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

Created: Jun 21, 2022

BAF_Protocol_001 In-gel Digestion

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Biomolecular Analysis Facility



xjbbf

ABSTRACT

This protocol is for in-gel digestion of proteins, including large mixtures, to produce peptides for mass spectrometry analysis. The gel method can be useful for getting rid of detergents or small molecules that might interfere with minimal loss. The input is a gel band up to 1cm x 1cm. The output is a relatively clean peptide digest that is ready for quick cleanup by C18 tip.

GUIDELINES

- 1. Wash hands before starting. Refrain from wearing heavy lotions or perfumes.
- 2. Do not wear clothing that contains wool or a lot of loose fibers.
- 3. Use nitrile gloves (not latex).
- 4. Perform all procedures under PCR clean hood cabinet.
- 5. All sample preparation must use polypropylene tubes cleaned by addition/removal of EtOH (pure ethanol), then H_2O (water), then EtOH. Dry in clean hood for several hours upside down. This strips off polymer coating.
- 7. All reagents must be prepared in small glass bottles (polypropylene lined caps no glue foil).
- 8. Solutions should be prepared fresh.

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PROTOCOL integer ID: 65051

Keywords: proteomics, mass spectrometry, digestion

MATERIALS

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

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Screw cap microtubes 1.5 mL - Fisher Brand, Conical Screw Cap Tube, color: natural, part number: 02-681-339.

20mL liquid scintillation vials PE cone lined cap - Wheaton DWK986756

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

Micropipettes

ABC - Fluka analytical, Ammonium Bicarbonate, Sigma Aldrich, part number: 09830.

DTT - Fisher BioReagents, Dithiothreitol, part number: BP 172-5.

Iodoacetamide - Sigma Aldrich, Iodacetamide, part number: 1149.

Promega Trypsin: Promega, Sequencing grade modified, frozen, part number: V511C.

FA - Fisher Chemical, Formic Acid, Optima™ LC/MS Grade, part number: A117-50.

MeOH - Fisher Chemical, Methanol, Optima™ LC/MS Grade, part number: A456-4.

ACN - Fisher Chemical, Acetonitrile, Optima™ LC/MS Grade, part number: A955-4.

TFA - Pierce[™], Trifluoroacetic Acid, part number: 28903.

Water - Fisher Chemical, Optima™ LC/MS Grade, part number: W6-4.

Syringe - Unimetrics PKS250, 250µL Peek Laboratory Syringe

2 to 20 µL Micropipette - Gilson™ F144056MT

10 to 100 C Micropipette - Gilson™ F144057MT

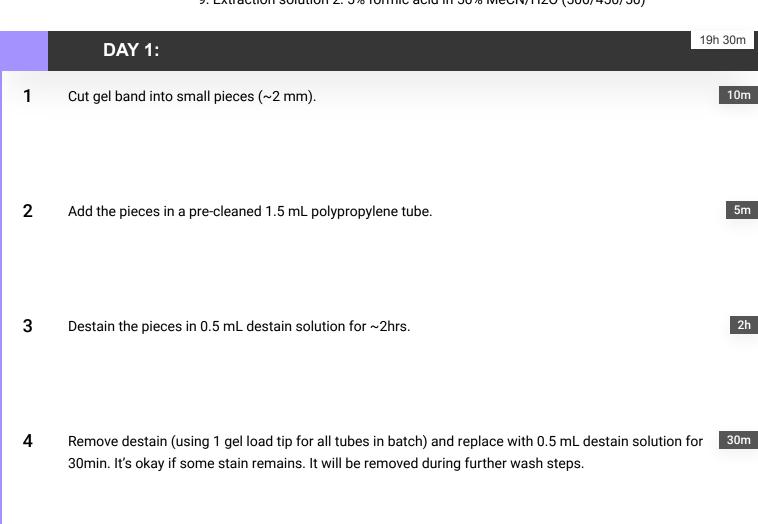
20 to 200 µL Micropipette - Gilson™ F144058MT

100 to 1000 µL Micropipette - Gilson™ F144059MT

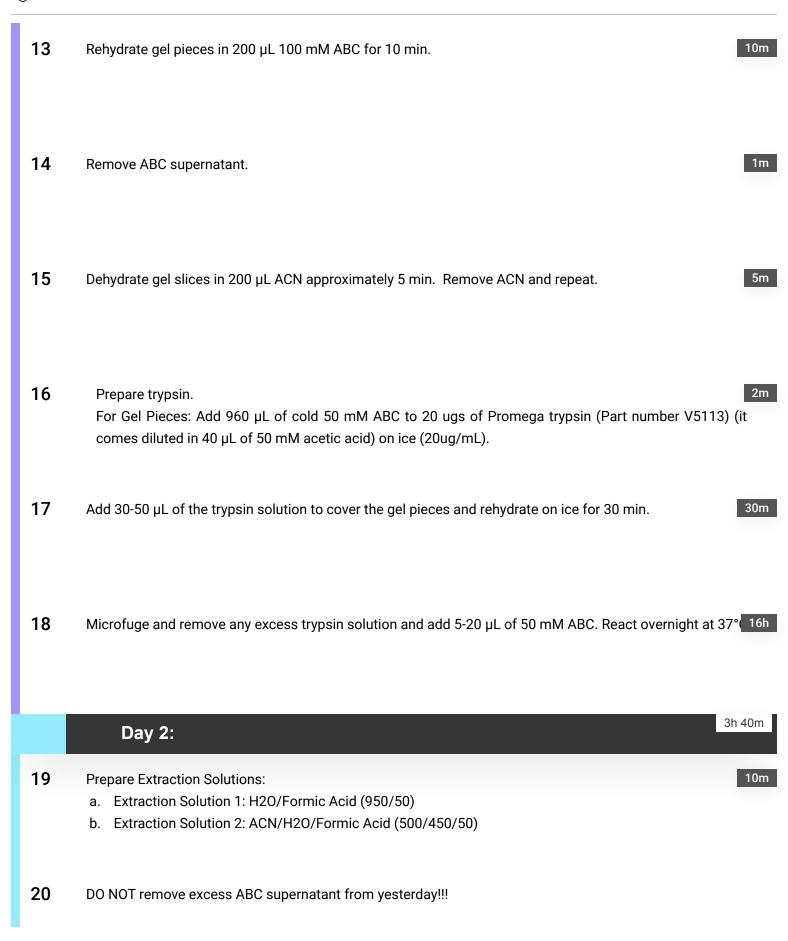
BEFORE START INSTRUCTIONS

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

- 1. Destain solution: 10 mL MeOH (methanol), 10 mL H2O (water), 500 μ L Acetic Acid (glacial)
- 2. 100 mM ABC (ammonium bicarbonate): 0.158 g in 20 mL distilled H20
- 3. 50 mM ABC: 0.079g in 20 mL distilled water
- 4. ACN (Acetonitrile)
- 5. 10mM DTT: 0.0015 g in 1 mL of 100 mM ABC (Use screw caps microtubes. DO NOT mix until directly before you are ready to use)
- 6. 50 mM lodoacetamide: 0.01 g in 1 mL of 100 mM ABC (Use screw caps microtubes. DO NOT mix until directly before you are ready to use)
- 7. Trypsin solution: Keep on ice. Promega sequencing grade trypsin (cat. # V5113 (porcine)) dilute when ready to use in 50 mM ABC. It comes frozen in $40\mu L$ of 50 mM Acetic Acid
- 8. Extraction solution 1: 5% formic acid in H2O (950/50)
- 9. Extraction solution 2: 5% formic acid in 50% MeCN/H2O (500/450/50)



5 Remove the destain solution and dehydrate gel slices in 200 µL ACN (Acetonitrile) for 5 min. 5m 6 Remove ACN and repeat. 5m 7 Reduce the gel pieces in 30 µL of 10 mM DTT (dithiothreitol) for 0.5 h at room temperature. 30m 8 Remove the DTT solution. 1m Alkylate in 30 µL 50 mM IA (iodoacetamide) at room temperature, in dark for 0.5 h. (get ice for 50 mM AB 30m 9 10 Remove the IA solution. 1m 11 Wash gel pieces with 100 µL 100 mM ABC (ammonium bicarbonate) for 10 min and remove. 10m 12 Dehydrate gel pieces in 200 µL ACN for approximately 5 min. Remove ACN. 5m



- 21 Add 10 µL Extraction Solution 1 to each tube (or more if needed to cover the gel pieces) Let rest for 10 minutes. Take off supernatant to a clean 0.5 mL tube. 22 Add 10 µL Extraction Solution 2. Incubate for 10 min. Take off supernatant to the same 0.5 mL tube. 23 24 of total protein) with 0.1% Formic acid.
- 10m
- 10m

- Repeat the 10 µL Extraction Solution procedure. Take off supernatant to the same 0.5 mL tube.
- 10m
- Evaporate the sample via speed vac and reconstitute to 10-100 µL total volume (depending on the amount 2h

1h

- 25 Perform desalting using C18 tips (BAF_Protocol_003):
 - 10 µL tips for a small amount of proteins
 - 100 µL for a higher amount of proteins.