In-Solution Digestion of Purified Yeast Protein for LC-MS

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[Abstract] This method describes the preparation of total yeast protein extract for mass spectrometry analysis. The protein extract is digested by trypsin in a solution with strong denaturants. The digested sample is dried and re-constituted in a mixture compatible with HPLC separation. Samples of isobaric labels should be processed in parallel experiments starting from trypsin digestion.

Materials and Reagents

1. Purified protein
2. Sequencing grade modified trypsin (Promega Corporation, catalog number: V5113)
3. 6 M guanidine HCl
4. Tris HCl (pH 8.0)
5. 1 M DTT
6. Triethylamine (TEA) (Sigma-Aldrich)
7. HPLC solvent A (usually 10% acetonitrile in water)
8. Acetic acid

Equipment

1. Amicon Ultra centrifuge filters Ultracel 10 k MWCO (EMD Millipore)
2. SpeedVac
3. Heat block
4. High Performance Liquid Chromatography (HPLC)
5. Amicon filters

Procedure

1. Concentrate purified protein on Amicon filters to 20 µl.
2. Take 20 µl protein solution (~100 µg), add to final of 6 M guanidine HCl, 50 mM Tris-HCl (pH 8.0), 2-4 mM DTT. Heat at 95 °C for 20 min.

3. Cool the reaction, then add 200 mM TEA. Final guanidine HCl concentration should be below 1 M.
4. Dissolve a vial of trypsin (20 µg) in 20 µl 50 mM acetic acid.
   a. Add trypsin to target protein solution in a ratio of 1:50. Incubate at 37 °C for 1 h or longer.
   b. SpeedVac the reation to dryness, then re-suspend with solvent A in HPLC.

References

1. Empirical lab protocol from Thermo Fisher.