Filter Aided Sample Preparation (FASP)

1. Materials

Solutions and Reagents

- SDT: 4%(w/v) SDS, 0.1M Tris/HCl pH 7.6, 0.1M DTT
- UA: 8 M urea in 0.1 M Tris/HCl pH 8.5. Prepare 1 ml per 1 sample by mixing 9 parts 8.88M urea with 1 part 1M tris 8.5.
- IAA solution: 0.05 M iodoacetamide in UA. Prepare 0.1 ml per 1 sample. Usually start with ~ 1 mL of 0.5M IAA in UA; dilute 10-fold in UA to make the working solution.
- Trypsin (Promega V5111 or V5280), Stock 0.4 µg/µL in resuspension buffer (0.1M acetic acid; supplied with trypsin)
- 0.5 M NaCl in water. Prepare 0.05 ml per 1 sample
- ABC: 0.05M NH_4HCO_3 in water. Prepare 0.25 ml per 1 sample

Notes:

1. ABC should be less than 2 weeks old.
2. IAA solutions must be prepared within minutes of use.
3. Minimize freeze-thawing cycles of trypsin. After dissolving the stock solution, separate into 1-2 µg aliquots and freeze at -20°C.

Equipment

- Pall Nanosep 10k Omega centrifugal devices (Pall Life Sciences, OD010C34 or OD010C33)
- Refrigerated Bench-top centrifuge (Eppendorf 5415R), temperature 20°C
- Dry incubator set to 37°C

2. Protocol

2.1 Sample lysis

Lyse cells or tissues in SDT-lysis buffer (omit DTT if performing BCA protein quant.; add DTT and boil after BCA assay) using 1:10 sample to buffer ratio for at 95°C for 3-5 min. The DNA can be sheared by sonication to reduce the viscosity of the sample. Liquid samples (saliva, serum, pure protein solutions, etc.) do not require sonication.

Before starting sample processing, cell/tissue lysates have to be clarified by centrifugation at 16,000 x g for 10 min.

Notes:

1. Tissue samples have to be homogenized with a blender in the lysis solution before heating.

2. Avoid temperatures below 15°C and potassium salts to avoid precipitation of concentrated SDS.

2.2 FASP

1. Mix up to 300µL of a sample (10-250µg) with 200µL of UA in the filter unit and centrifuge at 14,000 x g for at least 15 min; until filter unit is just dry.

2. Add 200µL of UA to the filter unit and centrifuge at 14,000 x g for 15 min.

3. Discard the flow-through form the collection tube.

4. Add 100 µL IAA solution and mix at 600 rpm in a thermo-mixer for 1 min and incubate without mixing for 20 min in the dark (usually in the centrifuge without spinning)

5. Centrifuge the filter units at 14,000 x g for 15 min.

6. Add 100 µL of UA to the filter unit and centrifuge at 14,000 x g for 15 min. Repeat this step twice.

7. Add 100 µL of ABC to the filter unit and centrifuge at 14,000 x g for 10 min. Repeat this step twice.

8. Add 40 µL ABC, with trypsin (enzyme to protein ratio 1:100) and mix at 600 rpm in thermomixer for 1 min.

9. Incubate the units in a wet chamber at 37°C for 4 -18 h.

10. Spin down the condensate from the filters: 1000 x g for ~ 10s.

11. Empty out and thoroughly rinse the collecting tubes. Replace the filter in its tube.

12. Centrifuge the filter units at 14,000 x g for 10 min.

13. Add 50 µL 0.5 M NaCl and centrifuge the filter units at 14,000 x g for 10 min. For small samples, repeat this step.

14. Acidify with CF_3COOH and desalt the filtrate. For samples > 100µg, a tC18 Sep-pak is probably best. For smaller samples, desalt in aliquots of up to 10µg on a 2-punch Stage Tip.

15. If no BCA protein quantitation was performed, quantify peptides with the peptides BCA assay.