



SenTragor™ troubleshooting guide Tissue

Problem: No Staining or Low Signal

Possible Cause:

1. Low duration of SenTraGor™ reagent incubation

Solution:

- Increase in a step-wise manner incubation time of SenTraGor™ reagent.
- Incubate SenTraGor™ reagent for 10 min at 37°C.

2. Non-sufficient penetration into the cells

Solution:

- 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 stirrer.
- If tissue has been in fixative solution for more than 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 stirrer.

3. Reduced anti-biotin antibody reaction

Solution:

- Increase incubation time with anti-biotin antibody.
- Increase anti-biotin antibody concentration. (Decrease the dilution of the anti-biotin solution)

4. Insufficient DAB exposure

Solution:

- Increase the DAB incubation time.

5. Absence of Lipofuscin

Solution:

- Choose an appropriate positive control.



Problem: High Background

1. Endogenous Biotin present

Solution:

- 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).

2. Insufficient washings

Solution:

- 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.

3. Detergent solution

Solution:

- Instead of Triton X use Tween 20.

4. Concentrated anti-biotin antibody

Solution:

- Decrease the anti-biotin antibody concentration. (Increase the dilution of the anti-biotin solution)

5. Increased SenTraGor™ reagent incubation

Solution:

- Reduce SenTraGor™ reagent incubation time in a step-wise manner.

6. Concentrated secondary antibody

Solution:

- Decrease the concentration of the secondary antibody. (Increase the dilution of the secondary antibody)



SenTraGor™

Antibody - enhanced detection of Senescent cells

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7. Increased DAB exposure

Solution:

- Reduce DAB exposure time.