



FIRST TIME USER INSTRUCTIONS BEFORE SUBMITTING TISSUES FOR PARAFFIN EMBEDDING

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.

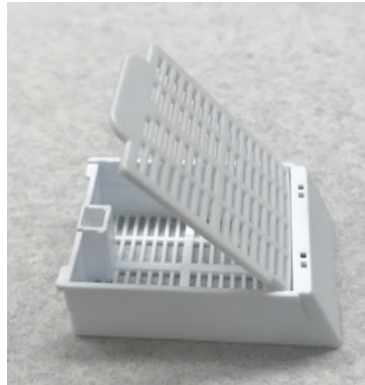
The duration of fixation is a very important step and should not be compromised. Usually we recommend fixation for 24-48 H depending on the tissue and type of fixative used. You can procure fixative from the histology core facility for a cost recovery fees. You can store tissues in fixative at room temperature.

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



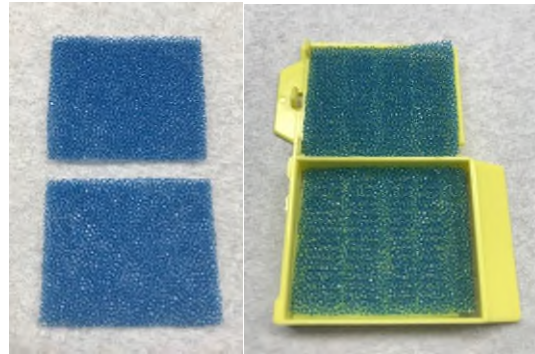
Microsette (has multiple slots and much smaller pore size for tiny and delicate tissues – e.g. mouse embryo, early life stage zebrafish)



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 train first time users on the different techniques involved with tissue embedding. If you embedding tissues for the first time, we recommend on processing more than 25 tissues.