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Tissue Preparation for Intact Proteoform MALDI-MSI on Human Tissue

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Human BioMolecular Atl...

PNNL-TTD



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We use this protocol and it's working

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Abstract

Scope:

A detailed protocol entailing the overall protocols developed for human tissue sections for the UHMR HF Orbitrap platform. This includes QC metrics for tissue sectioning, an overview of different sample washing steps, an optional tissue acidification protocol, as well as matrix application.

Expected Outcomes:


Tissue sections fully prepared for MALDI imaging using UHMR HF Orbitrap.

Troubleshooting


Safety warnings

- ⚠ All steps of this protocol working with solvents should be performed within a fume hood as to minimize exposure to fumes from volatile organic solvents.

Before start

Prepare  100 mL of all necessary solvents for the tissue washing steps and clean Coplin jars, turn on the HTX M5 Sprayer and purge the sample loop and sprayer head, and ensure the incubation chamber is equilibrated at the proper temperature for recrystallization.

Tissue sectioning and metrics

- 1 Tissue either sectioned by PNNL-TTD or provided by others and are stored at  -80 °C , these slides have undergone either high-resolution bright-field (BF) light microscopy or auto-fluorescence (AF) microscopy prior to cold storage (e.g., AF for kidney) for the evaluation of tissue integrity.

The general overview of preparation of cryosectioned tissue mounted onto slides has been outlined by the Vanderbilt Biomolecular Imaging Center below:


Citation

David Anderson, Elizabeth Neumann, Jamie Allen, Maya Brewer, Danielle Gutierrez, Jeff Spraggins
. Cryostat Sectioning of Tissues for 3D Multimodal Molecular Imaging.

[dx.doi.org/10.17504/protocols.io.7ethjen](https://doi.org/10.17504/protocols.io.7ethjen)

LINK

In addition to sectioning on indium-tin oxide (ITO) slides for MALDI-MSI and histological staining post-acquisition, polyethylene naphthalate (PEN) membrane slides for laser capture microdissection (LCM) are also processed if needed.

- 1.1 Note: Fresh tissue sections and tissue stored for up to one year have been analyzed by using the outlined protocols with success in proteoform mapping, slight decrease within proteoform intensity and sensitivity can be expected with increased tissue age.
- 2 Once mounted onto the slides, we perform visual inspection of the tissue sections.
- 2.1 We evaluate the integrity, lack of cracks, folds, detachment and tears in the tissue section after microtome sectioning, washing and rehydration steps by visual inspection of the AF and BF microscopy images. Cracks, folds, detachments and tears should be < 10% of the section surface to pass tissue QC.
- 3 After inspection, tissue sections are packaged for cold storage within slide carriers with desiccant, care is taken to reduce the number of freeze/thaw cycles as up to five slides can be held but five slides may not be imaging during a singular day. Once packaged, these slides are sealed under vacuum using a commercial vacuum sealer and put into a  -80 °C freezer.





Sample preparation and metrics

30m

- 4 Outlined below is the general workflow for intact proteoform mapping via MALDI imaging. These steps are highly dependent upon sample type, tissue adhesion to the slide, and the instrumentation used.

This section can be broken down into three or four major steps including:

- 1) sample washing post desiccation
- 2) an optional pre-extraction step via HTX M5 Sprayer
- 3) matrix application via HTX M5 Sprayer
- 4) tissue rehydration or recrystallization

- 4.1 Note: A test should be completed prior to full sectioning of a new block as tissue can detach from the slide it is mounted on, and while conductive ITO slides are the standard, in certain cases the creation of poly-lysine coated ITO slides is beneficial for better adherence of the tissue.



- 5 Samples to be prepared are removed from the freezer and put into a vacuum desiccator which is held at 0.5 Bar for 00:30:00 , any additional slides within the carrier are vacuum sealed and returned to the freezer.

30m

Sample washing protocols

- 6 After drying the samples, a series of clean Coplin staining jars are prepared with a series of ethanolic solutions, polar aprotic solvents, and water. This changes based upon the targets of proteoform imaging and the tissue type, below are two examples created for the optimized mapping of histone proteoforms within human kidney:

Protocol

NAME

Washing Protocol for Intact Proteoform MALDI on Human Kidney

CREATED BY

Kevin J Zemaitis

[Preview](#)

Or alternatively human pancreas:



Protocol

NAME

Washing Protocol for Intact Proteoform MALDI on Human Pancreas

CREATED BY

Kevin J Zemaitis

Preview

For other smaller proteoforms and targets sample washes will vary.

- 6.1 Note: Some tissue types will require an extensive optimization based on targets and mass range, these protocols were optimized for this platform specifically for histone targets and would need to be validated on other MALDI instrumentation and other targets.
- 7 After visual inspection of the tissue after sample washing, prepare the HTX M5 Sprayer for an optional tissue acidification. This pre-extraction step has been shown to increase sensitivity of analyses, this may be required based upon the spatial resolution and focus of the MALDI laser beam.

This can be an optional portion of the protocol. Follow the outlined section within the below protocol for tissue acidification with an HTX M5 Sprayer:

Protocol

NAME

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

CREATED BY

Kevin J Zemaitis

Preview

Tissue pre-extraction by acidification

- 8 Directly following acidification (if completed), the HTX M5 Sprayer loop is flushed and prepared for deposition of matrix. The matrix used for the intact proteoform mapping is



2,5-dihydroxyacetophenone (DHA). This matrix was found to be optimal for the UHMR Q Exactive HF Orbitrap with a Spectroglyph EP-MALDI-2 source.

Follow the outlined section within the below protocol for DHA matrix application with an HTX M5 Sprayer:

Protocol

NAME

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

CREATED BY

Kevin J Zemaitis

Preview

- 8.1 Note: Depending on the tissue type or target proteoforms this may change, however, for each matrix the QC metrics for application is the same.

Recrystallization of matrix

- 9 After the completion of the matrix application, tissue sections are then placed within the apparatus outlined within the protocol for recrystallization of the matrix.

This is also an optional portion of the protocol. Follow the outlined section within the below protocol for recrystallization of matrix immediately following matrix application with an HTX M5 Sprayer:

Protocol

NAME

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

CREATED BY

Kevin J Zemaitis

Preview



- 10 Directly following the completion of this step the sample can be loading into the spectrometer for MALDI mapping of intact proteoforms following the below protocol. Efforts to verify the tissue integrity are taken along the way and described in greater depth within the sub-protocols.

Citation

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>

LINK

Citations

Step 1

David Anderson, Elizabeth Neumann, Jamie Allen, Maya Brewer, Danielle Gutierrez, Jeff Spraggins. Cryostat Sectioning of Tissues for 3D Multimodal Molecular Imaging
[dx.doi.org/10.17504/protocols.io.7ethjen](https://doi.org/10.17504/protocols.io.7ethjen)

Step 10

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>