

Urea Solution Digest Protocol

Solutions Needed:

8M Urea/2M Thiourea/400mM Ammonium Bicarbonate

480mg Urea

152mg Thiourea

32mg Ammonium Bicarbonate in 1mL water **final volume**

50mM Ammonium Bicarbonate

20mg Ambic in 5ml water **final volume**

100mM Dithiothreitol

15.4mg DTT in 1mL 50mM Ambic

100mM Iodoacetemide

18.5mg IAA in 1mL 50mM Ambic

Procedure:

1. Dissolve protein pellet in the minimum volume necessary of 8M Urea/2M Thiourea/400mM Ambic. Volume should be no more than 50-100 μ L.
2. Add DTT to 5mM. Pipette 5 μ L of the DTT solution into the protein solution if your volume is 100 μ L, 2.5 μ L if volume is 50 μ L.
3. Incubate solution at 50°C for 30min.
4. Allow the solution to cool to room temp and add IAA to 12.5mM. Pipette 12 μ L of the IAA solution to the protein solution if volume is 100 μ L, 6 μ L if volume is 50 μ L. Incubate the solution at room temp in the dark for 20min.
5. Quench the reaction by adding another 5 μ L DTT to the solution, 2.5 μ L if 50 μ L volume.
6. Dilute solution 8x with 50mM Ambic so final concentrations are 1M Urea/250mM Thiourea/50mM Ambic.
7. Add trypsin 1:50 (trypsin to protein). If protein concentration is not known add 100ng of trypsin solution in 50mM Ambic. Incubate overnight at 37°C.
8. Acidify the solution to inactivate Trypsin by adding TFA to 0.5% final concentration.
9. Desalt peptides and inject on the mass spectrometer.