

In-Solution Digestion

A. Reduction

1. Take the proper volume from the extracted protein sample according that contains 15-30 μg .
2. Unify the volume.
3. Add 2 μl of 200mM DTT, vortex, and then spin down.
4. Incubate 30 min at room temperature.

B. Alkylation

5. Add 2 μl of 1M IAA, and then incubate at room temperature for 45 min to 1 hour in dark.
6. Add 102 μl of 100mM Tris pH 8.5.

C. Trypsinization

7. Add 1 $\mu\text{g}/\mu\text{l}$ Trypsin solution.
8. Incubate overnight at 37°C in shaker.
9. Add 6 μl of 100% Formic acid.
10. Spin down for 30 min at max speed at room temperature.

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