



Aug 05, 2020

Trypsin digestion

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DOI

dx.doi.org/10.17504/protocols.io.bjdpki5n

DOCUMENT CITATION

avinash.kale 2020. Trypsin digestion. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bjdpki5n>

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CREATED

Aug 05, 2020

LAST MODIFIED

Aug 05, 2020

DOCUMENT INTEGER ID

40079

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