EXTRACTION AND HYDROLYSIS OF PROTEINS

(Courtesy from Ana Paula Alonso)

I. Extraction of proteins

1. Dry pellet from previous lipids extraction under a nitrogen flow.
2. Add 0.5 mL of extraction buffer (20 mM Tris-HCl, pH 7.5, 150mM NaCl, and 1% SDS) previously pre-warmed at 42°C.
3. Vortex (on position 7) for 15 minutes at 42°C (push samples half way down into the foam; start at 10 then reduce speed).
4. Centrifuge at 17,000 x g for 10 minutes.
5. Transfer supernatant into a 2 mL tube using a 1000 µL pipet.
6. Add 0.5 mL of pre-warmed extraction buffer.
7. Vortex (on position 7) for 10 minutes at 42°C.
8. Centrifuge at 17,000 x g for 10 minutes.
9. Pipet supernatant into a 2 mL tube.
10. Repeat step 6-9 one more time.
11. Sample are ready for protein hydrolysis.

II. Hydrolysis of proteins:

Note:

Prepare the following:

- 300 mL of 8 N HCl solution (original HCl stock at 12.1N): 200 mL of concentrated HCl solution are added to a graduated cylinder containing 100 mL of double distilled water ***(Always add the water first and work under a fume hood to avoid toxic fume).***
- 500 mL of 1 N HCl solution from 8 N HCl: 62,5 mL of 8 N HCl solution are added to a graduated cylinder containing 400 mL of water. Complete to 500 mL mark ***(Always add the water first and work under a fume hood to avoid toxic fume).***
- 50 mL of 0.01 N HCl solution from 1 N HCl: add 500 µL 1N HCl to a 50 mL tube and complete to the 50 mL mark with ddH_2O.

1. Add 750 µL of 8 N HCl ***(Perform under fume hood, use a p1000)*** to 250 µL of protein sample to be hydrolyzed, purge with nitrogen ***(Purge with adapted Pasteur pipette and cut bulb as drying needle)*** for 5-10 seconds and close the glass test tubes. Vortex ***(position 5-6; up to middle of the glass tube).***
2. Heat at 125°C for 24 hours with occasional shaking.
3. Remove samples and evaporate with nitrogen at 60°C until dry ***(For approx. 45 min.; with adapted Pasteur***

pipette and cut bulb as drying needle). Sample at this point can be store at -20°C until amino acid purification step.

III. Sample resuspension:

1. Add 500 µL of 0.01N HCl to the dried sample. Resuspend amino acids by vortexing for 10-20 seconds. Let stand tubes for 5 minutes on your bench.
2. Spin samples at 1,000 g at room temperature for 30 seconds.
3. Transfer sample (using a Pasteur pipette) to a pre-labeled 0.2 micron filtering device.
3. Centrifuge samples at 17,000 x g for 10 minutes at 25°C.
4. Discard filter and put tube on ice or store at -20°C until LC-MSMS experiment.