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## Automatic Deposition of DAN Matrix using a TM Sprayer for MALDI Analysis of Lipids

Jamie Allen<sup>1</sup>, Elizabeth Neumann<sup>1</sup>, Jeff Spraggins<sup>1</sup>

<sup>1</sup>Vanderbilt University

**1** Works for me [dx.doi.org/10.17504/protocols.io.4srgwd6](https://doi.org/10.17504/protocols.io.4srgwd6)

VU Biomolecular Multimodal Imaging Center

Human BioMolecular Atlas Program (HuBMAP) Method Development Community



### 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.

### 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<sub>2</sub>O), 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. Tetrahydrofuran (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<sub>2</sub>O

3. Stock of 80% Acetonitrile:

Add 400 mL Acetonitrile to 100 mL Milli-Q H<sub>2</sub>O

4. 50mM Ammonium Formate

Add 1.5765 g of Ammonium Formate to 500 mL Milli-Q H<sub>2</sub>O

Keep bottle at 4°C

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.

Wash/Scan




- 1 Remove slides from freezer and place in desiccator for 🕒 00:30:00 .
- 2 Scan for autofluorescence on Zeiss Axio Scanner
- 3 Use a transfer pipet to add cold 50mM ammonium formate to slides, covering tissue. Allow to sit for 🕒 00:00:05 , and gently tip slide to allow the ammonium formate to fall onto kim wipes.

TM Sprayer Setup


- 4 Change nitrogen to 6 psi and turn on sprayer.
- 5 Open software and set nozzle temperature to 🌡 40 °C
- 6 Change LC solvent to A1 to 100% acetonitrile at 0.05 mL/min.
- 7 "Load" sprayer loop with 📦 5 ml acetonitrile.
- 8 Switch sprayer to "Inject" and spray for 2 minutes.
- 9 "Load" 📦 6 ml of matrix solution into sprayer loop.
- 10 Switch sprayer to "Inject" and spray for ~1 minute. Use slide to check that matrix is flowing.

- 11 Tape slide(s) onto  75 °C heated block on top of stage and adjust software scanning area.
- 12 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
- 13 Save method and make sure it is highlighted.
- 14 Under Cycle, click "Start," then click "Continue."
- 15 When finished, remove slide and place in slide box.

#### Cleanup

- 16 Set TM sprayer temperature to  30 °C .
- 17 Switch A1 solvent line to 100% ACN and set flow rate to 1 mL/min for three minutes then return to 50% ACN 0.05 mL/min
- 18 "Load" loop with  10 ml acetonitrile and "Inject" loop for  00:10:00 .
- 19 Spray tip of nozzle with 5 mL of MEOH
- 20 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.
- 21 Change the HPLC flow rate to 0.05 mL/min.
- 22 Spray nozzle with methanol.
- 23 Remove the foil and wipe stage down with methanol.

- 24 Replace wypall and bench diapers.
- 25 Place all syringes in the biohazard container and empty the solvent waste.
- 26 Shut down the software and turn off the TM Sprayer.
- 27 Shut the nitrogen off.

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