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MALDI-MSI Lipidomics of Lung Tissue V.1

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Christopher Anderton¹, Brittney Gorman¹

¹Pacific Northwest National Laboratory

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

Tech. support email: Jeff.spraggins@vanderbilt.edu



Brittney Gorman

Pacific Northwest National Lab

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the procedure to obtain MALDI mass spectrometry images of lipids using a high mass resolution FTICR. This protocol is optimized for 12 μ m sections of agarose-inflated and carboxymethyl cellulose-embedded fresh-frozen lung tissues.

The embedding method is described in Lukowski et al. doi.org/10.3389/fmolb.2022.1022775

Guidelines

Always wear appropriate PPE.

Cryosectioning requires safety glasses, gloves, and a lab coat

All other activities require safety glasses and gloves while handling samples and chemicals

Materials

Instruments:

Bruker Solarix FTICR

HTX M5 Sprayer

Slides:

Fisherbrand™ Superfrost™ Plus Microscope Slides

Indium Tin Oxide-coated glass slides (Delta Technologies; Part #: CG-80IN-S115)

Chemicals:

Sodium trifluoroacetate (1 mg/ml)

2,5-dihydroxybenzoic acid

N-(1-naphthyl-) ethylenediamine dihydrochloride

Paraformaldehyde

Ethanol

Methanol



Safety warnings

! BSL-2 controls should be used for Human tissue samples

Before start

Label and number all slides before beginning. Place tissue and slides into the crystat and allow temperature to equilibrate before mounting the tissue (~20 min).

Put up BSL-2 Lab postings on doors

Tissue sectioning

- 1 Place sample in cryostat (allow to thermally equilibrate ~20 min)
- 1.1 Set blade temperature -16 °C and specimen temperature of -18 °C
- 2 Mount tissue on chunk using a small droplet of water
- 3 Cut sections (12 µm) and thaw mount on slides
- 3.1 Mount serial sections on slides:
 1. H&E staining (Superfrost plus microscope slide)
 2. MALDI (ITO-coated glass slide) - positive ion mode lipidomics
 3. MALDI (ITO-coated glass slide) - negative ion mode lipidomics
 4. MALDI and Multimodal optical imaging (Superfrost plus microscope slide)
 5. MALDI and Multimodal optical imaging (Superfrost plus microscope slide)
- 4 Remove slides from cryostat and place in vacuum desiccator (~20 min)
- 5 Slide 1 is vacuum-sealed and placed at -80 °C until staining
- 6 Section 4×50 µm additional tissue and place in a Sorenson tube for MPLEx (dx.doi.org/10.17504/protocols.io.36wgqd15ovk5/v1)

Autofluorescence Imaging

- 7 **Autofluorescence Microscopy (AF)** is collected on all each tissue section (dx.doi.org/10.17504/protocols.io.q26g75z23lwz/v1)

Matrix Application

- 8 Measure out matrix and solvent



Positive ion mode analysis: 2,5-dihydroxybenzoic acid (DHB; 40 mg/mL in 70% MeOH:H₂O)

Negative ion mode analysis: N-(1-naphthyl-) ethylenediamine dihydrochloride (NEDC, 7 mg/mL in 70% MeOH:H₂O)

8.1 Vortex matrix solutions briefly and sonicate for 15 min

9 On the HTX M5 Sprayer program methods with the following settings:

9.1 **Positive ion mode (DHB):**

Spray temperature: 75 °C

0.05 mL/min flow rate

12 passes

velocity of 1300 mm/s

3 mm offset

40 mm nozzle height

9.2 **Negative ion mode (NEDC):**

Spray temperature 75 °C

0.12 mL/min flow rate

8 passes

Velocity of 1300 mm/s

3 mm offset

40 mm nozzle height

10 Remove the needle tip and fasten the syringe to the M5 Sprayer line going to the 6-port valve

11 Move the switch to "LOAD" and steadily depress the syringe until all the sample is loaded. Note: Do not load air bubbles or undissolved matrix.

11.1 Make sure that the pump is flowing and pump pressure is 30-40 psi

12 Place the tissue mounted slides in the M5 Sprayer tray, fasten them with tape.

13 Move the 6-port valve switch to "Spray". (Wait for 1-2 min, or until the M5 Sprayer nozzle is spraying the solution)



- 14 Once matrix is detected as an opaque solution on a dummy slide, press Start on the M5 Sprayer
- 15 When finished, matrix coated slides may be imaged immediately or stored in a desiccator

MALDI imaging MS Acquisition

- 16 Put the slide in the MALDI *MTP Slide Adapter II* and load into Bruker 12T Solarix FTICR
- 17 Load the method for Lipid analysis

Note

Parameters of the method:

Step size: 35 μm

Laser focus: Minimum

Ion polarity: Positive

Scan begin: 250 m/z Scan end: 1200 m/z; Time domain 2 M; Resolving power: ~180,000 at 400 m/z; 120 shots with 2000 Hz frequency

Ion polarity: Negative

Scan begin: 200 m/z Scan end: 1200 m/z; Time domain 2 M; Resolving power: ~240,000 at 400 m/z; Ion polarity: positive; 100 shots with 2000 Hz frequency

- 18 Calibrate method in ESI mode

Note

- Change from MALDI to ESI
- Turn on the nebulizer

- 18.1 Load syringe with sodium + trifluoroacetate (1 mg/ml) and infuse calibrant



Note

Na-TFA Peaks			
Negative		Positive	
Molecular Formula	m/z	Molecular Formula	m/z
CF ₃ CO ₂	112.986	Na ₂ (CF ₃ CO ₂)	158.964
Na(CF ₃ CO ₂) ₂	248.960	Na ₃ (CF ₃ CO ₂) ₂	294.939
Na ₂ (CF ₃ CO ₂) ₃	384.935	Na ₄ (CF ₃ CO ₂) ₃	430.914
Na ₃ (CF ₃ CO ₂) ₄	520.910	Na ₅ (CF ₃ CO ₂) ₄	566.888
Na ₄ (CF ₃ CO ₂) ₅	656.885	Na ₆ (CF ₃ CO ₂) ₅	702.863
Na ₅ (CF ₃ CO ₂) ₆	792.860	Na ₇ (CF ₃ CO ₂) ₆	838.838
Na ₆ (CF ₃ CO ₂) ₇	928.834	Na ₈ (CF ₃ CO ₂) ₇	974.813
Na ₇ (CF ₃ CO ₂) ₈	1064.809	Na ₉ (CF ₃ CO ₂) ₈	1110.788
Na ₈ (CF ₃ CO ₂) ₉	1200.784	Na ₁₀ (CF ₃ CO ₂) ₉	1246.762
Na ₉ (CF ₃ CO ₂) ₁₀	1336.759	Na ₁₁ (CF ₃ CO ₂) ₁₀	1382.737
Na ₁₀ (CF ₃ CO ₂) ₁₁	1472.734	Na ₁₂ (CF ₃ CO ₂) ₁₁	1518.712
Na ₁₁ (CF ₃ CO ₂) ₁₂	1608.708	Na ₁₃ (CF ₃ CO ₂) ₁₂	1654.687
Na ₁₂ (CF ₃ CO ₂) ₁₃	1744.683	Na ₁₄ (CF ₃ CO ₂) ₁₃	1790.662
Na ₁₃ (CF ₃ CO ₂) ₁₄	1880.658	Na ₁₅ (CF ₃ CO ₂) ₁₄	1926.637
Na ₁₄ (CF ₃ CO ₂) ₁₅	2016.633	Na ₁₆ (CF ₃ CO ₂) ₁₅	2062.611
Na ₁₅ (CF ₃ CO ₂) ₁₆	2152.608		
Na ₁₆ (CF ₃ CO ₂) ₁₇	2288.583		
Na ₁₇ (CF ₃ CO ₂) ₁₈	2424.557		
Na ₁₈ (CF ₃ CO ₂) ₁₉	2560.532		
Na ₁₉ (CF ₃ CO ₂) ₂₀	2696.507		
Na ₂₀ (CF ₃ CO ₂) ₂₁	2832.482		
Na ₂₁ (CF ₃ CO ₂) ₂₂	2968.457		

Na-TFA peak list

- 18.2 Calibrate (Enhanced Quadratic)
- 18.3 Change settings back to MALDI and save method
- 19 Set up imaging run in Flex imaging
- 20 Check signal-to-noise ratio on QC-tissue

**Note****Positive peaks:**

723.493 m/z; S/N ~1e5

796.53 m/z; S/N ~1e6

835.663 m/z; S/N ~1e6

Negative peaks:

716.524 m/z; S/N ~1e6

766.539 m/z; S/N ~1e7

788.545 m/z; S/N ~1e5

21 Start Flex imaging run

Matrix Removal

22 Rinse slides by emerging into fresh 70% methanol for 3 min

23 Continue on to other imaging modalities

23.1 *Optional Before U-FLIP imaging:

1. 30 min fixation with 4% paraformaldehyde
2. Dehydrated in graded ethanol (75%, 96%, 100%)
3. Dried under desiccation and vacuum sealed until imaging