

## OVER STAINING

Sources	Possible Solutions
<b>High concentration of primary and / or secondary antibodies.</b>	Reduce antibody concentration. Do a titration to determine the optimal dilution for primary and secondary antibodies.
<b>Long incubation time.</b>	Reduce incubation time.
<b>High incubation temperature.</b>	Reduce incubation temperature.
<b>Extended substrate incubation time.</b>	Reduce substrate incubation time.
<b>Sections dried out.</b>	Avoid sections being dried out. Keep tissue sections and cells at high humidity.

## HIGH BACKGROUND

Sources	Possible solutions
<b>Inadequate washing of sections.</b>	Wash at least 3 times between steps.
<b>Tissue contains endogenous enzyme.</b>	Block endogenous enzyme activities.
<b>Tissue contains endogenous biotin activity.</b>	Block endogenous biotin activity using the avidin/biotin blocking reagent prior to incubation of primary antibodies.
<b>Non-specific binding of primary antibodies to tissue or high antibody concentration.</b>	Non-specific binding may be reduced by using higher dilution of primary antibodies.
<b>Non-specific binding of secondary antibodies to tissue.</b>	Treat tissue with normal serum block from the same species as secondary antibodies.
<b>Diffusion of tissue antigen due to inadequate fixation.</b>	Increase duration of post fixation.