Immunohistochemistry FAQ Sheet

Q: What is the Core's standard IHC technique?

A: We routinely offer bright field IHC using secondary antibody polymers conjugated to Horseradish Peroxidase (HRP) developed with a DAB chromogen, resulting in an insoluble colored brown precipitate.

Q: Does the Core offer IHC techniques other than bright field with DAB?

A: Yes, several other IHC techniques can be performed by the Core, including but not limited to immunofluorescence, double-labeling and multiplexing using Opal technology. Please contact Dr. Dafydd Thomas for non-standard IHC technique requests: thomasda@med.umich.edu.

Q: Can the Core use antibodies provided by a researcher?

A: Yes. Please contact the Core before ordering an antibody to see if we already have a working protocol/antibody for your target of interest. When ordering an antibody, look for an antibody that will stain the species, format and fixative of the samples you will be submitting. Avoid ordering same species antibodies (example: monoclonal mouse antibody for staining mouse or mouse xenograft tissue).

Q: Do I need to provide a positive control tissue?

A: For antibodies offered on our core antibody list you do not, we will provide a positive control with your stained slides. For PI provided antibodies we do ask if possible to provide us with positive control tissue. We do have access to a wide variety of FFPE human tissue, however if you are studying non-human tissue (rat, mouse, rabbit, etc.) we ask that you provide a suitable positive (WB or PCR) control tissue fixed and processed in a similar manner to your experimental tissue. If you are studying a rare human disease, we also ask that you provide a suitable control tissue. **We will not accept tissue blocks for Transmissible Spongiform Encephalopathies (TSEs), also known as prion diseases.**

Q: What is antibody titration?

A: Antibodies supplied to the core by a PI for testing need to be optimized on known control tissues before staining of the PI's samples can be done. There is a per slide antibody titration charge; an average titration will use 2-4 slides. There will also be an unstained slide charge if control slides for the titration are sectioned by the core. Antibody titration charges will be charged for the titration/optimization slides only. After optimal conditions are achieved, samples stained will be charged at the rate for Group I antibody charges (Antibodies provided by PI).

Q: Are the completed IHC slides QC'd by the Core before being sent to the PI?

A: Yes, the technician performing the IHC and Dr. Thomas will review the stained IHC slides for quality control before they leave the core.

Q: Can you use the same immunohistochemistry protocols as the clinical immunohistochemistry lab?

A: Currently, the Core has DAKO and Ventana Discovery Ultra autostainers. Because our Ventana autostainer is a different model than the clinical Ventana, there will be a round of optimization required to adapt the clinical immunohistochemistry protocol to our platform. In most cases we will be able to use the same antibody and a similar protocol to the clinical immunohistochemistry lab, although this can be antibody- and project-dependent.

INFORMATION WE WILL NEED ABOUT YOUR SAMPLES:

Q: What format are your samples?

A: We need to know if your samples are frozen in OCT (fixed or unfixed) or paraffin-embedded, as this will determine what protocols to use. Our list of available antibodies is optimized for 10% NBF fixed paraffin embedded tissue (FFPE); any other sample will have to be optimized.

Q: What fixative was used on your samples?

A: We need to know the fixative you used to fix your tissue samples as this will determine what protocols to use. Our list of available antibodies is optimized for 10% NBF fixed tissue; any other fixative used will have to be optimized.

Q: What species are your samples?

A: We need to know what species your tissue samples are, as this will determine what protocols to use. Our list of available antibodies is optimized for human tissue (unless otherwise specified); any other species submitted for staining will have to be optimized.

Q: What species is your investigator-supplied antibody made in? Was the antibody produced commercially or non-commercially?

A: We need to know what species your investigator antibody is made in, as this will determine what protocols to use. If your antibody is commercially made, send us a copy of the antibody specification sheet along with your antibody. If your antibody was non-commercially made, send us a sheet listing antibody name, antibody type (monoclonal or polyclonal), species antibody was made in (antibody host), antibody clone, antibody format (unconjugated, biotinylated, etc.), antibody isotype (IgG class) and if it is known to work on FFPE tissue.