



INSTRUCTIONS FOR PROCESSING AND PARAFFIN EMBEDDING TISSUES UNDER COVID-19 RESTRICTIONS.

Under the current Covid-19 restrictions, all histology core facility users (new or experienced) will have to drop off tissues for processing in a jar of 70% ethanol in the fumehood and Adi will do the embedding.

You **MUST** wash your hands first and wear gloves for anything you need to do in the histology core facility. **NO EXCEPTIONS!!!** It is your responsibility to have your own gloves. We will not provide them. Wearing a mask in the histology core facility is not mandatory. However, if you are at a risk of working less than 6 feet away from another person in the lab, you **MUST** wear a mask. If you forget a mask, we can sell you a disposable mask for \$1 per mask.

You must ensure that the histocassettes used for housing these tissues are labelled with a pencil and ensure that you provide Adi with embedding rings with matching labels. Please read instructions below for details.

BEFORE HARVESTING ORGANS FOR HISTOLOGY/IHC:

Make sure that you harvest organs in a process causing minimum damage to tissues. Tissue harvesting is the first and most important step in histology. Forceful handling or immense pressure on tissue can cause physical damage, especially to delicate tissues. Talk to Adi for further clarification.

You must plan to procure the right fixative and store tissues appropriately before they are submitted to the histology core facility for processing followed by paraffin embedding.

FIXATIVES

The two most commonly used fixatives are

- 10 % Neutral buffered formalin (NBF)
- 4 % Paraformaldehyde (PFA)

You must ensure that the surface to volume ratio for fixative to tissue is at least 20:1 For example, if your tissue weighs 1 gram, you need to use at least 20 ml of fixative in a vial/tube/container.

The duration of fixation is a very important step and should not be compromised. Usually we recommend fixation for 24-48 H depending on the tissue and type of fixative used. You can procure fixative from the histology core facility for a cost recovery fees. You can store tissues in fixative at room temperature.

We provide a variety of sizes of histocassettes which are used to store tissues for fixation and processing. Some groups also use histocassettes to embed their tissues in paraffin wax for sectioning. When selecting histocassettes, you must consider the following points:

1. Size of your tissue
2. Physical state of the tissue, e.g. delicate tissue such as ovary vs. kidney

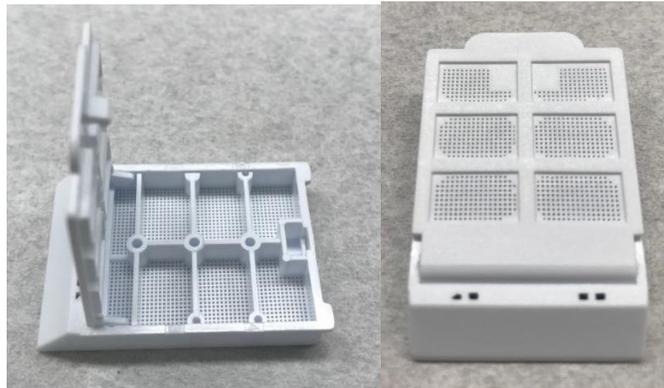
3. If you need to divide a tissue into smaller pieces

We provide the following options:

Macrosette (for larger tissues, e.g. rat liver) which is deeper



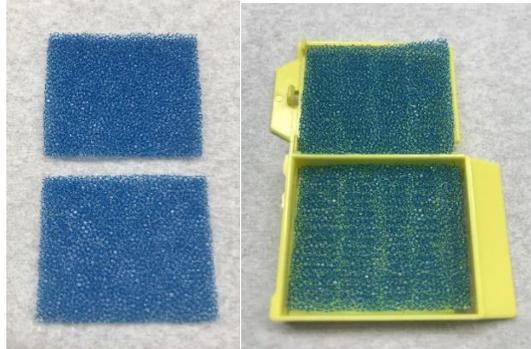
Microsette (has multiple slots and much smaller pore size for tiny and delicate tissues – e.g. mouse embryo, early life stage zebrafish)



Regular histocassettes for general use and widely acceptable for most tissues



If your tissue is fragile or can possibly curl up during storage and processing, consider using biopsy pads to protect them during processing



POST FIXATION IN EITHER 10% NBF OR 4% PFA

Once your tissue is fixed, you will have to ensure that the tissue undergoes at least 3 washes (ideally 24 H for each wash in different containers) in 70% ethanol (made with distilled water).

The first step in tissue processing is exposure to 70% ethanol and hence the washes will ensure that you have minimised the transfer of the initial fixative in the final wash. If you are storing tissues in biopsy pads, we recommend a 4th wash since the biopsy pads retain more of the solution.

Use the online booking calendar to book the tissue processor to book a slot for processing and embedding your tissues. Here is the link to the online booking calendar: <http://healthsciences.usask.ca/booking/equipment-hcf/week.php>. If you have not communicated with Adi about the booking calendar or availability yet, please remember that we prefer that you book at least 48 H in advance. For first time users, the processor must be booked between Monday-Thursdays only.

TISSUE PROCESSING

Adi will set up the tissue processor for you and depending on the type of tissue, will recommend the most appropriate program for processing the tissues. Tissue processing is conducted overnight followed by embedding the next day.

TISSUE EMBEDDING

Adi will be embedding the tissues for you (THIS IS TEMPORARY). You need to discuss tissue orientation (transverse vs. longitudinal vs. sagittal) beforehand. If you have multiple tissues requiring multiple orientations, please talk to Adi in advance and he will co-ordinate this with you.

If you want to use histocassettes for embedding tissues, please communicate with Adi beforehand.

TISSUE STORAGE

After the paraffin wax has solidified, Adi will ensure that the tissue blocks are detached from the metal moulds and leave them next to the cryo section of the embedding station. It is your responsibility to ensure that the tissue blocks are stored in your designated drawers.

LOGGING USAGE IN THE TISSUE PROCESSOR BINDER

Adi will log usage in the binder for you. If you need to add any specific information in the binder, communicate with Adi.