In-solution trypsin digestion

1. If the protein sample was precipitated with trichloroacetic acid (TCA) or acetone, resuspend the pellet in 50 mM ammonium bicarbonate (ABC) containing 10% acetonitrile (ACN). Adjust to pH 8.0 using ammonium hydroxide. If the protein sample is in solution: add ABC to 50 mM and CAN to 10% final concentration.

2. Add TCEP (TRIS (2-carboxyethyl) phosphine hydrochloride) to 10 mM final concentration. Boil for 5 minutes and allow it to cool to room temperature.

3. Add fresh iodoacetamide solution to 15 mM final concentration and incubate in the dark for 1 hour at room temperature.

4. Add DTT to 5 mM to quench the iodoacetamide for 10 minutes at room temperature.

5. Add trypsin to a ratio of 1:30-50.

6. Incubate for 4 hours to overnight at 37°C waterbath. (Incubation time will depend on the specifications of trypsin used.)

7. Add equal volume of 5% formic acid and sonicate for 10 minutes.

8. Sample desalt with ZipTip is REQUIRED before LC-MS analysis.

**NOTE:** Keep the volume as small as possible. You can dry or concentrate the digested sample in a speed-vac before desalting with ZipTip. It is important to make sure that proteins from precipitation are thoroughly solubilized and pH is readjusted to 8.0, specially if TCA precipitation was performed.