A Guide to Proper Fixation of Tissue Specimens for Eventual Immunostaining (09/2015)

1. First and most important - the original tissue sample must be of good quality. Factors such as warm ischemic time, the time delay between tissue excision and fixation, etc. are important. Ideally, tissues should be acquired as close to still being viable as possible, and put into fixative as soon as possible following excision. Delays lead to cell death, autolysis, and loss of tissue and cell integrity with concomitant losses of immunostaining (e.g. due to proteolysis of the antigen). If acquiring animal tissues, consider performing perfusion fixation prior to organ/tissue removal if it is an option.

2. Once the tissue sample is obtained, proper fixation technique is the next critical component of generating high quality fixed tissues that have the best chance to succeed in immumostaining. There are a number of important guidelines here:

(a) Formalin solution slowly inactivates over time, thus fresh formalin should always be used. Freshly prepared buffered formaldehyde solution is the absolute best, but good results can certainly be obtained with commercial formalin solutions that are not too old.

(b) Diffusion of formalin solution into the tissue is relatively slow- a rate of approximately 1 mm per hour. Thicker specimens take longer to fix, and such specimens fix in a gradient fashion - fixing fastest and most completely from the tissue surface towards the interior. The practical impact of this is that if a specimen is too thick, then the interior may not become fully fixed, or that significant autolysis can occur before the fixative diffuses into the area and finally does fix the interior. In this case, immunostaining will be heterogeneous throughout the sample due to spatial differences in antigen preservation. To help assure proper fixation, keep the maximum physical dimensions of the tissue to 2 mm or less in all directions. To facilitate this, either cut larger specimens into thinner sections (each < 2mm in thickness), or make several slices deep into the body of an intact tissue to allow for good flow of the fixative.

(c) Use plenty of fixative. The general rule is to use at least 15 volume equivalents of formalin per volume of tissue. A higher formalin-to-tissue ratio certainly won't hurt, and just requires a larger container. Formalin is relatively cheap, so don’t skimp on this step.

(d) Gentle agitation of the tissue in the formalin during fixation (e.g. on a slowly moving platform shaker or tube roller) will foster good exposure and help maximize diffusion. If instead fixation is static, areas of the tissue in contact with the walls of the container can become "dead zones" with poor local fixation that will behave poorly in immunostaining.

(e) Fixation is usually done at room temperature.

3. Time of fixation. Be sure to allow adequate time for thorough fixation. If the guidelines above are followed, then 48 hours in fixative should suffice, although an additional day or two won’t hurt. Unlike in the past, in the modern era of immunostaining, under-fixation is much more of a worry than over-fixation. This is because we now employ antigen retrieval techniques just prior to immunostaining that act to efficiently reverse some of the chemical cross-links formed during fixation; thus removing their antigen-masking effects.

4. Once tissues are fixed, remove them from the fixative and rinse in either 1X PBS or water, then store refrigerated, immersed in 1X PBS until they will be delivered to the histology lab for embedding and sectioning.