

## CellTracker™ Probes for Long-Term Tracing of Living Cells

### Quick Facts

#### Storage upon receipt:

- ≤-20°C
- Desiccate
- Protect from light

**Abs/Em:** See Table 1

**Solvent for stock:** DMSO

**Precaution:** Avoid amine- and thiol-containing buffers.

chloromethyl group that reacts with thiols (Figure 1), probably in a glutathione *S*-transferase-mediated reaction, since this has been shown to occur *in vitro*<sup>1</sup>. In most cells, glutathione levels are high (up to 10 mM) and glutathione transferase is ubiquitous. The reagent is transformed into a cell-impermeant fluorescent dye-thioether adduct that can be fixed with aldehyde fixatives, permitting long-term sample storage. Excess unconjugated reagent passively diffuses to the extracellular medium.

Fluorescent CellTracker reagents include the blue-fluorescent chloromethyl derivatives of amino-, hydroxy- and difluoro hydroxycoumarin (CMAC, CMHC and CMF<sub>2</sub>HC), the green-fluorescent chloromethyl derivatives of fluorescein diacetate (CMFDA) and a BODIPY® dye, the orange-fluorescent CMTMR and CMRA and the red-fluorescent CMTPX. CellTracker Blue CMAC, CMHC and CMF<sub>2</sub>HC, CellTracker Green BODIPY, CellTracker Orange CMTMR, and CellTracker Red CMTPX do not require enzymatic cleavage to activate their fluorescence, whereas the green CMFDA and orange CMRA do.<sup>4</sup> The impermeable reaction products of the chloromethyl coumarins have excellent retention, strong fluorescence and relatively uniform cytoplasmic staining, making these derivatives potentially useful for correcting motion artifacts in imaging. CMFDA is colorless and nonfluorescent until cytosolic esterases cleave off the acetates, releasing a brightly fluorescent product (see Figure 1).

The blue (CMAC, CMHC and CMF<sub>2</sub>HC), green (CMFDA), orange (CMTMR and CMRA) and red (CMTPX) fluorescence in the cells is reasonably photostable during microscopic examination. Cells stained with CMAC, CMHC, CMFDA and CMTMR probes were brightly fluorescent for at least 72 hours after incubation in fresh medium at 37°C and through at least four cell divisions (retention time of these probes within the cell can vary

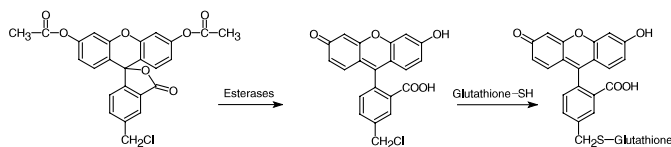
### Introduction

Molecular Probes has developed a series of fluorescent probes that are retained in living cells through several generations. They are inherited by daughter cells after cell fusion and are not transferred to adjacent cells in a population. These CellTracker™ reagents can be loaded into cells by adding the reagent to the culture medium and then washing the cells briefly with fresh medium before analysis. These reagents pass freely through cell membranes, but once inside the cell, are transformed into cell-impermeant reaction products. The CellTracker dyes contain a

**Table 1.** Spectral characteristics of the fluorescent CellTracker probes.

Cat #	CellTracker Probe	Abs* (nm)	Em* (nm)
C2110	CellTracker Blue CMAC (7-amino-4-chloromethylcoumarin)	353	466
C12881	CellTracker Blue CMF <sub>2</sub> HC (4-chloromethyl-6,8-difluoro-7-hydroxycoumarin)	371	464
C2111	CellTracker Blue CMHC (4-chloromethyl-7-hydroxycoumarin)	372	470
C2925, C7025	CellTracker Green CMFDA (5-chloromethylfluorescein diacetate)	492 †	517 †
C2102	CellTracker Green BODIFY (8-chloromethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene)	522	529
C2927	CellTracker Orange CMTMR (5-(and-6)-((4-chloromethyl)benzoyl)amino) tetramethylrhodamine	541	565
C34551	CellTracker Orange CMRA	548	576
C34552	CellTracker Red CMTPX	577	602

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



**Figure 1.** The two intracellular reactions of the CellTracker Green CMFDA reagent. Although the dye may react with intracellular glutathione first, the product is nonfluorescent until acted upon by intracellular esterases, shown here as the first reaction.

dependent upon the cell type, incubation conditions and other factors and should be empirically determined prior to any tracing experiments). No other permeant dyes of this type, including the widely used calcein AM and BCECF-AM, are retained in viable cells for more than a few hours at such physiological temperatures.

Co-incorporation of a CellTracker reagent and a calcium indicator, for example the incorporation of CMAC with fluo-3 or Calcium Green™ or the incorporation of CMFDA with Calcium Orange™ or Calcium Crimson™, may permit ratio measurements of intracellular calcium, if the distribution and compartmentalization of the dyes are similar. The eosin–glutathione conjugate is likely to be phototoxic, and may be used for cell ablation studies. Anti-fluorescein, anti-BODIPY FL or anti-tetramethylrhodamine antibodies can be used to amplify the fluorescence signal of CellTracker Green CMFDA, CellTracker Green BODIPY CellTracker Orange CMTMR, respectively.

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<sup>2</sup> and for investigating cellular thiol levels,<sup>3,4</sup> cell viability and cytotoxicity,<sup>5</sup> transplantation and cell fusion.<sup>6,7</sup>

## Materials

### Contents

The three CellTracker Blue dyes and the CellTracker Green BODIPY dye are all supplied in unit sizes of 5 mg dry powder. CellTracker Green CMFDA and CellTracker Orange CMTMR are supplied in unit sizes of 1 mg. CellTracker Green CMFDA, CellTracker Orange CMRA and CellTracker Red CMPTX are supplied in our special packaging that consists of 20 vials each with 50 µg of dry powder.

### Storage and Handling

Upon receipt, store these products at  $\leq -20^{\circ}\text{C}$ , desiccated and protected from light. Divide DMSO stock solutions (see below) into single-use aliquots, and store them at  $\leq -20^{\circ}\text{C}$ , 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.

### 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 epifluorescence microscopes with both standard optics and video enhancement. Optical filters should be selected according to the dye. Table 1 summarizes the spectral characteristics of the CellTracker probes.

## 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
C2110	CellTracker™ Blue CMAC (7-amino-4-chloromethylcoumarin).....	5 mg
C12881	CellTracker™ Blue CMF <sub>2</sub> HC (4-chloromethyl-6,8-difluoro-7-hydroxycoumarin).....	5 mg
C2111	CellTracker™ Blue CMHC (4-chloromethyl-7-hydroxycoumarin).....	5 mg
C2102	CellTracker™ Green BODIPY® (8-chloromethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene).....	5 mg
C2925	CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate).....	1 mg
C7025	CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate) *special packaging*.....	20 x 50 µg
C34551	CellTracker™ Orange CMRA *special packaging*.....	20 x 50 µg
C2927	CellTracker™ Orange CMTMR (5-(and-6)-((4-chloromethyl)benzoyl)amino)tetramethylrhodamine) *mixed isomers*.....	1 mg
C34552	CellTracker™ Red CMTPX *special packaging*.....	20 x 50 µg

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