Matrix Sublimation via In-House Developed Sublimation Apparatus

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

Scope:
This protocol describes the use of an in-house developed sublimation device to deposit small molecule matrices onto tissue sections for high resolution MALDI imaging mass spectrometry (IMS). Concentrations will vary based on matrix type.

MATERIALS

Slides with sample affixed
Matrix of choice
Solvents: acetone, isopropyl alcohol, ethanol
Dry ice

Keywords: MALDI IMS, matrix, Sublimation

Sample Section Preparation

1 Chill three solutions of [M] 150 millimolar (mM) ammonium formate On ice for 30 minutes.

2 Wash sectioned tissue slides with three separate solutions of [M] 150 millimolar (mM) ammonium formate for 00:00:45 each. 45s

3 Dry tissue using a stream of nitrogen gas.

4 Store in a vacuum desiccator for 00:20:00 . 20m

Cold Trap Preparation

5 Ensure the cold sleeve is at least 2/3 full of ethanol or isopropyl alcohol when trap is fully seated.

6 Carefully add dry ice chips to the ethanol bath in the sleeve and allow chips to sublimate off. Repeat until minimal sublimation of dry ice is observed (ethanol has reached equilibrium temperature).

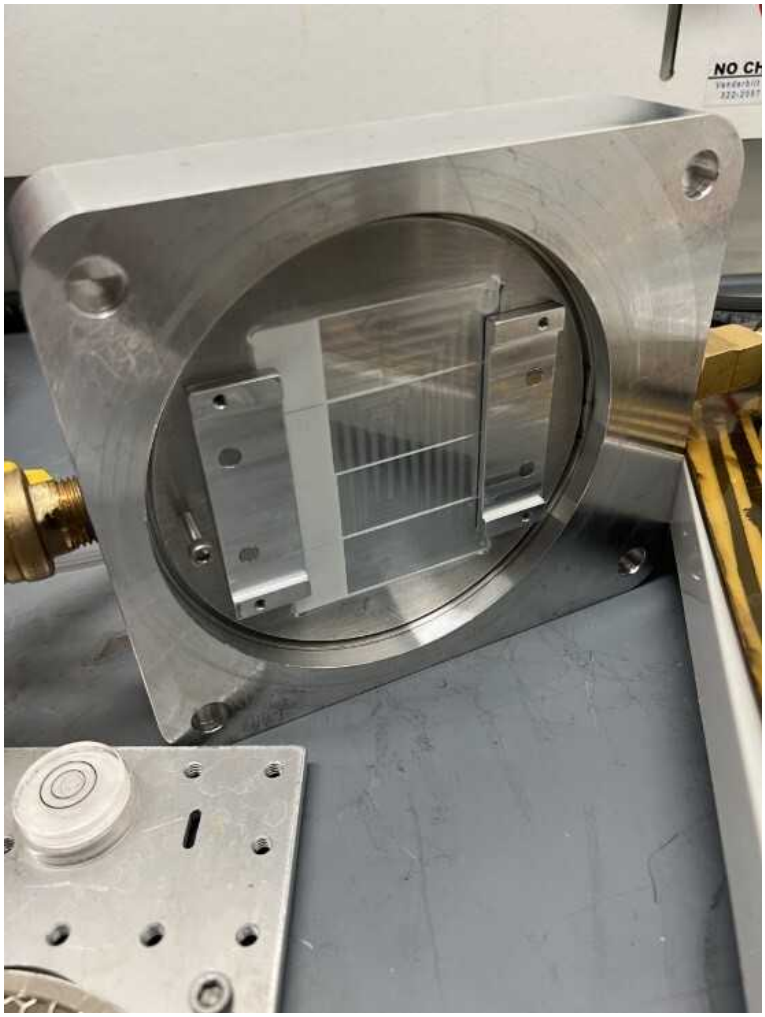
Sublimation Apparatus Set up

7 Ensure that the vacuum line is securely installed between the cold trap and the apparatus lid.

8 Ensure that the electrical connections between the heater controller and the passthrough in the apparatus base are secure and remain dry.

9 Place sample slides in the sample holder indentations.
Use copper tape or magnetic holder as needed to secure samples as close to the center of the holder as possible.

10 Place sample holder in the lid using the embedded magnets.



Matrix Preparation

5m

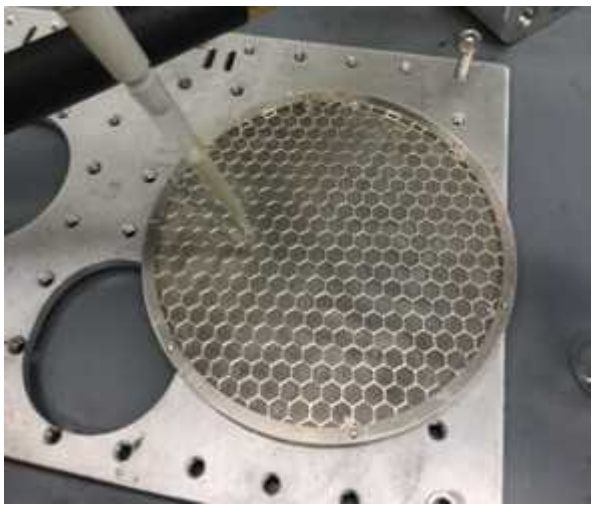
11 Dissolve 5mg of matrix in 2 mL of acetone for a total matrix concentration of [M] 2.5 mg/mL .

> **Note**
>
> Concentration will vary depending on matrix used.

12 Place matrix pan on a level surface and evenly distribute the matrix solution by dispensing the solution in an equilateral triangle around the center of the matrix pan.

Note

Dispense three solutions of 0.667 mL each.



13 Wait for solvent to dry (00:03:00). 3m

Sublimation Procedure 30m

14 Place matrix pan on the heat plate in the apparatus base.

15 Check that mating surfaces of the lid, base, and the O-ring are clean, and carefully seat the O-ring in the groove in the base.

16 Place apparatus lid on the base, ensure that base is fully seated in the indentions on the induction heater top cover. Start pulling vacuum.

17 Once it is clear that vacuum is established, ensure that bath drain stopcock is closed. Add a small amount of acetone (20 mL) into the well than fill with dry ice and allow to cool for 00:03:00 . 3m

18 Wait for the pressure to fall to < 50 mTorr measured at the pump (usually ~25 mTorr).
Note the pressure at the pump and at the apparatus.

19 Turn on internal thermocouple. 10m
Turn on induction heater, set heater to 390F temperature setting, and start a Start 00:10:00 timer.

Note

Temperature will fluctuate. Make sure ice bath is stocked with dry ice and that there is sufficient melt to cover the bath surface.

20 Turn off the heater and remove dry ice chips from the ice bath. Replace ice bath with warm water and open to drain excess water. Repeat warm water exchanges until the apparatus is at room temperature.

Note

A heat gun can be used to help warm up the valve prior to turning it.
The entire process may take up to 15 minutes to complete.

21 Turn off the vacuum pump and break vacuum with the leak valve on the vacuum manifold and remove matrix-coated samples.

Clean Up 5m

22 Clean matrix pan and sample holder with acetone.

Annealing

1m

23 Place samples on a heating plate (130^{o}C) for 30 seconds.