

## Trypsin Digestion Protocol

### **Necessary reagents and instrumentation:**

200 mM iodoacetamide (IAM)  
1.5 M tris pH 8.8  
200 mM TCEP  
25 mM  $\text{NH}_4\text{HCO}_3$   
MeOH  
200 mM DTT  
Speedvac

### **Protocol**

For 300 ul protein mixture

1. Make protein solution to 6M urea (e.g. add 108 mg to 300 ul)
2. Add 20 ul of 1.5 M tris pH 8.8 (this raises the pH since TCEP is so acidic)
3. Add 7.5 ul of 200 mM TCEP (Tris(2-Carboxyethyl) phosphine Hydrochloride) and incubate 1 hr, 37 deg C (5 mM TCEP)
4. Add 60 ul of 200 mM iodoacetamide (IAM)
5. Vortex- sit for 1 hr, room temp, in the dark
6. Add 60 ul of 200 mM DTT
7. Vortex- sit for 1 hr, room temp (this soaks up all excess IAM)
8. Aliquot 150 ul into each of three 1.5 ml eppendorf tubes
9. Add 800 ul of 25 mM  $\text{NH}_4\text{HCO}_3$  to each tube to dilute the urea
10. Add 200 ul of MeOH (HPLC grade) to each tube
11. Add trypsin to protein at a ratio of 50 protein to 1 trypsin
12. Incubate overnight at 37 deg C
13. Speedvac to near dryness (pool your three tubes and wash wall with water)
14. Add 200 ul of water to the tubes three times and speedvac to reduce  $\text{NH}_4\text{HCO}_3$
15. Speedvac to dryness
16. Store at -80 deg C