

## Fixation

While we can not provide our clients with one protocol for fixation that will encompass all types of tissue we strongly recommend each lab develop a set protocol for tissue preparation and fixation. Without a standard protocol for fixation an investigator can not obtain reproducible and consistent results. Two of the biggest problems we encounter with samples are tissue that is inadequately fixed or is overfixed. If you have any questions about how best to prepare your tissue please feel free to ask the IHC Core staff for more information.

Please remember:

\*Fixation will affect not only processing and sectioning of your sample, but also routine, special, and immunohistochemical staining. ***Faults in improper fixation cannot be corrected at any later stage of processing and staining.***

\*The purpose of fixation is to stop autolysis and putrefaction. Tissue begins breaking down as soon as the animal dies so tissue should be harvested, grossed, and placed into fixative as quickly as possible.

\*The most commonly used fixative is 10% formalin. The IHC Core keeps 10% formalin and it is available at no cost to our clients.

Factors that affect fixation

\*Thickness/type of tissue

-Kidneys should always be sliced in half to allow penetration of formalin.

-For the best fixation of liver the lobes should be sliced into strips before being placed in fixative. If you need to submit a whole liver you should score the top to allow for penetration of fixative.

-Heart fixes more thoroughly when cut in half.

-Large tumors must be cut in half before being put into fixative. If large tumors are placed into fixative as one piece the outside will fix before the inside. The outside will form a type of capsule and fixative will not be able to reach the middle of the tissue which will remain unfixed.

-Brain should be either cut into 2-3 coronal sections or into 2 longitudinal sections.

-Both the intestines and the stomach need to be free of debris before fixation. Gently pushing saline through the tissue using a syringe works well. Intestines can then be rolled up and stomach should be laid flat between two dampened sponges.

-Smaller lungs such as those from a mouse can be submitted whole. Larger lungs will need to be trimmed due to size.

-Skin needs to be laid flat between two dampened sponges. Without sponges skin tends to roll up and can not be embedded for a clean cross-section of tissue.

\*Amount of fixative:tissue

-Samples should be placed into 10-20 times the volume of fixative to tissue. If your sample needs to fix for more than a day then the fixation solution should be changed daily.

\*Temperature

-In most cases samples should be fixed at room temperature. One of the goals of fixation is to fix tissue quickly as refrigeration will slow down the fixation process.

\*Length of time

-The length of time tissue needs to be fixed varies between types of tissues and with tissue sizes. A good rule of thumb for most tissues is overnight. If you have an extremely small piece of tissue such as a biopsy you may only need to fix for a few hours. On the other hand, large pieces of tissue may need to be fixed longer.

-Determining when tissue is properly fixed takes a trained eye and many years of training. If you have any questions about how long your tissue should be fixed please contact the IHC Core staff.