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High Resolution Intact Proteoform Mass Spectrometry Imaging using UHMR HF Orbitrap V.1

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community

PNNL TTD

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ABSTRACT

Scope:
A detailed protocol entailing the calibration, operation, and verification of the custom UHMR Q Exactive HF Orbitrap with a Spectroglyph EP-MALDI-2 source for high-spatial resolution imaging. This entails various procedures for instrument calibration and checks for the MALDI source and spectrometer for the intact proteoform analyses at near-cellular spatial resolution (> 15 µm) for human tissue sections without oversampling the tissue.

Expected Outcomes:
Creation of several dozen ion images which correspond to proteoforms visually localized within physiological regions of the profiled tissue sections.

PROTOCOL CITATION

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Pre-Extraction of Proteoforms Through Tissue Acidification and Matrix Deposition via a HTX M5 Sprayer

Tissue Preparation Overview for Intact Proteoform MALDI-MSI on Human Tissue

SAFETY WARNINGS

This instrument has three lasers; a 349 nm MALDI-1 laser, a 266 nm MALDI-2 laser, and a 193 nm UVPD laser. When aligning the beams follow all proper guidelines for PPE, laser safety, and sign posting the lab.

BEFORE STARTING

Prepare slides per outlined protocols, these will depend on the tissue type as well as the mass range targeted, proper sample preparation is the most important step to ensure success of expected outcomes.

Instrument calibration

1 Prior to acquisition the instrument is calibrated or evaluated with a fresh solution of 2 mg/mL cesium iodide (CsI) made in 50% aqueous isopropanol.

1.1 ⚠

Note: CsI solution must be made fresh, and the instrument cleaned properly in

order to obtain stable clusters for calibration.

1.2 Both the heated electrospray ionization (HESI) probe and ion transfer tube (ITT) are removed and cleaned thoroughly. This occurs with continually flushing for the HESI probe via an Agilent 1200 series pump, and sonication of the ITT in 10-25% formic acid in methanol after which the ITT is rinsed several times with HPLC grade nanopure water and methanol.

2 At this time preset instrument methods are loaded onto the MALDI source for electrospray operation for high mass range detection, manual adjustments include pressure modulation within the MALDI source to between 7-9 Torr, adjustment of in-source trapping (IST), and testing of HCD collision voltages.

3 The syringe and sample line is then loaded and primed with the aforementioned 2 mg/mL CsI solution and run at 5 µL/min for several minutes until clusters are formed at a stable rate.

4 

Once clusters are stable, calibration peaks are determined based upon the targeted mass range, an evaluation of the calibration is performed prior to calibrating the instrument per manufacturers recommendations if within the week of previous mass calibration. For best instrument performance and stability of the measurements a calibration is accepted when error is returned at sub-2 ppm RMS with no outliers.

4.1 Default calibration list includes *m/z* 912.3346, 1691.7643, 2471.1941, 3510.4337, 4549.6734, 5588.9130, 6628.1526, 7407.5824, 8706.6319, and 11304.7310. This list has been expanded to include up to *m/z* 16241.1193.

4.2 Phasing of the instrument and line shape of the peaks throughout the mass range is also checked at this time as the UHMR Q Exactive HF is a custom instrument platform. Other pertinent parameters including quadrupole isolation and calibration are also evaluated and completed per manufacturers recommendations. Calibration and optimization of all these protocols ensures instrument drift is minimized.

Instrument setup

5 At this time the sample is then loading into the MALDI source, this includes closing the beam valve and venting the source. After loading the sample both the MALDI source roughing pump is started, the beamvalve opened, and fore vacuum roughing pump is regulated to the proper pressure regimes as outlined within the methods.

5.1 Note: The MALDI source voltages are pressure regulated, ensure the vacuum pressure does not drift throughout the course of the method if using max

voltages within the extraction region.

6 The instrumental method for MALDI imaging is then loaded on the UHMR Q Exactive HF and MALDI source for the tissue type and mass range previously optimized. The frequency of the radio-frequency (RF) heads which drive the dual ion funnels are also inspected, minor changes can occur.

7 Laser energy is then monitored within a test measurement region. This can either be within a subset of the tissue and/or matrix on the slide. After a test of the method, visual inspection of the desorption crater formed within matrix alone and matrix on-tissue can also be inspected at this time by microscopy.

7.1 The laser can either be focused or defocused as to not oversample the tissue (i.e., a beam size of less than 20 µm x 20 µm is used for 20 µm spatial resolution imaging). This is completed manually and is optimized per each matrix and sample preparation.

For broad proteoform profiling a beam diameter of roughly 30 µm is used, and for near-cellular resolution the minimum focus is roughly 12 µm. All tissues are inspected post-MALDI-MSI acquisition.

7.2 If no alteration to the beam focus was made during the instrument setup, only current supplied to the laser diode is adjusted as to maintain a previously calculated fluence for operation, this is noted within metadata.

7.3 ⚠

Note: When changing the focus, external verification and measurement of the desorption crater is a necessary step for instrument qualification prior to imaging any tissue. This can be completed by high-resolution bright-field (BF) microscopy.

8 After ensuring proper laser fluence and that signal meets expectations based upon serial sections or previous analyses (dependent on tissue type and wash protocols). A measurement region is selected for the analysis.

8.1 Note: Care is taken to ensure that space outside off tissue is included within the measurement region to inspect for proteoform leakage and delocalization of proteoforms from sample preparation.

Instrument testing

9 Signal intensity and output variables depends upon several factors within the experiment, and these as well as the number of unique proteoforms and proteoform fingerprints (i.e., histone H4) are inspected for consistency between 2D MALDI-MSI experiments for batch variation. A

pixel-by-pixel inspection for dynamic range can also be completed, however, normalized levels and intensities can vary based upon noise thresholding and care must be taken during this post MALDI-MSI inspection.

9.1 ⚠

Any MALDI-MSI dataset which does not meet any criteria, shows signs of delocalization, or has any issues with tissue integrity or adherence post-MALDI-MSI previously outlined is not to be submitted to HuBMAP for data ingestion.

Post MALDI staining

10 After the imaging run is completed the samples are removed, high-resolution microscopy with and/or without the matrix is completed and then appropriate staining for histological analyses is performed per standard protocols.

10.1 For example kidney is stained used periodic acid-Schiff (PAS) stain and pancreas is stained via hematoxylin and eosin (H&E).

Exemplary protocol for post MALDI imaging staining is cited below.

Jamie Allen, Jennifer Harvey, Maya Brewer, Mark De Caestecker, Jeff Spraggins. PAS Staining of Fresh Frozen or Paraffin Embedded Human Kidney Tissue.
http://dx.doi.org/10.17504/protocols.io.4qngvve

Angela Kruse, Diane Saunders, Jamie Allen, Carrie Romer, Danielle Gutierrez, Alvin Powers, Jeff Spraggins. Post-IMS H&E Staining for Pancreas or Eye Cryosections.
https://protocols.io/view/post-ims-h-e-staining-for-pancreas-or-eye-cryosect-b9ywr7xe

Visualization

11 After imaging the data goes through several semi-automated steps before dissemination and submission to the ingestion pipelines.