# 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 µL 500 mM DTT with 980 µL 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 µL 100 mM IAA with 400 µL 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 µg per vial
  - Dissolve vial contents in 200 µL 1 mM HCl solution (0.1 µg/µL) 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 µL sample from this sample solution and add 5 µL 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 (µg) | Volume 6M Urea (µL) *-optional-* | Volume 10mM DTT (µL) | Volume 20mM IAA (µL) | Volume 200mM AmBiC (µL) | Trypsin amount (µg) | Trypsin Volume (µL) | Final volume (µL) |
|---|---|---|---|---|---|---|---|
| 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 |

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