BioTracker™ Green H_2O_2 Live Cell Dye

Live Cell Dye

Cat. # SCT039

pack size: 30 nmol x 3

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

Store at -20ºC

Data Sheet

Background

Hydrogen peroxide (H_2O_2) is involved in therapeutic processes such as wound healing, anti-bacterial defense, stem cell proliferation, and an adaptive response in astrocytes that leads to neuronal protection. However, over-production of H_2O_2 exerts toxic effects on the cell and its surrounding environment. Over-production of H_2O_2 is connected to serious pathological conditions such as cancer, ageing, diabetes, and neurodegenerative diseases.

BioTracker™ Green H_2O_2 live cell dye (SCT039) is a fluorescent probe that fluoresces upon reaction with H_2O_2 but does not react with other ROS such as hydroxyradical (-OH), superoxide (O2−), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$), and nitric oxide (NO). SCT039 dye is cell permeable and initially has low reactivity with H_2O_2, but it is quickly hydrolyzed by intracellular esterases to generate a reactive and cell impermeable product that fluoresces upon reaction with H_2O_2.

Storage

Store BioTracker™ Green H_2O_2 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: 492 nm
Emission maximum: 516 nm (Green)

Quality Control

Purity: ≥ 90% confirmed by LC.

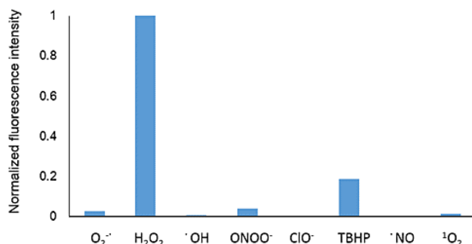

Figure 1: Reaction of BioTracker™ Green H_2O_2 dye with various reactive oxygen species. Only H_2O_2 increases the fluorescence of the dye at physiological pH of 7.4 or higher. Fluorescence intensity of 10 μM dye was measured after addition of each ROS (final conc. 50 μM) in 0.1 M sodium phosphate buffer at pH 7.4 containing 0.1 % DMF as a cosolvent. Fluorescence intensities were measured at 520 nm, with excitation wavelength of 490 nm.

Protocol

Reagent Preparation

1. Prepare N,N-dimethylformamide (DMF) as a solvent.
2. BioTracker™ Green H_2O_2 live cell dye is a colorless solid. Before opening the cap, warm the vial to the room temperature and use micro-centrifuge to spin down the solid that might be adhered on the cap.
3. Add 30`μL of DMF to one vial to prepare 1 mM solution. Dissolve the solid completely by pipetting for more than five times. The dye solution will be colorless.

Detection of Intracellular H_2O_2

1. Dilute the 1 mM dye solution with observation buffer or culture media to 1−5 μM (Cell staining solution).
2. Remove the culture medium on the dish and wash twice with the observation buffer or the culture medium.
   Note: We recommend optimizing the dye concentration and the incubation time. In our experience, incubation in 5 μM dye at 37°C for 20 min gave good results for HeLa cells, A431 cells, and 1 μM dye at 37°C for 20 min for RAW276.4 cells.
3. Add the cell staining solution to the dish and incubate at 37°C for 20 min.
4. After the staining, wash 2 times with the observation buffer.
5. Induce the production of H_2O_2 by the addition of PMA and start observation under a microscope. A dose response curve with PMA and vehicle (blank/control solution without PMA) is recommended.
   Note: We detected the fluorescence signal by microscope, 30 min after stimulation.

Fluorescence observation

Use 488 nm blue light for excitation. Maximum emission is observed at 516 nm. For fluorescence microscopy, blue excitation filter sets for GFP or FITC is appropriate.

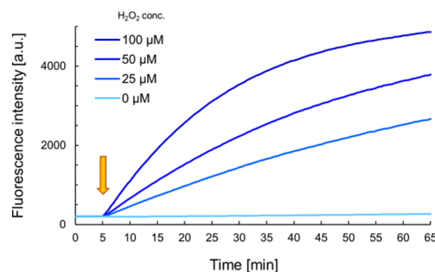

Figure 2: Quick reaction with H_2O_2. Fluorescence increase can be observed just after the addition of H_2O_2. Fluorescence intensity increases as the incubation time becomes longer.

Rev.1.0/2020-04-01/SCT039/MD

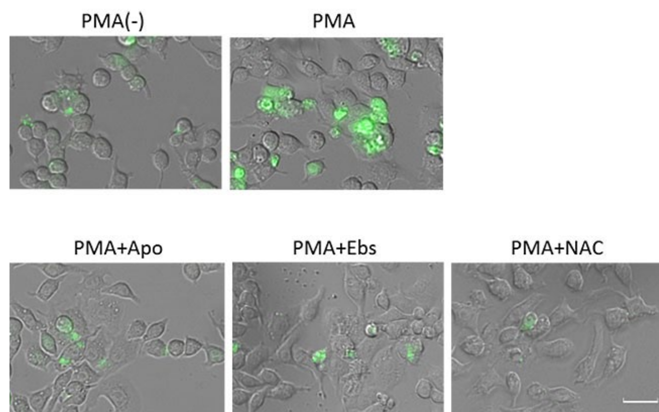

Figure 3: In RAW264.7 cells stimulated with PMA, more spot-like signals of the SCT039 dye was observed compared to intact cells (top). Addition of antioxidants inhibits H_2O_2 productions (bottom). Scale Bar: 25 µm.

Live Cell Imaging of intracellular H_2O_2 production in RAW 246.7 cells (Figure 3)

1. Seed RAW264.7 cells (5 × 10^4 cells/mL) on glass bottom dishes and culture the cells in DMEM without FBS at 37°C in 5% CO_2 for overnight.
2. Dilute the 1 mM dye-DMSO solution 1000 times with DMEM without FBS to prepare 1 µM reacting solution.
3. Add each of the ROS inhibitors (antioxidant) to the reacting solution (Apo; final 5 mM of apocynin, Ebs; final 5 µM of ebselen, NAC; final 10 mM of N-acetyl-L-cysteine).
4. Remove the culture media and rinse cells once with HBSS.
5. Add the reacting solution and incubate for 20 minutes at 37°C, 5% CO_2.
6. Induce hydrogen peroxide production by the addition of final 1 ng/mL of phorbol myristate acetate (PMA) in DMEM without FBS, and incubate for 30 minutes at 37°C, 5% CO_2.
7. Remove the PMA solution from the dish and rinse cells twice with HBSS. Observe cells with fluorescence microscope.
   Note: The optimal concentration of reagents and the reaction time could vary depending on the cell type and culture conditions.

References

Abo M et al. *Development of a Highly Sensitive Fluorescence Probe for Hydrogen Peroxide*. J. Am. Chem. Soc. 2011. 133, 27, 10629-10637

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