Urea-FASP (Filter Aided Sample Preparation)

Overview:

In-solution protein digestion on a molecular cutoff spin column [1–4]. Its two major advantages are the possibility to pre-concentrate proteins from dilute mixtures (large volumes) and to remove mass spectrometry incompatible substances (SDS, Triton) before digestion. FASP should only be used with relatively large protein sample amounts (>= 50µg), since it suffers from peptide losses at the spin filter membrane [4].

Material:

- Vivacon 500 30.000 MWCO spin columns (Sartorius, max. capacity = 500 µl, dead stop volume = ~5 µl, use a fixed-angel rotor at max. 10.000 g [Attention: Membrane might break at higher velocities!])

- Urea Wash Solution
  8M Urea
  100 mM TEAB
  pH 8.5

- Urea Reduction + Alkylation Solution (10 ml)
  8 M Urea (60.06 g/mol) ➔ 4.85 g
  10 mM TCEP ➔ 100 µl from 1 M stock
  40 mM CAA (Chloroacetamide, 93.5 g/mol) ➔ 0.037 g
  100 mM TEAB pH 8.5 ➔ 1 ml from 1 M stock
  fill up to 10 ml

- Lys-C stock solution
  200 ng/µl solution (in 0.01% TFA), store at -20°C

- Trypsin stock solution
  200 ng/µl solution (in 0.01% TFA), store at -20°C

Procedure:

1. Preparation of Vivacon 500 30.000 MWCO ➔ Wash **twice** with 500 µl 70% isopropanol or 70% ethanol. At all steps always centrifuge 15 min at 10,000 g.
2. Membrane check & wash:

- Add 500 µl Urea wash solution ➔ Centrifuge 1 min ➔ Check amount of passed trough solution. The membrane is broken, if the majority has passed trough already.
- If the filter is ok proceed with the centrifugation (10,000 g, 15 min)➔ Discard flowtrough (FT)

3. Apply protein sample (50 - 100 µg). It should contain at least 6 M urea (if necessary by adding solid urea) ➔ Centrifuge ➔ Discard FT
4. Add 450 µl Urea Reduction + Alkylation solution, mix and incubate for 30 min in the dark at room temperature ➔ Centrifuge ➔ Discard FT
5. Add 500 µl Urea wash solution ➔ Centrifuge ➔ Discard FT
6. Check remaining volume on membrane. If it is still larger 10 µl extend centrifugation.
7. Add Lys-C at a Lys-C : protein ratio of 1:100 (500 ng per 50 µg), mix by mild orbital shaking and incubate for 3 - 4 h at 37°C.
8. Add 80 µl 100 mM TEAB and mix to reduce the urea concentration to at least 1.6 M.
9. Add trypsin at a trypsin : protein ratio of 1:50 (1 µg per 50 µg), mix by mild orbital shaking and incubate over night at 37°C
10. Replace collection tube and discard the old one.
11. Centrifuge to transfer the peptides to the collection tube. (Undigested proteins will be retained.)
12. To elute remaining peptides from the spin column add 100 µl, incubate for 5 min under mild orbital shaking and elute by centrifugation.
13. Acidify sample (pH <=2) and proceed with peptide desalting using C18-StageTips.

References:

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2. Wi[niewski, J. R., Zielinska, D. F. & Mann, M. Comparison of ultrafiltration units for proteomic and N-glycoproteomic analysis by the filter-aided sample preparation method. *Analytical biochemistry* **410,** 307 9
3. Wi[niewski, J. R., Zougman, A., Nagaraj, N. & Mann, M. Universal sample preparation method for proteome analysis. *Nature Methods* **6,** 359 362
4. Liebler, D. C. & Ham, A.-J. L. Spin filter-based sample preparation for shotgun proteomics. *Nature Methods* **6,** 785; author reply 785 6