCA - Micro BCA Protein Assay kit, Thermo Scientific: 23235.

ABC - Fluka analytical Ammonium Bicarbonate, Sigma Aldrich, 09830-500G

DTT - Fisher Bioreagents Dithiothreitol, C₄H₁₀O₂S₂ F.W. 154.24

IA (Iodoacetamide) - Sigma Aldrich 1149-5G PCode:1002138224

HALT Protease and phosphatase inhibitor cocktail, EDTA free (100x), Thermo Scientific: PI78443.

SDS - Sodium dodecyl sulfate, Sigma-Aldrich: L4509.

Promega Trypsin: Sequencing grade modified, frozen, V511C, Promega

FA - Fisher chemical A117-50, Formic Acid, optima LC/MS

Methanol - A456-212, Methanol, optima LC/MS.

Acetone - A929-4, Acetone, optima LC/MS.

Micropipettes:

2 to 20 µL Micropipette - Gilson™ F144056MT

10 to 100 µL Micropipette - Gilson™ F144057MT

20 to 200 µL Micropipette - Gilson™ F144058MT

100 to 1000 µL Micropipette - Gilson™ F144059MT

VWR Analog Vortex mixer - CAT No: 58816-121

Centrifuge 5427 R, Eppendorf.

Bead Mill 24 Fisher Brand.

Troubleshooting



Before start

REAGENTS: (All reagents to be prepared fresh for each digestion)

1. 100 mM ammonium bicarbonate (ABC): 0.158 g in 20 mL distilled water
2. 50 mM ABC: 0.079g in 20 mL distilled water
3. Acetone
4. Methanol
5. 10% sodium dodecyl sulfate (SDS): 1g in 10 mL distilled water.
6. 100 mM DTT: 0.0015g in 100 μ L of 100mM ABC (DO NOT mix until directly before you are ready to use)
7. 500 mM Iodoacetamide: 0.01g in 100 μ L of 100 mM ABC (DO NOT mix until directly before you are ready to use)
8. Trypsin solution: Keep on ice. Promega (cat. # V5113) is already diluted in 50 mM acetic acid.



Solution Tissue Digest with Precipitation

2d

- 1 Add 5X (volume to mg tissue) of lysis buffer (1% SDS, 100mM AmBiC) to tissue and transfer to Bead Mill 24 reinforced tube with 5 stainless steel balls. 1m
- 2 Lyse the cells with Fisher Bead Mill 24 (speed: 5m/s, time: 20 sec, number of cycle: 3, dwell/pause between runs: 10 sec). 1m
- 3 Centrifuge lysate at 16,000 x g for 10 min at 4°C. 10m
- 4 Carefully separate the supernatant and transfer into a new 1.5mL Eppendorf tube. 1m
- 5 Add 5µL of 10 mM dithiothreitol (DTT) solution for 30 min at RT to reduce. 30m
- 6 Add 5µL of 50 mM iodoacetamide (IA) solution for 30 min at RT to alkylate. 30m
- 7 Transfer sample to 15 mL conical tube. 1m
- 8 Perform precipitation by adding -20C acetone (1x volume of the sample) and -20C methanol (9 x volume of the sample) and incubate overnight at -80C. 10h
- 9 Centrifuge sample 1h at 4C at 3200 x g. 1h
- 10 Remove supernatant, wash pellet with 1 mL of -20C cold methanol and centrifuge. 2m
- 11 Remove supernatant, air-dry pellet in the PCR workstation at RT till just damp. 1m
- 12 Solubilize proteins in 50 uL (more depending on amount of tissue) of 50 mM Ammonium Bicarbonate 5m



- | | | |
|----|---|-----|
| 13 | Determine the protein concentration of the supernatant using BCA (5uL of 10X dilution) | 5m |
| 14 | Transfer 20ug into a new, pre-washed tube and adjust to a final volume of 100uL with 100mM AmBiC. | 1m |
| 15 | 12. Add 1 ug of modified trypsin solution overnight at 37°C. | 10h |
| 16 | 13. Add 8uL of formic acid to the sample to quench the reaction. | 1m |
| 17 | Speedvac to dry peptide sample. | 2h |
| 18 | Samples can be cleaned up using C18 (Protocol 003) and/or Magnetic beads (Protocol 006). | 1h |

Cell Pellet Digest with Precipitation

- | | |
|----|--|
| 19 | Add lysis buffer to final concentration 1% SDS using 100mM AmBiC and protease inhibitor cocktail (1:100 dilution) in 1.5 mL tubes. This is generally 10E6 cells but can be adjusted for 10E4-10E7. |
| 20 | Submit samples to 3 cycles at 90C and RT incubation as follows: shake at 800 rpm for 10 min at 90C in temperature controlled thermal shaker and incubate at RT for 10 min. |
| 21 | Add ~60 uL of 100 mM DDT (final conc. 10 mM) to samples and incubate for 30 min at RT |
| 22 | Add ~60 uL of 500 mM IA (final conc. 50 mM) to samples and incubate for 30 min at RT in the dark. |
| 23 | Transfer samples to 15 mL tubes (~700 uL). |
| 24 | Perform precipitation by adding 700 ul of -20C cold acetone (same volume of the sample) and 7200 mL of -20C cold methanol (9 volumes of the sample) and incubate overnight at -80C |
| 25 | Centrifuge samples 1h at 4C at 3200 xg |



- 26 Remove supernatants, wash pellets with 1 mL of -20C cold methanol and centrifuge.
- 27 Remove supernatants, air-dry pellets in the PCR workstation at RT
- 28 Solubilize proteins in 50-100 uL of 50 mM Ammonium Bicarbonate (~1ug/uL concentration).
- 29 11. Determine the protein concentration of the supernatant using BCA (use 5uL of 10X dilution sample).
- 30 12. Transfer 20ug per condition into a pre-washed tube and adjust to a final volume of 100uL with 100mM AmBiC
- 31 13. Add 1 ug of modified trypsin solution overnight at 37°C.
- 32 14. Add 8uL of formic acid to the sample to quench the reaction.
- 33 15. Speedvac to dry peptide sample.
- 34 Samples can be cleaned up using C18 (Protocol 003) and/or Magnetic beads (Protocol 006).