Immunohistochemistry TroubleShooting Guide

Problem	Possible Cause	Solution
No signal or weak signal	Epitope recognized by antibody not expressed in tissue sample	Verify expression of protein/antigen by immunoblotting or other method. Primary antibody may not react with the protein of interest from the species being studied. Check the literature/data sheet and protein sequence information.
	Epitope recognized by antibody destroyed by excessive antigen unmasking/retrieval	Determine optimal digestion procedure
	Antibody concentration not optimal	Determine optimal working dilution for primary antibody by titration. Consider using more antibody if no signal or weak signal is detected.
	Incubation time with antibody not adequate	Increase incubation time.
	Primary antibody does not have access to epitope recognized by antibody due to cross-linking caused by aldehyde fixation.	Consider unmasking with proteases. Optimal conditions must be determined empirically
	Enzyme activity not optimal	Increase incubation time with the substrate. For peroxidase make sure sodium azide is not present in substrate solution
	Substrate or conjugate is weak or no longer active due to age or improper storage	Test conjugate and substrate for activity. For example, add enzyme conjugate to substrate solution. It should change color.
	Incorrect substrate for application or substrate prepared incorrectly	Make sure that the substrate selected is appropriate for the enzyme conjugate (see substrate selection guide).Follow instructions that accompany the substrate. Make sure that fresh H_2O_2 is added if necessary.
High Background	Aggregates	Centrifuge antibody conjugate briefly in microcentrifuge at highest speed to remove antibody aggregates.
	Antibody concentration not optimal	Determine optimal working dilution for primary and secondary antibody by titration. Consider using less antibody if background is too high.
	Antibody binding to Fc receptors on cell surface, or is binding non-specifically to cell components.	Incubate sample with 10% serum (from same species as the host of the secondary antibody) to occupy Fc receptors prior to applying antibody conjugate.Use a labeled secondary antibody that is selective for Fab fragment to help reduce background.
	Wash steps not adequate	Increase number or length of washes.
	Blocking is not optimal	Increase length of incubation with BSA/normal serum (from the same species as the host of the secondary antibody)or increase their concentration. Use a different blocker or fresh batch of the same blocker.
	Secondary antibody cross-reacts	Use secondary antibodies that have been absorbed with immunoglobulins or serum from the same species as the tissue samples.
	Slides have dried during the procedure	Incubate in humidified chambers and avoid allowing the sections to dry.
	Incubation with substrate is too long	Shorten substrate reaction time.
	Endogenous enzyme inhibition is not adequate	Use new hydrogen peroxide (peroxidase system) or levamisole (alkaline phosphatase system).