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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 - GilsonTM F144056MT

10 to 100 µL Micropipette - GilsonTM F144057MT

20 to 200 µL Micropipette - GilsonTM F144058MT

100 to 1000 μL Micropipette - GilsonTM 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

- Add 5X (volume to mg tissue) of lysis buffer (1% SDS, 100mM AmBiC) to tissue and transfer to Bead Mill 2 1m reinforced tube with 5 stainless steel balls.
- 2 Lyse the cells with Fisher Bead Mill 24 (speed: 5m/s, time: 20 sec, number of cycle: 3, dwell/pause betweer runs: 10 sec).
- 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  $5\mu$ L of 10 mM dithiothreitol (DTT) solution for 30 min at RT to reduce.

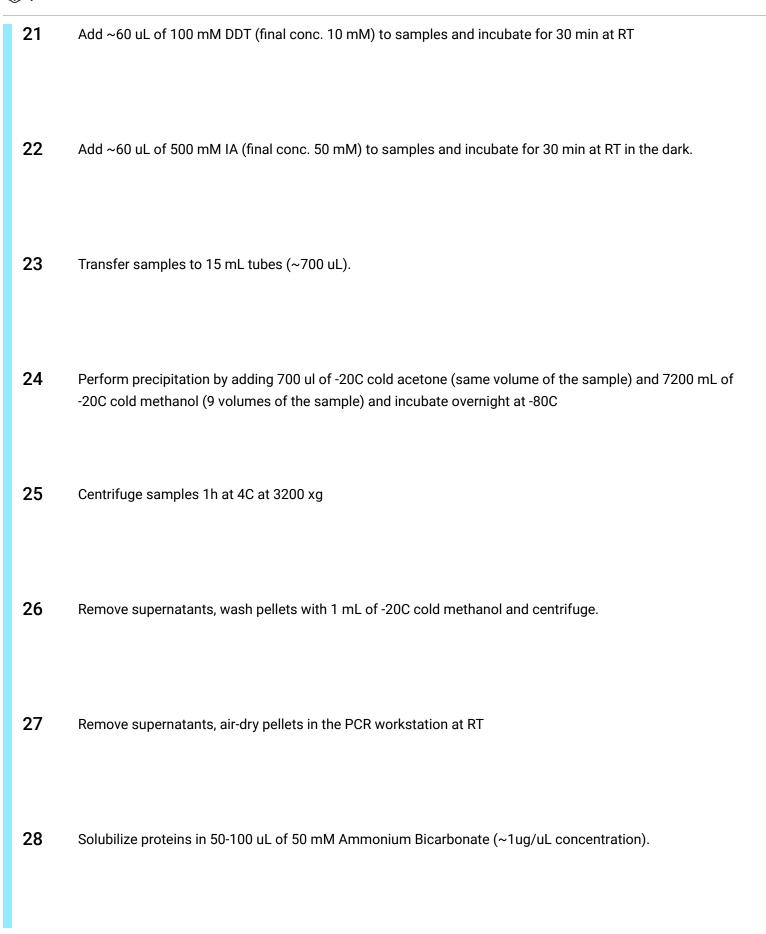
30m

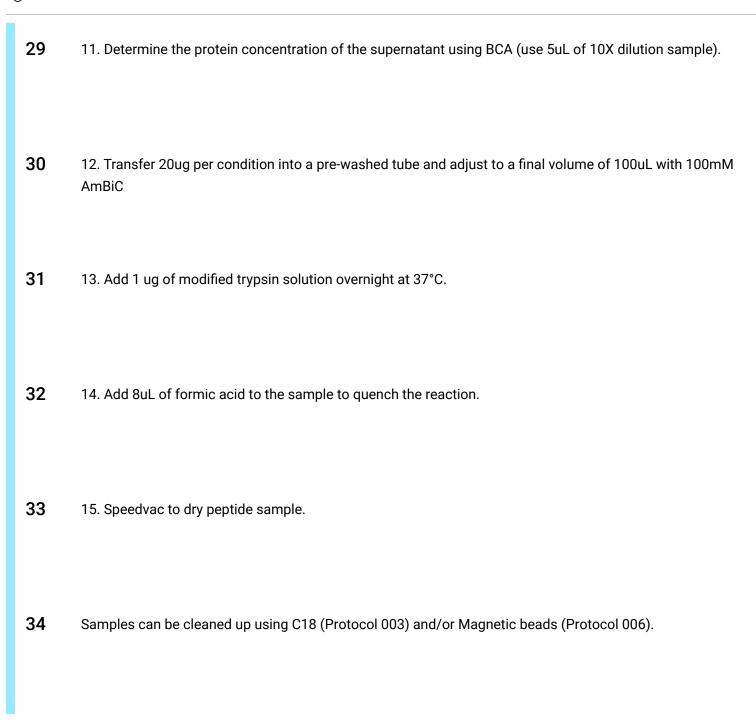
6 30m Add 5µL of 50 mM iodoacetamide (IA) solution for 30 min at RT to alkylate. 7 Transfer sample to 15 mL conical tube. 1m 8 Perform precipitation by adding -20C acetone (1x volume of the sample) and -20C methanol (9 x volume of 10h line) the sample) and incubate overnight at -80C. 9 1h Centrifuge sample 1h at 4C at 3200 x g. 10 Remove supernatant, wash pellet with 1 mL of -20C cold methanol and centrifuge. 2m 11 1m Remove supernatant, air-dry pellet in the PCR workstation at RT till just damp. 12 5m Solubilize proteins in 50 uL (more depending on amount of tissue) of 50 mM Ammonium Bicarbonate 13 Determine the protein concentration of the supernatant using BCA (5uL of 10X dilution) 5m

# **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.

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