Preparation of HeLa peptides for LC-MS

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Method Article

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Abstract

A protocol for the generation of a tryptic peptide mixture from HeLa cell culture is described. The primary purpose of the sample is to evaluate, optimize and validate LC-MS/MS procedures.

Reagents

Acetone, Sigma-Aldrich Caution\! It is flammable\! Handle it a fume hood and wear gloves and safety goggles Ammoniumbicarbonate, Sigma-Aldrich Beta-glycerophosphate, Sigma-Aldrich Chymostatin, Sigma-Aldrich DL-Dithiothreitol \(DTT), Sigma-Aldrich Dimethyl sulfoxide \(DMSO), Fluka Ethylenediaminetetraacetic acid \(EDTA), BioChemika Glycerol, BioChemika High glucose Dulbecco's modified Eagle's medium \(DMEM), \(Sigma-Aldrich) supplemented with 10% \(v/v) fetal bovine serum, \(Gibco, Invitrogen) Iodoacetamide, Sigma-Aldrich Caution\! It is toxic and corrosive. Handle in a fume hood and wear gloves and safety goggles. KCl, Sigma-Aldrich KOH, Merck Leupeptin, Sigma-Aldrich L-glutamine, Sigma-Aldrich Lys-C, Wako Chemicals N-\(2-Hydroxyethylpiperazine-N'-\(2-ethanesulfonic acid) \(HEPES), Sigma-Aldrich NaF, Sigma-Aldrich Na-pyrophosphat, Sigma-Aldrich Na3VO4, Sigma-Aldrich Nocodazol, Sigma-Aldrich Penicillin, Sigma-Aldrich Pepstatin, Sigma-Aldrich Phenylmethylsulfonylfluorid, Sigma-Aldrich Streptomycin, Sigma-Aldrich Trifluoroacetic acid \(TFA), Pierce Caution\! It is corrosive. Handle in a fume hood and wear gloves and safety goggles. Triton X-100, Fluka Trypsin Gold Mass spectrometry grade, Promega Urea, Sigma-Aldrich REAGENT SETUP **HeLa culture medium** High glucose Dulbecco's modified Eagle's medium \(DMEM) supplemented with 10% \(v/v) fetal bovine serum, 0.2 mM L-glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin. **Protease Inhibitor Mix \(1000× stock solution)** 10 mg/mL each of leupeptin, pepstatin and chymostatin, in DMSO **Lysis buffer** 50 mM HEPES-KOH pH 7.5; 5 mM EDTA, 15mM KCl; 10% glycerol, 1% Triton X-100, 20 mM beta-glycerophosphate 10 mM Na-pyrophosphat, 10 mM NaF, 1 mM DTT, Na_3VO_4 \(200mM), 0,1 mM Phenylmethylsulfonylfluorid, and 2 x Protease Inhibitor Mix.

Procedure

HeLa Protein Preparation 1. Grow HeLa Kyoto cells in DMEM and arrest the growth with nocodazol over night. 2. Harvest cells with a scraper and wash three times with PBS. One tray \(25 cm x 25 cm; 100 mL medium) generates approximately 4 mg of protein. 3. Suspend the cell pellet in lysis buffer using twice its volume and disrupt the cell membrane by pulling the suspension through a thin needle \(first 21 then 27 gauge). Centrifuge the suspension at 500 g in a cooling centrifuge \(4°C) for 15 min. **Acetone precipitation of proteins** 4. Add 5 times the volume of chilled acetone to the protein solution and incubate the sample at minus 30 °C. 5. Centrifuge the suspension at 500 g in a cooling centrifuge \(4°C) for 30 min. 6. Discard the supernatant and carefully suspend the protein pellet with 80% chilled acetone and repeat the centrifugation. 7. Air-dry the pellet avoiding complete desiccation and dissolve the protein pellet in the digestion buffer \(8 M urea, 0.5 M ammoniumbicarbonat) to a protein concentration of 5 µg/µL. The protein solution should have a pH of 8.0. in our laboratory we measure protein concentration using a Bradford assay. **Reduction and alkylation of proteins** 8. Add 1 µg DTT stock solution up to a

final concentration of 0.05 µg DTT per µg protein and incubate the sample for 30 min at 56 °C, shaking. 9. Next add iodoacetamide to a final concentration of 0.25 µg per µg protein and incubate the sample for 30 min at room temperature in the dark. 10. Quench the reaction by adding DTT stock solution up to a final concentration of 0.25 µg DTT per µg protein **Digestion with Lys-C** 11. Dilute the sample to 6 M urea with 50 mM ammoniumbicarbonat buffer and add Lys-C to a final concentration of 1 µg per 50 µg protein. 12. Incubate the sample at 30 °C for 2 h. Next, digest the predigested protein sample with trypsin starting with diluting the solution to 0.8 M urea with 50mM ammoniumbicarbonat buffer. 13. Add 1 µg trypsin per 60 µg protein and incubate the sample for 2 h at 37 °C. 14. Add another aliquot of 1 µg trypsin per 60 µg protein and incubate the sample at 37 °C over night. The digest is stopped by acidifying the sample to a pH of 2 by adding TFA. 15. Peptide mixtures should and stored in aliquots at minus 80º C.