SOP	department of protein science (prot)		
TITLE	**In-Solution-Digest**		
CATEGORY	**Digestion**	*AUTHOR*	**Core Facility Proteomics**
VERSION #	**4.1**	*DATE*	**1.12.2010**

REAGENTS

Use HPLC water (Merck) for the preparation of all solutions.

Amonium bicarbonate (ABC) (Sigma; A61 41 -500G)

- Dissolve 198 mg ABC in 25 ml HPLC water for a 100mM solution.

RapiGest SF Surfactant (Waters; 1 86001 861)

- Dissolve in 50 µl HPLC water for a 2 % solution and homogenize the solution
- Store at 4 °C for 1 week or store aliquots at -20 °C

Dithiothreitol Solution (DTT), 1,4-Dithiothreitol (100 mM; DTT; Merck; 1 .11 474.0025)

- Always prepare a fresh solution.
- Prepare 1M stocks and freeze 50 µl aliquots at -20 °C
- Dissolve 50 µl in 500µl HPLC water to gain a 100 mM DTT Solution.

Iodacetamide Solution (IAA), 2-Iodacetamide (300 mM; IAA; Sigma; 8.04744.0025)

- Dissolve 55.5 mg IAA in 1 ml HPLC water and homogenise the solution carefully before use.
- Store in darkness until you use it, since IAA is light sensitive.
- Always prepare a fresh solution.

Trypsin Solution (T)

- Trypsin, sequencing grade (Sigma, proteomics grade, T6567-5x20ug). Dissolve lyophilized Trypsin in one aliquot (20 µg) by applying 40 µl H_2O, 1mM; HCl. → This can be used as a stock solution with a concentration of 0,5 µg/µl. Dilute one Trypsin aliquot (2,5 µg) with 245 µl 50 mM ABC for LC-MS/MS approaches This will result in a final concentration of 0.01 µg/µl of Trypsin.

1 mM HCl, Hydrochloric acid (32 %); Merck; 1 .0031 9.2500)

TFA Solutions (Trifluoracteic Acid, protein sequencing grade; AB Sciex; 400003; 40ml)

- 0,5 % TFA solution: Dilute 500µl TFA in 100ml HPLC water.

PROCEDURE

Tip:

- It is very important to measure the protein concentration before you start with the digest
- After the digest, the sample is directly injected into the LC and the MS. Substances, possibly interfering with the LC, have to be removed before the run. Using the speed-vac can reduce volume and remove ethereal substances, but will concentrate e.g. salt. A precipitation or dialysis of the sample should be carried out.
- If necessary, precipitate your protein as shown in the SOP - Protein Precipitation.
- The amounts of this protocol are calculated for a total protein amount of around 10 to 20 µg. If necessary, adjust the DTT and IAA amounts.

In Solution Digest

- Re-suspend the protein pellet in 30 µl to 100 µl 50 mM ABC or dilute the sample with 100 mM ABC. The pH should be > 7. Vortex strongly and spin down.
- Add 1 µl 100 mM DTT and incubate at 60 °C for 10 min in order to break existing disulfide bonds of cysteine.
- Cool down to RT, add 1 µl 300 mM IAA, vortex and incubate protected from light at RT for 30 min.
- Add 5 % (1:20; enzyme amount: protein amount) of Trypsin to your sample and incubate at 37 °C over night
- Add 5 µl 0,5 % TFA, vortex and incubate for 10 min to stop the Trypsin activity

In Solution Digest with RapiGest

- Re-suspend the protein pellet in 30 µl to 100 µl 50 mM ABC / 0,2 % Rapigest, vortex strongly and spin down.
- Add 1 µl 100 mM DTT and incubate at 60 °C for 10 min
- Cool down to RT, add 1µl 300 mM IAA, vortex and incubate protected from light at RT for 30 min.
- Add 5 % (1:20; enzyme amount: protein amount) of Trypsin to your sample and incubate at 37 °C over night
- Add 2 µl HCl (37 %) and quickly check the pH by using the drop at the end of your tip on indicator-paper. The pH should be around 2, in order to stop Trypsin activity and precipitate the detergent
- Transfer the sample into 0.2 ml polypropylene inserts (Sigma: #24722) and centrifuge for 30 min (13.000 rpm, 4 °C),
- Retain the supernatant (between upper phase and pellet!) It is important to ensure that no pellet is carried over!