BioTracker™ 625 Red GSH Dye

Live Cell Dye

Cat. # SCT036

pack size:2X25nmol

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

Store at -20ºC

Data Sheet

Background

Glutathione (GSH) is an important antioxidant preventing damage to important cellular components caused by reactive oxygen species (ROS) including free radicals, peroxides, lipid peroxides, and heavy metals. Alterations in glutathione homeostasis are associated with a variety of diseases; therefore, real-time live-cell imaging and quantification of GSH dynamics are important for understanding pathophysiological processes.

The BioTracker™ 625 Red GSH Dye is probe for glutathione (GSH) in living cells. The dye is formed with an orange fluorescent dye (T) and a deep-red fluorescent dye (S). At a low GSH concentration, green light excitation of T, leads to an energy transfer to S and a subsequent emission of deep-red fluorescent light. At a high GSH concentration, the energy transfer does not occur, so the probe emits the orange fluorescent light from the emission of T. The dye produces a quick and reversible response to the GSH concentration changes, relatively slow photobleaching and low cytotoxicity.

Storage

Store BioTracker 625 Red GSH 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: 520nm
Emission: 625nm and 582nm

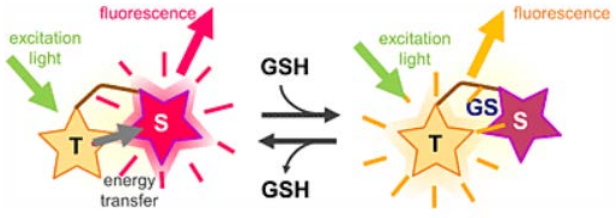

Figure 1. Mechanism of BioTracker 625 Red GSH Dye.

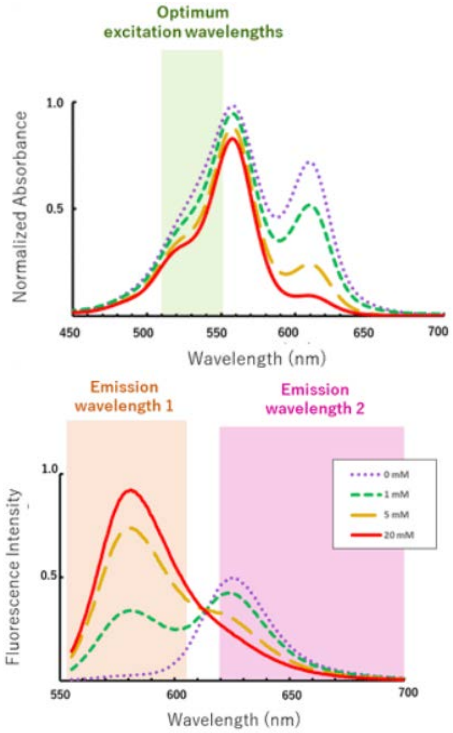

Figure 2. Fluoirescence spectra of BioTracker 625 Red GSH Dye. The emission maximum is 625 nm in the absence of GSH, wheras it shifts to 582 nm upon addition of GSH.

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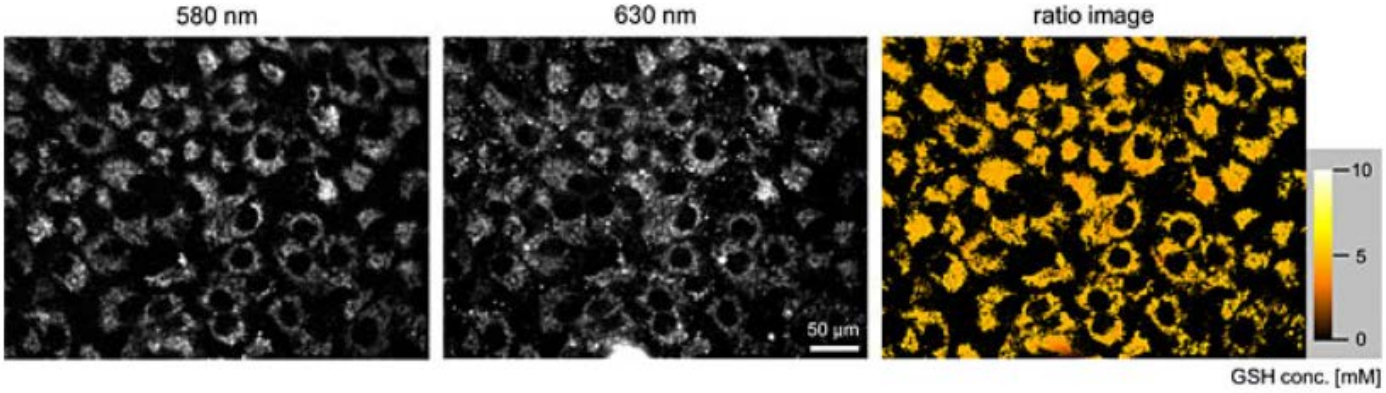

Figure 3. 2 µM BioTracker 625 Red GSH Dye was added to A549 cells and incubated for 10 minutes. The intracellular concentration of GSH (right) was calculated from images captured in the two wavelength ranges.

Protocol

Reagent Preparation
1. Before opening the vial, spin down the solid to the bottom by a microcentrifuge or by a desktop centrifuge.
2. Dissolve 1 vial in 25 µL DMSO to prepare 1 mM solution

Prepare Standard Curve
A standard curve of ratiometric fluoresnce intensity vs GSH for different imagaing instruments corresponding to excitation in the region of 530-550nm is required.

Protocol for Cell Staining
1. Prepare 1 µM of dye staining solution by diluting 1 mM dye solution with the 1XPBS. Add Pluronic F-127 to the final concentration of 0.01%.
2. Remove culture medium from the cells and add the staining solution into the culture dish. Incubate at 37ºC for 10 minutes.
3. Wash the cells twice with the 1XPBS.
4. Observe the cells with the fluorescence microscope. Observe cells at the two wavelengths and capture images in the same conditions that used to measure the image intensities for the standard curve.
5. Calculate FI ratios from the images.
6. Calculate GSH concentration using the standard curve.