BioTracker™ Orange OH and HClO Live Cell Dye

Live Cell Dye

Cat. # SCT038

pack size: 100 nmol x 5

Store at -20ºC

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

Data Sheet

Background

Reactive oxygen species (ROS) are important mediators in a variety of biological and pathological events. For example, ROS generated in mitochondria play important roles in the modulation of signal transduction cascades and transcription factors. However, oxidative stress due to excessive generation of ROS is implicated in many human diseases, including acute and chronic inflammatory processes, ischemic stroke, diabetes, sepsis, ischemia-reperfusion injury, atherosclerosis, and neurodegenerative disorders.

BioTracker™ Orange OH and HClO Dye is an orange fluorescent probe to detect hydroxy radical (OH) or hypochlorous acid (HClO) in live-cell imaging. It can detect -OH and HClO among other reactive oxygen species (O^{2-}, H_2O_2, NO, $ONOO^-$). Its nearly red fluorescence spectrum allows multicolor imaging with green (ex. GFP, FITC) and blue (ex. Hoechst 33342) fluorophores. This dye, because of its positive charge, tends to localize within mitochondria. It has high photostability and is suitable for time-lapse imaging of intracellular hROS generation. Its fluorescence is stable after mild fixation (3-4% PFA, 20 min).

Storage

Store BioTracker™ Orange OH and HClO 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: 553 nm
Emission maximum: 577 nm

Quality Control

Purity: ≥ 95% confirmed by LC.

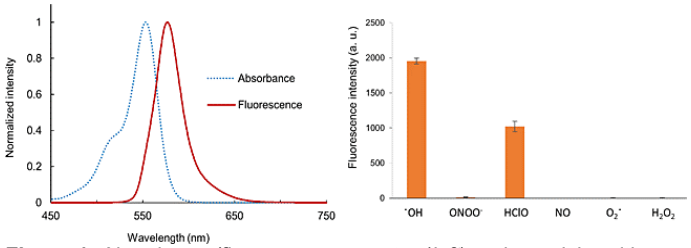

Figure 1: Absorbance/fluorescence spectra (*left*) and reactivity with various ROS (*right*). About 30 times fluorescence increase after the reaction with hydroxy radical (-OH) can be detected.

Protocol

Materials required but not provided

1. N,N-dimethylformamide (DMF)
2. Observation buffer (1×PBS pH 7.4, HBSS, Krebs-Ringer phosphate (KRP) buffer, etc.)

Time-lapse imaging of hROS production in HeLa cells

1. Warm one vial of the dye to room temperature. Before opening the vial, spin down the solid to the bottom by a microcentrifuge.
2. Dissolve the dye in 1 vial (100 nmol) in 100 µL DMF to prepare 1 mM solution.
3. Dilute the 1 mM dye solution with observation buffer or culture media to 1 µM (cell staining solution).
   Note: Optimization of dye concentration and the incubation time is required. In our experience, incubation in 1 µM dye at 37ºC for 20 min gave good results for HeLa cells, RAW264.7 cells and HL-60 cells.
4. Remove the culture medium from the glass bottom dish and wash cells once with the observation buffer or with the culture medium.
5. Add the cell staining solution to the dish and incubate at 37°C for 20 min.
6. After the staining, wash cells 2 times with the observation buffer. Add new observation buffer.
   Note: We recommend using cell culture medium without phenol red instead of using buffers, because starved HeLa cells are known to produce ROS.
7. Induce the production of hROS by the addition of 500 µM H_2O_2 (final conc.) and start observation under microscope.
   Note: We detected the fluorescence signal 15 min after the stimulation.

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Fluorescence observation

Use 532 nm or 543 nm light source for excitation. Maximum emission is observed at 577 nm. In fluorescence microscopy, use green excitation filter cube such as Cy3, G-2A, G-2E/C, TRITC (Nikon) or U-FGW, U-FGWA, U-FGNA, U-MWIGA3, U-MNIGA3 (Olympus).

References

Koide Y et al. *Design and Synthesis of Fluorescent Probes for Selective Detection of Highly Reactive Oxygen Species in Mitochondria of Living Cells.* J. Am. Chem Soc. 2007, 129, 34, 10324-10325.

Rev.1.0/2020-03-31/SCT038/MD

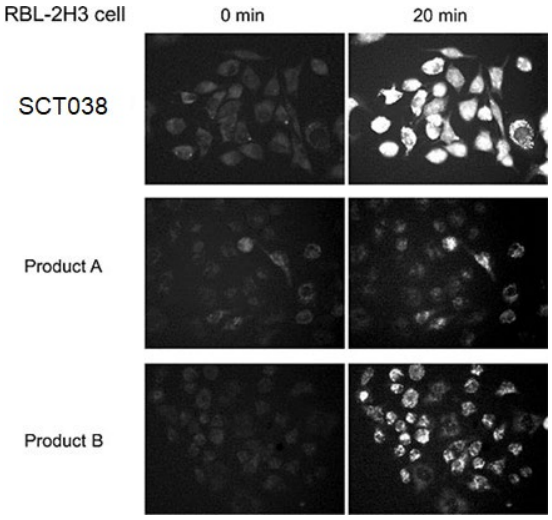

Figure 2: Bright and stable fluorescence. Comparison between mitochondria-localizing probes to detect oxidative stress. RBL-2H3 cells loaded with 1 µM of SCT038 dye (OxiORANGE, above), product A (center), or product B (bottom) were stimulated by the addition of 0.5 µM H_2O_2. Photos were taken just after the addition of the probes (left) and 20 minutes later (right) in the same excitation/observation conditions. SCT038 dye shows the brightest fluorescence among these products. Product B migrated into nucleus. In contrast, localization of SCT038 dye was stable.

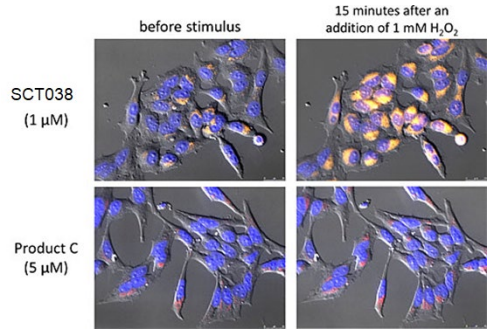

Figure 3: Comparison with a ROS-detecting probe. SCT038 (OxiORANGE™, 1 µM, top, orange) or other product C (5 µM, bottom, deep red) was added to the medium and incubated for 30 minutes. After the medium was exchanged to HBSS, 1 mM H_2O_2 was added to stimulate ROS production. Bright signal from OxiORANGE™ was detected. DIC image (gray), Hoechst 33342 (blue), and OxiORANGE™ (orange), or Product C (red) is overlaid.

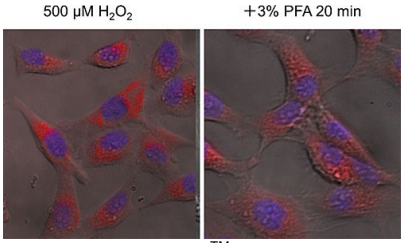

Figure 4: BioTracker™ Orange OH and HClO dye fluoresces after reaction with ROS. The reaction is irreversible, and the fluorescence remains after mild fixation with 3-4% PFA for 5-20 minutes.

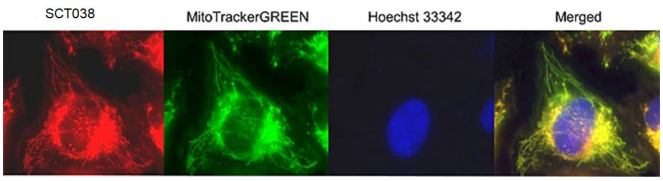

Figure 5: SCT038 (OxiORANGE™) dye tends to localize within mitochondria if the concentration of the reagent is low enough. SCT038 dye can also distribute to some other parts of cells, especially when the concentration of the dye is higher, or when other mitochondria-localizing reagents was added. HeLa cells were loaded with 0.5 µM of SCT038, 0.25 µM of MitoTrackerGREEN, and 0.2 µg/mL of Hoechst33342, then stimulated with 100 µM of hydrogen peroxide, for 30 min.