

In-Solution Digestion Protocol using TCEP & IAA

Materials:

Note: Make 8M Urea in Tris and IAA fresh!

8M Urea in 100 mM Tris-HCl, pH 8.5

Dissolve 480 mg of Urea in 700 ul of 100 mM Tris-HCl (pH 8.5). Make Fresh.

1M TCEP (reducing agent)

Dissolve 287 mg TCEP in 1ml HPLC water. Aliquot (10 ul) and store at -20C.

500 mM Iodoacetamide (IAA) (alkylating agent)

Dissolve 92 mg in 1 ml HPLC water. Make Fresh

1 ug/ul Trypsin solution

5 ul aliquots of 1 ug/ul Trypsin in 0.5 ml microtubes are stored in the -80C. [To make the 1 ug/ul stock, mix 100 ul HPLC water with 100 ug of Trypsin Gold from Promega (V5280).]

Digestion Procedure

- 1) Reconstitute dried protein sample in 40 ul of **8.0 M urea in 100 mM Tris-HCl (pH 8.5)**
- 2) Dilute the 1M TCEP 1:10 in HPLC water and add 1.2 ul of **100 mM TCEP** (5 mM final concentration) and shake for 20 minutes at room temperature.
- 3) Add 0.88 ul of **500 mM IAA** (10 mM final concentration) and shake in the dark for 15 minutes.
- 4) Add 120 ul of **100 mM Tris-HCl (pH 8.5)** solution to dilute the urea to 2M.
- 5) Add Trypsin in appropriate ratio (1:30) to approximate amount of protein by weight. (Generally speaking, for a complex sample, such as a whole cell or whole organelle lysate, a good starting point is 0.5 ug.)
- 6) Digest overnight at 37°C.
- 7) Follow C18 spin column protocol to clean up sample (remove urea) before running on Mass Spec.