Filter Aided Sample Preparation (FASP) Method

Adapted from Dr Matthias Mann laboratory

Introduction

The following protocol describes a method for generation of tryptic peptides from crude lysates for LC-MS analysis. The method allows analysis of detergent lysed cells and tissues. Therefore it is particularly suitable for studying entire proteomes and fractions containing biological membranes. Since the essential steps of the method occur within a filtration device the methods has been termed Filter Aided Sample Prep (FASP). The key features of the method making it superior over other sample preparation methods are:

1. The method provides protein digest that are free from nucleic acids and other cell components.

2. The method can be applied to samples containing high concentrations of detergents.

3. There are no precipitation and the concentration of sample is kept high.

4. In a single filter device 0.2-200 µg of total protein can be processed. Thus, FASP can be used upstream of separations of peptides such as OFFGEL and 2D- LC.

5. The yield and purity of peptides can be monitored by Nanodrop absorbance at 205nm allowing QC of the digest.

1. Materials

Solutions and Reagents (use MS-grade water for all solutions)

UA: 8 M urea (Sigma, U5128) in 0.1 M Tris/HCl pH 8.5. Prepare 1 ml per 1 sample.

ClAA solution: 0.05 M chloroacetamide in UA. Prepare 0.1 ml per 1 sample.

Trypsin, Stock 0.4 µg/µl.

0.5 M NaCl in MS-grade water. Prepare 0.05 ml per 1 sample.

ABC: 0.05 M NH_4HCO_3 in MS-grade water. Prepare 0.25 ml per 1 sample.

Note: UA and ClAA solutions must be freshly prepared and used within a day.

Equipment

1.5 ml lobind tubes.

Microcon YM-30 (Millipore, Cat. MRCF0R030).

Pierce C18 tips, 100 ul (VWR, Cat. PI87784).

Refrigererated Bench-top centrifuge (Eppendorf 5415R), temperature 20°C.

Wet chamber with a rack for Eppendorf tubes.

Thermo-mixer set to 20°C.

Nanodrop Spectrophotometer.

2. Methods

2.1 Sample preparation

Lysis and thiol-reduction

There is no universal protocol to lyse any kind biological material but heating in the presence of high concentrations of SDS and reduction of disulfide bridges usually results in total or at least high extent of lysis.

Examples for total lysate preparation:

50 µl HeLa cell pellet (one ø 15 cm dish), 25 mg mouse liver or cerebellum are lysed by mixing with in 150 µl of solution containing 4% SDS, 100 mM Tris/HCl pH 7.6, 0.1 M DTT (lysis solution) and incubation at 95°C for 3 min (caution should be taken here if you use BCA for protein quantification as DTT is not compatible with BCA). 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.

3. 50 µl HeLa pellet or 25 mg brain or liver contains roughly 2 mg proteins.

2.2 Sample processing

1. Mix up to 30 µl of a protein extract with 200 µl of UA in the filter unit and centrifuge at 14,000 x g for 15 min.

2. Add 200 µl 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 ClAA solution and mix at 600 rpm in a thermo-mixer for 1 min and incubate without mixing for 20 min.

5. Centrifuge the filter units at 14,000 x g for 10 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 (for a total of 3).

7. Add 100 µl of ABC to the filter unit and centrifuge at 14,000 x g for 10 min. Repeat this step twice (for a total of 3).

8. Add 40 µl ABC with trypsin (enzyme to protein ratio 1:100) and mix at 600 rpm in thermo-mixer for 1 min.

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

10. Transfer the filter units to a new 1.5 ml lobind tube.

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

12. Add 40 µl ABC and 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. Speedvac to dryness.

15. Resuspend in 300 µl 0.1% TFA and desalt the filtrate using C18 tips.

2.3 Yield determination

Concentration of the peptides can be estimated by Nanodrop absorbance at 205 nm.