Protocol for in Solution Tryptic Digestion:

Sample preparation:

1. 50 µg of *Ecoli* crude cell extract in 5µl of 6M Guanidine/50mM Tris-HCl pH 8.0 buffers.

   Note: The proteins have already been denatured in 6M Guanidine.

Reduction and Alkylation:

1. Add **1.5 µL of 50 mM TCEP stock solution**
2. Mix and spin down sample
3. Incubate at 95°C for 10 min
4. Allow sample to cool down to room temperature and spin down to collect any condensation
5. Add **1.2 µL of 100 mM IAM into sample**
6. Mix and spin down sample
7. Incubate at room temperature for 1 hour in dark condition.
8. Add **1.2 µL of 100 mM DTT** to quench the IAM and wait for 10 min at RT

Trypsin Digestion:

1. Add 36 µl of 10 mM Tris-HCl pH8.0 buffers to dilute Guanidine concentration
2. Add **5uL of 1µg/ µl Trypsin** to the sample in a **1:10 ratio of Trypsin:protein**
3. Mix and spin down sample
4. Incubate at **37°C** for 5 hour

Stop trypsin reaction:

1. Add 150 µl of 0.5% formic acid to inactivate trypsin activity
2. Inject 2 µl of above sample for nanoLC-MS/MS analysis in either 4000 Q Trap or Synapt Q-TOF HDMS analysis

Stock solutions:

TCEP [Tris(2-carboxyethyl) phosphine]: 50 mM in H_2O
IAM (iodoacetamide): 100 mM in H_2O
DTT: 100 mM in H_2O
Trypsin: 1µg/µl in 50 mM acetic acid
Tris-HCl buffer: 50mM pH8.0/10% ACN
0.5% Formic acid