**Solution Digestion Protocol (tissue culture/animal tissue):**

**Lysis buffer:**

8.5M urea solution in 150mM NaCl
50mM Tris buffer, pH 8.1- 8.7
Roche EDTA-free protease inhibitor cocktail tablets
10mM sodium beta-glycerophosphate
1mM sodium fluoride
1mM sodium molybdate
1mM sodium orthovanadate, prepared per established protocols and stored at -20°C

**Reagents:**

500mM DTT stock solution stored frozen at -20°C
300mM iodoacetamide stock solution (prepared fresh in 8.5M urea/150mM NaCl, 55.8mg/1ml).

**To prepare:**

Turn a heat block or water bath on and allow to warm up to ~55°C.

1. Obtain cell pellet or tissue sample in a 15 or 50mL conical vial. An ideal target protein concentration is 3mg protein/ml of lysis buffer. For HeLa cells for example, 10 million cells is roughly 4mg total protein, so an aliquot of 25 million cells would be lysed in ~3.5ml lysis buffer in a 15ml conical vial.
2. Add lysis buffer to pellet and place on ice.
3. Immediately sonicate/dounce the cell pellet/tissue sample. Sonication is performed on a Branson microtip sonicator at power level 1.5 – 2.5 for 3 x 10 second bursts per sonication with incubation on ice for a minute or so in between to allow any heat to dissipate. When sonicating, move the tip of the sonicator up and down in solution to disperse any cell pellet debris and evenly distribute the lysing energy throughout the lysis solution. Take care, however, not to allow the sonication probe tip to rise too high to the top of the solution, or foaming will occur.
4. After sonication, mix the lysate thoroughly and remove ~200ul to an Eppendorf tube for BCA total protein analysis.
5. Add 500mM DTT solution as a 100x stock solution (e.g. 50ul in 5ml lysate) and mix thoroughly to distribute.

**At this point we would take over the sample.**

6. Place lysate in a 55°C water bath for 30 minutes. Swirl the lysate container occasionally to distribute heat to the sample. Take care not to allow the water bath temp to rise above 60°C, where inadvertent carbamoylation of lysine residues can occur.
7. ***IMPORTANT*** Remove the sample from the water bath and allow to completely cool to room temperature. A tap water bath can be used for this. If alkylation reagent is added when the sample is too warm, alkylation of lysine residues can occur. Occasional swirling of the lysate will assist in heat redistribution during cooling.
8. Once completely cooled, add iodoacetamide solution (prepared fresh) as a 20x stock to the lysate and mix thoroughly. Remove to the dark for 30 – 60 minutes.
9. Quench the alkylation with an additional 100x aliquot of DTT – mix and allow to stand for 15 minutes.
10. Dilute the sample by a factor of at least 5 in 25mM Tris, pH 8.7 in 50ml conical vials. This brings the urea concentration down to around 1.5M, where trypsin can still function without being denatured. Add trypsin in roughly 1 part trypsin to 200 parts protein in lysate (w/w) (4mg lysate in 3.5ml is diluted to ~20ml in Tris buffer, followed by addition of 20ug of trypsin). Remove to 37°C incubator overnight.