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Trypsin digestion

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Trypsin digestion

In solution trypsin digestion of the TRIzol extracted protein sample was carried out by using Trypsin (Promega). Briefly, 15 μL of Digestion Buffer (50 mM Ammonium bicarbonate) and 1.5 μL of Reducing Buffer (100mM Dithiothreitol) were added to 10 μL of the protein solution to the micro-centrifuge tube and the final volume was adjusted to 27 μL with ultrapure water. The samples were incubated at 56 °C for 45 minutes and allowed to cool at room temperature. 3 μL of freshly prepared alkylation buffer (100mM Iodoacetamide) was then added to the tube and kept for incubation in the dark at room temperature for 20 minutes. Finally, 10 μL of Trypsin (20 $\mu\text{g}/\text{ml}$) solution was added in the tube and kept for incubation at 37°C for 15 hours (overnight)(25).