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Laser Capture Microdissection of Tissue Functional Units for microPOTS Top-Down Proteomics

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ABSTRACT

In this protocol, we describe a detailed procedure for high-resolution top-down proteomic analysis of human pancreatic and kidney tissue sections using a spatial proteomic platform by combining laser capture microdissection (LCM), microPOTS (microdroplet processing in one pot for trace samples), and nanoflow liquid chromatography mass spectrometry (LC-MS). This protocol has been applied to human pancreatic and kidney specimens for HubMAP Tissue Mapping Centers.

Expected outcome:
Identification of >300 proteoforms at 100-μm spatial resolution depending on sample types and LC-MS instrumentation.

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MATERIALS TEXT

1. Reagents

1.1. LCM collection

• Dimethyl sulfoxide (DMSO)

1.2. Proteomic sample processing

• Nanopure water
• LC-MS grade DMSO
• n-Dodecy-ß-D-maltoside (DDM) (e.g. Thermo Fisher Scientific Cat# 89902)
• Ammonium bicarbonate (ABC)
• Magnesium chloride ($MgCl_2$)
• 250 units/µL benzonase (EMDMillipore, Cat# 71206-3)
• 0.5 M Neutral pH, TCEP (tris(2-carboxyethyl)phosphine)-HCL (Thermo Fisher scientific, Cat# 77720)

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• Urea BioUltra (Sigma Aldrich, SKU 51456-500G)
• Formic acid (Thermo Fisher scientific, Cat# 28905)
• Acetonitrile (MeCN)

2. Equipment

• Laser Capture Microdissection System (Zeiss PALM MicroBeam)
• Home-built nanoPOTS sample preparation system
• polypropylene microPOTS chips

Weke K, Singh A, Uwugiaren N, Alfaro JA, Wang T, Hupp TR, O'Neill JR, Vojtesek B, Goodlett DR, Williams SM, Zhou M, Kelly RT, Zhu Y, Dapic I (2021). MicroPOTS Analysis of Barrett's Esophageal Cell Line Models Identifies Proteomic Changes after Physiologic and Radiation Stress.. Journal of proteome research. https://doi.org/10.1021/acs.jproteome.0c00629

• LC-MS/MS system (Orbitrap Lumos Tribrid MS, Thermo Scientific)

3. Buffer preparation

3.1. Extraction buffer (Desired conc.: ~4 M Urea, 10%DMSO, 5 mM TCEP, 0.1% DDM in 50 mM ABC)
• Weight 240 mg urea into 1.5 mL Eppendorf Tube
• Add 530 µL 50 mM ABC (pH 7.8)
• 100 µL 100% LC-MS grade DMSO
• 100 µL 1% DDM
• 10 µL 0.5 M TCEP

3.2. Benzonase buffer (Desired conc.: 1 mM $MgCl_2$ in 50 mM ABC with 2.5 unit/µL benzonase)
• 98 µL of 50 mM ABC
• 1 µL of 100 mM $MgCl_2$
• 1 µL of 250 units/µL benzonase

3.3. Quenching buffer (Desired conc.: 94.9% water, 4.9% MeCN, 0.2% FA)
• 949 µL LC-MS grade water
• 49 µL MeCN
• 2 µL formic acid

Tissue collection

1 Load DMSO as capturing media onto the microPOTS chip. Microdroplet processing in one pot for trace samples (microPOTS) chips are polyproplyene chips designed for benchtop manipulation of low sample volumes (< 5 µL).

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Xu K, Liang Y, Piehowski PD, Dou M, Schwarz KC, Zhao R, Sontag RL, Moore RJ, Zhu Y, Kelly RT (2019). Benchtop-compatible sample processing workflow for proteome profiling of < 100 mammalian cells.. Analytical and bioanalytical chemistry. https://doi.org/10.1007/s00216-018-1493-9

1.1 Pipette 1 µL of DMSO onto each microPOT chip well

1.2 Cover the chip with the glass slide and enclose with aluminum foil. Store at 4°C until ready for use.

2 Tissue section with LCM

2.1 Turn on the LCM (PALM MicroBeam).

2.2 Scan the tissue slide.

2.3 Find the target region on scanned image

2.4 Navigate to the region of interest (ROI).

2.5 Draw grid lines (1 voxel size= 2500 µm2) on ROI.

2.6 Load the laser setting (Speed 1, Cut energy 40, LPC Energy delta15).

2.7 Load the microPOTS chip on the collector (slide collector 48). Align A1 corner of slide collector and A2 well corner of the microPOTS chip.

2.8 Start the collection with CenterRoboLPC function.

2.9 After collecting all voxels, check on the microscope if every DMSO droplet contains the tissue voxel.

2.10 Remove the microPOTS chip carefully from the collector and cover it with the glass slide.

3 Dry DMSO from microPOTS chip

3.1 Set incubator temperature to 70 °C.

3.2 Place uncovered chips into incubator for 60 minutes

3.3 After DMSO has evaporated, check the microPOTS chip under optical microscope to perform a QC check for missing tissue pieces.

Protein extraction

4 Tissue protein extraction

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4.1 Set incubator to 37 °C and place microPOTS chips on ice into a biological safety cabinet (BSC)

4.2 Add 1 µL of extraction buffer (4 M Urea, 5 mM TCEP, 10% DMSO, 0.1% DDM in 50 mM ABC) to each well containing tissue.

4.3 Place microPOTS chip with cover slide into incubator at 37 °C for 30 minutes

4.4 Place the microPOTS chip into vacuum chamber and slowly introduce vacuum to evaporate remaining droplet.

5 Nuclease-based histone extraction

5.1 To each well on the microPOTS chip, add 1 µL of benzonase buffer (1 mM $MgCl_2$ in 50 mM ABC with 2.5 unit/µL benzonase).

5.2 Incubate microPOTS chip with cover slide at 37 °C for 60 minutes

6 Transferring sample for LC-MS analysis

6.1 To an LC-MS vial, add a PCR tube with cap cut off.

6.2 Add 10 µL of quenching buffer (94.9% water, 4.9% MeCN, 0.2% FA) to each PCR-insert LC-MS vial.

6.3 Transfer 1 µL of sample from each well into a PCR-insert LC-MS vial

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containing 10 µL of quenching buffer.

6.4 Re-pipette 2 µL of quenching buffer from the LC-MS vial back into the well to extract protein that may be adsorbed to the microPOTS surface. Remove the 2 µL back into the PCR-insert LC-MS vial

7 Samples are now prepared and ready for LC-MS analysis.

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