

Protein Digestion Utilizing Trypsin

Introduction

Characterization of protein structure and function is critical for development of effective pharmaceuticals and often involves the use of techniques such as mass spectrometry (MS). This type of analysis requires a specific preparation of the target protein that includes digestion using trypsin. However, denaturation, reduction of disulfide bonds, and alkylation of reduced cysteine residues must occur, prior to digestion. Here, we outline a general procedure for a complete protein digestion.

Materials

- Ammonium Bicarbonate (NH_4HCO_3)
- Urea Solution (GoldBio Catalog # [U-200](#))
- Tris (GoldBio Catalog # [T-400](#))
- Iodoacetamide
- Methyl Alcohol
- Trypsin (GoldBio Catalog # [T-161](#))

Reducing Agents

- TCEP (GoldBio Catalog # [TCEP](#))
- DTT (GoldBio Catalog # [DTT](#))

Method

1. Dissolve the protein in 300 μl of 50mM ammonium bicarbonate, pH 7.8.
2. Make 6M urea solution by adding 108.11 mg of urea to the 300 μl of 50mM ammonium bicarbonate containing the protein.

Note: Urea denatures the protein.

3. Add 20 μl of 1.5M Tris at pH 8.8.
4. Add 7.5 μl of 200mM TCEP and incubate for 1 hour at 37°C.

Note: TCEP reduces the disulfide bonds.

5. Add 60 μ l of 200mM iodoacetamide and vortex. Incubate for one hour at room temperature in a dark area.

Note: Iodoacetamide is the alkylating agent.

6. Add 60 μ l of 200mM DTT and vortex. Incubate for one hour at room temperature.

Note: DTT quenches the alkylation reaction.

7. Aliquot 150 μ l each into three sterile 1.5 ml centrifuge tubes.
8. Add 800 μ l of 25mM ammonium bicarbonate to each tube to dilute the urea.
9. Add 200 μ l of MeOH to each tube.
10. Add trypsin to protein at a ratio of 50 protein to 1 trypsin and incubate overnight at room temperature. Speedvac until dry.
11. Add 200 μ l of molecular biology grade water to the tubes three times and speedvac to reduce the ammonium bicarbonate.
12. Store at -80°C .

Associated Products

- [Urea Solution \(GoldBio Catalog # U-200\)](#)
- [Tris \(GoldBio Catalog # T-400\)](#)
- [TCEP \(GoldBio Catalog # TCEP\)](#)
- [DTT \(GoldBio Catalog # DTT\)](#)
- [Trypsin \(GoldBio Catalog # T-161\)](#)

References

Goodlett, D. R. (n.d.). Trypsin Digestion Protocol. Goodlett Laboratory Protocols. University of Maryland School of Pharmacy, Baltimore Maryland.

Rebecchi, K. R., Go, E. P., Xu, L., Woodin, C. L., Mure, M., and Desaire, H. (2011). A general protease digestion procedure for optimal protein sequence coverage and post-translational modifications analysis of recombinant glycoproteins: application to the characterization of human lysyl oxidase-like 2 glycosylation. *Analytical Chemistry*, 83(22), 8484-8491.
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