

Protein in-solution digestion protocol

Chemicals

- DL-Dithiothreitol (DTT) (Sigma Aldrich, cat# 43815-5G)
 - Dissolve 77 mg DTT in 1 mL 200 mM AmBiC (500 mM DTT)
 - Dilute 20 μ L 500 mM DTT with 980 μ L AmBiC (10 mM DTT)
- Iodoacetamide (IAA) (Sigma Aldrich, cat # I1149-5G)
 - Dissolve 18.5 mg IAA in 1 mL of 200 mM AmBiC (100 mM IAA)
 - Dilute 100 μ L 100 mM IAA with 400 μ L AmBiC (20 mM IAA)
- Ammonium bicarbonate (AmBiC) 200 mM (Sigma Aldrich, cat # 09830-500g)
 - Dissolve 160 mg AmBiC in 10 mL H₂O (200 mM)
- Hydrochloric acid (HCl) solution, 1 mM
- Trypsin Sequencing Grade (Promega, cat # V5111), 20 μ g per vial
 - Dissolve vial contents in 200 μ L 1 mM HCl solution (0.1 μ g/ μ L) and store in fridge at 4 °C until used
- 6M Urea solution (Sigma, cat # U5128-500G) in 200 mM AmBiC

Procedure

1. Dissolve your protein sample in 6M Urea solution.
2. Reduce the sample by adding the appropriate volume of 10 mM DTT (see Table 1, based on BCA assay or any protein concentration determination) and let the reaction proceed for 60 minutes at 37 °C
3. Alkylate the sample by adding the appropriate volume of 20 mM IAA (see Table 1) and let the reaction proceed for 30 minutes at RT in the dark (samples wrapped in aluminium foil).
4. Dilute sample to Urea concentration < 1 M with 200 mM AmBiC (see Table 1)
5. Take ~150 μ L sample from this sample solution and add 5 μ L trypsin solution (sample ratio approximately 1:25 to 1:100 enzyme:protein)
6. Digest at 37 °C overnight. Reaction can be stopped by placing the sample in the fridge 2 - 8 °C and following solid phase extraction.

Table 1. Overview of amounts of protein and volumes of reaction solutions to be added.

Protein amount (μ g)	Volume 6M Urea (μ L) -optional-	Volume 10mM DTT (μ L)	Volume 20mM IAA (μ L)	Volume 200mM AmBiC (μ L)	Trypsin amount (μ g)	Trypsin Volume (μ L)	Final volume (μ L)
5	20	2	2	96	0.5	5 - 10	130
10	20	3	3	94	1	5 - 10	130
50	90	15	15	420	2	5 - 10	550
100	100	30	30	440	10	5 - 10	610