Plattform für explorative, zielgerichtete und ortsaufgelöste Proteomik
Platform for explorative, targeted and spatially resolved proteomics
Core Facility (ProtCF)

We hereby provide information on how to prepare tissue slides from FFPE blocks for MALDI Imaging with the Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer.

Overview

a) Tissue block sectioning and mounting

Materials: ITO slides (labelled (with about 8mm empty space at the bottom)

1. Make sure the FFPE block is stored in -20°C freezer before sectioning for at least 1 h
2. Fill water bath with destilled H_2O and heat it up to 37°C
3. Take the FFPE block out of the freezer and directly mount it onto the sample holder of the microtom
4. For a new FFPE block: Set the slice thickness to 20 µm and remove excess paraffin slices just until the first tissue layer is removed
5. Then set the thickness to 6 µm and cut multiple tissue slices
6. Transfer them carefully onto the water surface of the water bath with a needle, allowing flattening and smoothing of the slice
7. When flattened, dive an ITO slide into the water beneath the tissue slice and lift it up gently, so that the slice attaches to the slide
8. Remove water from the back of the ITO slide with a paper towel
9. Put the ITO slide on a heating plate that's heated up to 37°C
10. Let it dry overnight
11. Store the slide in a slide box at room temperature.

b) Deparaffinization

Device:

1. Place ITO slides into slide holder (max. 6 slides per holder)
2. Attach the mounting and place it into the deparaffinization device
3. Switch it on, a whole cycle takes around **40 min** (continue with step 3 but make sure to be back in time)
4. Take the slide holder out and put it into destilled water (green box) until next step

c) Antigen retrieval

Preparations during deparaffinization:

→Prepare solutions:

AmBC solution 10 mM	0.8 g ammonium bicarbonate 1 L distilled H_20
Citrate buffer pH 6	1.05 g citrate acid 494 ml H_20 distilled 6 ml NaOH 2M for adjusting to pH 6

→Fill steamer with enough water and fill a slide container of the steamer with citrate buffer, put it into the steamer and preheat it to 100°C for around 20 min, control temperature before proceeding

1. Rinse slides twice in 10 mM AmBC solution for 2x1 min
2. Place the slides into the slide holder for the steaming container and put in into the container with preheated citrate buffer
3. Adjust clock to 1 hour, after 30 min refill water (in the meantime prepare bombesin, trypsin and matrix sprayer – see step 4 and 5)
4. Take the slides out, let them cool down for a short time (1 min) and rinse slides in 10 mM AmBC solution 2x1 min
5. After rinsing let the slides dry on a towel (5min)

d) Digestion control spots

Bombesin solution: 0.01 mg/ml in H_20 distilled (1:100)

1. Spot 4 spots of 1 µL Bombesin solution (1:100) in each corner of the tissue
2. Let the spots dry

e) Trypsin digestion

Solutions

Trypsin solution	8,6 mL H_20 deion. 1 mL ACN 0.4 mL AmBC 1M 1 mg Trypsin
K_2SO_4 saturated solution	Weigh again!!! 20 ml H_2O distilled

1. Freshly prepare Trypsin solution on ice
2. Preheat an incubation chamber to 50 °C
3. Detailed SOP for iMatrix Sprayer is in the drawer under the hood
4. Clean the sprayer tubes by letting the sprayer run 2 cycles with 70% EtOH as load using the correct loading tube
5. Thaw 1.6 ml of Trypsin solution at room temperature and and place the Eppendorf vial into a water-ice-bath to prevent early activation
6. Place the ITO slide onto the spray area and secure the edges with adhesive tape
7. Insert the loading tube into the Trypsin vial
8. Start the spraying procedure using the web-based control panel with settings shown below. Monitor the whole spraying procedure

iMatrixSprayer Settings Trypsin

height	60 mm
line distance	1 mm
speed	180 mm/s
density	0.5 µL/cm^3
cycles	10
delay	15 s
pressure	1.6 bar

9. Prepare the digestion chamber by adding saturated K_2SO_4 solution (20 ml) to a Tupperware box for optimal humidity and to prevent condensation
10. After the spraying procedure is complete, put the slide into the digestion chamber
11. Close the lid and put the box into the incubation chamber for 2 hours
12. Prepare the LenMix/Matrix mix
13. After incubation check for condensation or water droplets on the slide

f) Calibrants preparation

Calibrants = LenMix

	m/z	concentration in µg/ml LenMix diluted 1:10	concentration in µg/ml in END MIX
Angiotensin I	1296.69	1	0.08
Substance P	1347.72	0.4	0.04

[Glu]-Fibrinopeptide B	1570.68	2	0.15
ACTH 18-39	2465.19	4	0.30

LenMix preparation

1. Use 1 mg/ml stock solutions for calibrants *(out of the freezer -20°C)*
2. Mix 5 µL Substance P, 10 µL Angiotensin I, 20 µL [Glu]-Fibrinopeptide B and 40 µL ACTH 18-39 with 925 µL H_2O

Dilute to 1:10 with deion. water. This is your LenMix solution for further usage. This can be stored in the freezer at -20°C

g) Matrix preparation and application

Matrix solution	100 mg a-CHCA 5 ml H_2O 5 ml CAN 10 µL TFA (PROZENT)

1. Freshly prepare Matrix solution and sonicate it for 20 min before usage
2. Mix LenMix 0.2 ml LenMix solution to 2.4 ml Matrix solution. This is sufficient for 30 spray cycles
3. Clean the sprayer tubes by letting the sprayer run 2 cycles with 70% EtOH as load using the correct loading tube (C)
4. Place the slide onto the spraying area and secure the edges with adhesive tape
5. Insert the loading tube into the Matrix/LenMix mixture
6. Start the spraying procedure using the web-based control panel with settings shown below. Monitor the whole spraying procedure

iMatrixSprayer Settings Matrix

height	60 mm
line distance	1 mm
speed	180 mm/s
density	0.5 µL/cm^3
cycles	20
delay	5 s
pressure	1.6 bar

7. After spraying remove Matrix from top and bottom part of the slide to ensure optimal contact with the clamps of the MALDI plate. This is done by wiping carefully with a paper towel soaked in 70% EtOH

h) Teach-marks application

1. Add teach-marks by carefully scratching little crosses next to the tissue using a ceramic cutter (see image)
2. Add markings with a xylene-resistant pen (SECURE LINE or comparable)