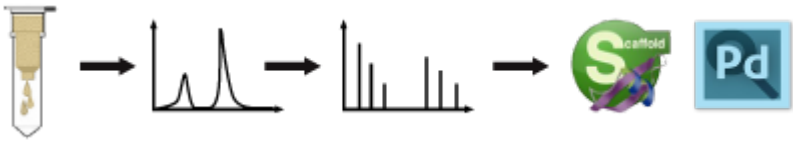
Filter Aided Sample Preparation – FASP



What is this method?

In-solution protein digestion on a molecular cutoff spin column.

When to use this method?

FASP should only be used with relatively large protein sample amounts (> 50µg). Choose this method every time you are concerned about SDS, salts and other contaminants, that might interfere with trypsin digest efficiency.

Advantages:

Possibility to pre-concentrate proteins from dilute mixtures (large volumes) and to remove mass spectrometry incompatible substances (SDS, Triton) before digestion.

Disadvantages:

Possibility of some peptide loss at the spin filter membrane.

Material:

- MWCO spin columns
- Triethylammonium bicarbonate (TEAB) or Ammonium bicarbonate (ambic)
- Tris(2-carboxyethyl) phosphine (TCEP)
- Iodoacetamide (IAA)
- Urea Wash Solution - 8M Urea in 100 mM TEAB (50 mM ambic), pH 8.5
- Urea Reduction & Alkylation Solution

  8 M Urea
  10 mM TCEP
  50 mM IAA
  100 mM TEAB (50 mM ambic)

- Trypsin in ambic: 200-400 ng/µl solution, store at -20°C
- Trufluoroacetic acid (TFA) or formic acid (FA)

Procedure:

1. Wash your column twice with 500 µL 70% isopropanol or 70% ethanol. At all steps always centrifuge 15 min at 10000 g.

2. Add 500 µL Urea wash solution and centrifuge for 1 min. Inspect the column membrane: the membrane is broken if most of the solution has passed through already. If the filter looks fine, proceed with the centrifugation for 15 min. Discard your flow through (FT)

3. Add protein sample (50 - 100 µg). It should contain at least 6 M urea (add a solid urea if necessary). Also, you can mix 30 µL of your protein sample with 200 µL of 8 M urea in 0.1 M Tris, pH 8.5, centrifuge and discard FT.

4. Add 450 µL Urea Reduction & Alkylation solution, mix and incubate for 30 min in the dark, at room temp, centrifuge and discard FT

5. Add 500 µL Urea wash solution, centrifuge and discard FT

6. Check remaining volume on membrane. If it is more than 10 µL, extend centrifugation.

7. Add trypsin in ambic, at trypsin: protein ratio of 1:50 (1 µg per 50 µg), mix and incubate overnight at 37°C.

8. Replace a collection tube and discard the old one.

9. Centrifuge to transfer the peptides to the new collection tube. (Undigested proteins will be retained.)

10. To elute remaining peptides from the spin column add 100 µL of ambic, incubate for 5 min and elute by centrifugation.