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MALDI-MSI Lipidomics of Lung Tissue V.2

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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 µm 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** and protocol.io **dx.doi.org/10.17504/protocols.io.j8nlkyzx6g5r/v1**

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

Ethics statement

The protocols.io team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

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
CF3CO2	112.986	Na2(CF3CO2)	158.964
Na(CF3CO2)2	248.960	Na3(CF3CO2)2	294.939
Na2(CF3CO2)3	384.935	Na4(CF3CO2)3	430.914
Na3(CF3CO2)4	520.910	Na5(CF3CO2)4	566.888
Na4(CF3CO2)5	656.885	Na6(CF3CO2)5	702.863
Na5(CF3CO2)6	792.860	Na7(CF3CO2)6	838.838
Na6(CF3CO2)7	928.834	Na8(CF3CO2)7	974.813
Na7(CF3CO2)8	1064.809	Na9(CF3CO2)8	1110.788
Na8(CF3CO2)9	1200.784	Na10(CF3CO2)9	1246.762
Na9(CF3CO2)10	1336.759	Na11(CF3CO2)10	1382.737
Na10(CF3CO2)11	1472.734	Na12(CF3CO2)11	1518.712
Na11(CF3CO2)12	1608.708	Na13(CF3CO2)12	1654.687
Na12(CF3CO2)13	1744.683	Na14(CF3CO2)13	1790.662
Na13(CF3CO2)14	1880.658	Na15(CF3CO2)14	1926.637
Na14(CF3CO2)15	2016.633	Na16(CF3CO2)15	2062.611
Na15(CF3CO2)16	2152.608		
Na16(CF3CO2)17	2288.583		
Na17(CF3CO2)18	2424.557		
Na18(CF3CO2)19	2560.532		
Na19(CF3CO2)20	2696.507		
Na20(CF3CO2)21	2832.482		
Na21(CF3CO2)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