BioTracker™ Green Free H_2O_2 Dye

Dye for Solutions

Cat. # SCT040

pack size: 30 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

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 free H_2O_2 dye cannot penetrate cell membrane and so can be used only for detection of extracellular H_2O_2 and free H_2O_2 in solutions. This dye 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).

Storage

Store BioTracker™ Green free H_2O_2 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: ≥ 85% confirmed by LC.

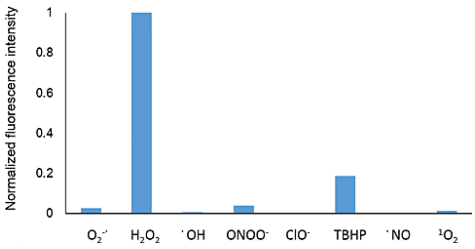

Figure 1: Reaction of BioTracker™ Green free H_2O_2 dye with various reactive oxygen species. Only H_2O_2 increases the fluorescence of the dye at physiological pH of 7.4 or higher. Fluorescence intensity of 10 µM dye was measured after addition of each ROS (final conc. 50 µM) in 0.1 M sodium phosphate 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. (Data was kindly provided by Prof. Dr. Y. Urano, Univ. Tokyo)

Protocol

Reagent Preparation

1. Prepare N, N-dimethylformamide (DMF) as a solvent.
2. BioTracker™ Green free H_2O_2 dye is an orange-colored 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 orange.

Quantification of H_2O_2 in solutions

1. Prepare hydrogen peroxide (H_2O_2) solutions to generate a standard curve. Commercially available 30−35% H_2O_2 solution is about 10 M. Dilute the solution 1000 times with pure water and measure absorbance at 240 nm (A240).
2. Concentration of the H_2O_2 can be obtained by the equation: C = A240 / ε × dilution factor (M), whereas ε = 43.6 ($M^{-1}cm^{-1}$) is the molar extinction coefficient of H_2O_2.
3. Dilute the H_2O_2 to the final concentration of 0−100 µM using a solution. The diluting solution condition should be equivalent to that in which you measure H_2O_2 concentrations. Add the dye to final concentration of 1−10 µM, react at 37ºC for 1 hour. These concentrations and reaction conditions should be adjusted to the expected conditions to be measured.
4. Measure fluorescence intensities using a fluorescence spectrometer or a fluorescence microplate reader. Before the measurement, dilute the solution to make the final dye concentration to be <1 µM, or the absorbance of the solution at 490 nm should be less than 0.1. We recommend setting the excitation wavelength within 470−490 nm and emission wavelength to be 520−530 nm.
5. Plot the measured fluorescence intensity (I) against H_2O_2 concentration (C) and fit using the following equation, to obtain the parameters, a, b, and k.

$$I = \frac{ac}{k - c} + b$$

6. Add the same concentration of HYDROP-EX to the solution you would like to measure, mix well and incubate in the same conditions (temperature and time). After the incubation, measure fluorescence.
7. Obtain H_2O_2 concentrations using the following equation,

$$C = \frac{I-b}{I+a-b}\mathrm{k}$$

Rev.1.0/2020-04-01/SCT040/MD

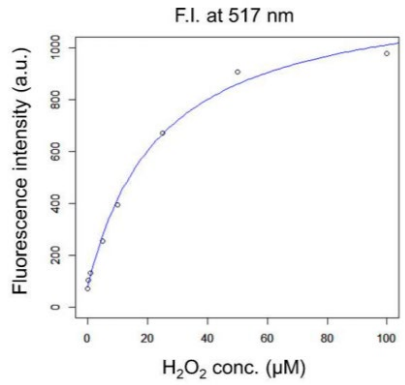

Figure 2: An example of standard curve of BioTracker™ Green Free H_2O_2 dye.

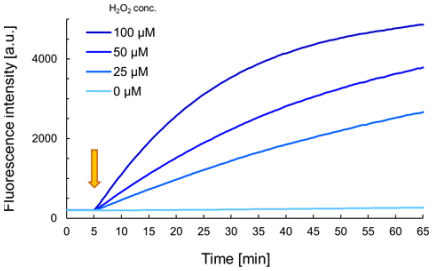

Figure 3: Quick reaction with H_2O_2. Fluorescence increase can be observed just after the addition of SCT040 with H_2O_2. Fluorescence intensity increases as the incubation time becomes longer.

References

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