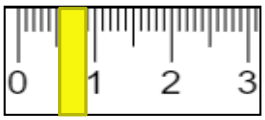
Don't waste your money and time! Use this IHC check list to identify the right antibody for your study!

FIRST – TISSUE PRESERVATION

Even the *best* antibody won't work if the tissue is not correctly collected.

Click here to get additional details on appropriate tissue collection and fixation/freezing.



- ⊘ Never rinse your tissues in anything but isotonic solutions (no water rinse!)
- ✓ Always trim tissues to < 4 mm in one plane (less for anti-Phospho Ab)
- ✓ Always fix tissue at least 72 hours (prolonged fixation is not a problem for most IHC)
- ✓ Always use at least 10 volumes of fixative to 1 volume of tissue

Fixative considerations

Usually 10% neutral buffered formalin (10% NBF) or 4% paraformaldehyde (4% PFA)
Formalin-fixed tissues work well for *most* IHC

- 10% NBF fixation is at room temperature
- 4% PFA fixation is a 4C

Place in 70% EtOH *only when ready to transfer* to the PSC facility

Frozen sample considerations

Click here to see how to properly freeze tissues for histology endpoints

Consider fresh frozen samples for IHC using 1) newly generated antibodies or 2) antibodies reported only for flow cytometry or western blots

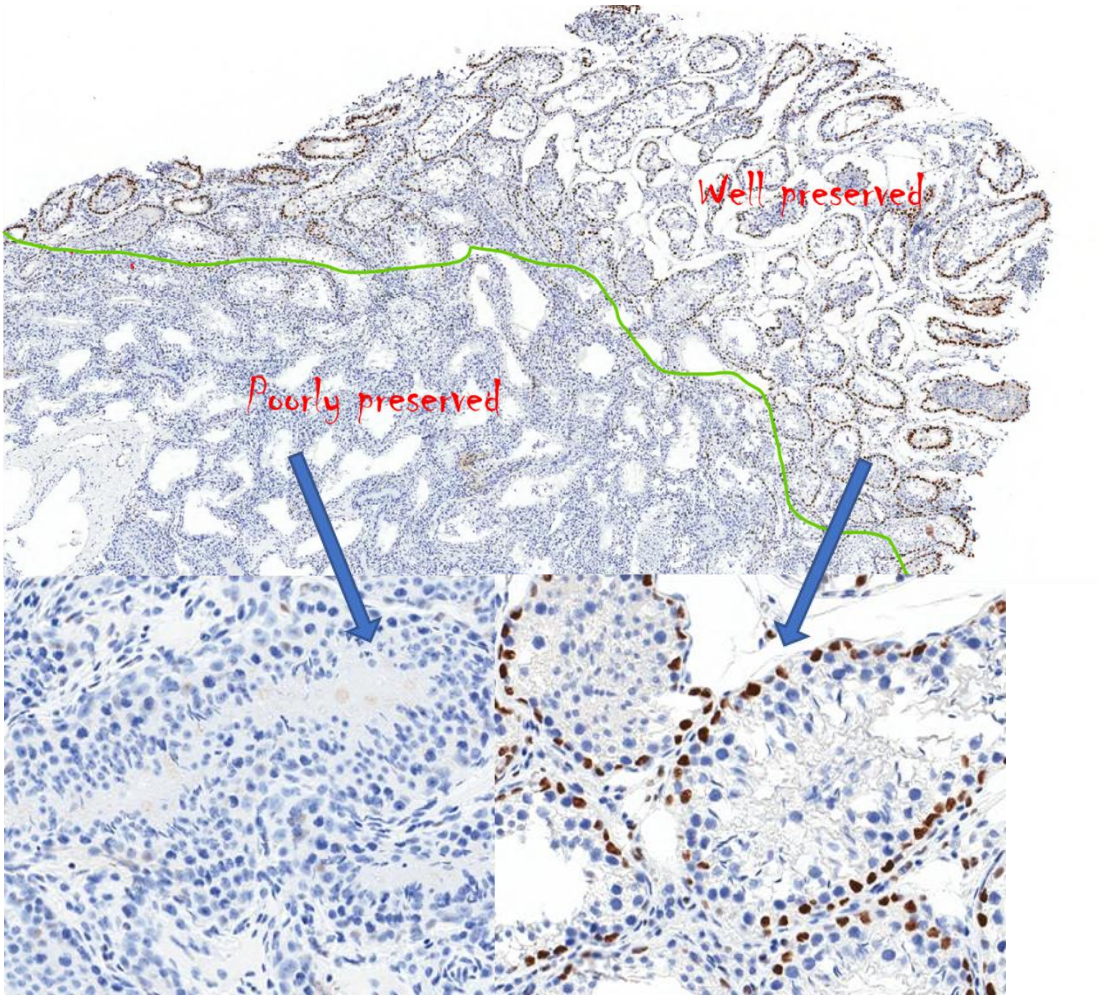
- Except for muscles, freeze tissues in OCT medium in cryomolds (freeze muscle directly in N_2)
- Freeze tissue rapidly (in 2-methylbutane or liquid nitrogen) and store wrapped at -80C
- Transport to facility on DRY ICE!!! *Never* let samples melt

Fixing or freezing – how to decide

TISSUE COLLECTION	PROS	CONS
Fixed Tissues	Excellent morphology Easy Works for most IHC/IF Works for ISH Fixed/stored at RT Works well for bone	IHC may require antigen retrieval Some antibodies for IHC/IF don't work Chemical irritant
Frozen Tissues for Cryosectioning	Required for enzyme-based stains Good for IHC for problem antibodies	Suboptimal morphology Time consuming Not good for bone Storage at -80C

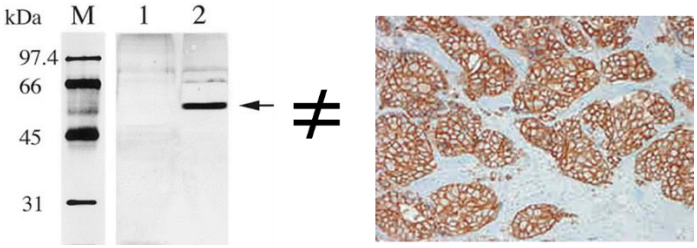
MYTH: Tissues should be placed in fixative for the shortest time possible to optimize immunogenicity

TRUTH: Incompletely fixed tissues will have less consistent IHC results and more background artifacts



SECOND – SELECTING THE PERFECT ANTIBODY

One antibody doesn't fit all



Great antibodies for western blot or flow cytometry may not make great IHC antibodies

✓ Always check the vendor site to confirm it can be used for IHC-P (paraffin) or IHC-F (frozen)

Vendors are a great resource – if you call, you may get additional information on the antibody that is not on the website

Buyer Beware

Not all vendors are equal – You often get what you pay for

The Good: Some guarantee their antibodies (ie, you get your money back if it doesn't work)

- Abcam, Novus, Dako

The Bad and Ugly: Some antibodies are poorly purified, are inadequately tested, etc.

- There are lots of fly-by-night (and some legitimate) companies that will sell you a terrible antibody for a lot of money

Do your homework –

- Make sure the images on the vendor sites reflect accurately the subcellular distribution of the antibody - membrane, nuclear, cytoplasmic
- Make sure the images on the vendor sites reflect accurately the cell type specificity ~ *Lots of nonspecific staining is passed off as "real" (brown ≠ real)*
- Review the literature to identify normal tissue distribution in your species
- Review the literature to identify tested antibodies

Monoclonal vs Polyclonal Antibodies

- Monoclonal antibodies recognize single epitope
  - Greater specificity
  - Kits to reduce background in mouse tissues with mouse monoclonal antibodies
  - Rat and rabbit monoclonal antibodies may be better for use in mouse

- Polyclonal antibodies recognize multiple epitopes
  - Greater sensitivity than for monoclonal antibodies
  - Increased risk of binding non-specific molecules with similar epitope

- Significant batch to batch variation in staining – one batch might work great and another may not work at all (*If polyclonal antibody does not work as they say it should, call and request a different batch*)

THIRD – IHC OPTIMIZATION

IHC requires patience. And control tissues!

- ✓ Always use a positive control (tissue or cell pellet know to express the protein of interest)
- ✓ Always use a negative control (tissue or cell pellet which doesn't express the protein of interest)
- ✓ Always include an Ig Isotype control slide (used at same concentration as primary antibody)
- ✓ Always optimize using a range of concentrations around the recommended vendor concentration (e.g., vendor recommends 1:200, try 1:100, 1:200, 1:400)

Cell pellets

Click here to see how to properly

Collect cells expressing the protein of interest – generally scraping cells from the plate rather than using trypsin is preferred.

Can be used for frozen or fixed IHC testing

For detailed IHC protocols, click here.