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We use this protocol and it's working.

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Automatic Deposition of CHCA Matrix for MALDI Analysis of Lipids

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

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

[1]Vanderbilt University

VU Biomolecular Multimodal Imaging Center | Human BioMolecular Atlas Program (HuBMAP) Method Development Cor

Jamie Allen
Vanderbilt University

ABSTRACT

Scope:
To describe the procedure for spraying tissue sections with CHCA for lipids. CHCA is used as a safer matrix to ship coated slides to collaborators.

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

GUIDELINES

Definitions:
1. ACN is Acetonitrile
2. MeOH is Methyl Alcohol/Methanol
3. CHCA is α-cyano-4-hydroxycinnamic acid
4. TFA is trifluoroacetic acid

MATERIALS

Reagents:
1. Water: (H_2O), Milli-Q System Water
2. α-cyano-4-hydroxycinnamic acid, Sigma-Aldrich 70990
3. Methanol, Fisher A452
4. Acetonitrile, Fisher A9984
5. Trifluoroacetic Acid ampules, Fisher A116
6. 15mL Conical Tubes, Fisher 14-959-49B

Equipment:
1. Ultrasonic Cleaner, Branson
2. M3 TM Sprayer, HTX Imaging

Reagent Preparation:
1. Matrix prep:
   Add 100 mg CHCA to a 15mL conical tube
   Add 10mL 70% Methanol + 0.1% TFA to tube
   Sonicate for 10 minutes

2. Stock of 70% Methanol + 0.1% TFA
   Add 500uL Trifluoroacetic Acid to 350 mL of Methanol and 150mL Milli-Q H_2O

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: 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:** CHCA is an eye and skin irritant.

Autofluorescence Scan

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

2 Scan for autofluorescence on Zeiss Axio Scanner.

TM Sprayer Setup

3 Change nitrogen to 6 psi and turn on sprayer.

4 Open software and set nozzle temperature to 85 °C

5 Change LC solvent to 70% methanol at 0.1 mL/min.

6 “Load” sample loop with 5 mL methanol.

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) to the top of the stage and adjust software scanning area.

11 Set method in software:
700 mm/min nozzle velocity
2 mm track spacing

0.1 mL/min flow rate
CC Pattern
2 L/min flow rate
8 passes
40mm nozzle height
60 second drying time

12 Save method and make sure it is highlighted.

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

14 When finished, remove slide and place in slide box.

Cleanup

15 Set TM sprayer temperature to 30 °C .

16 Switch A1 solvent line to 100% ACN and set flow rate to 0.5 mL/min.

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

18 Spray tip of nozzle with 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.