Data Sheet

BioTracker™ TP-HOCl1 Live Cell Dye

Live Cell Dye

SCT043

Pack Size: 1 mg

Store at −20 °C

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

Hypochlorous acid (HOCl) is a highly potent reactive oxygen species (ROS) that helps to eliminate pathogens when produced by activated cells of the innate immune system. Mounting evidence indicates that intracellular HOCl plays additional important roles in regulating inflammation and cellular apoptosis. Subcellular detection of HOCl has historically been limited due to low concentration, strong oxidization, and short lifespan of the analyte.

The BioTracker™ TP-HOCl1 Dye is a live cell, two-photon green, fluorescent "turn-on" imaging probe for HOCl. The probe exhibits fast response time, good selectivity, and high sensitivity towards hypochlorous acid in living cells.

Spectral Properties

Excitation peak range: 340-380 nm

Emission peak range: 400-490 nm

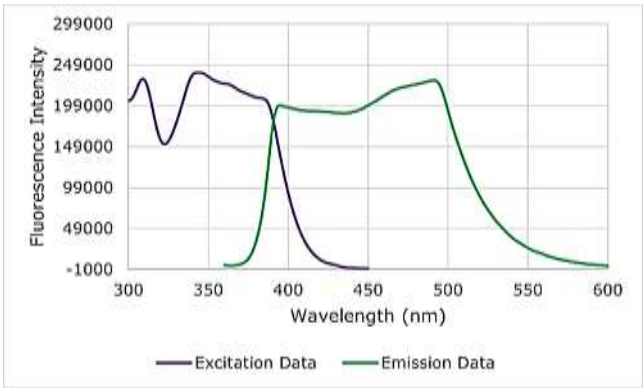

Figure 1. Probe excitation (purple) and emission (green) data. 7 µL of probe at stock concentration (10 mM) was diluted in 1 mL of DMSO before undergoing excitation and emission scans. Spectral scans were conducted using a PerkinElmer FL8500 Fluorescence Spectrophotometer.

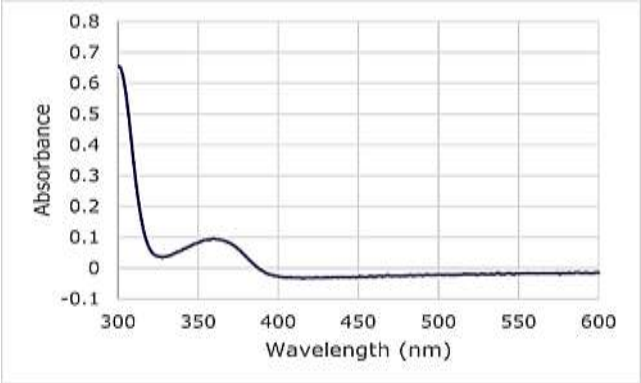

Figure 2. Probe absorbance data. 7 µL of probe at stock concentration (10 mM) was diluted in 1 mL of DMSO before undergoing an absorbance scan. Spectral scans were conducted using a PerkinElmer FL8500 Fluorescence Spectrophotometer.

Quality Control Testing

Purity: ≥ 98% confirmed by HNMR, LC-MS and HPLC and elemental analysis

Molar Mass: 258.36 g/mol

Storage and Handling

Store BioTracker™ TP-HOCl1 Live Cell Dye at −20 °C, desiccate and protect from light.

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

Representative Data

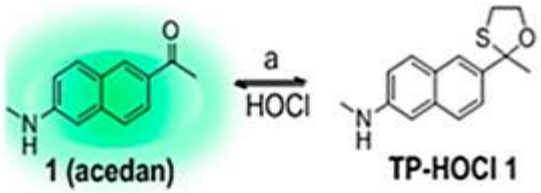

Figure 3. TP-HOCl1 Mechanism. Acedan was chosen as the fluorescence reporting group due to its excellent photophysical properties resulting from the typical "push−pull" (amineketone) structure. 2-mercaptoethanol and 1, 2-ethanedithiol were employed to protect the ketone of acedan in the design of HOCl probes. Reaction of the probe with HOCl, which deprotects the oxathiolane/mercaptal group to reveal the ketone, leads to fluorescence enhancement.

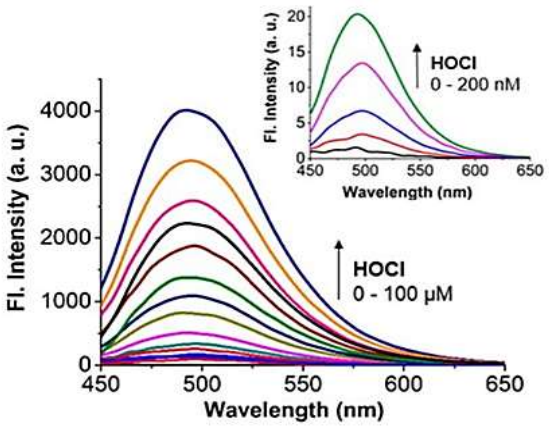

Figure 4. Fluorescence intensity of TP-HOCl1 (5 µM) as a function of HOCl concentration (0-100 µM). Inset shows Fluorescence spectra of TP-HOCl1 (0.5 µM) before and after adding HOCl at low concentrations (0-200 nM).

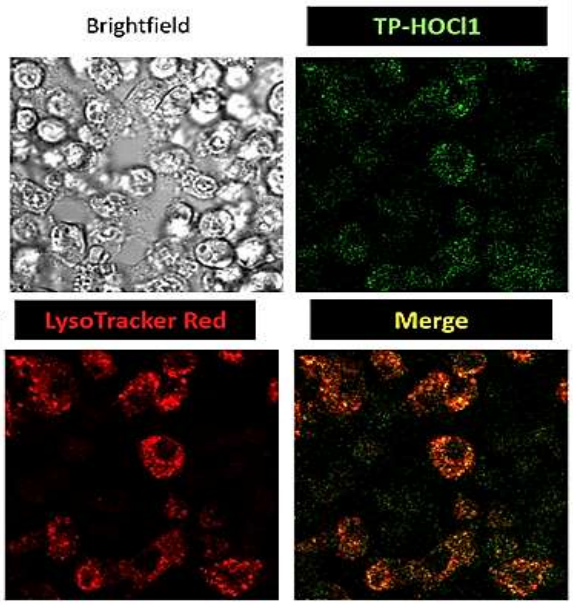

Figure 5. RAW264.7 mouse macrophage cells were treated with 120 ng/mL LPS, and 20 ng/mL IFN-gamma for 24 hour to induce intracellular HOCl production. Cells were then co-incubated with TP-HOCl1 dye (10 uM) and LysoTracker™ Red for 20 minutes before being imaged on a Nikon Eclipse® Ti confocal microscope.

Protocols

Reagent Preparation

1. Before opening the vial, spin down the solid to the bottom by a microcentrifuge or by a desktop centrifuge.
2. Warm the vial to room temperature and add DMSO to make a 1000X stock solution of 10-20 mM (freeze aliquots at −20 °C).
3. Dilute stock solution in cell culture media to a final concentration of 10-20 µM and add to cells in culture. Incubate at 37 °C for 20-30 minutes.
4. Wash cells with PBS buffer before imaging.

   Note: Optimal concentration must be determined by end user.

References

1. Chang YT et al. Development of Targetable Two-Photon Fluorescent Probes to Image Hypochlorous Acid in Mitochondria and Lysosome in Live Cell and Inflamed Mouse. Model. J Am Chem Soc. 2015 May 13;137(18):5930-8.

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