# BioTracker™ Green Sulfane Sulfur Live Cell Dye

Live Cell Dye

**Cat. # SCT211**

**pack size: 60 nmol x 3**

Store at -20ºC

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

**Data Sheet**



## Background

Intracellularly abundant sulfane sulfur species including persulfide (R-S-SH), polysulfide (R-S-$S_n$-S-R) and polysulfide ($H_2S_n$) play important roles to maintain intracellular reducing environments.

BioTracker™ Green Sulfane Sulfur Live Cell Dye is a FRET-based fluorescence probe to specifically detect the intracellular sulfane sulfur species. It shows only weak fluorescence without sulfane sulfur, and reversibly fluoresces in response to micromolar concentrations of sulfane sulfur. Its reaction is highly specific and has minimal to no reactivity with $H_2S$, cysteine residues, and sulfur oxides. The dye is a cell permeable diacetylated form which is hydrolyzed by intracellular esterases to generate a metabolite which is retained within the cells. Thus, it is suitable to monitor intracellular concentration of sulfane sulfur via live-cell imaging.

## Storage

Store BioTracker™ Green Sulfane Sulfur Live Cell Dye at -20°C, desiccate and protect from light

*Note: Centrifuge vial briefly to collect contents at bottom of vial before opening.*

## Spectral Properties

Absorbance maximum: 495 nm
Emission maximum: 525 nm

## Quality Control

Purity: ≥ 65% confirmed by LC.

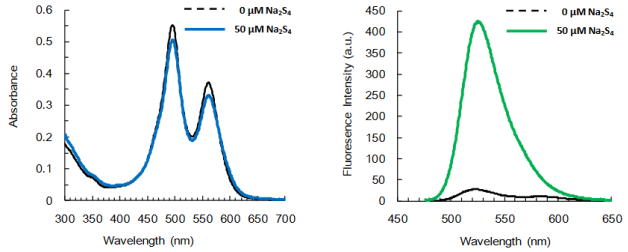


**Figure 1:** Absoprtion spectra (left) of 5 μM of BioTracker™ Green Sulfane Sulfur live cell dye, and fluorescence spectra (right) when excited at 470 nm.

## Protocol

**Materials required but not provided**

1. Dimethyl sulfoxide (DMSO)
2. A protein solution such as bovine serum albumin (BSA)
3. Observation buffer (Hank's balanced salt solution (HBSS), etc.). It should be a solution without phenol red.

**Reagent Preparation**

1. BioTracker™ Green Sulfane Sulfur live cell dye is a violet solid. Before opening the vial, first warm it to room temperature. Spin down the solid to the bottom by a microcentrifuge or by a desktop centrifuge.
2. Add 60 μL of DMSO to one vial to prepare 1 mM solution. Finally, dissolve the solid entirely by pipetting for more than five times. The dye solution will become reddish violet.
   Note: *This dye may aggregate in an aqueous solution. We recommend diluting the dye-DMSO solution with aqueous buffer solution containing proteins such as 1 mg/ml BSA.*

**Detection of intracellular sulfane sulfur in A549 cells**

1. Prepare a cell-staining solution by diluting 1 mM BioTracker™ Green Sulfane Sulfur live cell dye solution to 10 μM with observation buffer containing 10 mg/mL BSA.

**Note**: Fluorescence of the dye is diminished by reduced glutathione (GSH). Intracellular GSH concentration is varied among different kinds of cells. Therefore, we recommend optimizing dye concentrations and incubation time in your conditions. In our experience, incubating A549 cells with 1 μM dye solution at 37°C for 60 minutes gave good results.
**Note**: We recommend paying attention to intracellular nutrition because the intracellular GSH concentration depends on the nutritional conditions.

2. Remove culture media from the dish and rinse twice with the observation buffer.
3. Add the cell-staining solution to the dish and incubate at 37°C for 60 minutes.
4. After staining, wash twice with the observation buffer.
5. Observe with a fluorescence microscope. You may induce the production of sulfane sulfur by adding $Na_2S_4$ to the final concentration of 5 μM.

**Fluorescence observation**

Use 488- or 495-nm laser for the laser microscopy. Maximum emission is detected at 525 nm. For fluorescence microscopy, blue-excitation filter sets for GFP or for FITC are appropriate.

Rev.1.0/2020-04-01/SCT211MD

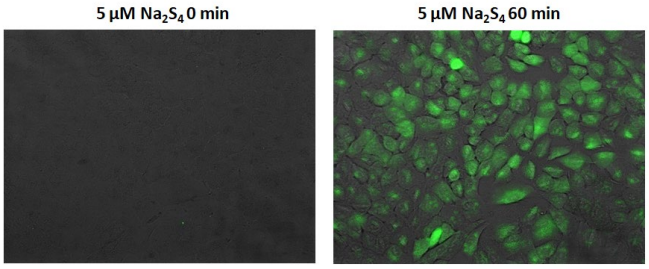


**Figure 2. Live cell imaging using SCT211 dye in A549 cells.** Intracellular sulfane sulfur was imaged with the dye, before (*left*) and after (*right*) a stimulation with 5 µM $Na_2S_4$ in A549 cells. Cells were treated with 10 µM of dye solution (containing 1 mg/mL BSA as additives) for 1 hour at 37ºC, 5% $CO_2$. After washing, the cells in HBSS were observed under a fluorescence microscope using 460—500 nm excitation filter and 512—542 fluorescence filter. Grayscale images of DIC and green pseudocolor images of fluorescence were overlaid. A549 cell line provided from JCRB cell bank was used.

## References

Takano Y et al. *Development of a reversible fluorescent probe for reactive sulfur species, sulfane sulfur, and its biological application*. Chem Commun. 2017. Jan 17; 53(6):1064-1067.