

## Filter aided proteome preparation (FASP)

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### INTRODUCTION

In-gel digestion for mass spectrometry-based proteomics is extremely robust whereas in-solution digestion is more easily automated and minimizes sample handling. Filter aided proteome preparation (FASP), combines these advantages by completely solubilizing the proteome in sodium dodecyl sulfate (SDS), which is exchanged by urea on a standard filtration device. Peptides eluted after digestion on the filter are pure, allowing single run analysis of organelles and unprecedented depth of proteome coverage.

### MATERIAL

- SDT-lysis buffer: 4%(w/v) SDS, 100mM Tris/HCl pH 7.6, 0.1M DTT
- UA: 8 M urea (Sigma, U5128) in 0.1 M Tris/HCl pH 8.5
- UB: 8 M urea (Sigma, U5128) in 0.1 M Tris/HCl pH 8.0
- IAA solution: 0.05 M iodoacetamide in UA
- Endoproteinase Lys-C from Wako Bioproducts (Richmond, VA) Stock 5 µg/µl
- Trypsin, Stock 0.4 µg/µl
- 0.5M NaCl in water
- ABC: 0.05M NH<sub>4</sub>HCO<sub>3</sub> in water.
- Microcon YM-30 (Millipore, Cat. No. 42410) or Microcon YM-10 (Millipore, Cat. No. number 42407)
- 3M Empore HP Extraction disk cartridge (C18-SD); 7mm/ 3 ml (Varian Cat. No. 12144002)

**Note:** UA, UB, and IAA solutions must be prepared freshly and used within a day.

### METHODS

#### Sample lysis

Lyse cells or tissues in SDT-lysis buffer using 1:10 sample to buffer ratio for at 95°C for 3-5 min. The DNA has to be sheared by sonication to reduce the viscosity of the sample. Before starting sample processing the lysate has to be clarified by centrifugation at 16,000 x g for 5 min.

#### Notes

1. The tissues 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.

#### Sample processing

1. Mix up to 30µl of a protein extract with 200µl of UA in the filter unit and centrifuge at 14,000 x g for 40 min.
2. Add 200µl of UA to the filter unit and centrifuge at 14,000 x g for 40 min.

3. Discard the flow-through from the collection tube.
4. Add 100  $\mu$ l IAA solution and mix at 600 rpm in thermo-mixer for 1 min and incubate without mixing for 5 min.
5. Centrifuge the filter units at 14,000 x g for 30 min.
6. Add 100  $\mu$ l of UB to the filter unit and centrifuge at 14,000 x g for 40 min. Repeat this step twice.
7. Add 40  $\mu$ l of UB with Lys-C (enzyme to protein ration 1:50) and mix at 600 rpm in thermo-mixer for 1 min.
8. Incubate the units in wet chamber overnight.
9. Transfer the filter units to new collection tubes.
10. Add 120  $\mu$ l ABC with trypsin (enzyme to protein ration 1:100) and mix at 600 rpm in thermo-mixer for 1 min.
11. Incubate the units at RT for 4 h.
12. Centrifuge the filter units at 14,000 x g for 40 min.
13. Add 50  $\mu$ l 0.5 M NaCl and centrifuge the filter units at 14,000 x g for 20 min.
14. Acidify with CF<sub>3</sub>COOH and desalt the filtrate.

### Desalting of peptides

Small amounts of digest for direct LC-MS analysis can be desalted on StageTips.

Large amounts of peptide mixtures have to be desalted on SPE cartridges according to the following protocol:

1. Place a 3 ml MILI-SPE Extraction disk cartridge (C18-SD) in and 15 ml conical tube.
2. Add 1 ml of CH<sub>3</sub>OH and centrifuge at 1,500 x g for 1 min.
3. Add 0.5 ml of 0.1% CF<sub>3</sub>COOH, 70% CH<sub>3</sub>CN in water and centrifuge at 1,500 x g for 1 min.
4. Add 0.5 ml of 0.1% CF<sub>3</sub>COOH in water and centrifuge at 1,500 x g for 1 min.
5. Load the filtrate (2.2 step 15) and centrifuge at 150 x g for 3 min.
6. Add 0.5 ml of 0.1% CF<sub>3</sub>COOH in water and centrifuge at 150 x g for 3 min.
7. Transfer the cartridge to anew tube, add 0.5 ml 70% CH<sub>3</sub>CN in water and centrifuge at 150 x g for 3 min.
8. The eluate contains desalted peptides.

### Yield determination

Concentration of the peptides can be estimated by UV spectrometer assuming that 0.1% solution of vertebrate proteins has at 280 nm an extinction of 1.1 absorbance units (1mg/ml solution has 1.1 au).

## BIBLIOGRAPHY

Wiśniewski JR, Zougman A, Nagaraj N, Mann M. (2009). *Nat Methods*. 6(5):359-62.