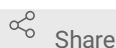protocols.io

🌐 Overall protocol for 2D intact proteoform mapping by MALDI imaging

Sep 01, 2022

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dx.doi.org/10.17504/protocols.io.q26g74qn8gwz/v1

Human BioMolecular Atlas Program (HuBMAP) Method Development Community
PNNL-TTD

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ABSTRACT

Scope:

An overall protocol outlining several nested sub-protocols for the 2D mapping of intact proteoforms from human tissue by MALDI imaging on an UHMR Q Exactive Orbitrap MS.

Expected Outcomes:

Annotated ion images of intact proteoforms ready to be ingested and disseminated.

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PROTOCOL INTEGER ID

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Sample sectioning and preparation

1 Tissue provided from TMCs are either shipped already cryo-sectioned, or shipped as tissue blocks and the preparation of tissues for MALDI imaging is performed similar to as described elsewhere. Once samples have been sectioned, tissue preparation has been outlined within the following protocol with several nested steps:

> Kevin J. Zemaitis, Dusan Velickovic, Ljiljana.PasaTolic.
> Tissue Preparation for Intact Proteoform MALDI-MSI on Human Tissue.
> http://dx.doi.org/10.17504/protocols.io.6qpvr61x2vmk/v1

Instrument preparation and operation

2 After tissue preparation the instrument is the optimized, calibrated, and MALDI imaging is performed according to the protocol below:

> Kevin J. Zemaitis, Dusan Velickovic, Mowei Zhou, Ljiljana.PasaTolic.
> High Resolution Intact Proteoform Mass Spectrometry Imaging using UHMR HF Orbitrap.
> http://dx.doi.org/10.17504/protocols.io.4r3l2onz4v1y/v1

Data visualization and annotation

3 Prior to this stage supplemental top-down proteomics by nanoPOTS/microPOTS LCMS/MS is

run, once the imaging is completed the image visualization pipeline is run according to the following protocol. Proteoform annotations are then output and manually verified:

David J Degnan, Kevin J. Zemaitis, Dusan Velickovic, Mowei Zhou, Ljiljana.PasaTolic. Image Visualization and Proteoform Assignment of MALDI-MSI from LCMS Experimental Databases. http://dx.doi.org/10.17504/protocols.io.4r3l2ode3v1y/v1