Sublimation/Rehydration of 2,5-DHA for imaging proteins

Health and Safety:

Wear nitrile gloves, laboratory coat and safety glasses. The process should be done with fume hood ventilation.

Equipment and Materials:

Equipment

Petri dish (plastic, Fisher, 08-757-100D, 100mm x 15mm); stainless steel plate (AB, 4347680); heat conductive copper tape (Electron Microscopy Sciences, 77801);Tape (Fisher, 35901R) or petri seal tape (Fisher, 50-820-970);Oven set at 37°C.

Chemicals

Trifluoroacetic acid; methanol; MilliQ water; Carnoy's fluid (6 ethanol, 3 chloroform, 1 acetic acid)

Procedure

1. Tissue preparation prior to sublimation.

   Washing conditions for detection of proteins: 70% ethanol (30s), 100% ethanol (30s), Carnoy's fluid (2min), 100% ethanol (30s), H_2O (30s), 100% ethanol (30s).

2. Sublimation of 2,5-DHA:

   DHA is very volatile, it can be sublimated at 105°C for 5 min to obtain ~0.15 mm/cm^2 at 50mm Torr.

3. Rehydration of sublimated DHA for imaging proteins (Figure 1).
   1) The slide with sublimated matrix is attached to a stainless-steel plate (used as a heat sink). (Note: this plate can be any type as long as it fits the size of the petri dish).
   2) The plate is attached to the inside of the top part of the Petri dish using a heat conductive copper tape.
   3) A piece of filter paper is placed in the bottom part of the Petri dish, and 500 µL of MilliQ water and 500 µL of TFA pipetted onto the paper.

4) The two parts of the Petri dish are reassembled so as to form a hydration chamber, sealed using a tape (Fisher, 35901R) or using petri seal tape (Fisher, 50-820-970) for the better sealing and left in the 37°C oven for 3 min.
5) The slide is taken out from the petri dish and is air dried.

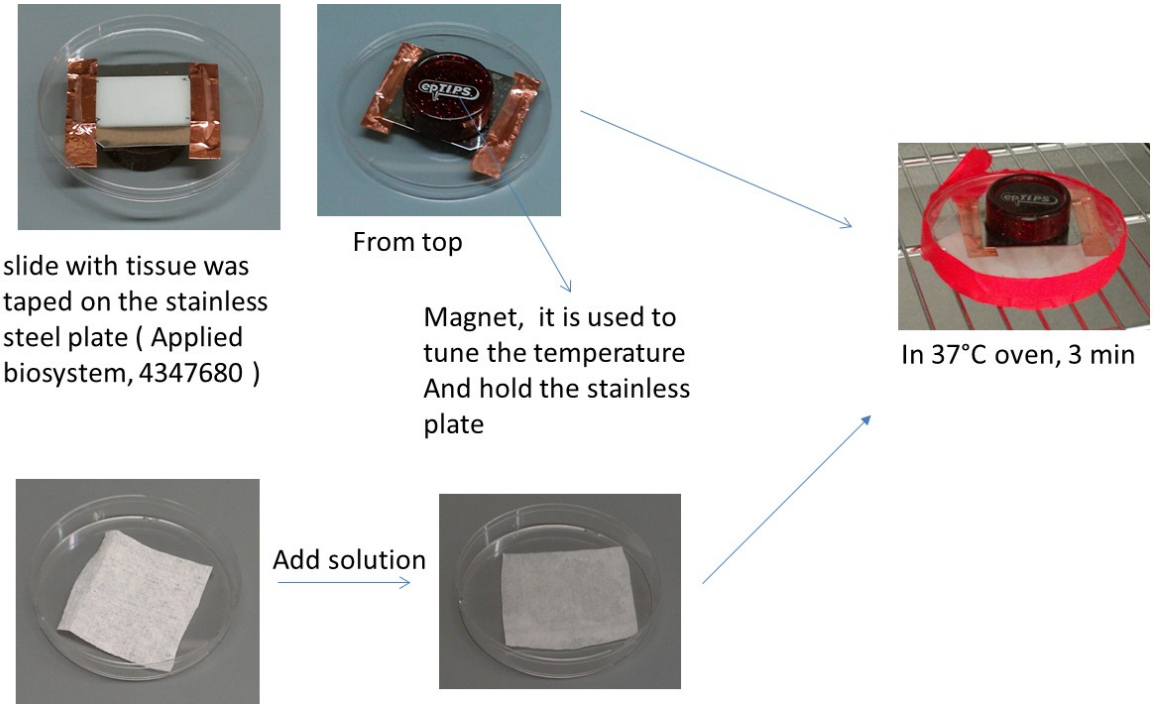

Figure 1 Construction of the rehydration/recrystallization chamber with a Petri dish, magnet, stainless steel plate and tape.

Expected outcome:

The sign of a successful rehydration is determined by a quick color change at the moment the Petri dish is dissembled. This phenomenon is caused by the evaporation of solvent condensed on the surface of matrices.

Reference: (this is a modified procedure from following references)

1. Matrix Sublimation/Recrystallization for Imaging Proteins by Mass Spectrometry at High Spatial Resolution, Junhai Yang and Richard M. Caprioli; *Analytical Chemistry* **2011** *83* (14), 5728-5734
2. High spatial resolution imaging mass spectrometry and classical histology on a single tissue section, F. Deutskens, J. Yang, R. M. Caprioli. *Journal of Mass Spectrometry* 2011, 46, 568-571.