

CellTracker™ Probes for Long-Term Tracing of Living Cells

Quick Facts

Storage upon receipt:

- -20°C
- Avoid freeze-thaw cycles
- Desiccate
- Protect from light

Abs/Em: See Table 1

Solvent for stock: DMSO

Precaution: Avoid amine- and thiol-containing buffers.

should provide an excellent means for long-term studies of normal and transformed cells in culture² and for investigating cellular thiol levels,^{3,4} cell viability and cytotoxicity,⁵ transplantation and cell fusion.^{6,7}

Fluorescent CellTracker reagents include: the green and yellow-green chloromethyl derivatives of fluorescein diacetate (CMFDA), eosin diacetate (CMEDA) and a BODIPY® dye; the blue fluorescent chloromethyl derivatives of amino, hydroxy and difluorohydroxycoumarin (CMAC, CMHC and CMF₂HC); and the orange fluorescent chloromethylbenzoylamino tetramethylrhodamine (CMTMR). All of these reagents can be loaded into cells by simply adding the CellTracker probe to the culture medium and then briefly washing with fresh medium before analysis. The blue (CMAC, CMHC and CMF₂HC), green (CMFDA) and orange (CMTMR) fluorescence in the cells is reasonably photostable during microscopic examination. Moreover, CMAC-, CMHC-, CMFDA- and CMTMR-stained cells were found to be brightly fluorescent for at least 72 hours after incubation in fresh medium at 37°C and through at least four cell divisions. No other permeant dyes of this type, including the widely used calcein AM or BCECF-AM, are retained in viable cells for more than a few hours at such physiological temperatures.

Furthermore, the fluorescent products formed by these chloromethyl derivatives are aldehyde-fixable, thus enabling long-term sample storage. Reagents such as CellTracker Green CMFDA, CellTracker Green BODIPY and CellTracker Blue CMF₂HC may also be probed with our anti-fluorescein, anti-BODIPY FL and anti-Marina Blue® antibodies, respectively, and developed for electron microscopy using standard immunochemical techniques. Similarly, CellTracker Orange CMTMR can be probed using our anti-tetramethylrhodamine antibody. The enzymatic products of the chloromethyl coumarins have excellent retention, strong fluo-

Introduction

Molecular Probes has developed a series of fluorescent probes that are retained in living cells through several generations, are inherited by daughter cells after cell fusion and are not transferred among adjacent cells in a population. These CellTracker™ reagents represent a major breakthrough in improving the cellular retention of vital probes. CellTracker probes freely pass through cell membranes, but once inside the cell, undergo what is believed to be a glutathione *S*-transferase-mediated reaction, producing a cell-impermeant reaction product.¹ Since glutathione levels in most cells are high (up to 10 mM) and glutathione transferase is ubiquitous, these CellTracker reagents

Table 1. Spectral characteristics of the fluorescent CellTracker probes.

Cat #	CellTracker Probe	Abs † (nm)	Em † (nm)
C-2110	CellTracker Blue CMAC (7-amino-4-chloromethylcoumarin)	354	466
C-12881	CellTracker Blue CMF ₂ (4-chloromethyl-6,8-difluoro-7-hydroxycoumarin)	371	464
C-2111	CellTracker Blue CMHC (4-chloromethyl-7-hydroxycoumarin)	372	470
C-2925, C-7025	CellTracker Green CMFDA * (5-chloromethylfluorescein diacetate)	492	516
C-2102	CellTracker Green BODIFY (8-chloromethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene)	522	528
C-2926	CellTracker Yellow-Green CMEDA * (5-chloromethyleosin diacetate)	524	544
C-2927	CellTracker Orange CMTMR (5-(and-6)-(((4-chloromethyl)benzoyl)amino)tetramethylrhodamine)	540	566

* CMFDA and CMEDA are nonfluorescent until their acetate groups are cleaved by intracellular esterases; hydrolysis of the acetates yields a product with the indicated wavelengths. † Absorption and fluorescence emission maxima, determined in aqueous buffer or methanol; values may vary somewhat in cellular environments.

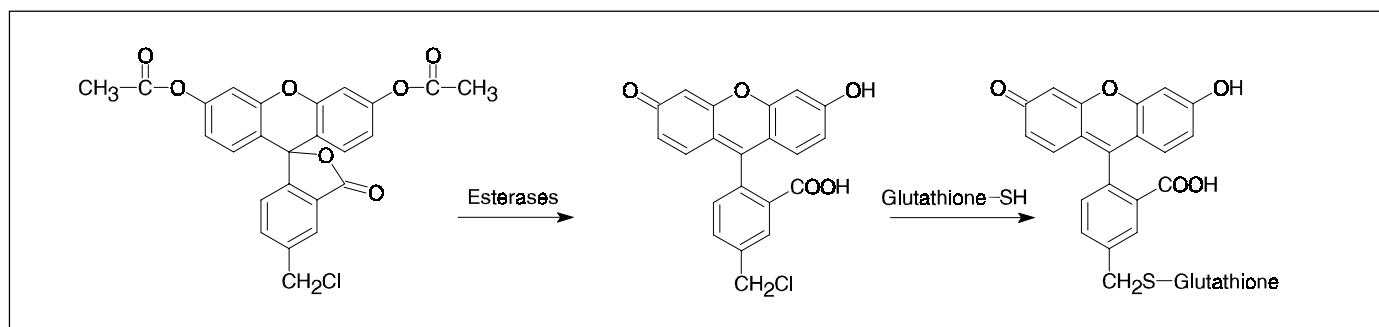


Figure 1. The two intracellular reactions of our glutathione-reactive dye, 5-chloromethylfluorescein diacetate (CMFDA). Although the dye may react with intracellular glutathione first, the product is nonfluorescent until acted upon by intracellular esterases.

rescence and relatively uniform cytoplasmic staining, making these derivatives potentially useful for correcting motion artifacts in imaging. Assuming the distribution and compartmentalization of the dyes are similar, then co-incorporation of CMAC with fluo-3 or Calcium Green™, and CMFDA with Calcium Orange™ or Calcium Crimson™, may permit ratio measurements of intracellular calcium. It is probable that the eosin–glutathione conjugate is phototoxic, and thus can be used for cell ablation studies.

Description of CellTracker Reagents

All of the CellTracker dyes contain a mildly thiol reactive chloromethyl reactive group. Once inside the cell, the chloromethyl group reacts with intracellular thiols, transforming the probe into a cell-impermeant fluorescent dye–thioether adduct that can be fixed with aldehyde fixatives. Excess unconjugated reagent passively diffuses to the extracellular medium. While the CellTracker probes may react with intracellular proteins to some extent, we believe they are primarily conjugated to the abundant tripeptide glutathione by glutathione *S*-transferase, a reaction that has been shown to occur *in vitro*¹ (Figure 1).

CMFDA and CMEDA are colorless and nonfluorescent until cytosolic esterases cleave off their acetates, releasing a brightly fluorescent product. The argon-ion laser–excitable eosin analog produces a less fluorescent product than does CMFDA, but has lower sensitivity to variations in intracellular pH. CellTracker Orange CMTMR, CellTracker Green BODIPY and CellTracker Blue CMAC, CMHC and CMF₂HC do not require enzymatic cleavage to activate their fluorescence.

Contents and Storage

Upon receipt, these products should be stored desiccated at -20°C until required for use. AVOID REPEATED FREEZING AND THAWING.

Before opening the vial, allow product to warm to room temperature. Dissolve the lyophilized product in high-quality, anhydrous dimethylsulfoxide (DMSO) to a final concentration of 10 mM; the molecular weight (MW) and unit size are indicated on the product label. To avoid repeated freezing and thawing, divide the stock solution into small aliquots and store frozen at -20°C, desiccated and protected from light. When stored properly, both the solids and the stock solutions are stable for at least six months.

Experimental Protocol

The following protocol describes the methodology for culturing cells, introducing the CellTracker reagent into the cultured cells and imaging the stained cells by fluorescence microscopy. Our suggested initial conditions may require modifications based on the cell type used and permeability of the dye into the cells or tissue, among other factors.

Cell Preparation and Staining

1.1 Prepare a sterile working solution: Dissolve the product in sufficient anhydrous DMSO to yield a 10 mM stock solution (see *Contents and Storage*). Dilute the stock solution to a final working concentration of between 0.5 and 25 μM in serum-free medium or the buffer of your choice. Avoid amine- and thiol-containing buffers.

1.2 The concentration of the probe necessary for optimal staining will vary depending upon the application; we recommend testing at least a tenfold range of concentrations. In general, long-term staining (more than about three 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 from overloading, the concentration of the dye should be kept as low as possible. Effects of overloading may not be immediately apparent. For example, peripheral blood lymphocytes treated with up to 1 μM dye respond normally to concanavalin A, while apparently healthy cells incubated with 5 μM dye or more do not.

1.3 For adherent cells, grow cells on coverslips inside a petri dish filled with the appropriate culture medium. When cells have reached the desired confluence, remove the medium from the dish and add the prewarmed (37°C) probe-containing (0.5–25 μM) medium. Incubate the cells for 15–45 minutes under growth conditions appropriate for the particular cell type. Then replace the loading solution with fresh prewarmed medium and incubate the cultures for another 30 minutes at 37°C. During this time, the CellTracker molecules will undergo modification of the chloromethyl group and, for some probes, acetate modification, or they will be extruded from the cell. If the cells are to be fixed and permeabilized, continue to *Fixation and Permeabilization*.

1.4 For suspended cells, centrifuge to obtain a cell pellet and aspirate the supernatant. Resuspend the cells gently in pre-

warmed (37°C) probe-containing (0.5–25 µM) medium. Incubate the cells for 15–45 minutes under growth conditions appropriate for the particular cell type. Re-pellet the cells by centrifugation and resuspend them in fresh prewarmed medium. Incubate the cells for another 30 minutes to ensure complete modification of the probe and then wash the cells again. If the cells are to be fixed and permeabilized, continue to *Fixation and Permeabilization*.

Alternatively, suspended cells may be attached to coverslips that have been treated with BD Cell-Tak® (Becton Dickinson; Franklin Lakes, NJ); in this case, see step 1.3.

Fixation and Permeabilization

2.1 Before fixation, the cells must be washed with phosphate-buffered saline (PBS) or any standard saline solution. This step is especially important if the cells are attached to a Cell-Tak-coated coverslip or any other amine-containing surface.

2.2 Standard fixation protocols using formaldehyde fixatives should effectively crosslink the amines of the protein- or pep-

tide-probe conjugate. Typically, we fix the cells for 15 minutes at room temperature using 3.7% paraformaldehyde.

2.3 After fixation, the cells should be rinsed in PBS.

2.4 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. Following permeabilization, the cells should be rinsed in PBS.

Fluorescence Microscopy

The CellTracker probes are designed to be used on a wide range of epifluorescence microscopes with both standard optics and video enhancement. Table 1 summarizes the spectral characteristics of the CellTracker Probes; optical filters should be selected accordingly.

References

1. FASEB J 6, A1835 (1992); 2. Nature 363, 549 (1993); 3. Cytometry 14, 747 (1993); 4. Cytometry 12, 184 (1991); 5. Toxicol Appl Pharmacol 112, 235 (1992); 6. Anal Biochem 216, 271 (1994); 7. Biophys J 67, 1574 (1994).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
C-2110	CellTracker™ Blue CMAC (7-amino-4-chloromethylcoumarin)	5 mg
C-12881	CellTracker™ Blue CMF ₂ HC (4-chloromethyl-6,8-difluoro-7-hydroxycoumarin)	5 mg
C-2111	CellTracker™ Blue CMHC (4-chloromethyl-7-hydroxycoumarin)	5 mg
C-2102	CellTracker™ Green BODIPY® (8-chloromethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene)	5 mg
C-2925	CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate)	1 mg
C-7025	CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate) *special packaging*	20x50 µg
C-2927	CellTracker™ Orange CMTMR (5-(and-6)-(((4-chloromethyl)benzoyl)amino)tetramethylrhodamine) *mixed isomers*	1 mg
C-2926	CellTracker™ Yellow-Green CMEDA (5-chloromethyleosin diacetate)	1 mg

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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