

Data Sheet

BioTracker™ BlueTFCH Oxidative Stress Probe

Live Cell Dye

SCT240**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

Drug-induced kidney injury—or nephrotoxicity—is an important aspect for consideration during development of new therapeutics. Currently available screening tools used to assess or detect damage have many potential downsides. Detection has been limited to changes in cell viability, morphology, and mitochondrial function which are not sensitive detection formats. These assays may only detect severe nephrotoxicity induced by high doses or long exposures to tested compounds.

An alternative method to improving testing sensitivity is to observe oxidative stress which is much more sensitive to mild drug-induced injury. While certain indicators of oxidative stress are transient, such as upsurges in reactive oxygen species (ROS) or depletion of reductants, one irreversible and non-transient change due to oxidative stress is the carbonylation of biomolecules post-injury.

The development of a new probe was aimed at identifying this new carbonylated biomolecule phenotype. Due to the need to identify more subtle levels of cell injury induced by nephrotoxicity, the probe must be highly sensitive in renal system applications. This novel sensor, 4-trifluoromethyl-7-hydrazinyl-2H-chromen-2-one (TFCH) meets a need for detection with enhanced sensitivity to nephrotoxicity secondary to oxidative stress in therapeutic development.

Source

SCT240 does not contain genetically modified organisms.

Spectral Properties

Fluorescence images obtained by λ_{exc} = 405 nm and emission at 550 to 650 nm.

Quality Control Testing

Purity: \geq 98% confirmed by HPLC, HNMR, LC-MS and elemental analysis. Molar Mass: 280.63 g/mol.

Storage and Handling

Store BioTracker™ TFCH Oxidative Damage Detection Live Cell Dye at -20 °C, desiccated and protected from light.

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

Presentation

Lyophilized. Yellow solid.

Representative Data

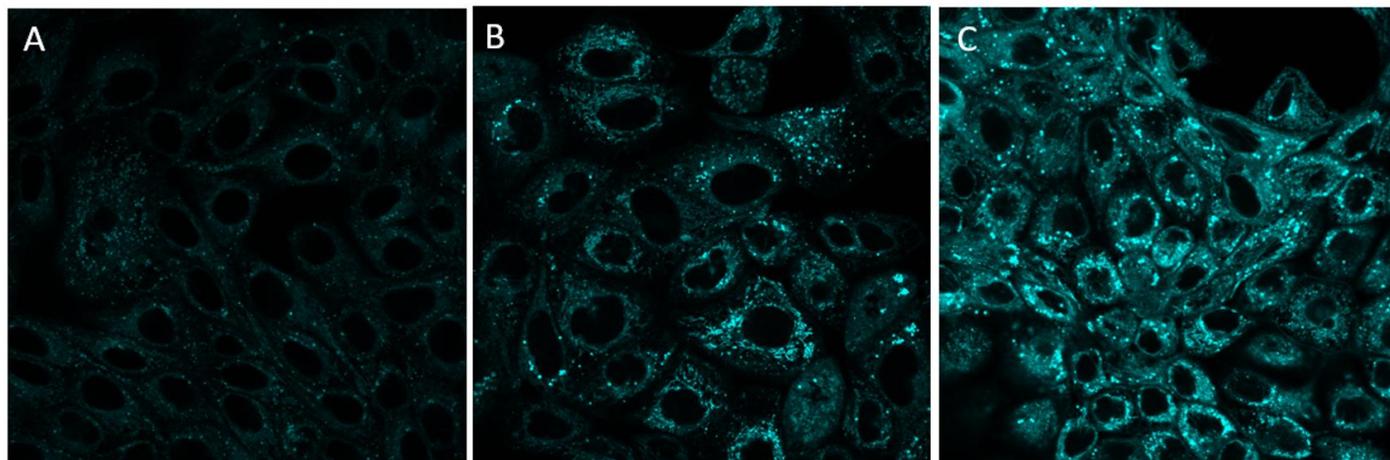


Figure 1: Confocal microscopy image of TFCH probe staining. MDCK cells were cultured and pre-treated with menadione before being stained with 20 μM TFCH probe for 1 hr. **A.** No menadione pre-treatment. **B.** Pre-treated with 100 μM menadione for 1 hr. **C.** Pre-treated with 200 μM menadione for 1 hr. All three samples were stained with 20 μM TFCH probe for 1 hr.

SCT240 TFCH Probe Spectral Data

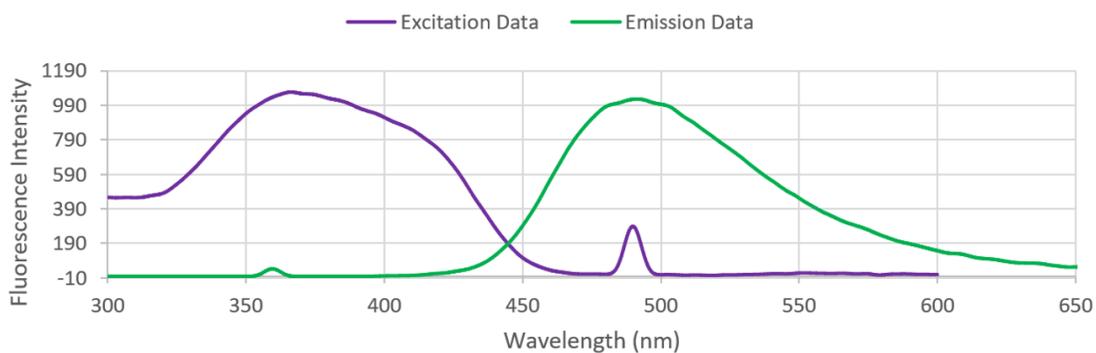


Figure 2: SCT240 spectral characteristics. 7 μL of probe at stock concentration (20 mM) was diluted in 1 mL of DMSO before undergoing excitation and emission scans. Spectral scans were conducted using a Fluorescence Spectrophotometer (PerkinElmer FL8500).

SCT240 TFCH Probe Absorbance

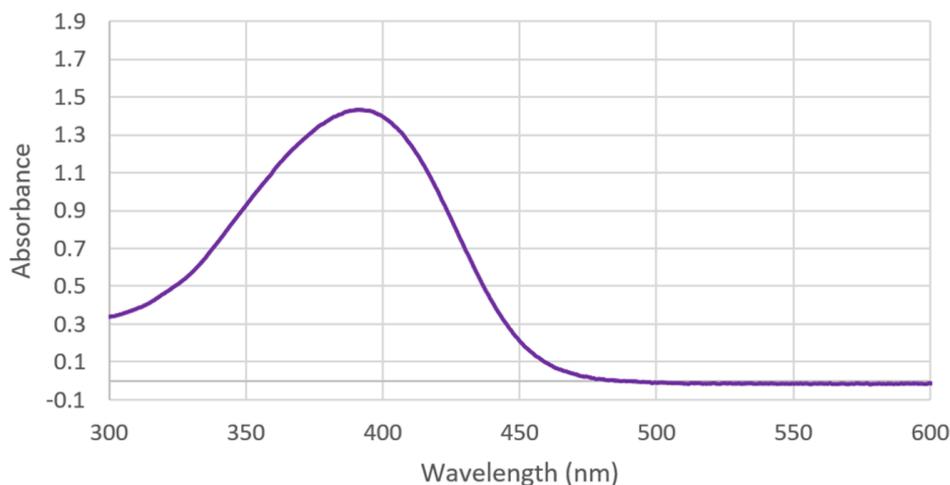


Figure 3: Probe excitation and emission data. 7 μL of probe at stock concentration (10 mM) was diluted in 1 mL of DMSO before undergoing excitation and emission scans. Spectral scans were conducted using Fluorescence Spectrophotometer (PerkinElmer FL8500). **A.** Mitochondrial probe spectral data. **B.** Lysosomal probe spectral characteristics.

Protocols

Preparing BioTracker™ TFCH Oxidative Damage Detection Live Cell probe stock solution

1. Before opening the vial, spin down the solid to the bottom by a microcentrifuge or desktop centrifuge.
2. Warm the vial to ambient temperature. Prepare the TFCH (Molecular Weight: 280.63 g/mol) dye stock solution by dissolving the contents of one vial (1 mg) in 180 μL of DMSO to create a 20 mM solution.
3. Aliquot and store stock solution stored at $-20\text{ }^{\circ}\text{C}$ or below for longer storage.

Labeling and imaging cells

1. Culture cells in an appropriate medium and vessel for fluorescence microscopy.
2. Prepare the TFCH staining solution by diluting the TFCH stock solution 1:1000 in culture medium.
3. Remove the cell culture medium from the cells.
4. Add sufficient staining solution to cover the cells.
5. Incubate for 30-60 minutes, protected from light.
6. Observe the cells under fluorescence microscope for the following fluorescence: $\lambda_{\text{ex}} = 405\text{ nm}$, $\lambda_{\text{em}} = 550\sim 650\text{ nm}$. Emission filters of shorter wavelength may also be suitable based on probe spectral characteristics.

Note: Optimal concentration must be determined by end user.

References

1. Mukherjee, K., Chio, T. I., Gu, H., Sackett, D. L., Bane, S. L., & Sever, S. (2021). A Novel Fluorogenic Assay for the Detection of Nephrotoxin-Induced Oxidative Stress in Live Cells and Renal Tissue. *ACS sensors*, 6(7), 2523-2528.

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Document Template 20306518 Ver 6.0

00153747 Ver 1.0, Rev 21NOV2023, RC, AB

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