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IN-SOLUTION DIGESTION

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

- **Clean your bench**
- **Try to avoid contact of samples and solutions with dust, skin or hair**
- **Wear gloves at all times**
- **All reagents should be prepared fresh**
- **Use ultra-pure water for all solutions**

REAGENTS

(All reagents should be prepared fresh)

Tris stock (0.4 M, pH 7.8):
Dissolve 12.1 g of Tris base in 200 ml of MilliQ-H_20. Adjust pH to pH 7.8 with 6 M HCl. Add MilliQ-H_20 to a final volume of 250 ml, store @ 4°C.

6 M Urea in Tris buffer, pH 7.8:
Place 2.0 g of urea in a 15 ml falcon tube. Add 1.25 ml of 0.4 M Tris stock. Adjust the total volume to 5 ml with MilliQ-H_20.

Reducing agent: **200 mM DTT in 0.1 M Tris buffer, pH 7.8**
Dissolve 0.031 g of DTT in 750 µl of MilliQ-H_20. Add 250 µl of 0.4 M Tris stock and vortex.

Alkylating reagent: **200 mM iodoacetamide in 0.1 M Tris buffer, pH 7.8**
Dissolve 0.037 g of iodoacetamide in 750 µl of water.
Add 250 µl of the Tris stock and vortex.

Trypsin solution
Add 25 µl of ice-cold Tris stock and 75 µl of ice-cold MilliQ-H_20
water to 20 µg of sequencing-grade modified trypsin (Promega) and re-suspend carefully
The final concentration is 0.2 µg/µl. Keep on ice until use.

This protocol is designated for a sample volume of **200 µl** and optimal for 10 µg of total protein. Reagent volumes may be adjusted for different sample sizes.

Day One

1. Add 5 µl of the DTT reducing reagent (final concentration 5 mM) and vortex. Incubate for 30-60 min at room temperature.

2. Add 20 µl of the iodoacetamide alkylating reagent (final concentration 20 mM) and vortex. Incubate the mixture for 30-60 min at room temperature.

3. Precipitate the protein sample via Methanol / Chloroform Extraction for Proteins (*see separate protocol*).

4. Resuspend the protein pellet in 50 µl 6 M urea buffer by vortexing and sonication.

5. Reduce the urea concentration to a final concentration of < 1M by diluting the reaction mixture with 250 µl MilliQ-H_20 and vortex.

6. Add trypsin in a 1:50 ratio regarding the total protein content of your sample. Mix carefully and carry out the digestion overnight at 37 °C.

Day Two

1. go to SEP-PAK C18 PURIFICATION or ZIP TIP protocol (*see separate protocol*)