UNIT FOR LABORATORY ANIMAL MEDICINE
PATHOLOGY CORE
UNIVERSITY OF MICHIGAN

Immunohistochemistry (IHC) Sample Guidelines

What immunohistochemical (IHC) standard protocols are offered through the Core?

We provide brightfield immunohistochemistry on paraffin-embedded or frozen tissues (with some exceptions) using an automated platform (Biocare Intellipath) and polymer-based, biotin-free detection reagents. Detection is based on horseradish peroxidase (HRP) catalysis of a DAB (brown) chromogenic reaction, with a hematoxylin (blue) nuclear counterstain.

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Can modifications be made to the Core's standard protocol (e.g. different chromogen, dual-stains)?

We offer customized protocols such as dual-labeling and alkaline phosphatase conjugated polymers with a red chromogen. Note these will incur additional charges and may extend turnaround time. Contact ULAM-PathologyCore@umich.edu for more details. Currently, we only offer brightfield IHC detection.

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Do you offer immunofluorescence (mono-plex or high-plex)?

At the current time we do not offer immunofluorescence.

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What tissue formats (FFPE, OCT) and fixative types do you accept?

We accept both FFPE and OCT (frozen) samples. For frozen samples we strongly recommend that the sample quality of each block be checked in an HE slide prior to IHC. You can supply previously generated HEs (or images) or we can generate these for you. This is because IHC will not work appropriately with freeze artifact, and it is a waste of your time and money to proceed with IHC on freeze-damaged tissues.

Most of our antibodies have been titrated on Formalin fixed, Paraffin embedded (FFPE) samples, however we have several antibodies that are known to work on other fixatives such as Zinc-tris, Zinc-Formalin, Methacarn as they may require different protocols. Be sure to indicate your specific fixative at submission. Tissues submitted in a new fixative may require titration even for established antibodies.

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What species will you accept?

We specialize in animal tissues and accept almost any type of animal tissues, including non-rodent species. For non-rodent species, control tissues may need to be provided by the client and additional titrations may be necessary.

Although we specialize in animal tissues, we do accept human tissues in the form of xenografts or de-identified human tissues. Human tissues must be fixed tissues or frozen non-CNS tissues. Remember to indicate when your samples are xenografts.

We do NOT accept frozen human CNS tissue. For fixed human CNS, we ONLY accept tissue that has documentation that the tissues do not originate from samples obtained from patients suspected or known to have a prion disease. Usually this is available as a statement from a Brain Bank or other clinical sample source. This should be a statement from the original sample provider, but not medical record info or other identifying patient information - remember that we only accept de-identified human samples.

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What primary antibodies are offered through the Core?

Core-provided antibodies are based on most frequent requests. Our emphasis is on antibodies designed to perform well on animal tissues. See our MiCORES list of frequently offered antibodies - please be sure to indicate your target species at submission since our antibodies only have defined, validated protocols for certain species (most commonly mouse and human, please inquire through MiCORES for others).

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Do you offer IHC using antibodies not on the Core's list?

The Core can run IHC with non-Core antibodies (with some exceptions) if the primary antibody is supplied by the client. We specialize in animal tissues but can run immunohistochemistry on fixed, de-identified human research tissues and on some frozen, non-CNS human tissues. We have titrated multiple antibodies on diverse species, fixatives, and sample types. Please inquire prior to purchasing a new antibody. (See also **How do I select the most appropriate new antibody for IHC titration?)**

- If the non-Core antibody has previously been titrated by the Core and falls under the same conditions as the initial optimization, no new titration is required. You must verify you are submitting the same species, processing conditions including fixative, format (FFPE vs OCT embedded), and the use of decalcification with the same decalcification reagent prior to submission. Your request may be rejected or result in a time delay if these criteria are not met. On occasion, a new titration may still be required if a different tissue type is submitted.
- If the new antibody does not meet these criteria, it will require a new titration. Antibody titration involves optimizing the appropriate dilution, incubation time, detection systems, and antigen retrieval procedures for the IHC reaction. A specification sheet (downloadable through the vendor) also must be uploaded via the "Upload specification sheet from manufacturer" field. (See also **Submitting IHC requests through MiCores** for more details**)**

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Do I still need a titration for a new antibody if I have literature, vendor, or experiential information on which titer has been previously used for this antibody?

For antibodies that we have not run before in the ULAM Pathology Core laboratory on our platform, a new titration will be required. Skipping this step can delay your project and increase costs if an entire set of slides is wasted on an antibody run under suboptimal conditions. These parameters can vary from laboratory to laboratory even for antibodies from the same vendor and lot number depending on detection platforms, instrumentation, tissue type, tissue preparation differences, species differences, and other factors

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How do I select the most appropriate new antibody for IHC titration?

- Select a reputable supplier. Our preferred vendors are Cell Signaling Technolgy and Abcam.
- Carefully review the spec sheet from the vendor. Ensure the antibody is designed specifically for IHC on paraffin and is known to cross-react with the experimental species. While other antibodies designed for applications such as western blots, Flow Cytometry, ICC (immunocytochemistry) or IHC-Frozens may occasionally work on FFPE samples and/or cross react with other non-tested species, selecting an antibody that has been validated by the vendor increases the chance of a successful titration. In most cases, the vendor will refund your money if the antibody does not perform to expectations.
- Prior to purchase, we recommend to send the spec sheet to our Core's immunohistochemical team (ULAM-PathologyCore@umich.edu) for review.

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What if I have to use an antibody made in the same species as my target species (e.g. mouse-on-mouse IHC)?

To avoid non-specific labeling by the secondary reagents, we recommend trying to find an antibody made in a non-target species wherever possible. If not possible, there are specialized methods that we can employ to try to minimize non-specific staining in "mouse-on-mouse" IHC reactions, but the results can be variable and careful interpretation with appropriate controls is required. Optimization as for a new antibody will be required and additional fees will apply.

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What controls are run with IHC reactions? Do I need to supply control samples?

Positive controls

For Core-offered antibodies, we provide positive control tissue for Core-offered antibodies run on species that we have previously validated. The purpose of this control is to determine whether the antibody is staining appropriately in a tissue with known expression pattern previously validated preparation conditions.

For investigator-provided antibodies, you should provide your own positive control in the form of a tissue anticipated to be positive for the target expression. Ideally this should be from the same species as your experimental slides but if your antibody has not been tested against your species, it may require a sample from a species for which the antibody has been previously validated. We may or may not be able to supply this, depending on the species.

Negative controls-

For all IHC reactions, reagent negative controls are performed for each run. These consist of an extra slide cut from one of your experimental blocks and stained with commercially obtained naive serum from the same host species in place of the primary antibody. The purpose of this control is to ensure that the secondary detection reagents do not non-specifically bind the target tissues.

In some instances, a second negative control type is useful. This is most frequently used during new titer optimization for investigator-supplied antibodies where the expected staining pattern is uncertain. For antibodies that show diffuse staining in the target experimental tissue, another tissue type anticipated to be negative can be run to ensure that staining is tissue-specific. This can be run either as a reagent negative control (naive serum in place of the primary) and/or a tissue negative control (run using the primary antibody). These samples may be provided by the client or by the Core depending on the circumstances. Please inquire if you have questions about appropriate controls for your submission.

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What quality control (QC) measures are employed and how are new IHC reactions validated?

Routine Core antibodies with previously determined protocols and staining patterns are QC'd by the senior IHC technician prior to release. Staining is assessed in terms of positive and negative controls and expected staining as described below.

New antibody or new tissue/condition titrations are validated by our board-certified veterinary pathologists in terms of:

- Appropriate staining of positive controls: Expected positive staining distribution, cell and compartment types and the absence of positive staining in cells/compartments that should not express the target; if non-specific staining is present, it should be easily distinguishable by site and/or intensity from true positive staining (ex. non-specific staining of mucin, erythrocytes, or keratin)
- Appropriate staining of negative controls: no or minimal staining when non-immune sera is used in place of the primary antibody
- Appropriate intensity/specificity of staining for the type of analysis intended (if known): IHC intended for automated analysis of digital slides may need a different intensity and specificity of staining than slides intended for manual, descriptive interpretation

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How do I submit IHC requests through MiCORES?

Standard Core antibodies and previously titrated slides require the following entries per antibody:

- Unstained slides, paraffin (or frozen) for Core to stain:
  - The number of experimental IHC reactions you would like us to run PLUS 2 unstained slides for positive and negative controls
  - If you are submitting unstained slides previously sectioned, enter only 2 unstained slides for positive and negative controls
- For Core antibodies, enter the number of experimental slides PLUS 1 slide for a positive control in the desired primary antibody field. In the negative control field, enter 1 slide
- For previously titrated antibodies, enter the number of experimental slides PLUS 1 slide for a positive control in the IHC Stain "Other" field. Indicate the name of the non-Core antibody in the "Specify the antibody (customer supplied)" field. In the negative control field, enter 1 slide

Antibodies requiring new titrations require the following entries per antibody (limit 3 titrations per request):

- Attach the antibody spec sheets through the "Upload specification sheet from manufacturer" link.
- Unstained slides, paraffin (or frozen) for Core to stain:
  - Five (5) slides for titration PLUS
  - The number of experimental IHC reactions to be run PLUS 2 unstained slides for positive and negative controls
- In the "IHC New Antibody optimization slide" field, enter 5 slides. More OR less slides may be used depending on the complexity of the titration, but the Core team will update this later as needed.
- In the "IHC Stain other (provided by PI) field, enter the number of experimental slides to be stained after a successful titration, PLUS 1 slide for a positive control. In the negative control field, enter 1 slide