

Standard Global Tryptic Digest of Mammalian Cells

- 1) Sample size: 300.0 μ L of cells suspended in 1xPBS.
 - 2) Wash cells 3 times with 2.0 mL of ice-cold PBS. Pellet the cells after each wash by centrifugation at 10 000 rpm for 2 min.
 - 3) Add 1.5 mL of Lysis Buffer (10 mM NaPO₄, pH 7.0; 0.5% SDS) to the cells. Gently rock (agitate) to resuspend cells in Buffer. Do not vortex.
 - 4) Sonicate cells for 10 min in cold sonication bath containing water and a layer of ice.
 - 5) Perform BCA Protein Assay to check protein concentration.
 - 6) Add powdered form of Urea to sample to a target concentration of 8 M (484.6 mg/mL solution).
 - 7) Add efficient amount of 200 mM stock TBP solution to target a final concentration of 5 mM TBP in the sample.
 - 8) Incubate the sample at 37°C for 1 hour.
 - 9) Dilute the sample 8-fold with 50 mM NH₄HCO₃, pH 7.8 to reduce the salt concentration.
 - 10) Add sufficient amount of a 1M solution of CaCl₂ to obtain a sample concentration of 1 mM CaCl₂.
 - 11) Digest sample for 5 hours with Trypsin @ 37°C at a concentration of 1 unit trypsin/50 units protein.
 - 12) After trypsin incubation, immediately place sample on ice or snap freeze it. Sample was stored ON at -80C.
 - 13) Clean up sample by putting through 3 mL C18 SPE column using the following steps:
 - 14) Condition column with 6 mL of MeOH
 - 15) Rinse column with 6 mL nanopure water
 - 16) Slowly put sample solution through the column
 - 17) Wash column containing sample with 8 mL of 95:5 H₂O: ACN with 0.1% TFA.
 - 18) Allow column to go to dryness and blot "needles" below columns dry.
 - 19) Put collection tubes under column add 2 mL of 80:20 ACN:H₂O with 0.1% TFA.
 - 20) Open tubes to vacuum, and slowly elute sample from column, let column go dry.
 - 21) Concentrate sample in Speed-Vac. And perform BCA protein assay on sample.
- Quick-freeze sample in liquid nitrogen and store at -80°C until needed for analysis.