Urea Solution Digest Protocol

Modified from Simpson, R., Proteins and Proteomics: A Laboratory Manual, 2003

Solutions Needed:

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

480mg Urea

152mg Thiourea

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

50mM Ammonium Bicarbonate (Ambic)

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 uL.
2. Add DTT to 5mM. Incubate solution at room temperature for 30min.
3. Add IAA to 12.5mM. Incubate the solution at room temp in the dark for 20min.
4. Quench the reaction by adding another 5mM DTT to the solution.
5. Dilute solution 8x with 50mM Ambic so final concentrations are 1M Urea/250mM Thiourea/50mM Ambic.
6. Add trypsin, in 50mM Ambic, 1:50 (trypsin to protein). If protein concentration is not known add 100ng of trypsin solution. Incubate overnight at 37^{o}C.
7. Acidify the solution to <pH3 to inactivate Trypsin by adding TFA to 0.5% final concentration.
8. Desalt peptides and inject on the mass spectrometer.

Alternative Method

From Mann, et.al. MCP 2007,6,4,697

Reagents

6M Urea

2M thiourea,

1mM DTT

5mM IAA

Lys-C

Trypsin

1. Dissolve protein in a minimum volume of 6M Urea/2M Thiourea.
2. Reduce sample by adding DTT to 1mM. Let sit at R.T. for 45min.
3. Carbamidomethylate sample by adding IAA to 5mM. Let sit at R.T. in the dark for 45min.
4. Add Lys-C to sample at a ratio of 1:50. Let sit at R.T. for 3Hr.
5. Dilute sample 4x with ddH20 to lower Urea concentration below 2M.
6. Add Trypsin to sample at a ratio of 1:50 and incubate at 37C overnight.
7. Acidify sample to <pH3 by adding TFA.
8. Desalt and analyze.