Data Sheet

BioTracker™ SiRNO Nitric Oxide Near Infrared Live Cell Dye

Live Cell Probe

SCT053

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

Nitric oxide (NO) is a reactive nitrogen species which is produced by nitric oxide synthase (NOS). NO is involved in many physiological processes including regulation of blood pressure, homeostasis, activation of immune system, neural communication, and contraction of both smooth muscle and vascular tissue. Therefore, detection and quantification of NO is critical to understanding health and disease.

The BioTracker™ SiRNO Nitric Oxide Dye (BioTracker™ SiRNO NO Dye) is a live cell near-infrared fluorescent imaging probe for detecting nitric oxide (NO). The probe targets NO specifically in the mitochondria and lysosome of living cells. The probe shows no cross reactivity to a variety of biomolecules and other reactive species including ascorbic acid (AA), dehydroascorbic acid (DHA), methylglyoxal (MGO), GSH, Cys, Hcy, H_2O_2, ClO^-, OH^-, O_2, NO_2^-, and $ONOO^-$. The BioTracker™ SiRNO NO dye is pH insensitive in the range of 5.5-8.0, demonstrating its compatibility with the physiological pH range. The SiRNO dye has been successfully used to detect NO in live cells, animal tissue, and *in vivo* experiments.

Source

The BioTracker™ SiRNO NO Dye (SCT053) does not contain genetically modified organisms.

Spectral Properties

Excitation max: 670 nm, 633 nm

Emission max: 690-700 nm

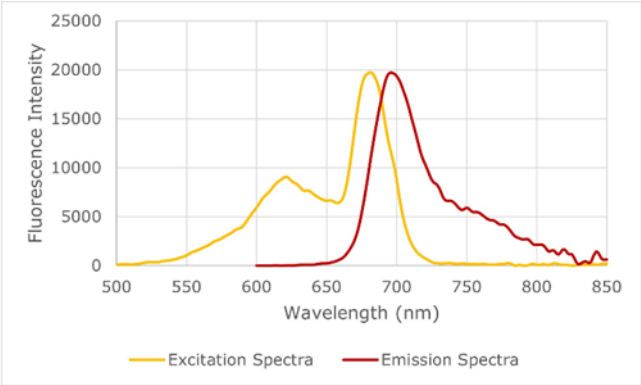

Figure 1: Probe excitation and emission data. 7 µL of probe at stock concentration (10 mM) was diluted in 3 mL of DMSO before undergoing an absorbance scan. Spectral scans were conducted using a PerkinElmer FL8500 fluorescence spectrophotometer.

Quality Control Testing

- Purity: ≥ 98% confirmed by HNMR, LC-MS and HPLC and elemental analysis
- Molar Mass: 758.34 g/mol

Storage and Handling

Store BioTracker™ SiRNO NO Dye at –20 °C, desiccated and protected from light.

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

Representative Data

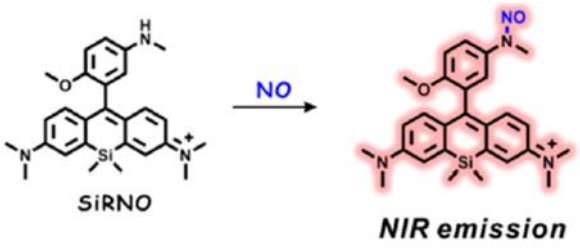

Figure 2: Schematic of SiRNO probe reacting with nitric oxide.

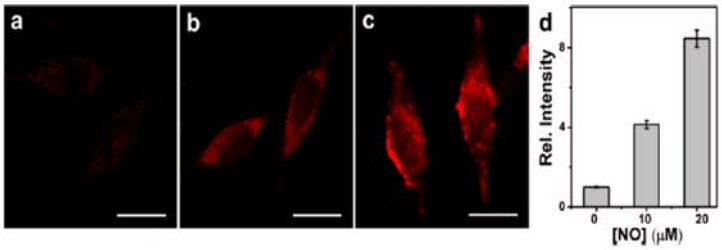

Figure 3: Two photon images of 5.0 µM SiRNO-stained HeLa cells incubated with various concentrations of NO for 30 min. (**a**) 0 µM NO; (**b**) 10 µM NO; (**c**) 20 µM NO; (**d**) Relative fluorescence intensity of images a-c. The fluorescence emission was collected at 650-750 nm. Scale bar=20 µm.

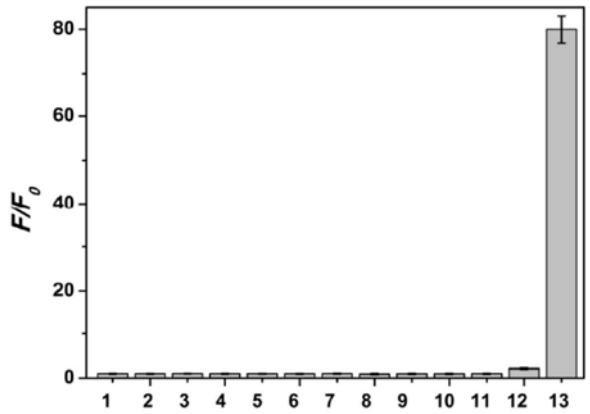

Figure 4: Relative fluorescence intensity of BioTracker™ SiRNO NO Dye in the presence of NO and various biomolecules, ROS and RNS. 1-6 (1.0 mM for AA, DHA, MGO, GSH, Cys and Hcy), 7-12 (30 μM for H2O2, ClO^-, ·OH, O_2^-, NO_2^- and $ONOO^-$) and 13 (20 μM for NO).

Protocols

Reagent Preparation and Cell Culture (Sample Protocol)

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 the room temperature and add DMSO to 1 mg of BioTracker™ SiRNO NO Dye to make a 1 mM stock solution (freeze aliquots at –20 °C).
3. Dilute the BioTracker™ SiRNO NO Dye DMSO stock solution with cell culture medium to prepare 5.0 μM BioTracker™ SiRNO NO Dye working solution.
4. Prepare cells for the assay. HepG2 cells were cultured with DMEM supplemented with 10% (v/v) newborn calf serum, 100 $U \cdot mL^{-1}$ penicillin, and 100 $\mu g \cdot mL^{-1}$ streptomycin at 5% CO_2 and 37 °C.
5. One day before imaging, cells were detached with a treatment of 0.2% (w/v) trypsin-EDTA solution and suspended in culture media. The cell suspension was then transferred to confocal dishes to grow with adherence.
6. For probe loading, the growth medium was replaced with 5.0 μM of BioTracker™ SiRNO NO Dye (SCT053) in culture media and incubated at 37 °C under 5% CO_2 for 30 minutes.

   Note: Optimal concentration must be determined by end user.
7. Next, the cells were washed three times with serum-free DMEM. Various concentrations of NO solution were added to the samples and incubated at 37 °C under 5% CO_2 for 30 minutes to test fluorescence in the presence of NO.
8. The cells were washed three times with PBS and imaged under confocal microscope.

References

1. Mao Z, Jiang H, Song X, Hu W, Liu Z. 2017. Development of a Silicon-Rhodamine Based Near-Infrared Emissive Two-Photon Fluorescent Probe for Nitric Oxide. Analytical Chemistry. 89(18):9620–9624.

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