Stony Brook Medicine
Department of Pathology

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Research Histology Core Laboratory
Procedures and Tips

Tissue Fixation

Time The amount of time a sample spends in fixative is important to monitor to prevent under or over fixation. A ratio of 5:1 of fixative to tissue is recommended and the time frame depends on the size of the sample.

Size Example: if a mouse organ needs to be fixed, 8-24 hours in fixative should be sufficient to penetrate the tissue compared to something that has a larger surface area (diameter of a quarter) which could be fixed from 24-48 hours. It is important to expose as much of the tissue to fixative as possible (cut sample in half or 'bread loaf' the sample). This will ensure that the fixative has fully penetrated the sample. Cutting a larger sample before fixation may also be necessary to fit into the standard cassettes that are used for processing.

Solutions After the sample has been fixed for the appropriate amount of time, it can be transferred to PBS or 70% ethanol. This will prevent the sample from becoming over fixed and will not affect the sample. This can be done for up to one week in refrigeration. If you wish to keep the sample in solution for a longer period of time, it is important to prevent bacterial growth on the sample. A very small amount of sodium azide can be added to prevent this.

Samples left in fixative too long When a sample is left in fixative for an extended period of time (one week or longer), histology may be affected. For example, if only an H&E is required, the histology may be slightly altered but can still be distinguished and interpreted. However if an immune stain is required, the excess number of cross links formed from the over fixation may mask the epitopes needed to react with the antibody.

Gross Preparation

Small Samples Preventing small samples from falling out of its cassette during processing: we have small bags that are made to contain samples in the cassettes, while still allowing the processor to filter through the material.

Thin samples (membranes, skin) that need to be kept straight: Since tissue tends to slightly shrink and crinkle after processing, we have biopsy papers that allow us to wrap the sample to preserve the shape. If there is a specific need for how you would like to request your sample, we will be happy to comply.

Maintaining Orientation of Sample during Processing If the sample is not secure in the cassette, it will be free to move around during tissue processing and lose its initial, intended orientation. This can be prevented by inserting foam pads inside the cassette to ensure that the sample does not move from the initial placement.

Marking Samples Samples may be marked so that one can identify a particular region later without affecting the histology. This can be done by applying india ink to the sample at the region requested. We have multiple colors and it is used solely for this purpose. The ink will only be external to the sample and will not penetrate the sample. This can also be seen after the tissue is processed, embedded, sectioned and stained (H&E, immunostain, etc.).

Paraffin Embedding

Orientation Specification Orientation of the sample and specific requests are part of the initial discussion of every order.

Clients embedding samples in paraffin blocks Upon request, we can arrange for clients to embed their samples.

Samples Containing Sutures It is required that all sutures be removed from samples prior to embedding and sectioning. To mark a particular region of interest, we can apply india ink.

Sectioning

Best thickness to use for sections Sections are generally cut between four and five microns to best visualize the histology however thicker sections may be done upon request.

Sterile Precautions/DNA Extraction While it is common practice to take precautionary measures to ensure no cross-contamination between samples and that materials used are sterilized between each sample, we encourage our clients to share specific project needs, requests and concerns with us.

Microtome Use Some clients have requested to use the microtome to learn how to section, however due to risk of injury, only trained technicians may use the microtomes. Clients, however, are welcome to observe the tissue being sectioned by the technician.

Slide Boxes The price charged for sections does not include cost of the slide boxes that contain the cut slides. We provide initial boxes and request they be returned to the Research Histology Core facility. If you would like to provide your own boxes we will gladly use them for your order.

Bone sectioning vs. Tissue Sectioning Pricing Generally bone sectioning requires extra decaling steps during the process of sectioning, therefore a higher price is charged for this service.

Serial/Step Sectioning may be requested at no additional cost.

Frozen Sectioning

Thawed Samples Once you snap freeze tissue, it is essential that it is kept frozen. Freeze/thawing your sample can cause deterioration and loss of preservation.

Transferring Frozen Samples When transferring frozen samples between locations, samples should be kept on dry ice.

Hematoxylin and Eosin Staining

Intensity of hematoxylin and/or eosin Upon request, the intensity of chemicals in the stain can be altered.

Cover Slipping The core facility coverslips H&E slides, however upon request, they do not need to be cover slipped.

Specialized Stains

Masson's trichrome and mucicarmine staining are provided by the Research Histology Core Lab.

Requests for specialized stains not listed on the website Specific stains not included in our list of services may be requested. We may be able to acquire the reagents needed or refer the request to the clinical histology lab.

Immunostaining

Cost of optimization / immunohistochemistry on slides for a specific antigen The price listed is the maximum amount that would be charged for an optimization. If the antibody is able to be optimized relatively quickly, the price will be substantially lowered. (The percentage lowered depends on multiple factors such as reagents used, added blocking steps and overall complexity of the optimization.)

Slide evaluation by a Pathologist Kenneth Shroyer, MD, PhD, is the Director of the Research Histology Core facility and a board certified anatomical and clinical pathologist. Upon request, he will evaluate any staining done by the Research Histology Core facility at no additional cost.

Rates for Staining (manual vs. standard) Manual staining indicates staining will be performed manually without the use of automated machines. Standard staining is performed by an automated autostainer. Rates differ accordingly.

Providing Antibodies Clients must provide their own antibody for staining.

Immunofluorescence Staining Our facility provides immunofluorescence staining.