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Tissue Sample Preparation for LC-MS Analysis

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This protocol describes the procedure for protein extraction, enzymatic digestion, sample cleanup an dTMT labeling for LC-MS analysis. Proper sample preparation and clean-up are extremely important to ensuring quality LC-MS data and reproducibility. Tissues tend to vary more than cells and extra care should be taken as MS is sensitive to any sample variations. In addition, proper clean-up is a preventative measure to less damage to the instruments and superior data. Here, we described each step in detail on how to process tissue samples prior to analysis.

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HuBMAP, BIOMIC, MSRC, Stanford, Proteomics, Peptides, Protein Precipitation, Desalting, Tissue Lysis, Protein Digestion, TMT labeling

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Please make sure at any point in the experiment, do not leave the samples unattended. If not actively performing a step, put your samples on ice.

MATERIALS

Lysing matrix D tubes **MP Biomedicals Catalog #6913100**

2-Chloroacetamide (CAA) **Sigma Aldrich Catalog #22790**

Trizma® base **Sigma Aldrich Catalog #93350**

Acetone **Sigma Aldrich Catalog #179124**

Guanidine-HCl **Thermo Fisher Catalog #24110**

Pierce™ Trypsin Protease, MS Grade **Thermo Fisher Catalog #90059**

TMT10plex™ Isobaric Label Reagent Set, 1 x 0.8 mg **Thermo Fisher Catalog #90110**

Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) **Sigma Aldrich Catalog #C4706**

Lysyl Endopeptidase Mass Spectrometry Grade (Lys-C) **Fujifilm Wako Pure Chemical Catalog #12505061**

Oasis HLB 1 cc Vac Cartridge 10 mg Sorbent per Cartridge 30 µm 100/pk **Waters Catalog #186000383**

All the waste needs to go to biohazard.

Ensure you have enough tissue before preparation. We recommend using at least 30 mg of tissue to start with.

Protein Lysate Extraction 4h

1 Place the tissue chunk onto a cell culture plate on ice and mince with disposable scalpels to the best of your abilities.

2 Add **500 µL** lysis buffer (**6 Molarity (M)** GdmCl, **10 Milimolar (mM)** TCEP, **40 Milimolar (mM)** CAA, **100 Milimolar (mM)** Tris **8.5**) to the minced tissue sample and transfer it to a Matrix D tube with ceramic beads.

3 Beat the tissue using FastPrep-24™ at 4.5 M/S, **00:00:40** at **4 °C** 2 times .

4 Centrifuge the sample for **00:05:00** at **7000 x g** , and transfer the sample to a new tube, and sonicating at 40% and 60% for **00:00:20** each, respectively on ice.

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5 Heat for **00:05:00** at **95 °C** and vortex every **00:01:00** .

6 Centrifuge for **00:05:00** , **00:10:00** , and **00:10:00** minutes respectively at **12000 x g** , and collect the supernatant at each step.

Enzymatic Digestion 16h

7 Take **5 µL** of the sample out and dilute to measure its protein concentration using BCA kit.

8 Take **100 µg** - **300 µg** of the sample to perform a two-step enzymatic digestion using **25 Milimolar (mM)** Tris **pH 8.5** as the dilution buffer.

9 Endoproteinase Lys-C digestion: Add 3x volume of dilution buffer to the original lysate. Add Lys-C to lysate at 1:100 (w:w) and incubate at **37 °C** for **02:00:00** . 2h

10 Trypsin digestion: Add an additional 6x volume of dilution buffer to the original lysate volume for an overall 9x dilution of the original sample volume. Add Trypsin to lysate at 1:50 (w:w) and incubate at **37 °C** **Overnight** . 14h

11 Digested samples can be stored at **-80 °C** .

Desalting the Peptide 8h

12 Desalt the digest with Waters Oasis® HLB Cartridge.

12.1 Condition the column with **1 mL** each of **100 % (v/v)** acetonitrile, **80 % (v/v)** acetonitrile with **0.1 % (v/v)** acetic acid, and **0.1 % (v/v)** acetic acid sequencially.

12.2 Adjust the sample to **1 % (v/v)** TFA, and load the sample onto the desalting column.

12.3 Wash the sample with **200 µL** of **0.1 % (v/v)** acetic acid 3 times.

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12.4 Elute peptides off the column with 150uL of [M]**40 % (v/v)** acetonitrile with [M]**0.1 % (v/v)** acetic acid, followed by 150uL of [M]**80 % (v/v)** acetonitrile with [M]**0.1 % (v/v)** acetic acid.

13 Put the sample in the SpeedVac until dry.

TMT Labeling 4h

4h

14 The TMT10plex™ Isobaric Label Reagents were prepared as suggested by the manufacture. https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0016969_2162457_TMT10plex_UG.pdf&title=VXNlciBHdWlkZTogVE1UMTBwbGV4IE1hc3MgVGFnIExhYmVsaW5nIEtpdHMgYW5kIFJlYWdlbnRz

14.1 Resuspend the sample in [M]**100 Milimolar (mM)** TEAB pH**8.5** and then add assigned TMT tag to the sample.

14.2 Incubate the sample with its associated TMT tag for **01:00:00** at room temperature.

14.3 Quench the reaction with [M]**5 % (v/v)** hydroxylamine.

14.4 Mix each set of 10 samples together in equal amounts.

15 Put the sample in the SpeedVac to dry.

16 Resuspend the sample with [M]**200 Milimolar (mM)** ammonium formate and perform LC-MS analysis.

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