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CellTracker® Orange Fluorescent Probe

Cat. No. PA-3012
Storage upon receipt: -20ºC and Desiccate
Protect from light
Solvent for stock: DMSO
Precaution: Avoid amine- and thiol-containing buffers
CellTracker® Orange CMTMR (5-(and-6)-(((4-chloromethyl)benzoyl)amino) tetramethylrhodamine
Molecular Weight: 554.04

*Abs/Em (nm): 541 / 565
* *Absorption and fluorescence emission maxima, determined in aqueous buffer or methanol; values may vary somewhat in cellular environments. CellTracker® Orange CMTMR does not require enzymatic cleavage to activate its fluorescence.*

Introduction

CellTracker® Orange is a thiol-reactive Fluorescent Probe suitable for long-term cell labeling and has been used in a wide variety of applications, including studies of cell adhesion[1,2], migration[3], cell tracing in mixed cultures[1,4], and long term viability and cytotoxicity assays[5,6]. CellTracker® Orange passes freely through cell membranes, but once inside the cell, is transformed into cell-impermeant reaction products that may be retained in living cells through several generations without transfer to adjacent cells in a population.
CellTracker® Orange may be loaded into cells simply by adding the reagent to the culture medium and then washing the cells briefly with fresh medium before analysis. The CellTracker® Dyes contain a chloromethyl group that reacts with thiols, probably in a glutathione S-transferase. mediated reaction, since this has been shown to occur *in vitro*[7]. In most cells, glutathione levels are high (up to 10 mM) and glutathione transferase is ubiquitous. Excess unconjugated reagent passively diffuses to the extracellular medium.
The impermeable reaction products have excellent retention, strong fluorescence and relatively uniform cytoplasmic staining. Cells labeled with CellTracker® Orange were brightly fluorescent for at least 72 hours after incubation in fresh medium at 37℃ and through at least four cell divisions. Most other cell permeant dyes, including the widely used calcein AM and BCECF-AM, are retained in viable cells for no more than a few hours at physiological temperatures. Additionally, the protein- or peptide-dye conjugates may be crosslinked by aldehyde fixatives in labeled cells, permitting long-term sample storage.

The CellTracker® Reagents represent a major breakthrough in the cellular retention of vital probes and are excellent tools for long-term studies of normal and transformed cells in culture[8] and for investigating cellular thiol levels, [9, 10] cell viability and cytotoxicity, [5, 6] transplantation and cell fusion.[11, 12]

Storage and Handling

Upon receipt, store these products at -20℃ desiccated and protected from light. Divide DMSO stock solutions (see below) into single-use aliquots, and store them at -20℃, desiccated and protected from light; avoid freeze-thaw cycles. When stored properly, both the solids and the stock solutions are stable for at least 6 months.

Materials Required but Not Provided

. Anhydrous dimethylsulfoxide (DMSO)
. Phosphate-buffered saline (PBS)

Protocol

The following protocol describes preparing the CellTracker® Reagent, culturing the cells, introducing the CellTracker® Reagent into the cultured cells and imaging the stained cells by fluorescence microscopy. Various factors, such as penetration of the dye into the cells or tissue, may require that some conditions be modified for particular cell types.

Cell Preparation

1.1 Grow the cells. Grow cells in an appropriate culture medium. Adherent cells can be grown on coverslips inside a petri dish filled with culture medium.

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Prepare the CellTracker® Reagent

2.1 Prepare a working solution. Before opening the vial, allow the product to warm to room temperature. Dissolve the lyophilized product in high-quality DMSO to a final concentration of 10 mM. Dilute the stock solution to a final working concentration of 0.5-25 µM in serum-free medium. Avoid amine- and thiol- containing buffers. Warm the working solution to 37°C.

Cell Staining

The optimal concentration of the probe for staining will vary depending upon the application. Testing at least a tenfold range of concentrations is recommended. In general, long-term staining (more than about 3 days) or the use of rapidly dividing cells will require 5-25 µM dye. Less dye (0.5-5 µM) is needed for shorter experiments, such as viability assays. To maintain normal cellular physiology and reduce potential artifacts, the concentration of the dye should be kept as low as possible. The effects of overloading may not be immediately apparent. For example, peripheral blood lymphocytes respond normally to concanavalin A when treated with up to 1 µM dye, but not with more than 5 µM dye.

3.1 Stain the cells. For cells in **suspension**, centrifuge the cells to pellet them and aspirate the supernatant. Resuspend the cells gently in prewarmed working solution. Incubate the cells for 15-45 minutes under growth conditions appropriate for the particular cell type. Centrifuge the cells. For **adherent** cells, when the cells have reached the desired confluence, remove the medium from the dish and add the prewarmed probe. Incubate the cells for 15-45 minutes under growth conditions appropriate for the particular cell type.

3.2 Replace the working solution with culture medium. Replace the probe solution with fresh, prewarmed medium and incubate the cultures for another 30 minutes at 37°C. During this time, the chloromethyl group (and for some probes, the acetate group) of the dye undergo modification or are secreted from the cell.

3.3 Attach suspended cells to coverslips, if desired. Attach suspended cells to coverslips treated with BD Cell-Tak® (Becton Dickinson; Franklin Lakes, NJ).

3.4 Wash the cells. Wash the cells with PBS. This step is especially important if the cells are attached to a Cell-Tak® coated coverslip or any other amine-containing surface.

3.5 Fix the cells, if desired. Fix the cells with 3.7% formaldehyde in PBS for 15 minutes at room temperature. The formaldehyde used in standard fixation protocols crosslinks the amines of the protein.or peptide.dye conjugate.

3.6 Wash the cells with PBS.

3.7 Permeabilize the cells, if desired. When the cells are going to be subsequently labeled with an antibody, a permeabilization step is often required to enhance the antigen's accessibility. Cells can be permeabilized by incubating them in ice-cold acetone for 10 minutes.

Fluorescence Microscopy

The CellTracker® Probes can be used on a wide range of epi-fluorescence microscopes with both standard optics and video enhancement. Optical filters should be selected according to the dye absorption and emission maxima of 541nm and 565nm, respectively.

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References

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