



## SenTragor™ troubleshooting guide Cells

### 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 at 37°C for respective time.

#### 2. Non-sufficient penetration into the cells

##### Solution:

- 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.

#### 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. Absence of Lipofuscin

##### Solution:

- Increase anti-biotin antibody concentration. (Decrease the dilution of the anti-biotin solution)
- Choose an appropriate positive control.



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## Problem: High Background

### 1. Endogenous Biotin present

Solution:

- 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.

### 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. Concentrated anti-biotin antibody

Solution:

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

### 4. High duration of SenTraGor™ reagent incubation

Solution:

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

### 5. Concentrated secondary antibody

Solution:

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

### 6. Increased DAB exposure

Solution:

- Reduce DAB exposure time