Filter-Aided Sample Preparation (FASP)

Adapted from Wisniewski et al. Nat. Methods, 2009.

Materials

1. FASP filters (30Kda cutoff; Millipore or Sartorious). Protocol is adjusted to conical filters. For flat filters, volumes should be adjested.
2. Proteomics-grade modified Trypsin (Promega).
3. **Urea buffer (UA)**: 8M urea in 0.1M Tris/HCL (pH-8.5). **Prepare fresh** (4.84 gr urea up to 10ml Tris).
4. **Iodoacetamide (IAA)**, 0.5M. Note: light sensitive.
5. **Digestion buffer**: 10% ACN, 25mM Tris pH-8.

Sample preparation

1. Extract cells or tissues in 0.1 M Tris-HCl pH-7.5, 4% SDS, 0.1 M DTT
2. Dilute lysates with UA, 5-7 fold (vol/vol).
3. Place a FASP filter inside a collection tube, and load up to 500µl of lysate solution onto the filter. Pipette well while being careful not to damage the filter.
4. Centrifuge at 8000 x g for 10 minutes.
5. Add 400µl UA to the filter, pipette well and centrifuge at 8000 x g for 10 min.
6. Repeat step 5 again.
7. Discard the flow-through from the collection tube.
8. Add 270µl of UA and 30µl of IAA 0.5M (final IAA concentration 0.05M). Mix and incubate in the dark for 20 min.
9. Centrifuge at 8000 x g for 10 min.
10. Add 400µl UA to the filter, pipette well and centrifuge at 8000 x g for 10 min.
11. Repeat step 10 again.
12. Discard the flow-through from the collection tube.
13. Add 400µl digestion buffer to the filter, pipette well and centrifuge at 8000 x g for 10 min.

14. Repeat step 13 again.
15. Add 300µl digestion buffer with trypsin at a concentration of 1:50 w/w trypsin to protein. Mix at 600rpm at room temp for 1 min.
16. Incubate in a wet chamber at 37°C for 4-18h.
17. Transfer the peptide-containing filter to a new collection tube.
18. Centrifuge at 8000 x g for 10 min.
19. Add 250µl digestion buffer to the filter, pipette well and centrifuge at 8000 x g for 10 min.
20. Concentrate the sample in a vacuum-concentrator for 1h.
21. Determine peptide concentration with NanoDrop. Yield should be 50-70% of the original amount.