MALDI Imaging Mass Spectrometry on Formalin-Fixed Paraffin-Embedded Tissues

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49.1 Introduction and Purpose

Most of the tissue samples in pathology are formalin-fixed and paraffin-embedded for preservation. This huge reservoir cannot be accessed by standard MALDI Imaging Mass Spectrometry since the proteins in the FFPE samples are cross-linked [1–5]. Therefore the proteins have to be made accessible by digestion into peptides before Imaging Mass Spectrometry.

The required time for the sample preparation is one working day (6–8 h) and the time for the sample measurement is between 1 and 12 h, depending on its size.

49.2 Protocol

49.2.1 Reagents[1]

- *Acetic acid* (HPLC grade, Sigma-Aldrich)
- *Acetonitrile (ACN)* (HPLC grade, Sigma-Aldrich)
- *Ammonium bicarbonate* (reagent grade, Sigma-Aldrich)
- *α-Cyano-4-hydroxycinnamic acid (CHCA)*[2] (MALDI TOF-MS grade, Sigma-Aldrich)
- *EDTA* (reagent grade, Sigma-Aldrich)
- *Ethanol* (HPLC grade, Sigma-Aldrich)
- *Trifluoroacetic acid (TFA)*[2] (HPLC grade, Sigma-Aldrich)
- *Tris base* (reagent grade, Sigma-Aldrich)
- *Trypsin Gold*[2] (Promega)

[1] If not differently stated, the reagents can be stored at room temperature.

[2] Store at 4°C.

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- *Water* (HPLC grade, Sigma-Aldrich)
- *Xylene* (HPLC grade, Sigma-Aldrich)
- Ammonium bicarbonate solution 100 mM in ddH_2O
- CHCA matrix solution[3] (7 g/L in ACN:H_2O 1:1 with 0.2% TFA)
- Trypsin stock solution (0.5 µg/µL trypsin in 50 mM acetic acid)
- Trypsin solution[3](dilute 50 µL trypsin stock solution with 500 µL 100 mM ammonium bicarbonate solution)
- Tris-EDTA pH 9.0 solution (10 mM Tris Base, 1 mM EDTA in ddH_2O)

49.2.2 Equipment

- *Cuvettes* (100 mL)
- *Hot plate* (40°C)
- *ImagePrep spray roboter* (Bruker Daltonics)
- *Incubator* (65°C)
- *Ultraflex III MALDI-TOF/TOF mass spectrometer* (Bruker Daltonics)
- *FlexControl 3.0, FlexImaging 2.1, and ClinProTools 2.2 software* (Bruker Daltonics)
- *Indium-tin-oxide (ITO)-coated slides* (Bruker Daltonics)
- *Portrait 630 reagent multispotter* (Labcyte)
- *Microtome*
- *Microwave*

49.2.3 Method

49.2.3.1 Tissue Preparation

- Cool the FFPE tissue block at −20°C before cutting.
- Using a clean and sharp microtome blade make a 5 µm section of FFPE sample and mount it onto an ITO-coated slide.
- Incubate for 10 min on a hot plate (60°C).
- Remove the paraffin by washing twice with xylene in a 100 mL cuvette for 20 min.
- Rehydrate the sample by washing with 100%, 95%, 80%, and 70% ethanol for 5 min each (in 100 mL cuvettes).
- Warm up 1 L of Tris-EDTA pH 9.0 buffer to boiling temperature in the microwave.
- Transfer the sample slide on a rack into the pre-heated buffer and boil it for 20 min in the microwave (350 W). [4]
- Wash with double-distilled water for 5 min in 100 mL cuvette.
- Dry for 10 min on a hot plate (40°C).

49.2.3.2 On-Tissue Digestion

- Spot trypsin solution onto the sample using a multispotter. The spots have a centre to centre distance of 250 µm and each spot is 175 µm in diameter. On each spot 30 cycles of trypsin solution are added. In each cycle 160 pl are added.
- After each cycle, the sample is incubated for 5 min at room temperature.
- After digestion continue directly with the matrix coating.

49.2.3.3 Matrix Coating

- The sample is spray-coated with CHCA matrix solution using the ImagePrep spray roboter. For this, the ImagePrep is set up according to the manufacturer's protocol and the standard programme for CHCA is used.

49.2.3.4 MALDI Imaging Mass Spectrometry and Data Analysis

Analysis is performed using an Ultraflex III MALDI-TOF/TOF mass spectrometer equipped with a smart beam N_2 laser. The spectrometer is operated using the Flex Control 3.0 software in positive polarity in reflectron mode. The acquisition range is 700–5,000 *m/z*. 200 spots per spot position are collected.

For data analysis the Flex Imaging 2.1 and ClinProTools 2.2 software are used.

[3]Store the solution for less than 1 week.

[4]In order to prevent evaporation of large amount of water, loosely place a lid onto the containment.

References

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