ULAM IN-VIVO ANIMAL CORE
UNIVERSITY OF MICHIGAN

Histology Sample Guidelines

How do I make a request?

Samples may be submitted through MiCORES: https://umich.corefacilities.org/
See services to select for common submission types. Make sure to click "Add Selected Services" located beneath each group of services, and thoroughly review the charges and payment section (including shortcode) before you submit your request. Indicate your preferred sample drop-off location and time at the bottom of your MiCORES request. Wait until you receive confirmation of your drop-off time, then drop off your samples labeled with your MiCORES Service ID at your requested location. Please be punctual.

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What is the "Customer Experiment ID (Optional)" field in MiCORES?

This field is for your own internal experiment ID number or project name. It is optional but may help you better track your submissions. It is different from the MiCORES Service ID, which is the accession number that our core gives your samples – please use the MiCORES Service ID for communications with the core about your samples.

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What services should I request in MiCORES if I want HE slides (or unstained sections, special stains, or IHC) from tissues in fixative (or cassettes)?

The basic services necessary to generate paraffin HE slides (or special stains, or IHC) from tissues or cassettes are given below.

A. "Trim/Cassette": Cutting your tissues into pieces small enough and of appropriate orientation for tissue cassettes. Select as the TOTAL number of cassettes (with 1-4 tissues per cassette).

B. "Paraffin Processing and Embedding": Tissue dehydration through a series of alcohols and solvents and infiltrating and embedding in a hard cutting medium (paraffin wax). The selected quantity should be the SAME as the number of cassettes.

C. "Paraffin H&E Slide (section & stain)": Select TOTAL number of paraffin slides to be sectioned and subsequently stained with H&E

D. "Paraffin Unstained slides (returned unstained)": Select TOTAL number of unstained slides you wish to have returned to you, unstained

E. "Paraffin Unstained slides (for IVAC to stain, Specials or IHC)": Select as the TOTAL number of unstained slides for any special histochemistry or immunohistochemical stains.

F. "Special Stain, xxxx": Select as the TOTAL number of special stains required. See MiCORES for list.

G. "IHC, xxxx" (for IVAC antibodies) or "IHC, paraffin (other, provided by PI)": Select as TOTAL number of IHC stains using our listed antibodies or using an antibody that you provide. See MiCORES for list.

<u>For tissues submitted in fixative:</u>

- for HE slides only: select A, B, C
- for HE and unstained slides (returned unstained): select A, B, C, D
- for HE and special stains or IHC: select A, B, C, E AND F or G

<u>For tissues submitted in cassettes:</u>

- Same as above but omit A (Trim/Cassette). **Note** that if your cassetted tissues are trimmed improperly or are too large, our staff may re-trim and add cassettes, with Trim/Cassette charge.

<u>For previously cut slides submitted to the core for **staining only:**</u>

- select "H&E only" or the appropriate Special stain or IHC.

NOTE: Additional charges (Histology technician time) may be added for special instructions such as "2 sections per slide" or "take a section every 500 microns until block is exhausted". Additional charges may also apply to account for extra time and effort needed if our trimming and cassetting guidelines were not followed (e.g., too many pieces of tissue were put into a single cassette).

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How should my samples be packaged for submission?

Samples may be submitted in fixative or as fresh or frozen tissue, depending on the type of request (specify fixative in MiCORES request). Samples should be in secure primary containers* within secure, clean secondary containment (e.g., plastic bag or biohazard bag, sample-dependent). For safety, please do NOT use glass containers and do not submit liquids in bags as primary containment.

*FOR TISSUE CASSETTES: WIDE-mouthed plastic containers should be used. Narrow-mouthed containers make it difficult for our staff to retrieve cassettes. Samples submitted in narrow receptacles will be returned for appropriate packaging. Please ask us if you need appropriate sample containers – we have some (limited) supply available for clients. Tissue cassettes should be labeled in #2 pencil or histology pen (NEVER sharpie or regular pens).

**FOR TISSUES: Ensure that you are using a volume of fixative to tissue ratio of ≥10:1. Further ensure that the container does not deform the tissue – containers with a flat bottom are preferable to conical bottoms. Tissues tend to sink to the bottom of a conical tube and deform. Commercially available pre-filled formalin biopsy containers are available from major medical supplies (Fisher, etc.).

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How should my tissues be trimmed?

Tissues should be trimmed so that they are no thicker than 4 mm (a little thicker than a nickel) and so that they can comfortably fit within a histology cassette without touching the exterior sides (this will ensure that they will be small enough to be supported on all sides by wax at embedding).

Tissues should be trimmed so that they have at least one flat surface (for solid organs) or into flat strips or rolls that will allow all relevant layers of the tissue to be visualized (skin, gut). A good reference for organ-specific trimming are the standardized tissue trimming guidelines issued by the Registry of Industrial Toxicologic Pathology (RITA) https://reni.item.fraunhofer.de/reni/trimming/index.php?lan=en We also have organ-specific guidelines for particular projects within our core – please inquire for organ or project-specific tissue trimming guidance. The core reserves the right to return or re-trim (with charge) improperly trimmed tissues. Improper trimming will delay your project and/or generate unsatisfactory results.

See question below on tissue types and numbers that can be cassetted together.

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What types of tissues can be grouped together? How many tissues can I put in one cassette?

Tissues of similar density/hardness should be grouped (ex. muscle with muscle, gut with gut, bone with bone). The more tissues placed within a cassette, the greater likelihood that one or more tissue will not be at the optimal sectioning plane in your slides. We recommend no more than 2-4 tissues (or pieces of tissue) in a cassette. Project-specific tissue grouping recommendations and/or cassetting schemes can be generated for you upon request. Improper tissue groupings or excessive tissue within your cassettes will delay your project and may result in additional charges or sample return.

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How do I ensure that my tissues are oriented to show my target structure?

If you have a particular target within your tissues (tumor, structure, etc.) it is your responsibility to communicate this to the core so that we can appropriately orient your tissues during embedding and appropriately section them. Please include the preferred orientation in the free text fields in your MiCORES request or upload a diagram. For some targets, serial or step sectioning may be required (with charge).

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Should I use tissue dye?

In general, no. Please discuss your project with us before using tissue dye as a marker, since it may not be necessary and requires proper application to avoid bleed-off between samples. Following standardized trimming guidelines https://reni.item.fraunhofer.de/reni/trimming/index.php?lan=en and the communication of your tissue target to the core are usually sufficient to ensure that your target is visible.

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What types of tissues will the core accept? Will the core work with human tissues? Will the core accept biohazardous or chemically hazardous tissues?

Our core will accept any animal tissue or organoids/cell lines from any animal species. We will accept non-CNS, de-identified human tissues or organoids/cell lines for research use only. No samples will be accepted with any clinically identifying information. We do not accept unfixed human CNS samples. We will accept fixed human CNS tissues if they are documented as not having been obtained from patients with indication or suspicion of prion disease. If your tissues contain a known biohazard or chemical hazard, please indicate the specific hazard on your MiCORES submission form and indicate the appropriate precautions our staff should take to work with your tissues. You can find this information in the EHS recommendations for your IBC protocol or by contacting your EHS representative. We reserve the right to refuse samples that cannot be safely accommodated within our laboratory space and equipment.

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What if I have larger tissues (eyes*, large animal tissues) that do not fit in standard histology cassettes?

Large tissues are usually submitted as tissue in fixative and trimmed/cassetted by our core. If our core is trimming tissues for you, please describe the target structure(s) for your tissues at submission or set up a time to be present during trimming. If you are trimming your own tissues, please ensure that trimming guidelines are followed. Formalin is a slow-penetrating fixative – for proper fixation please ensure that tissues in fixative are no thicker than 5 mm-1 cm and that the fixative to tissue volume ratio is ≥10:1. Making several cuts into the surface of larger organs to allow fixative penetration is typically necessary. The core reserves the right to return tissues of improper thickness or in insufficient fixative. Instruction in collection/fixation of larger tissue is available from core staff – please inquire.

*Specialized, species-specific guidelines for the fixation and trimming of eyes are available upon request. In general, mouse and rat eyes are not injected or trimmed and may be submitted whole in cassettes. Larger species eyes should be injected with ~0.3-0.5 ml formalin at the limbus, directed to posterior chamber, and submitted whole in fixative (allow our core staff to trim AFTER fixation). Please specify the target of evaluation at the time of submission.

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What if I have tiny tissues that may slip through the holes in the cassette?

Tissue sponges may be used within the cassette to make a “sandwich” that holds the tissue in place. Biopsy bags or specialized biopsy cassettes may also be used. The core has some of these supplies – please inquire (extra charges may apply).

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How should I label my cassettes and how will the core label my slides?

Always use #2 pencil or histology pen to label cassettes, NEVER use a sharpie or regular pen since solvents used during processing will wash these labels off the cassettes. The core is not responsible for loss of identification of improperly labeled cassettes during processing. For best results, enter or upload a printed list of your desired sample IDs at the time of request submission in MiCORES. Even the neatest of handwriting can be smeared during processing and embedding, so uploading this printed list will save time and avoid mistakes. For your slides, we will label your slides exactly as labeled on your cassettes (8-character limit) unless you specify otherwise on your sample ID list in MiCORES.

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What processing schedule will be used for my samples? Do you use eosin during processing?

Our core has optimized 3 different standard tissue processing schedules based on the tissue size and species of origin. Short schedules are used for delicate mouse tissues or biopsies of similar delicacy. Longer schedules are used for rat and for larger animal tissues. Details of the processing schedules are available upon request. Customized processing schedules other than these 3 are not available. We do not include eosin in our processing solutions.

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What tissue thickness will be used for sectioning my paraffin-embedded samples and how many sections are placed per slide?

Our standard paraffin sectioning thickness is 4 microns. Other thicknesses are available upon request, but keep in mind that our staining procedures are optimized for standard, 4-micron-thick sections. Our standard sectioning protocol involves one tissue section per slide. Additional sections per slide may be placed (tissue size permitting) but additional charges may apply (Histology technician time).

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When will my samples be completed? How do I check submission status?

Turn-around times vary depending on the queue and the project type. We also batch-run similar submissions to ensure species-appropriate processing schedules and to maximize reagent utilization. In general, routine histology requests run between 1-4 weeks. Special stains or immunohistochemistry can take 4-8 weeks or longer for specialty projects. You can follow the progression of your samples through trimming, processing/embedding, sectioning, staining, scanning or analysis by following the milestones within MiCORES.

Please check MiCORES before emailing the core about your submission status – our techs are bench techs and time spent answering emails takes away from time spent working on your submission. You can also enter a comment within MiCORES to ask a specific question about your submission.

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How will I pick up my samples?

You will get an email message from within MiCORES when your submission has been completed. This message will ask you to contact us with a requested day/time/location for pickup. Once we hear from you, we will schedule your pickup and send you a link to the appointment. Please be punctual and contact us if you need to reschedule. We have limited storage space and samples left in the core after completion for >1 month without communication are subject to discard.

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Why did my submission cost more than the initial quote in MiCORES? Can I get a more precise estimate ahead of time?

The MiCORES quotes generated at submission are a rough guideline. Actual charges may be higher or lower depending on the request. The core will add necessary services omitted in the initial submission or that become evident upon sample receipt. Complex trimming or sectioning requests (very precise tissue targets, multiple sections per slide, serial or step sectioning) will necessitate additional technician time added. Supplies (slide boxes for sample return) will also be added. You may supply your own slide box with your submission if you wish.

You can follow your project costs within MiCORES and ask questions if you have concerns. For large projects we encourage you to request a project-specific estimate to help with planning purposes. This is generally the most accurate way to project expenses. Project-specific estimates are available upon request from the ULAM business office and are highly recommended for complex requests - please use the Consultation button within MiCORES or email us directly.

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Can someone help me assess histological findings in my samples?

Our board-certified veterinary pathologists are available to provide histology interpretation, lesion severity scoring, and/or quantitative digital pathology analysis. Pathology requests may be submitted through MiCORES. If this is your first pathology interpretation request, please request a consultation and see our Pathology FAQs.

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How can I take images of my histology samples or perform digital pathology analysis?

Digital slide scanning may be requested through our MiCORES site. Freely downloadable software (Leica Aperio ImageScope, QuPath) may be used to generate images or perform analysis from the digitized slide files. See our Digital Slides FAQs or request consultation.

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How can I be assured of sample and process quality for histology services?

Our technicians are highly trained and include individuals with ASCP(HT) certification. They have years of experience in the preparation of histological specimens in a research or industry setting and participate in continuing education through the National Society of Histotechnology and other resources. We follow standardized operating procedures developed and optimized for the tissue and species types processed by our laboratory. Slides are subjected to quality control inspection before leaving the laboratory. Our laboratory is overseen by board-certified veterinary pathologists experienced in standard models, nomenclature, and lesion criteria for the evaluation of preclinical models and animal models of human disease.

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Do you have a methods description for the generation of standard histological slides?

General wording for the generation of hematoxylin-and-eosin stained slides from tissues in fixative is provided below. Modifications for your particular stain or tissue type may be necessary. Please remember to mention our core in your Acknowledgments or Methods section. This metric is important for us to continue providing services.

“Histology preparation was performed in the ULAM In Vivo Animal Core pathology laboratory at the University of Michigan. After fixation for 48 hours in 10% neutral buffered formalin, tissues were trimmed, cassetted, and processed to paraffin in an automated tissue processor (TIssueTek, Sakura). Processed tissues were embedded in paraffin and sectioned at 4 microns on a rotary microtome (Leica Biosystems, Buffalo Grove, IL). Tissues were mounted on glass slides and stained with hematoxylin and eosin using routine protocols on an automated histostainer (Leica ST5010 Autostainer, Leica Biosystems), followed by coverslipping.”

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Can you support GLP (Good Laboratory Practice) studies?

Our laboratory is not a GLP-certified laboratory as might be found in industry. Our area of focus is quality support of academic research and early phase (non-GLP) translational research. For GLP studies you should consult an outside contract research organization.

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