Version 2

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Automatic Deposition of DAN Matrix using a TM Sprayer for MALDI Analysis of Lipids V.2

Elizabeth Neumann[1], Carrie Romer[1], Jamie Allen[1], Jeff Spraggins[1]

[1]Vanderbilt University

1 Works for me dx.doi.org/10.17504/protocols.io.bt8hnrt6

VU Biomolecular Multimodal Imaging Center
Tech. support email: jeff.spraggins@vanderbilte.du

Jamie Allen
Vanderbilt University

ABSTRACT

Scope:
To describe the procedure for spraying tissue sections with DAN for imaging lipids.

Expected Outcome:
Slides should be coated with DAN. Tissue sections should be imaged within 24 hours of matrix application.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

J Yang, JL Norris, R Caprioli. "Novel Vacuum Stable Ketone-Based Matrices for High Spatial Resolution MALDI Imaging Mass Spectrometry." Journal of Mass Spectrometry. 2018, 53 (10), 1005-1012.

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GUIDELINES

Definitions:

1. ACN is Acetonitrile
2. MeOH is Methyl Alcohol/Methanol
3. DAN is 1,5-diaminonaphthalene is DAN
4. THF is tetrahydrofuran

MATERIALS TEXT

Reagents:

1. Water: (H_2O), Milli-Q System Water
2. Ethyl Acetate, Fisher E195
3. 1,5-diaminonaphthalene (DAN), Sigma-Aldrich 56451
4. Ammonium Formate, Sigma-Aldrich 516961
5. Methanol, Fisher A452
6. Acetonitrile, Fisher A9984
7. Glacial Acetic Acid, Fisher A35
8. Tetrahydrofluran (THF), Sigma-Aldrich 401757

Equipment:

1. Ultrasonic Cleaner, Branson
2. TM Sprayer, HTX Imaging
3. HPLC, Agilent

Reagent Preparation:

1. Matrix prep:
   Add 200 mg DAN to a scintillation vial
   Add 10mL THF to vial
   Sonicate for 5 minutes
2. Stock of 90% Acetonitrile + 1% Acetic Acid
   Add 5mL Glacial Acetic Acid to 450 mL of Acetonitrile and 45mL Milli-Q H_2O
3. Stock of 80% Acetonitrile:
   Add 400 mL Acetonitrile to 100 mL Milli-Q H_2O
4. 50mM Ammonium Formate
   Add 1.5765 g of Ammonium Formate to 500 mL Milli-Q H_2O

   Keep bottle at 4^oC

SAFETY WARNINGS

Health and Safety:

1. Safety glasses or goggles, proper gloves, and a lab coat required. The area should be adequately vented and a lab mat placed underneath all solutions.
2. **Warning:** Trifluoroacetic Acid and Ammonium Hydroxide: HARMFUL or FATAL if swallowed. Vapor harmful. Affects the central nervous system. Causes severe eye irritation and respiratory tract irritation. May be harmful if absorbed through skin. Chronic exposure can cause adverse liver, kidney, and blood effects. Flammable liquid and vapor.
3. **Warning:** 1,5-diaminonaphthalene is a category 2 carcinogen.

Autofluorescence Scan

1 Remove slides from freezer and place in desiccator for **00:30:00** .

2 Scan for autofluorescence on Zeiss Axio Scanner.

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TM Sprayer Setup

3 Change nitrogen to 6 psi and turn on sprayer.

4 Open software and set nozzle temperature to **40 °C**

5 Change LC solvent to A1 to 100% acetonitrile at 0.05 mL/min.

6 “Load” sample loop with **5 mL** acetonitrile.

7 Switch sprayer to “Inject” and spray for 2 minutes.

8 “Load” **6 mL** of matrix solution into sprayer loop.

9 Switch sprayer to “Inject” and spray for ~1 minute. Use slide to check that matrix is flowing.

10 Tape slide(s) onto **75 °C** heated block on top of stage and adjust software scanning area.

11 Set method in software:
1350 mm/min nozzle velocity
1.5 mm track spacing
0.05 mL/min flow rate
CC Pattern
2 L/min flow rate
5 passes
40mm nozzle height
No drying time

12 Save method and make sure it is highlighted.

13 Under Cycle, click “Start,” then click “Continue.”

When finished, remove slide and place in slide box.

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Cleanup

15 Set TM sprayer temperature to **30 °C** .

16 Switch A1 solvent line to 100% ACN and set flow rate to 1 mL/min.

17 “Load” loop with **20 mL** acetonitrile and “Inject” loop for **00:10:00** .

18 Spray tip of nozzle with 5 mL of MEOH.

19 Log the pressure at your flow rate post cleaning. It must be within 3-4 psi of the starting pressure; if it is not, the cleaning procedure must be repeated.

20 Change the HLPC flow rate to 0.05 mL/min.

21 Spray nozzle with methanol.

22 Remove the foil and wipe stage down with methanol.

23 Replace wypall and bench diapers.

24 Place all syringes in the biohazard container and empty the solvent waste.

25 Shut down the software and turn off the TM Sprayer.

26 Shut the nitrogen off.

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