

Example of Reduction and Alkylation Protocol

1 mg Bovine serum albumin

----- Add 175 μL 500 mM Tris-HCL (pH 8.0) and 8 M urea (Solution 1).

----- Add 25 μL 40 mg/mL dithiothreitol in Solution 1.

----- Incubation at 37 °C for 90 mins

----- Add 50 μL 40 mg/mL iodoacetoamide in Solution 1.

----- Incubation at 37 °C for 30 mins without exposure to light.

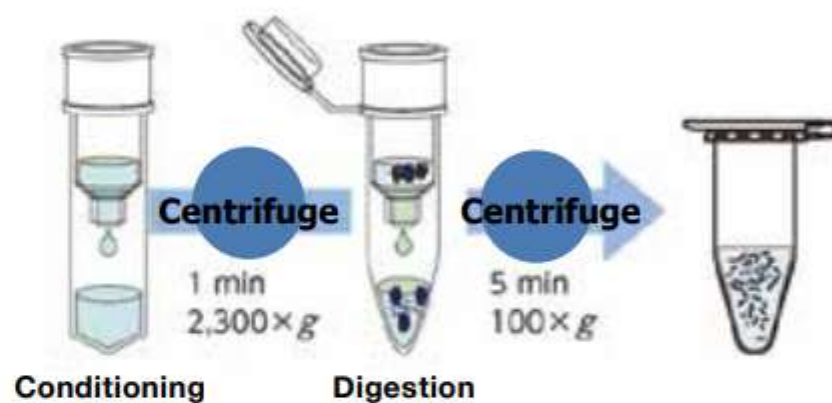
250 μL Reduced and alkylated protein

----- Add 50 mM ammonium bicarbonate to make the urea final concentration to 2 M and dilute it to 750 μL

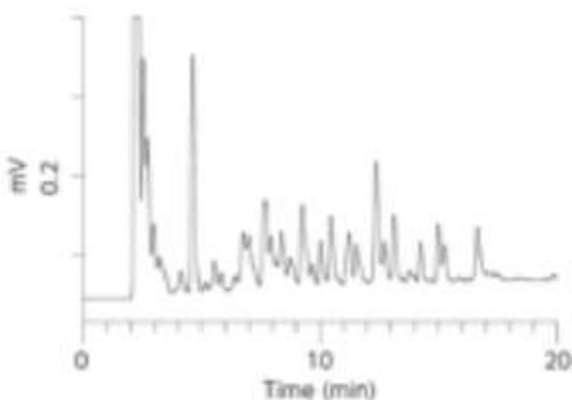
MonoSpin Trypsin

The protocol above is just an example.

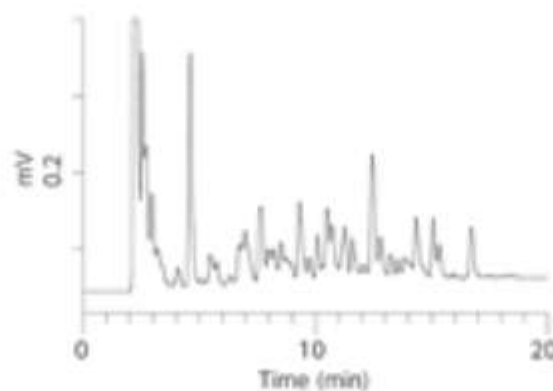
Optimize the protocol of preparation of reduced and Alkylated sample depending on the types of proteins.



Incubation at 37 °C
for 10 hours



Protein Digestion at 25 °C
for 10 minutes
using MonoSpin Trypsin



Conditions

Column : Inertsil ODS-3
(3 μm , 150 x 2.1 mm I.D.)
Eluent : A) H₂O (0.1 % HCOOH)
B) Acetonitrile (0.1 % HCOOH)
A/B = 90/10 - 20 min - 50/50
Flow Rate : UV 210 nm
Col.Temp. : 0.2 mL/min
Detection : 40 °C
Sample : Digested BSA 2 μL

MonoSpin Trypsin provide rapid and efficient protein digestion at room temperature in 10 mins.