C U S T O M E R S  T A L K ,  W E  L I S T E N

# HISTOLOGY TIPS & TRICKS

## QUESTIONS & ANSWERS

### How do you better ensure appropriate fixation and processing times for samples?

Tissue type, size and grossing methods will in part determine appropriate fixing time. Therefore, tissue processing should be optimized for each sample type.

Gross tissue should be of even thickness at no more than 3-4 mm to ensure sufficient fixation and reagent penetration.

Avoid overloading cassettes with tissue to ensure proper reagent flow through cassette and tissue.

Have adequate available fixing agent at 20:1 fixative-to-tissue ratio.

Cold fixation should be avoided. Room temperature or heated fixation allows for quicker penetration and fixation as the reaction is endothermic.

For manually prepared fixatives, confirm solution is at the appropriate concentration, optimal pH and limited exposure to oxidation.

### Battle of the fixative: Things to consider

Alcohol: A dehydrant/coagulant that destroys protein tertiary structure by displacing water, causing proteins to precipitate.

Formalin: An aldehyde that forms methylene bridge between proteins, forming covalent cross-links.

Coagulant fixatives are less toxic BUT can alter tissue morphology.

Cross-linking fixatives penetrate tissue quicker than alcohol BUT can distort or mask antigens for detection.

### Most common fixative & its optimal pH?

**Neutral buffered formalin**
**pH 6.8-7.2**

### How to reduce nonspecific background IHC staining?

Adjust antibody concentration and incubation time to optimize sensitivity and specificity while balancing signal to noise ratio.

Consider switching from biotin-based detection to polymer-based detection for increased sensitivity without endogenous biotin background staining.

Quench for endogenous peroxidases if not already part of the staining protocol.

Increase time and/or concentration of blocking agent; if using animal serum, ensure there is no cross-reactivity.

Increasing washing steps between each stage of IHC staining protocol can reduce background further.

Ensure sections do not dry out during the IHC process to avoid air-drying artifacts.

### How to get clearer tissue sections after staining?

For appropriate clearing of sections, optimize and ensure sufficient time and changes of:

Ascending alcohol concentrations for dehydration. The final alcohol container should not have any water present.

AND

Clearing agent (e.g. xylene/ xylene substitute.). Confirm there is no water contamination in these reagents, and it should not have any alcohol present.

Avoid drying out of tissue sections to prevent introduction of air-drying artifact.

Confirm compatibility of the stain with dehydrant, clearing agent and mountant, as well as the mountant with clearing agent.

Ensure mountant is free of crystalline structures.

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