Protein preparation for LC-MS/MS analysis

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ABSTRACT

This protocol is with regard to quantitative proteomics analysis to identify biomarkers of chronic myofascial pain and therapeutic targets of dry needling in a rat model of myofascial trigger points. For futher nanoscale liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, the first step is to extract and prepare the protein of the sample.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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PROTOCOL STATUS

Working

GUIDELINES

The animal experiments complied with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986, and associated guidelines.

SAFETY WARNINGS

BEFORE STARTING

After the treatment was terminated on the 5th day, all rats were deeply anesthetized using intraperitoneal sodium pentobarbital at 60mg/kg, and the left GM tissue was quickly removed and rapidly frozen in liquid nitrogen for 5min and then immediately stored at -80°C refrigerator. The whole procedure for removing the GM in vivo was completed within 1min from each rat, and air embolism was then administered.

1 The SDT lysis buffer was added to the sample and transferred to 2ml tubes with quartz sand consisting of 1/4-inch ceramic beads (MP 6540-424 for tissue samples).

2 The lysate was homogenized twice for 60 s using an MP homogenizer (24×2, 6.0M/S).

3 The homogenate was sonicated and then boiled for 15min.

4 After centrifuging at 14000 x g for 40min, the supernatant was filtered with 0.22μm filters. The filtrate was quantified with the BCA Protein Assay Kit (Bio-Rad Inc., Hercules, USA).

5 Protein samples of 20μg (each sample)were mixed with 5X loading buffer and the proteins were separated on 12.5% SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) gel for 90min with a constant current of 14mA after boiling for 5min.

6 200μg of proteins (each sample) were added to a sample of 30μl of SDT buffer (150mM Tris-HCl, 4% SDS, 100mM DTT, pH8.0) and then removed from the dithiothreitol (DTT), the detergent and other low-molecular-weight components, using uric acid buffer (150mM Tris-HCl, 8M urea, pH8.0) and 100μl iodoacetamide to block the reduced cysteine residues for Filter-aided sample preparation (FASP).

7 The samples were then incubated for 30min in the dark. Then, the filters were washed in triplicate with 100μl uric acid buffer and washed twice in100μl of 100mM tetraethyl-ammonium bromide (TEAB) buffer.

8 Finally, 4μg of trypsin in 40μl of TEAB buffer was used to digest the protein suspensions with overnight at 37°C.

9 Following sample digestion and immediately before use, the Tandem mass tag (TMT) labeled reagents were equilibrated to room temperature, and 4-plex TMT reagent was applied to label 100μg of the peptide mixture of each sample, according to the instructions of the manufacturer (Thermo Fisher Scientific Inc., Waltham, USA).

10 The protein peptides were labeled with TMT-126, TMT-127, TMT-128, and TMT-129 for the CG, MG, DG and NDG groups for three biological replicates.

11 Incubation for 60min was undertaken to allow the reaction to occur, and 8μl of 5% hydroxylamine was added to the sample, which was incubated for a further 15 minutes.

12 TMT-labeled samples were fractionated into 10 fractions using a Pierce high pH reversed-phase fractionation kit by an increasing acetonitrile step-gradient elution.

13 Before LC-MS/MS analysis, the fractions were dissolved in 0.1% formic acid, according to the instructions of the manufacturer (Thermo Fisher Scientific Inc.).