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IN-GEL DIGESTION

Guidelines for sample preparation
(How to protect your samples from contamination with keratin)

- **TRY TO AVOID ANY CONTACT OF SAMPLES AND SOLUTIONS WITH DUST, SKIN OR HAIR**
- **CLEAN YOUR BENCH**
- **WEAR GLOVES AT ALL TIMES**
- **ALL REAGENTS SHOULD BE PREPARED FRESH**
- **USE ULTRA PURE WATER FOR ALL SOLUTIONS**

SAMPLE SUBMISSION

- **PROVIDE SAMPLES IN 1.5 ML TUBES (NO SCREW CAPS)**
- **LABEL YOUR TUBE WITH YOUR NAME, SAMPLE NUMBER AND DATE**
- **PLEASE FILL IN ONLINE SAMPLE SUBMISSION FORM TO PROVIDE US WITH MORE INFORMATION ABOUT YOUR SAMPLE (PROTEIN ORIGIN, TAXONOMY, CONCENTRATION)**

REAGENTS

(All reagents should be prepared fresh)

Wash solution:
50 % methanol
5 % acetic acid
in MilliQ-H_20
we suggest total volumes of 50 ml

100 mM ammonium bicarbonate (79 g/mol):
0.79 g NH_4HCO_3 / 100 ml MilliQ-H_20

50 mM ammonium bicarbonate:
dilute 100 mM stock 1:1 in MilliQ-H_20

1 M DTT (154 g/mol):
1.54 g dithiothreitol in 10 ml MilliQ-H_20 (aliquot, keep at -20 °C)

10 mM DTT
1.0 ml 100 mM NH_4HCO_3
10 µl 1 M DTT

50 mM iodoacetamide (185 g/mol):
0.0092 g iodoacetamide in 1 ml MilliQ-H_20

Extraction buffer I:
50 % acetonitrile
5 % formic acid
in MilliQ-H_20
Extraction buffer II:

85 % acetonitrile
5 % formic acid
in MilliQ-H_20

Buffer A:
98 % MilliQ-H_20
2 % acetonitrile
0.1 % formic acid

DAY ONE

1. Cut out the protein bands with a clean scalpel, cut band into smaller pieces (1 – 2 mm^3) and place the gel pieces in a 1.5 ml tube.

2. Add **200 µl** of the wash solution and rinse the gel pieces overnight at room temperature while shaking

DAY TWO

1. Remove the wash solution and add another **200 µl** wash solution. Rinse the gel pieces for 2-3 hours at RT. Centrifuge shortly and remove the wash solution.

2. Add **200 µl** acetonitrile. Dehydrate the gel pieces for 5 min at RT. Remove acetonitrile from the sample. **REPEAT THIS STEP**

3. Add **30 µl** 10 mM DTT buffer and reduce for 30 minutes at RT. Remove the solution.

4. Add **30 µl** 50 mM iodoacetamide buffer and alkylate at RT for 30 minutes. Remove the solution.

5. Add **200 µl** acetonitrile. Dehydrate the gel pieces for 5 minutes at RT. They should be whitish. Remove the acetonitrile.

6. Rehydrate the gel pieces in **200 µl** 100 mM ammonium bicarbonate for 10 minutes at RT. Remove the solution.

7. Add **200 µl** acetonitrile. Dehydrate the gel pieces for 5 minutes at RT. Remove the solution.

8. Prepare the trypsin reagent: add **1 ml** of ice-cold 50 mM ammonium bicarbonate to 20 µg of trypsin [final concentration: 20 ng/µl]. The solution (10 x stock) should be kept on ice.

9. Dilute Trypsin stock 1:10 in 50mM ammonium bicarbonate (1x stock). Add **30 µl** (1 x stock) of the trypsin solution to the sample and rehydrate on ice for 10 min (DO NOT EXEED!) with occasional gentle mixing.

10. Centrifuge the gel pieces shortly and remove the excess trypsin solution.

11. Add **5 µl** of 50 mM ammonium bicarbonate to the sample. Carry out the digestion overnight at 37 °C.

DAY THREE

1. Add **50 µl** of 50 mM ammonium bicarbonate buffer to the tube containing the gel pieces. Incubate for 10 min with occasional gentle vortex mixing. Collect supernatant in a 1.5 ml tube

2. Add **50 µl** of the extraction buffer 1 to the tube containing the gel pieces. Incubate for 10 minutes with occasional gentle vortex mixing. Add supernatant to the collection tube.

3. Add another **50 µl** of the extraction buffer 2 to the tube containing the gel pieces. Incubate for 10 minutes with occasional gentle vortex mixing. Add supernatant to the collection tube.

4. Dry the sample down completely in a vacuum centrifuge. Resuspend in 20 µl buffer A

Footnote: Step 3 and 4 (reduction alkylation) from day two can be performed prior to loading your sample onto your gel. Please contact us for a protocol!