

**SeneTrace™ Blue**

 Catalog number: 23030  
 Unit size: 10 mg

Component	Storage	Amount (Cat No. 23030)
SeneTrace™ Blue	Freeze (< -15 °C), Minimize light exposure	10 mg

**OVERVIEW**

SeneTrace™ Blue is a biotinylated, lipophilic analog of Sudan Black B, engineered for the selective detection of lipofuscin—an autofluorescent, non-degradable lysosomal pigment that accumulates as a consequence of oxidative stress, impaired autophagic flux, and cellular senescence. The biotin moiety facilitates high-affinity detection via anti-biotin antibody-based assays, enabling robust quantification and spatial visualization of lipofuscin aggregates across diverse biological matrices.

Designed for both in vivo and in vitro applications, SeneTrace™ Blue is compatible with a wide range of sample types, including live and fixed cultured cells, fresh and cryopreserved tissues, as well as formalin-fixed, paraffin-embedded (FFPE) specimens. The reagent integrates seamlessly into immunohistochemistry (IHC), flow cytometry, and immunofluorescence (IF) workflows, making it an invaluable tool for investigating cellular aging, neurodegenerative disorders, and age-associated pathologies.

SeneTrace™ Blue is a valuable tool for evaluating lipofuscin accumulation in Vero E6 cells following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and in primary non-small cell lung carcinoma (NSCLC) specimens. Furthermore, it enables the quantification of soluble lipofuscin in human serum, providing mechanistic insights into oxidative damage, chronic inflammation, and age-related disorders, such as dementia.

**AT A GLANCE**
**Important**

Before first use, allow SeneTrace™ Blue to thaw at room temperature. Briefly centrifuge to collect any dried pellet.

**Protocol Summary**

1. Plate cells and treat them as desired.
2. Add SeneTrace™ Blue working solution.
3. Incubate cells for 5-10 minutes at room temperature.
4. Detect SeneTrace™ Blue using your preferred downstream application.

**KEY PARAMETERS**
**Fluorescence microscope**

Emission	Depends on the downstream detection system.
Excitation	Depends on the downstream detection system.
Recommended plate	Black wall/clear bottom

**PREPARATION OF STOCK SOLUTIONS**

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated*

*freeze-thaw cycles*

**SeneTrace™ Blue Stock Solution**

1. Prepare a 10–20 mM stock solution in DMSO. For example, to make a 20 mM stock solution, add 606 µL of DMSO to the SeneTrace™ Blue vial.

**Note:** For any unused SeneTrace™ Blue stock solution, prepare single-use aliquots and store them at ≤ -20°C. Keep protected from light and avoid repeated freeze-thaw cycles.

**PREPARATION OF WORKING SOLUTION**
**SeneTrace™ Blue Working Solution**

1. Prepare a 1 to 5 mM SeneTrace™ Blue working solution. For example, to make a 5 mM solution, mix 250 µL of a 20 mM SeneTrace™ Blue stock solution with 750 µL of 70% ethanol.

**Note:** Protect the working solution from light by covering it with foil or storing it in the dark.

**Note:** For optimal performance, use the solution within a few hours of preparation.

**SAMPLE EXPERIMENTAL PROTOCOL**
**Cell Staining with SeneTrace™ Blue**

1. Seed cells in a 96-well plate with black walls and a clear bottom according to your experimental needs.
2. Treat cells using your preferred method to induce senescence.
3. Remove the cell culture medium and wash the cells with HBBS buffer.
4. Fix the cells by adding a 4% formaldehyde solution and incubating them at room temperature for 20 minutes.
5. Remove the fixation solution, then wash the cells twice with PBS.
6. Wash cells once with TBS.
7. Add 50% ethanol to the cells and incubate for 5 minutes at room temperature.
8. Add 70% ethanol to the cells and incubate for 5 minutes at room temperature.
9. Add 100 µL of the SeneTrace™ Blue working solution to the cells.
10. Incubate the cells at 37°C in a 5% CO<sub>2</sub> incubator for 5–10 minutes at room temperature, keeping them protected from light.

**Note:** The optimal concentration and incubation time of SeneTrace™ Blue may vary depending on the cell line. It is recommended to test different concentrations to determine the best conditions.

11. Remove the dye working solution and wash the cells twice with 50% ethanol.
12. Remove and wash the cells twice with TBS.
13. Add 0.5% Triton X-100 in TBS to the cells and incubate at room temperature for 3 minutes.
14. Remove and wash the cells twice with TBS.

#### Detection of SeneTrace™ Blue Using Styramide™

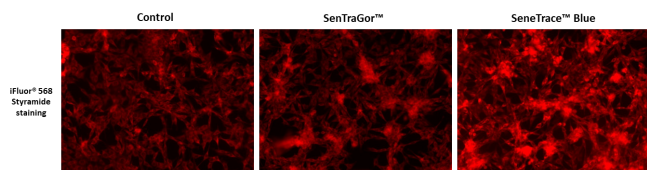
1. Block the sample with your preferred blocking solution (e.g., PBS with 1% BSA) at 4°C for 30 minutes.
2. Remove the blocking solution, then add the primary Anti-Biotin antibody diluted in the recommended antibody diluent at the suggested concentration. Incubate for 60 minutes at room temperature or overnight at 4°C.
3. Wash with PBS three times for 5 minutes each.
4. Apply the secondary antibody-HRP working solution to each sample at the desired concentration. Incubate at room temperature for 60 minutes.

**Note:** Adjust the incubation time and concentration as needed to achieve the desired signal intensity.

5. Wash with PBS three times for 5 minutes each.
6. Use the Styramide™ working solution as instructed. For a detailed protocol, visit: [AAT Bioquest - iFluor® 488 Styramide](#).

**Note:** Alternatively, the signal can be detected using a colorimetric detection system, such as DAB staining.

#### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Fluorescence imaging of iFluor® 568 Styramide (Cat# 45030) staining in 9L-LacZ cells (overexpressing  $\beta$ -Gal) treated with SenTraGor™ or SeneTrace™ Blue. Fluorescence intensities were observed using a Cy3 filter set.

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