**Histology training:** **Date and signature:**

**Access to the instruments and the online booking system is provided by the core facility managers, after training has been completed. The core facility is part of the "Department of Medical Biology".**

**Always wear gloves and a lab coat.**

## Room E-2.053:

- Store glasses with Xylol I and II in the chemical cabinet, which is normally locked.
- Key box of the chemical cabinet is on the left sided board.
- Xylol I and II incubation are performed under the hood, at room temperature, and no shaking.
- Transfer cassettes carefully to the first Xylol tank and avoid contamination of the 100% EtOH. Otherwise, Ethanol should be discarded. After Xylol incubation don't forget to lock the chemical cabinet again.

The heavy doors of the chemistry cabinet close automatically. Be careful that the heavy doors of the chemistry cabinet don´t knock the glasses filled with Xylol out of your hands.

| | | |
|---|---|---|
| 100% Xylol I | 40 mins | RT |
| 100% Xylol II | 40 mins | RT |

Then you use the top heating ovens: PP (Paraplast)/Xylol glass and 100% PP I glass is stored directly in top oven, where also sample incubation takes place (glasses with Xylol residues).

| | | |
|---|---|---|
| 1:1 PP/Xylol | 60 mins | 60°C top oven |
| 100% PP I | 60 mins | 60°C top oven |

Next incubation steps are done in the ground oven.

No Xylol anymore

| | | |
|---|---|---|
| 100% PP II | ON | 63°C ground oven |

- Reserve the embedding AND the microtome on the cluster market calendar.
- Account to Cluster market IHC is allocated after training by Gerti Achatz.
- Prepare 2 L Millipore water for the water bath at the Microtome room.

Embedding machine (E-2.053):

Turn on the machine 1 hour before starting the embedding to be sure that Paraplast is completely dissolved. Also start the cooling plate – takes approx. 10 mins to be cold. Place the glass with your samples into the PP reservoir to keep it warm.

- Take a plastic chamber from the box next to the machine. Fill it a bit with PP and place the chamber on the heated part of the machine. Open your cassette and place the sample into the plastic chamber filled with PP. Don't place the cassette immediately on the cold quadrant. Orientation of sample depends on the type of your sample. Place your sample to your satisfaction and then transfer the cassette to the cooled area.

- Then you put the grid of the cassette on top (the writing of the cassette is looking towards you!) and fill it up completely with PP.
- Then place the cassette on the cooling plate and wait for it to harden. You can remove the plastic chamber when the block is hardened, which takes about 15-20 minutes.

When you are done, turn off the embedding machine and the cooling plate. Clean both machines as good as possible and place two sheets of tissue on the cooling machine.

Paraplast, to fill up the embedding machine with fresh pellets, is stored in the grey cupboard (right to the entrance and left to the incubators).
It is not allowed to mix different types of Paraplast in the machine. If there is a different demand, ask the core facility manager.

Don't forget to fill in the list (left of the embedding machine) of how many samples you have embedded and the group name. The number of embedded samples determines the invoice amount the group must pay for.

**Room E-2.003**

Cutting at the Microtome:

- Bring your Millipore water. Put 1L in the water bath and turn it on. The temperature is adjusted at 37°C.
- Switch on the cooling plate next to the microtome and precool your samples.
- The knife should be positioned in the middle. Place your block in the metal holder. You can place it in the horizontal or vertical direction. Use the left side of the knife for trimming into the insert with 10-15 µm. Use the middle and right positions for sections with 4 µm. Cut 3-4 connected inserts and transfer them to the prewarmed water bath where the cuts have now the possibility to elongate. Thereafter fish the cuts with Superfrost or Ultrafrost slides out of the water bath. Label the slides beforehand with lead pencil (Number, date, and abbreviation of the name).

For your own safety: Between trimming or sectioning steps always close the protection gear of the knife and activate the safe lock!

After cutting, clean your workspace, turn off all devices. The slides dry best at room temperature overnight. Empty and clean the water bath - Pour water into the sink (Histo lab).

## How to acknowledge our core facility: PLUS Histology Core Facility

Further details are provided:

- User Manuals
  - 1_ Embedding Station Leica EG1150H User Manual
    https://www.plus.ac.at/wp-content/uploads/2023/07/1_Embedding-station-Leica-EG1150H-user-manual.pdf
  - 2_HistoCore_ArcadiaC_User Manual

https://www.plus.ac.at/wp-content/uploads/2023/07/2-HistoCore_ArcadiaC_User-Manual.pdf

- 3_HistoCore_AUTOCUT_Rotary Microtome User Manual
  https://www.plus.ac.at/wp-content/uploads/2023/07/3_-HistoCore_AUTOCUT_-Rotary-Microtome_User-Manual.pdf
- 4_Cryotome_CM1950_User Manual
  https://www.plus.ac.at/wp-content/uploads/2023/07/4_Cryotome_CM1950_User-manual.pdf