Protocol for Filter Aided Sample Preparation (FASP)

The use of detergents to lyse cells can be problematic for down-stream mass spectrometry. Routinely used detergents such as Triton-X and NP-40 can cause ion suppression in the mass spectrometer while SDS can additionally impair enzyme digestion. Filter aided sample preparation or FASP is a method for generating tryptic peptides (for LC-MS/MS) from detergent lysed cells and tissues, that incorporates a buffer exchange step for the removal of the detergent. (Matthias Mann, http://www.biochem.mpg.de/226356/FASP).

MATERIALS

NOTES

- The following items of electrical equipment are used in this procedure. You should ensure that you have been correctly instructed in their correct use prior to carrying out this procedure.
    - Bench-top microfuge
    - Heating Block
    - Sonicator
    - Speedivac
    - Thermomixer
    - Water Bath

- This protocol involves an overnight step
- Use Eppendorf® LoBind microfuge tubes Protein
- Filter units - Sartorius, Vivacon 500, Product number: VN0H22

Buffers

Note: Urea and Iodoacetamide buffers must be *freshly prepared*.
Use HPLC grade water.

Lysis Buffer	0.1% SDS; 50mM-Tris pH 7.5
Urea Buffer	8M-Urea; 0.1M-Tris pH 8.5 (4.8 g Urea per 10 ml, 1 ml 1M-Tris pH 8.5) *fresh*
IAA Buffer	0.05M-Iodoacetamide (5 mg in 500 μL 8M-Urea/Tris buffer) *fresh*
Ambic	0.05M-Ammonium bicarbonate (0.4 g in 100 ml water)

METHOD

Sample lysis

Methods of sample lysis other than the following are available. The biological sample (for example a single 50 μL cell pellet from a 15 cm dish) is first lysed in approximately 150 μL of a buffer containing SDS (1% SDS; 50 mM-Tris pH 7.5).

1. Incubate the sample at 95°c for 5 minutes.
2. Sonicate the sample on ice x 3 briefly to reduce viscosity and shear DNA
3. Clarify the lysate by centrifugation at 16,000 x g for 5 minutes
4. Estimate the protein content. In the following example 200 μg of protein in 100 μL was processed.

Reduction of sample

1. Add 1M-DTT to give a final DTT concentration of 0.1M in each sample
2. Incubate on Thermomixer at 90°c for 10 minutes
3. Spin briefly

Buffer exchange (Urea displaces SDS)

1. Take up to 100 μL of sample and bring volume up to 0.5 mL with Urea buffer (8M-Urea; 0.1M-Tris pH 8.5)
2. Load onto a 10 kDa filter unit
3. Spin at 14,000 x g for 15 mins
4. Discard the flow through
5. Add 200 μL of Urea buffer to filter
6. Spin at 14,000 x g for 15 mins
7. Discard the flow through

Alkylation of sample

1. Add 100 μL IAA buffer (0.05M Iodoacetamide in Urea Buffer) to filter and mix in Thermomixer at 600 rpm for 1 minute
2. Incubate for 30 minutes at room temperature in the dark
3. Spin at 14,000 x g for 15 mins
4. Discard flow through

Removal of IAA

1. Add 200 μL Urea buffer to the filter
2. Spin at 14,000 x g for 15 mins
3. Repeat the last three steps

Removal of Urea before trypsin digestion

1. Add 200 μL Ambic (0.05 M-Ammonium bicarbonate) to the filter
2. Spin at 14,000 x g for 15 mins

3. Discard flow through
4. Repeat the last two steps

Trypsin digestion (overnight step)

1. For every 100 μg of protein add 2 μg Trypsin in a total volume of 40 μL Ambic
2. Incubate filter units in water bath at 37°c overnight (units are too high to fit in thermomixer)
3. Transfer filter units to new collection tubes
4. Centrifuge the filters at 14,000 x g for 20 mins
5. RETAIN FILTRATE – THIS IS THE TRYPTIC PEPTIDE MIXTURE
6. Add 40 μL Ambic to filter
7. Spin at 14,000 x g for 15 mins
8. Transfer the collected peptide digest to clean Lo-bind tube
9. Determine concentration with Nanodrop at 280 nm (using Ambic as blank)
10. Dry the digest under vacuum
11. Resuspend the digest in 0.5% TFA to 2 μg/μL

The re-suspended tryptic digest should be de-salted using Zip tips as per protocol