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Denaturing On-bead Digestion for LC-MS/MS

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We use this protocol and it's working

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Abstract

A denaturing on-bead digestion for protein purification after pull-down using magnetic beads. The protocol is compatible with the cell membrane-permeable Ez-link NHS-SS-Biotin (ThermoScientific, PI21441) labeling and cell membrane-permeable DSP (ThermoScientific, PI22586). Step-by-step instructions for the pull-down are available in "Co-Immunoprecipitation/Pull-Down of C1q-Interacting Intracellular Proteins/Protein Complexes for Unbiased Forward Screen Using NanoLC-MS/MS"-protocol.

Guidelines

1. Wash beads with HPLC grade H2O and remove the supernatant.
2. Add 1 bed volume of 2M urea with 25 mM ammonium bicarbonate in water to the magnetic beads with protein.
   Note: Bed volume refers to the volume of the resin or magnetic beads. For example, if you have 100 µL of a 50% slurry, the bed volume is 50 µL once settled.
3. Add Dithiothreitol (DTT) to a final concentration of 2 mM and incubate for 15 minutes at 37°C.
4. Add iodoacetamide to a final concentration of 4 mM and incubate for 30 minutes in the dark at room temperature.
5. Add DTT to a final concentration of 2 mM and incubate for 15 minutes at 37°C to quench.
6. Add 1 bed volume of 25 mM ammonium bicarbonate to dilute the urea to a final concentration of 1M.
7. Add trypsin to a final concentration of 20 ng/µL. Incubate at 37°C for 8 hours to overnight. *Dissolved in 50 mM acetic acid.
8. Add formic acid to 0.1% to stop the digestion.
9. Collect the supernatant from the beads for a c18 cleanup.

Materials

- HPLC grade H2O (Fisher Scientific, Cat# W5-4)
- 2M urea (Fisher Scientific, Cat# BP169-500)
- Ammonium bicarbonate (Sigma-Aldrich, Cat# 09830)
- Dithiothreitol (DTT) (Fisher Scientific, Cat# BP172-25)
- Iodoacetamide (Sigma-Aldrich, Cat# I6125)
- Trypsin (Fisher Scientific, Cat# PRV5111)
- 50 mM acetic acid (Millipore-Sigma, Cat# 5330010050)
- Formic acid (Fisher Scientific, Cat# 60-006-17)

Proceed with the following steps for protein denaturation, reduction, and alkylation:

1 Wash beads with HPLC-grade H2O (Fisher Scientific, Cat# W5-4) and remove the supernatant.

2 Add 1 bed volume of 2M urea (Fisher Scientific, Cat# BP169-500) with 25 mM ammonium bicarbonate (Sigma-Aldrich, Cat# 09830) in water to the magnetic beads with protein.
Note: Bed volume refers to the volume of the resin or magnetic beads. For example, if you have 100 µL of a 50% slurry, the bed volume is 50 µL once settled.

3 Add Dithiothreitol (DTT) (Fisher Scientific, Cat# BP172-25) to a final concentration of 2 mM and incubate for 15 minutes at 37°C.

4 Add iodoacetamide (Sigma-Aldrich, Cat# I6125) to a final concentration of 4 mM and incubate for 30 minutes in the dark at room temperature.

5 Add DTT to a final concentration of 2 mM and incubate for 15 minutes at 37°C to quench.

6 Add 1 bed volume of 25 mM ammonium bicarbonate to dilute the urea to a final concentration of 1M.

7 Add trypsin* (Fisher Scientific, Cat# PRV5111) to a final concentration of 20 ng/µL. Incubate at 37°C for 8 hours to overnight. *Dissolved in 50 mM acetic acid (Millipore-Sigma, Cat# 5330010050).

8 Add formic acid (Fisher Scientific, Cat# 60-006-17) to 0.1% to stop the digestion.

9 Collect the supernatant from the beads for a C18 cleanup. See Sample Desalting Using OMIX C18 Tips for LC-MC-protocol for details.