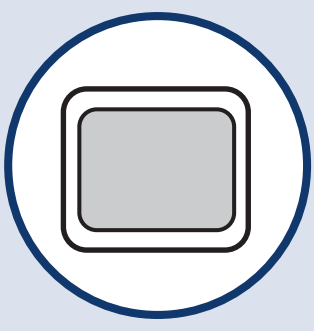
GENERAL ICC-P TROUBLESHOOTING TIPS

SYMPTOM	ISSUE	RECOMMENDATIONS
	Antibody Application	Increase the concentration or incubation time of the primary or secondary antibody.
	Permeabilization Buffer	Use the proper permeabilization reagent for the target protein's localization. Triton detergent is necessary for mitochondrial or nuclear proteins, but will dissolve the outer membrane and disrupt proper membrane localization.
		Increase incubation duration or detergent concentration.
	Cell Fixation	Over fixation can cause epitope masking. Decrease the time or concentration of the fixative.
	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 microscope's filter sets by using a positive control primary.
	Target Availability	Use an overexpression assay or positive control cell line known to express the protein of interest.
	Cells Drying	Fluorescent signal will be lost if the cells are allowed to dry. Ring coverslips with nail polish.
	Cell Viability	Confirm cell viability before starting the staining procedure.
	Microscope Adjustments	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. Use blocking buffer for primary and secondary antibody dilutions.
	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.
	Contamination Artifacts	Ensure slides are clean and free of dust. Buffers should be made fresh to prevent microbial contamination.
	Washing	Increase the amount of washes. Add very gentle agitation to the plates.
		Increase the concentration of Tween in the PBS-T.
	Spectral Overlap	If double or triple labeling the cells, confirm that the secondaries do not overlap into the same spectral range.

NOVUS BIOLOGICALS

phone - 1-888-506-6887 | fax - 303-730-1966 | novus@novusbio.com | www.novusbio.com