In-Solution digestion of protein mixture

Duration: 13-19 hrs, 2-days

Day 1

1. Sample selection

Starting material: Whole cell extract, cytoplasmic or membrane fraction, or any protein mixture/sample.

a) Measure protein concentration of the sample, and collect volume of sample containing 10 μg of protein in a 1.5 ml LoBind Eppendorf vial.

IF IT IS NECESSARY: Concentrate sample with Amicon filters (10 kDa cut-off) or by speed vac (not to complete dryness).

b) Dilute in 6 M Urea (7.21 gr/20 ml), 2 M Thiourea (3.04 gr/20 ml), V = 1.0 - 2.0 ml.

2. Reduction

(Duration: ~45 min)

Reduce with 1 mM DTT for 45 min at 56°C (add 20 μl of 100 mM -0.15 gr/10 ml- solution in 2 ml of sample solution).

3. Alkylation

(Duration: ~45 min)

Carbamidomethylate with 5 mM IAA for 45 min RT in the dark (add 100 μl of 100 mM -0.18 gr/10 ml- solution in 2 ml of sample solution).

4. Digestion

(Duration: ~8-14 hrs)

a) Collect volume of sample containing 1 μg protein and perform 4x dilution of the sample with deionized water.

b) Digestion with 5 ng trypsin overnight at 37°C (enzyme/protein : 1/50).

Trypsin solution preparation: Dissolve 20 μg Trypsin in 20 μl Storage buffer (=50 mM acetic acid/water).
Day 2

5. Lyophilisation
(Duration: ~3 hrs)

a) Dilute 2x with deionized water and acidify with TFA (pH ~ 3).

b) Use Speed Vac to evaporate the liquid until dry powder remains.

c) Store the dry samples in -20°C until LC-MS/MS analysis.