COLLEGE of AMERICAN PATHOLOGISTS
Laboratory Quality Solutions

NATIONAL SOCIETY FOR HISTOTECHNOLOGY
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IHC Troubleshooting

Problem	Cause	Solution
1. Absence of staining or weak staining of patient tissue with adequately stained positive control.	a. Loss of antigenicity due to delayed, inadequate or extended fixation.	i. Repeat using a different patient tissue block if available. ii. Repeat using a more aggressive epitope retrieval method (e.g. change buffer's pH).
	b. Patient tissue sections are allowed to dry after the heat induced epitope retrieval method.	i. Once deparaffinized, ensure that slides remain wet throughout entire protocol.
2. Absence of staining or weak staining with weakly stained known positive control.	a. Incorrect concentration of primary antibody.	i. Repeat using freshly prepared antibody at the previously determined dilution. ii. Confirm antibody has been stored at recommended temperature. iii. Check expiration date of antibody. iv. Prepare freshly cut positive control.
	b. Loss of immunoreactivity due to the use of aging antibody stock solution.	i. Re-titer primary antibody using at least three consecutive dilutions on a previously validated positive control tissue. ii. Replace with a new lot of antibody. Once validated, snap freeze concentrate, placing at -70°C for long term storage.
	c. Hematoxylin counterstain too dark, and masks positive staining of nuclei.	i. Decrease staining time in hematoxylin or use a weaker solution of hematoxylin.
3. No staining of the patient or control tissues.	a. Incorrect pretreatment is used.	i. Review antibody specification sheet and check the validation protocol to ensure correct pretreatment process or reagent was used.
	b. Incorrect pH of retrieval solution.	i. Check pH of retrieval solution.

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	c. Primary antibody not applied or wrong primary antibody applied.	i. Repeat, ensuring that sufficient antibody is applied (manual or automated). ii. Repeat, ensuring slide is correctly labeled to identify correct antibody application on automated staining platforms that use barcode technology. iii. Review run log for errors on automated staining platforms, service instrument if needed. iv. Review antibody bottle for date prepared to check for tech preparation error.
	d. Incorrect protocol is followed.	i. Confirm that the correct protocol is being applied (manual or automated) using the correct staining sequence and that individual steps or reagents are not missed or exchanged and repeat.
	e. Expired reagents.	i. Inventory all reagents used for expiration dates. ii. Replace expired reagents with fresh and repeat.
	f. Detection reagents are not applied or not allowed to incubate for sufficient time.	i. Repeat staining, ensuring that all reagents are applied in the correct order and incubated for the required time indicated on the protocol.
4. Uneven staining of patient tissue.	a. Inadequate primary fixation in formalin leading to secondary alcohol fixation of parts of the tissue block.	i. Repeat using a different tissue block, if available. ii. Repeat using different (or omit) epitope retrieval protocols.
	b. Incomplete deparaffinization of tissue sections.	i. Use fresh clearant and/or extend time in clearant during deparaffinization.
5. Diffuse nonspecific staining of patient tissue with adequate positive control.	a. Fixative used for patient tissue is not compatible with validated protocol.	i. Repeat staining with tissue block fixed with appropriate fixative for antibody to be used. ii. Validate a new protocol for fixative being used.
	b. Degenerating cells or necrotic tissue being evaluated.	i. Repeat using a different block that contains tissue away from the necrotic tumor.

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	c. Nonimmunological binding of detection reagents caused by pseudoperoxidase activity (high concentration of erythrocytes), endogenous peroxidase or endogenous biotin present in patient tissue.	i. Incorporate a blocking peroxidase pretreatment step in protocol. ii. Incorporate a protein blocking step, if not routinely applied. iii. Incorporate avidin-biotin blocking steps.
6. Nonspecific staining of the patient and control section.	a. Microbial or chemical contamination of the reagents.	i. Always dilute, dispense and store primary antibodies and reagents following manufacturer's instructions. ii. Ensure that equipment used to make and store all reagents is clean or sterile, if required. Rinse well with distilled water to remove detergents.
	b. Normal sera used for blocking is from the same species as the primary antibody.	i. Ensure that blocking sera is from the same species as the secondary antibody.
	c. Detection substrate is trapped under the tissue.	i. Repeat stain with sections that do not contain wrinkles, air bubbles, or water under the section. ii. Use a slide with a strong adhesive such as aminopropyltriethoxysilane or positively charged slides to ensure section attachment to the slide.
7. False positive staining of nontarget cells.	a. Cross reactivity of the antibody with tissue cellular components.	i. Use a purified primary antibody that has had nonspecific immunoglobulin removed. ii. Use a monoclonal antibody, if available. iii. Compare IHC stained slide to H&E slide and identify target vs. nontarget cells.
8. Nonspecific staining of patient tissue with over stained positive control and overstained specific targets in patient tissue.	a. Concentration of the primary antibody is too strong.	i. Check dilution of antibody and repeat using freshly prepared antibody. ii. Re-titer the primary antibody; testing at least three consecutive dilutions of the antibody on control and patient tissue.
	b. Section too thick.	i. Cut all sections at the same thickness as section used to validate antibody dilution.

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9. Tissue detachment.	a. Overly aggressive epitope retrieval.	i. Repeat using a less aggressive epitope retrieval protocol. ii. Increase slide drying time. iii. Use a slide with a strong adhesive such as aminopropyltriethoxysilane or positively charged slides to ensure section attachment to the slide.
	b. Poorly fixed and/or processed tissue.	i. Repeat using a different tissue block, if available. ii. Increase slide drying time. iii. Use a slide with a strong adhesive such as aminopropyltriethoxysilane or positively charged slides to ensure section attachment to the slide.