General Trypsin Digestion Using Urea Protocol for Cell Lysates; LCMS on LTQ

Procedure for Lysis of Monolayer-cultured Mammalian Cells

1. Carefully remove (decant) culture medium from adherent cells, wash cells once in wash buffer (e.g., PBS).
2. Lift cells by scraping in PBS.
3. Pellet the suspension of cells by centrifugation at 2,500 × *g* for 5 minutes. Discard the supernatant.
4. Add Urea stock solution containing HALT protease inhibitor mix to the cell pellet. Use at least 1 ml of either Reagent for cell pellet from a 100mm plate (~0.5 - 2 mg protein).
5. Pipette the mixture up and down to re-suspend pellet.
6. Shake mixture gently for 10 minutes. ***Dounce*** homogenize solution in 1ml unit and sonicate on ice for 4 x 15 second bursts
7. 7. Centrifuge sample at ~14,000 × *g* for 15 minutes. Allow sample to sit several minutes at 4°C then spin again at ~14,000 × *g* for 15 minutes.
8. Remove supernatant by submerging the pipet tip into the sample between the fat layer and the cellular debris pellet. Discard the fat layer and debris pellet.
9. Transfer the supernatant to a new tube for quantification and analysis

Digestions Procedure:

1. The sample from above is dissolved in 6 M Urea, 50 mM Tris-HCl, pH 8.0, the scale is based on 1 mg of protein in 1 ml of solution.
2. Add 5 µL of 200 mM DTT/ 50 mM Tris-HCl, pH 8.0, and incubate the mixture for 1 h at room temp.
3. Add 20 µL of 200 mM Iodoacetamide/ 50 mM Tris-HCl, pH 8.0, gentle vortex, and incubate the mixture for 1 h at room temp in dark.
4. Add 20 µL of 200 mM DTT/ 50 mM Tris-HCl, pH 8.0 to consume any un-reacted iodoacetamide. Incubate the mixture for 1 h at room temp in dark.
5. Add 775 µL of 50 mM Tris-HCl, 1 mM CaCl2 (pH 7.6) to reduce the urea concentration to ~0.6 M.
6. Add Trypsin solution to a final ratio of 1:50 (w/w, trypsin : protein). Gentle vortex and incubate at 37oC for 16-20 h.
7. 7. Add formic acid to adjust pH to 3-4. Test pH by placing 1µL aliquots onto a pH paper.
8. Store at -20°C.

Reagents: (prepare fresh right before the digestion)

(Use HPLC grade solvents, highest possible grade reagents and MilliQ water for all preparations)

6 M Urea, 50 mM Tris-HCl, pH 8.0 (360 mg/ml)

200 mM DTT, 50 mM Tris-HCl, pH 8.0 (30.8 mg/ ml)

200 mM Iodoacetamide, 50 mM Tris-HCl, pH 8.0 (37 mg/ml)

50 mM Tris-HCl (6.1 mg/ml), 1 mM CaCl2, pH 7.6 (0.11mg/ml)

Trypsin solution (0.1 µg/ µL): Reconstitute or dilute trypsin stock in resuspension buffer

(50 mM ammonium bicarbonate), keep on ice before use.