IHC FAQ Sheet

Q: What is the Cores 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](mailto:thomasda@med.umich.edu) .

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: 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: 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 known if it works on FFPE 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 - 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.