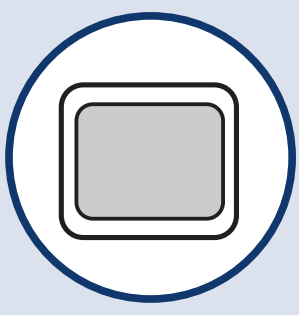
GENERAL IHC-P TROUBLESHOOTING TIPS

SYMPTOM	ISSUE	RECOMMENDATIONS
 No Signal	Antibody Application	Increase the concentration or incubation time of the primary or secondary antibody.
	Tissue Fixation	Over fixation can cause epitope masking. Decrease the time or concentration of the fixative. Under fixation can cause heavy edge staining with little to no positive signal in middle of your specimen.
	Antibody Compatibility	Confirm that your primary and secondary antibodies are compatible by checking the species reactivity.
		Confirm the antibody can be used for assays in which the protein is in its native conformation.
		Ensure that the secondary is working and compatible with your primary.
	Permeabilization	Use 0.5-1.0% Triton detergent in the buffers in order to allow full permeabilization of antibody and buffers into the tissue sections.
	Microscope Adjustments (Fluorescence)	Increase the exposure time of your camera.
 Background	Antibody Concentration	Decrease the concentration of the primary/secondary antibody.
	Blocking	Increase the incubation time or concentration of serum in the blocking buffer.
	Antibody Application	Always incubate primary antibodies overnight at 4° C. Room temperature incubation increases unspecific binding and causes higher background.
		Confirm that the secondary is not crossreacting with the cells by performing the assay without the primary.
	DAB Reaction	Do not overexpose the DAB reaction. Rinse DAB off slides sooner.
		Endogenous peroxidases are activating the reaction. Quench with hydrogen peroxide.
	ABC Method	Endogenous biotin is activating the complex. Block with avidin/biotin blocking kit.
	Cells Drying	Fluorescent signal will be lost if the cells are allowed to dry. Ring coverslips with nail polish.
	Microscope Adjustments (Fluorescence)	Increase the exposure time of your camera.
	Spectral Overlap (Fluorescence)	If double or triple labeling the cells, confirm that the secondaries do not overlap into the same spectral range.
	Washing	Increase the amount of washes. Add very gentle agitation to the plates.
 Sectioning Tissue	Holes in the tissue	Ensure the blade is adequately sharp. Adjust the cutting speed. Perfuse at a lower rate.
	Tissue Falling Off Slides	Use coated slides. Place slides in covered dish with a small amount of 16% formaldehyde on the bottom to fix the tissues to the slides. Be gentle when using an antigen retrieval method and avoid heavy agitation of the slides.

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