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General proteomics FASP (Filter-Aided Sample Preparation) V.1

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Spin-filter based protein digestion and purification for bottom-up proteomics

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It is important that nearly all liquid passes through spin filters after each centrifugation step. If a substantial amount of liquid (more than 75 µL) remains on filter after 10 minutes of centrifugation, spin for longer.

MATERIALS

Pierce trypsin Protease **Thermo Scientific**

Tween 20 **Bio-rad**

Laboratories Catalog #170-6606-MSDS

Optima LC/MS grade Water **Fisher**

Scientific Catalog #W6-4

Urea **Fisher**

Scientific Catalog #U15-500

Ammonium bicarbonate **Fisher**

Scientific Catalog #A643-500

Deoxycholic

Acid **bioworld Catalog #40430004-1**

Optima LC/MS grade Acetonitrile **Fisher**

Scientific Catalog #A955-212

Formic Acid (Optima LC/MS) **Fisher**

Scientific Catalog #A117-50

Ethyl Acetate **Fisher**

Scientific Catalog #E145-4

Passivate Vivacon filter unit (Satorius Vivicon 500, 30,000 MWCO) and collection tube overnight in 5% (v/v) Tween-20 in MilliQ water. The next day, rinse with nanopure water until no suds, then rinse 3x with LC-MS water.

Day 1

1 Prepare fresh buffers in LC-MS water:
Exchange buffer: 8M urea, 0.2% (w/v) deoxycholate, 1M ammonium bicarbonate (pH 8)
Digestion buffer: 0.2% (w/v) deoxycholate, 50 mM ammonium bicarbonate (pH 8)
Trypsin dissolution/peptide recovery buffer: 50 mM ammonium bicarbonate (pH 8)

2 Dispense 25-50µL (depending on concentration) reduced, alkylated protein extract to filter unit.

3 Fill filter units with 200 µL exchange buffer, mix with pipet.

4 Spin at 14,000g for 10 min, discard filtrate.

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5 Repeat steps 3 and 4 three more times.

6 Wash filter unit with 200 µL digestion buffer, and spin at 14,000g for 10 min. Do this a total of 2 times.

7 Transfer filter unit to passivated collection tube.

8 Add 110 µL digestion buffer and desired amount of trypsin (~1:50 trypsin/protein), dissolved in 50 mM ammonium bicarbonate, keeping trypsin on ice or at 4° C. Incubate at 37° C overnight. Parafilming tubes is recommended to prevent evaporation.

Day 2

9 Remove parafilm if applicable and centrifuge tubes at 14,000g for 10 min. **Do not discard filtrate!**

10 Add 50 µL of peptide recovery buffer to filters, spin at 14,000g for 10 min. Do this step a total of 2 times.

11 Transfer the filtrate to a LoBind tube.

12 Add 900µL ethyl acetate and 2.5 µL TFA to filtrate, vortex. Precipitate may be visible.

13 Sonicate in a bath sonicator for 10s, and centrifuge at 16,000g for 10 min.

14 Carefully remove upper organic layer with a pipet without disturbing phase boundary.

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15 Repeat steps 12, 13 and 14 two more times (Note: Only add 900µL ethyl acetate, no more TFA)

16 Place sample uncovered in Thermomixer and dry at 60° C for 5-10 minutes until ethyl acetate is gone.

17 Freeze sample solid at -80° C, Centrivap to dryness.

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