Filter Digestion

from Mann, et.al. 2009, Nature Methods, v6, p359

Solutions and Equipment

Sample Lysis Buffer (if needed): 4% (w/v) SDS, 100mM Tris-HCl pH 7.6, 0.1M DTT, Phosphatase Inhibitor Cocktail (PIC)* if needed.

*(50mM NaF, 50mM B-glycerophosphate, 1mM Sodium Orthovanadate, 10mM Sodium pyrophosphate, 1mM PMSF)

UA: 8M Urea, 0.1 M Tris-HCl pH8.5

UB: 8M Urea, 0.1 M Tris-HCl pH7.9

IAA solution: 0.05M Iodoacetamide in UA

0.5M NaCl in water

ABC: 0.05M Ambic in water

Formic Acid

Microcon YM-10 (10kDa cut off, Millipore #42407) or Microcon YM-30 (30kDa cut off, Millipore #UFC3LTK00)

Procedure

***Please note that listed spin times are an estimate based on the YM-10 filter and may differ in your centrifuge. You should determine actual spin times yourself before you begin the digestion with a real sample.**

1. Prepare filter unit by centrifuging 500uL of water through the cartridge at 14,000 x G for 20min. Do not let the filter dry out.
2. Mix up to 30uL of a protein extract with 200uL of UA in the filter unit and centrifuge at 14,000 x G for 30min.
3. Add 200uL of UA to the filter unit and centrifuge again, discard flow-through.
4. Add 100uL of IAA solution and mix in Thermomixer at 600rpm for 1min at room temp. Incubate without mixing for 5min.
5. Centrifuge units at 14,000 x G for 20min.
6. Add 100uL of UB to the filter unit and centrifuge at 14,000 x G for 30min. Repeat.
7. Transfer filter units to a new collection tube.
8. Add 120uL ABC with trypsin (1:100 ratio trypsin to protein) and mix in the Thermomixer at 600rpm for 1min.
9. Incubate at RT for min 4Hr to overnight.
10. Centrifuge filter unit at 14,000 x G for 30min, do not discard the filtrate as the peptides should now reside there.
11. Add 50uL of 0.5M NaCl and centrifuge at 14,000 x G for 20min.
12. Acidify filtrate with Formic Acid to 2% (v/v).
13. Freeze digests or continue to peptide desalting.