Washing Protocol for Intact Proteoform MALDI on Human Kidney

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ABSTRACT

Scope:
A detailed protocol entailing the sample washing protocols developed for human kidney, this includes several timed washing steps for fixing the tissue, removing small molecule interferents, and desalting the tissue surface optimized for use with a Spectroglyph EP-MALDI-2 source mounted on a custom Thermo Scientific UHMR Q Exactive HF Orbitrap.

Expected Outcomes:
Human kidney slides ready for pre-extraction and/or matrix application.

PROTOCOL CITATION

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PARENT PROTOCOLS

In steps of

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

MATERIALS TEXT

Chemicals:

- Ethanol (200 Proof)
- Nanopure water (LC-MS Grade)
- Chloroform (LC-MS Grade)
- Acetic acid (Glacial, LC-MS Grade)
- Trifluoroacetic acid (LC-MS Grade)

Equipment:

- Regulated nitrogen gas supply with nozzle
- Coplin jars

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 STARTING

Prepare **100 mL** of all necessary solvents for the tissue washing steps and clean Coplin jars prior to use.

Preparation

1 While the tissue is within the vacuum desiccator, pour all necessary solvents and solutions into the Coplin jar. These have a volume of approximately **75 mL** and to reduce the variability in extraction fill the jars consistently.

2 Prior to submerging the tissue section in solvent and solutions, ensure that the timers are set beforehand. While three timers are not necessary, it vastly improves the process.

3 ⚠

If any metadata is present on the slide written in permanent marker remove it at this time. Take a photo and note within a notebook prior to removal, this is necessary as this will contaminate the Coplin jars within all washes.

Tissue fixation wash 3m

4 Submerge the tissue section within a Coplin jar filled with a solution of **70% ethanol** for **00:00:30** 30s

5 Immediately move the tissue section with tweezers to then next coplin jar containing **100% ethanol** for **00:00:30** 30s

Lipid depletion wash 3m

6 After completion of the tissue fixation steps, immediately move the tissue section with tweezers to then next Coplin jar containing **Carnoy's solution** for **00:02:00** 2m

6.1 **Carnoy's solution** is a 6:3:1 volumetric solution of ethanol: chloroform: glacial acetic acid, while other solutions could be put within plastic staining jars a glass Coplin jar is highly recommended for this and other polar aprotic solvents.

6.2 ⚠

Note: dipping, agitation, and any movement of the slide within the Coplin jar will dramatically change outcomes of all washes. Tissue is prone to detach from the surface within this step.

7 Following exposure to Carnoy's solution, submerge the slide with **100% ethanol** for **00:00:30** 30s

Desalting wash 3m

8 After completion of the lipid depletion washes, submerge the tissue sections within **nanopure water with 0.2% trifluoroacetic (TFA) acid** for **00:00:15** 15s

8.1 ⚠

Note: dipping, agitation, and any movement of the slide within the Coplin jar will dramatically change outcomes of all washes. Tissue is prone to detach from the surface within this step.

9 After exposure to water, submerge the slide within **100% ethanol** for **00:00:30** 30s

9.1 Ensure this is a new solution of **100% ethanol** not used within previous steps.

Drying tissue 3m

10 After the completion of all steps, dry the tissue for **00:00:30** under a diffuse stream of nitrogen, take care to ensure that liquid does not pool within the surface. The tissue section is now prepared for the next steps within the tissue preparation protocol. 30s

11 Discard all solvents and solutions properly, and best practice is to use fresh solvents and solutions for subsequent washes.