



## SenTraGor™ Troubleshooting Guide

CELLS		
Problem	Possible Cause	Solution
No Staining or Low Signal	Low duration of SenTraGor™ reagent incubation	Increase in a step-wise manner incubation time of SenTraGor™ reagent.
		Incubate SenTraGor™ reagent at 37°C for respective time.
	Non-sufficient penetration into the cells	Use a permeabilization step before the addition of SenTraGor™ reagent. Incubate with 0.5% Triton X-100 / TBS for 5 min at RT. Continue with Step 3.3.
		Use a permeabilization step before the addition of SenTraGor™ reagent. Incubate with 1% Triton X-100 / TBS for 5 min at RT. Continue with Step 3.3.
	Reduced anti-biotin antibody reaction	Increase incubation time with anti-biotin antibody.
		Increase anti-biotin antibody concentration. (Decrease the dilution of the anti-biotin solution)
Absence of Lipofuscin	Choose an appropriate positive control.	
High Background	Endogenous Biotin present	Make sure your cells don't express endogenous biotin. In case there is endogenous biotin proceed with a streptavidin/biotin blocking kit before the incubation with SenTraGor™ reagent.
	Insufficient washings	Wipe thoroughly the excess of SenTraGor™ reagent right away from the incubation step.
		Increase washing time in EthOH and TBS solutions.
		Add several steps of washings and rinse out with the EthOH solution.
	Concentrated anti-biotin antibody	Decrease the anti-biotin antibody concentration. (Increase the dilution of the anti-biotin solution)
		Decrease the anti-biotin antibody incubation time.
	High duration of SenTraGor™ reagent incubation	Reduce SenTraGor™ reagent incubation time in a step-wise manner.
	Concentrated secondary antibody	Decrease the concentration of the secondary antibody. (Increase the dilution of the secondary antibody)
Increased DAB exposure	Reduce DAB exposure time.	



## SenTraGor™ Troubleshooting Guide

TISSUES		
Problem	Possible Cause	Solution
No Staining or Low Signal	Low duration of SenTraGor™ reagent incubation	Increase in a step-wise manner incubation time of SenTraGor™ reagent.
		Incubate SenTraGor™ reagent for 10 min at 37°C.
	Non-sufficient penetration into the cells	If tissue sections are thicker than 4 µm proceed with an antigen retrieval step before SenTraGor™ reagent. Incubate with citric acid solution (pH 6), for 10 min in a steamer.
		If tissue has been in fixative solution for more that 2 days before embedded in paraffin, proceed with an antigen retrieval step before SenTraGor™ reagent. Incubate with citric acid solution, for 10 min in a steamer.
	Reduced anti-biotin antibody reaction	Increase incubation time with anti-biotin antibody.
		Increase anti-biotin antibody concentration. (Decrease the dilution of the anti-biotin solution)
	Insufficient DAB exposure	Increase the DAB incubation time.
Absence of Lipofuscin	Choose an appropriate positive control.	
High Background	Endogenous Biotin present	Make sure your tissue doesn't express endogenous biotin. In case there is endogenous biotin proceed with a streptavidin/biotin blocking kit before the incubation with SenTraGor™ reagent. (For liver tissue is a prerequisite to use the streptavidin/biotin blocking kit. Other tissues that might express endogenous biotin are kidney and pancreas).
	Insufficient washings	Wipe thoroughly the excess of SenTraGor™ reagent right away from the incubation step.
		Increase washing time in EthOH and TBS solutions.
		Add several steps of washings and rinse out with the EthOH solution.
	Detergent solution	Instead of Triton X use Tween 20.
	Concentrated anti-biotin antibody	Decrease the anti-biotin antibody concentration. (Increase the dilution of the anti-biotin solution)
	Increased SenTraGor™ reagent incubation	Reduce SenTraGor™ reagent incubation time in a step-wise manner.
	Concentrated secondary antibody	Decrease the concentration of the secondary antibody. (Increase the dilution of the secondary antibody)
Increased DAB exposure	Reduce DAB exposure time.	