TRIPath
HISTOLOGY - IHC - MOLECULAR - IMAGING

Histology Specimen Submission Guide

All samples submitted for histology services to TRIPath Lab must be fixed, transferred to 70% ethanol and properly labelled in a well-sealed container within a biohazard transport bag. All human subjects research projects need to have approved and up-to-date IRB documentation submitted to trip@pathology.wisc.edu. **NO BSL-2 samples will be accepted for frozen sectioning (e.g. tissue and agents associated with human diseases, non-human primates, etc.). You must note on the request if tissues are unfixed, fresh-frozen.** These tissues are handled on a BSL-2 area following biosafety guidelines. Below are our guidelines for specimen submission that will assure the best experimental results. Failure to follow these guidelines will result in project completion delays.

Formalin-fixed Tissue – Cell Pellets – Spheroids and Organoids – Frozen Tissue

FFPE Submission
▪ **Tissues should be fixed 24-48hrs in 10% NBF (neutral buffered formalin) then transferred to 70% ethanol.**
▪ **Other fixatives are acceptable but may require different steps (e.g. PFA requires less fixation time, Bouin's requires rinsing of the tissue multiple times).**
▪ **Time can be shortened for small biopsy specimens (as little as 8hrs) and lengthened for large (whole organ) specimens; whole organ specimens should get further fixation after being grossed down to cassette-size (3-4mm thickness). Bloody specimens should have their fixative replaced with fresh fixative after 12-24hrs.**
▪ **Tissue should be fixed in at least 20-parts fixative to 1-part tissue (20:1).**
▪ **Tissue should not be fixed in conical tubes as the tissue will settle, preventing the fixative from permeating the tissue. Tissues stiffen upon fixation and can take the shape of the conical tube.**
▪ **Fixation in microcentrifuge tubes is not recommended unless specimen is very small (still maintain 20:1 ratio).**
▪ **If tissues are submitted in cassettes, which is preferred, they should be grossed down to the appropriate thickness (3-4mm). For example, tissue should not be "squished" into a cassette and should be free to move. If a desired orientation is needed, biopsy sponges can be used to maintain this orientation.**
▪ **If a desired orientation or face is required, please note this on the iLab project request. The desired region/face should be face-down in the cassette. Routine embedding procedures are followed unless notified. (e.g. skin samples are cut cross-section or "on edge"; intestinal samples are usually cut cross-section with visible lumen).**
▪ **Label cassette with a pencil or chemically-resistant marker – DO NOT USE SHARPIE!** **Example: Fisherbrand™ Fisherfinest™ Chemically Resistant Markers: cat # 22-026-700**
▪ **If tissue needs decalcification, it is recommended that it be decalcified appropriately before submission to the TRIP Lab. If decalcified in TRIP, routine decalcification is done using Formical 2000, but EDTA decalcification can be performed upon request.**
▪ **All samples MUST be submitted to the TRIP Lab in 70% ethanol after proper fixation. Do not submit samples in fixative.**
▪ **All samples should be submitted in a well-sealed container, appropriate for transport. We advise using specimen container jars in a biohazard specimen transport bag.**

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Cell Pellet Submission (all steps are performed in a 15ml conical tube)

- Spin cells down to a pellet
- Aspirate off the growth/support medium
- Wash cells with 10ml PBS and spin to a pellet
- Resuspend cells in 10ml 10% NBF (2-16hrs, preferably rocking to keep cells from settling)
- Make 1% agarose in PBS in 15 ml conical tube (microwave until melted and store in 60C water bath until use)
- Spin down cells, aspirate off NBF, wash PBS, aspirate, and spin again
- Aspirate off PBS and resuspend cells in 0.5ml 1% agarose (work quickly, keeping tube of cells in beaker of 60C water)
- Place on ice to solidify the agar pellet.
- Once solid, use blunt forceps to gently release and remove agar plug from the bottom of the tube.
- Transfer agar plug to larger container with 70% ethanol

Spheroids and Organoids Submission

There are many modalities as how these samples are grown in tissue culture (dishes, multiple-well plates; with or without coverslips, etc.). Preparation of these specimens in gel media for histology will differ. Please contact us directly before submitting your samples to suggest the best specimen submission method that meet your experimental needs.

Frozen Tissue Submission

- NO BSL-2 samples will be accepted for frozen sectioning.
- Unfixed, fresh-frozen BSL-1 tissues must be noted on the request.
- It is preferred that frozen samples actually be fixed (4% PFA for appropriate amount of time) before freezing. This results in better tissue morphology with minimal effects on antigenicity.
- It is preferred that frozen samples actually be fixed (4% PFA for appropriate amount of time) before freezing. This results in better tissue morphology with minimal effects on antigenicity.
- Fix tissue for desired time, then transfer to 30% sucrose. Tissue will initially float in the sucrose; once it sinks, it's ready to be frozen.
- Specimens can be submitted in sucrose at this point if the TRIP Lab will be freezing them into blocks.
- If you are freezing the blocks, it is recommended that the blocks be frozen using dry ice. Liquid nitrogen has several detrimental effects on tissue blocks (extreme cold cracks the blocks, contact with OCT media causes bubbles, etc.)
- Blot off any excess sucrose from the tissue then submerge it into an appropriate sized mold containing OCT. Avoid any bubbles, especially those caught between tissue and OCT. Avoid tissue touching the sides of the mold; give a "border" of OCT surrounding the tissue.
- Avoid any OCT to come in direct contact with liquid (isopentane, ethanol). These solvents can quickly penetrate the OCT block, making it too soft to cut.
- Please see the TRIP Lab for advice on freezing your own samples if you are unsure.