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

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# BAF_Protocol_007 Solution Digest with Protein Precipitation

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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.

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**Keywords:** Cells, Solution digest, mass spectrometry, protein precipitation, tissue

## 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.

BEFORE START INSTRUCTIONS

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 uLof 100mM ABC (DO NOT mix until directly before you are ready to use)
7. 500 mM Iodoacetamide: 0.01g in 100 uL 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 2 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 o 10h
the sample) and incubate overnight at -80C.

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).