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Pre-Extraction and Matrix Application via a HTX M5 Sprayer for Intact Proteoform MALDI Imaging

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ABSTRACT

Scope:

Using an HTX M5 sprayer this protocol outlines a pre-extraction step for increased sensitivity for intact proteoform analyses, this is an optional step within the MALDI-MSI pipeline for intact proteoform mapping using a Spectroglyph EP-MALDI-2 source coupled to a custom UHMR HF Orbitrap. This is followed by the coating and optional recrystallization of MALDI matrix on the sample, all steps have been completed on both human kidney and human pancreas. Other tissue types should go through validation.

Expected Outcomes:

A tissue section prepared for intact proteoform analyses.

PROTOCOL CITATION

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In steps of

Tissue Preparation for Intact Proteoform MALDI-MSI on Human Tissue
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BEFORE STARTING

Ensure that the MALDI matrix is prepared fresh according to the set protocol, and that the incubation chamber is equilibrated at the proper temperature based on the recrystallization steps. The status of the HTX M5 sprayer and other equipment including the incubation chamber, and UHMR HF Orbitrap should be checked prior to engaging in sample preparation protocols.

Preparing the HTX sprayer

1 The preparation of the HTX M5 sprayer employed is similar to other protocols, it has an external pump and 5 mL sample loop which must be purged prior to analyses.

> Angela Kruse, Martin Dufresne, Jamie Allen, Danielle Gutierrez, Jeff Spraggins. Deposition of matrix using an M5 TM sprayer for high resolution MALDI analysis. https://protocols.io/view/deposition-of-matrix-using-an-m5-tm-sprayer-for-hi-b9wyr7fw

2 House nitrogen gas flow is regulated to 10 PSI prior to turning on the pump power supply and initializing the HTX M5 sprayer once opening the application on the controlling laptop.

3 After initialization the pump line is purged while the sample valve is set to load similar to the aforementioned protocol, and allowed to spray through the nozzle.

3.1 After purging the sample line, switch the valve to spray. Pump flow rate is changed to 0.3 mL/min, and allowed to purge for roughly **00:10:00** , then sprayer is now ready for use. 10m

Preparing the MALDI matrix

4 ⚠

Prior to starting this protocol 2,5 dihydroxyacetophenone (DHA) should be prepared as outlined within the sub-steps below, matrix application was optimized within several minutes of tissue acidification.

4.1 DHA is stored under roughly **0.5 Bar** in a vacuum desiccator, at temperatures per manufacturers recommendations.

4.2 Weigh out DHA into a scintillation vial which can hold at least **10 mL** , the target concentration is **15 mg/mL** and target between **7-10 mL** of total matrix volume.

4.3 Calculate and add the specific volume of **90% acetonitrile** to the scintillation vial and then vortex the solution for several minutes.

4.4 At this time the scintillation vial is placed into a ultrasonic sonicator for **00:15:00** , after which the volume is split evenly into **2 mL** Eppendorf tubes and then centrifuged for several minutes. 15m

4.5 A black precipitant is formed, and the solution will no longer be opaque. Decant the solution into a clean scintillation vial. The matrix is now prepared for spraying within the HTX M5 sprayer.

Tissue acidification

5 ✳

Once the HTX and matrix is prepared, the sample loop is then purged with **5% acetic acid (AA) in 50% ethanol**, this is then run through the nozzle for several minutes at the set flow rate and the loop replenished if volume remaining is less than the volume to be sprayed.

6 A slide is positioned on the sprayer bed, and secured with tape on the top and bottom edges (for use with Spectroglyph source) or on the sides (for use with Bruker sources).

7 The method is loaded for tissue acidification, this includes the following parameters:

1250mm/min nozzle velocity
0.150 mL/min flow rate
30 °C nozzle temperature

3 mm track spacing
"CC" pattern

40 mm nozzle height
5 seconds drying time

7.1 Note: This method and number of passes (4, 8, 12, etc.) should be evaluated on different tissue types and for different proteoforms, this pre-extraction was chosen as it demonstrated an improvement within histone intensity, however, for smaller proteoforms and on other instrumental platforms this may not be a necessary step.

8 After the HTX method is loaded, the slide position is set and the method is started within the software. Nitrogen pressure is checked to ensure no fluctuation throughout the process, and solution is sprayed for several minutes and stability of pump pressure and spray is checked.

8.1 Note: Pressure varies based upon flow rate, and the pump used, internal QC records are kept for various methods.

9 The method is then started, the sprayer is monitored throughout the process to ensure viable acidification.

10 Once the method is finished, switch the valve from spray to load, and the line and valve is purged with the solvent system of the matrix to be applied.

The sample integrity is monitored visually at this time.

11

11.1 ⚠

We evaluate the integrity, lack of cracks, folds, detachment and tears in the tissue section after this step by visual inspection and/or bright-field (BF) microscopy images. Cracks, folds, detachments and tears should be < 10% of the section surface to pass tissue QC.

If tissue does not pass tissue QC at this stage, the run is aborted.

Application of MALDI matrix

12 After acidification is complete, the method is loaded for DHA deposition, this includes the following parameters:

1300 mm/min nozzle velocity
0.150 mL/min flow rate
30 °C nozzle temperature

2 mm track spacing
"CC" pattern

40 mm nozzle height
0 seconds drying time

4 passes for 277 µg/cm^2 coverage

13 Following loading the matrix into the loop with the injection valve on load, the pump is turned on and the valve set to spray.

14 Nitrogen pressure is checked to ensure no fluctuation throughout the process, and matrix is sprayed for several minutes and stability of pump pressure and spray is checked.

14.1 Note: Pressure varies based upon flow rate, and the pump used, internal QC records are kept for various methods.

15 The same application region is used as within the tissue acidification step and the method is started. The sprayer is monitored throughout the process to ensure viable matrix application.

15.1 ⚠

As 2,5-DHA is slightly volatile and will sublime off within extended exposure to elevated pressure experienced within the MALDI source sample preparation should be minimized to samples being analyzed that day. Storage of matrix coated slides at room temperature, or within reduced temperatures from 🌡 **4 °C** down to 🌡 **-80 °C** is not advisable.

16 The sample integrity is monitored visually at this time.

16.1 We evaluate the integrity, lack of cracks, folds, detachment and tears in the tissue section after this step by visual inspection and/or bright-field (BF) microscopy images. Cracks, folds, detachments and tears should be < 10% of the section surface to pass tissue QC.

If tissue does not pass tissue QC at this stage, the run is aborted.

17 The sample loop is then flushed in the same manner as within earlier steps to prepare the HTX M5 sprayer. Power for the pump and the sprayer is then turned off, and the nitrogen flow is stopped.

Recrystallization of matrix 5m 30s

18 ✱

Directly following the matrix application an optional recrystallization step can be achieved using various parameters. Prepare an apparatus similar to that described.

Yang J, Caprioli RM (2011). Matrix sublimation/recrystallization for imaging proteins by mass spectrometry at high spatial resolution.. Analytical chemistry.
https://doi.org/10.1021/ac200998a

18.1 This apparatus uses an aluminum plate attached to the top of a petri-dish with double sided copper tape, the sample is mounted onto this plate. Within the bottom of the petri dish a Kimwipe is placed and 🫙 **1 mL** of the solution is pipetted evenly within to saturate the Kimwipe. Do not pipette the solution until directly prior to use.

19 The sample mounted onto the top plate is placed within the incubation chamber at 2m **38.5 °C** for **00:02:00**

20 **1 mL** of **5% acetic acid in nanopure water** is then pipetted directly before the timer 3m 30s expires, and the sample is placed on top of this bottom layer containing the kimwipe. This is allowed to incubate for **00:03:30**

21 After the time has elapsed the bottom plate is removed, and the sample is placed back within the incubation chamber to dry. After drying the sample is removed from the incubator, and removed the sample from the top plate.

22 Final tissue QC is completed as previously mentioned, this step in various forms is prone to causing loss of tissue adherence to the conductive slide. This can dramatically effect results and expected outcomes of proteoform informed MALDI-MSI.

22.1 ⚠

We evaluate the integrity, lack of cracks, folds, detachment and tears in the tissue section after this step by visual inspection and/or bright-field (BF) microscopy images. Cracks, folds, detachments and tears should be < 10% of the section surface to pass tissue QC.

If tissue does not pass tissue QC at this stage, the run is aborted.

23 If tissue is intact, the samples are immediately taken to the UHMR HF Orbitrap for intact proteoform mapping outlined within a separate protocol.

Kevin J. Zemaitis, Dusan Velickovic, Mowei Zhou, Ljiljana.PasaTolic. High Resolution Intact Proteoform Mass Spectrometry Imaging using UHMR HF Orbitrap.
https://protocols.io/view/high-resolution-intact-proteoform-mass-spectrometr-b793rr8n