Proteomics: In-solution protein digest

REAGENTS

(All reagents should be prepared fresh and sterile filtered)

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 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 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**. Dissolve the trypsin.
The final concentration is 0.2 µg/µl. Keep on ice until use.

DIGESTION

This protocol is optimal for 1 mg of total protein material. Volumes can be adjusted to lower amounts of protein (down to 300 µg). If less than 300 µg of proteins digested, the same volumes should be kept as for 300 µg.

Day One

1. Precipitate the protein sample via Methanol / Chloroform Extraction for Proteins (*see separate protocol*) and add 100 µl 6 M urea buffer; vortex and sonicate for 2 min. Do not resuspend with the pipette.
2. Add **5 µl** of the DTT reducing reagent and vortex the sample. Incubate the mixture for 30-60 min at room temperature.
3. Add **20 µl** of the iodoacetamide alkylating reagent and vortex the sample. Incubate the mixture for 30-60 min at room temperature.
4. Add **20 µl** of the DTT reducing agent. Vortex the sample and allow the reaction to stand at room temperature for 30-60 min.
5. Reduce the urea concentration by diluting the reaction mixture with **775 µl** MilliQ-H_20. Vortex the solution.
6. Add trypsin in a 1:50 ratio regarding the total protein content of your sample (for total protein amounts of less than 10 µg use a 1:10 ratio) (generally 20 µl is sufficient). Mix carefully and carry out the digestion overnight at 37 °C.

Day Two

7. Stop the reaction and adjust the pH of the solution to <6 by adding concentrated acetic acid (use an appropriate pH paper)

SEP-PAK C18 PURIFICATION or ZIP TIP Protocol

1. Attach a syringe to the Sep-Pak column (both should be attached to a vacuum manifold)

2. Equilibrate the column:
– flush the column with **5 ml** of solution **B**
– flush the column with **10 ml** of solution **A**

3. Add the peptide digest sample onto the column and let it into the Sep-Pak column

4. Wash the sample with **10 ml** of Solution **A**
5. Elute with **2 x 1 ml** of solution **B** (collect your purified sample in a 2 ml tube)

6. Dry down purified peptides completely in a speed-vac. Resuspend in Solution A (up to 20µl)

MATERIAL AND SOLUTIONS

Waters C18 Sep-Pack cartridges: Sep-Pak Plus™, WAT020515
Solution A:
98% MilliQ-H_20
2% CH_3CN
0.1% FA
Solution B:
65% CH_3CN
35% MilliQ-H_20
0.1% FA
Use HPLC-grade Acetonitrile and FA, and MilliQ-H_20.