

## CellTracker™ Fluorescent Probes

Catalog nos. C2102, C2110, C2111, C2925, C2927, C7025, C10094, C12881, C34551, C34552, C34565

Table 1 Contents and storage

Material	Amount	Storage	Stability
CellTracker™ Blue dyes and CellTracker™ Green BODIPY® dyes	5 mg dry powder	<ul style="list-style-type: none"> <li>• ≤-20°C</li> <li>• Desiccate</li> <li>• Protect from light</li> </ul>	When stored as directed the product is stable for 1 year
CellTracker™ Violet BMQC dye	5 × 0.1 mg dry powder		
CellTracker™ Green CMFDA and CellTracker™ Orange CMTMR dyes	1 mg dry powder		
CellTracker™ Green CMFDA, CellTracker™ Orange CMRA, and CellTracker™ Red CMPTX dyes	20 × 50 µg dry powder		
CellTracker™ Deep Red dye	20 × 15 µg dry powder		
Approximate fluorescence excitation/emission maxima: See Table 2 (page 2).			

## Introduction

Cell movement and location studies require specialized probes that are nontoxic to living cells and are available in a range of fluorescent colors to match instrument lasers and filters and to accommodate co-staining with antibodies or other cell analysis probes. The CellTracker™ fluorescent probes are excellent tools for monitoring cell movement, location, proliferation, migration, chemotaxis, and invasion.

The CellTracker™ fluorescent probes have been designed to freely pass through cell membranes; however, once inside the cell are transformed into cell-impermeant reaction products. The CellTracker™ fluorescence probes (except for CellTracker™ Deep Red) contain a chloromethyl or bromomethyl group that reacts with thiol groups, utilizing a glutathione S-transferase-mediated reaction. In most cells, glutathione levels are high (up to 10 mM) and glutathione transferase is ubiquitous. CellTracker™ Deep Red probe contains a succinimidyl ester reactive group, which reacts with amine groups present on proteins.

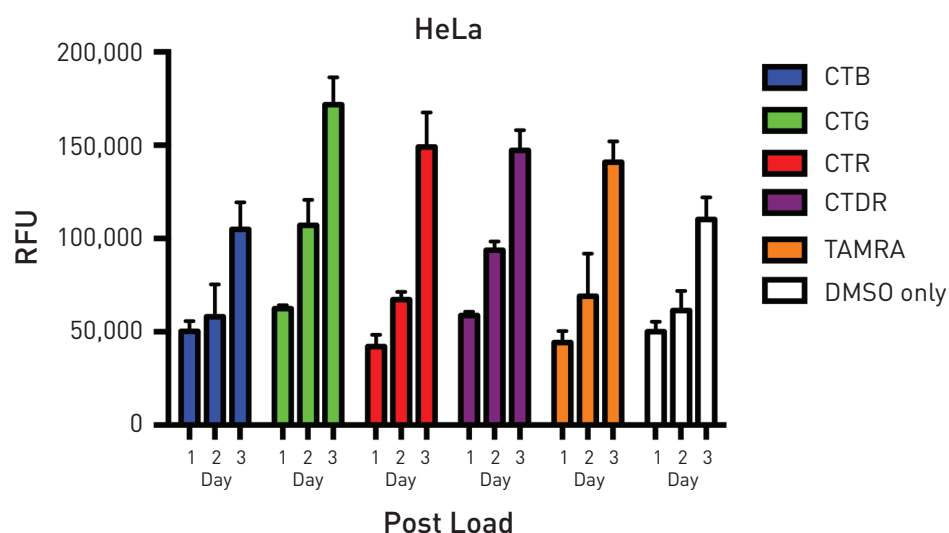
After conversion to impermanent versions, the CellTracker™ fluorescent probes are well retained in living cells through several generations. The probes are transferred to daughter cells, but are not transferred to adjacent cells in a population. Cells loaded with the CellTracker™ fluorescent probes display fluorescence for at least 72 hours and exhibit ideal tracking dye properties—they are stable, nontoxic at working concentrations (Figure 1, page 2), well retained in cells, and brightly fluorescent at physiological pH. Additionally, several CellTracker™ fluorescent probes with various excitation and emission spectra are available (Table 2, page 2) allowing for multiplexing.

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Fluorescent CellTracker™ reagents include the blue-fluorescent chloromethyl derivatives of amino-, hydroxy-, and difluorohydroxycoumarin (CMAC, CMHC and CMF2HC), 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 CMF2HC, CellTracker™ Violet, the violet-fluorescent bromomethyl derivative of coumarin (BMQC), 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 require enzymatic cleavage. The impermeable reaction products of the chloromethyl or bromomethyl 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 non-fluorescent until cytosolic esterases cleave off the acetates, releasing a brightly fluorescent product.

In addition to bright fluorescence and excellent retention the CellTracker™ fluorescent probes do not contribute to cytotoxicity (Figure 1, below).

**Figure 1** CellTracker™ fluorescence probes display no cytotoxicity. CellTracker™ Blue (CTB), CellTracker™ Green (CTG), CellTracker™ Red (CTR), and CellTracker™ Deep Red (CTDR) were loaded into HeLa cells following manufacture instructions. Time points of Day 1, 2, and 3 post-loaded were harvested and proliferation was measured using the CyQUANT® Direct Cell Proliferation Assay. RFU = Relative Fluorescence Units.



**Table 2** Spectral characteristics of the fluorescent CellTracker™ probes.

Cat. no.	CellTracker™ Probe	MW	Ex * (nm)	Em * (nm)
C2110	CellTracker™ Blue CMAC [7-amino-4-chloromethylcoumarin]	209.6	353	466
C12881	CellTracker™ Blue CMF <sub>2</sub> HC [4-chloromethyl-6,8-difluoro-7-hydroxycoumarin]	246.6	371	464
C2111	CellTracker™ Blue CMHC [4-chloromethyl-7-hydroxycoumarin]	210.6	372	470
C10094	CellTracker™ Violet BMQC [2,3,6,7-tetrahydro-9-bromomethyl-1H,5H-quinolizino[9,1-g]coumarin]	334.2	415	516
C2925, C7025	CellTracker™ Green CMFDA [5-chloromethylfluorescein diacetate]	464.9	492 †	517 †
C2102	CellTracker™ Green BODIPY [8-chloromethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene]	296.6	522	529
C2927	CellTracker™ Orange CMTMR [5-(and-6)-[[[4-chloromethyl]benzoyl]amino]tetramethylrhodamine]	554.0	541	565
C34551	CellTracker™ Orange CMRA	550.4	548	576
C34552	CellTracker™ Red CMTPX	686.3	577	602
C34565	CellTracker™ Deep Red	698.3	630	660

\*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. MW = Molecular weight.

## Before Starting

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### Materials Required but Not Provided

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

### Preparing Cells

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

### Caution

No data are available addressing the mutagenicity or toxicity of CellTracker™ reagents (Component A). Handle the DMSO dye solution with caution because DMSO is known to facilitate the entry of organic molecules into tissues. Always wear suitable protective clothing, gloves, and eye/face protection when handling this reagent. Dispose of the reagents in compliance with all pertaining local regulations.

### Important

Avoid amine- and thiol-containing buffers.

## Experimental Protocols

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The following protocol describes introducing the CellTracker™ reagent into 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.

The optimal concentration of the probe for staining varies depending upon the application. We recommend testing at least a ten-fold range of concentrations. In general, long-term staining (more than about 3 days) or the use of rapidly dividing cells requires 5–25  $\mu\text{M}$  dye. Less dye (0.5–5  $\mu\text{M}$ ) is needed for shorter experiments, such as viability assays. Due to the high fluorescent signal resulting from staining with CellTracker™ Deep Red dye, the optimal concentration for this dye is 250 nM–1  $\mu\text{M}$ . To maintain normal cellular physiology and reduce potential artifacts, keep the dye concentration 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  $\mu\text{M}$  dye, but not with more than 5  $\mu\text{M}$  dye.

### Prepare Working Dye Solution

- 1.1 Before opening the dye vial, allow the product to warm to room temperature. Dissolve the lyophilized product in high-quality DMSO to a final concentration of 10 mM. For CellTracker™ Deep Red dye, add 20  $\mu\text{L}$  of DMSO per vial to make a 1 mM (1000X) solution. Dilute the stock solution to a final working concentration of 0.5–25  $\mu\text{M}$  in serum-free medium. Warm the CellTracker™ Working Solution to 37°C.

**Note:** For the molecular weight of CellTracker™ reagents, see Table 2 (page 2).

## Staining Protocol – Cells in Suspension

- 1.2 Harvest cells by centrifugation and aspirate the supernatant. Resuspend the cells gently in pre-warmed CellTracker™ Working Solution (prepared in step 1.1).
- 1.3 Incubate 15–45 minutes under growth conditions appropriate for the particular cell type.
- 1.4 Centrifuge the cells and remove the CellTracker™ Working Solution.
- 1.5 Add culture media of choice and dispense the labeled cell onto slide or into a culture vessel of choice.
- 1.6 Image using the appropriate emission and excitation filters for the CellTracker™ probe (Table 2, page 2).

## Staining Protocol – Adherent Cells

- 2.1 Remove culture media.
- 2.2 Gently add the pre-warmed CellTracker™ Working Solution (prepared in step 1.1).
- 2.3 Incubate 15–45 minutes under growth conditions appropriate for the particular cell type.
- 2.4 Remove the CellTracker™ Working Solution.
- 2.5 Add culture media of choice.
- 2.6 Image using the appropriate emission and excitation filters for the CellTracker™ probe (Table 2, page 2).

## Fluorescence Microscopy

The CellTracker™ probes can be used on a wide range of epifluorescence microscopes with standard optics and video enhancement. Select optical filters according to the dye. Table 2 (page 2) summarizes the spectral characteristics of the CellTracker™ probes.

## References

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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 website or from our Customer Service Department.

Cat. no.	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
C10094	CellTracker™ Violet BMQC [2,3,6,7-tetrahydro-9-bromomethyl-1H,5H-quinolizino[9,1-g]coumarin]. . . . .	5 × 100 µg
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 × 50 µg
C34551	CellTracker™ Orange CMRA *special packaging*. . . . .	20 × 50 µg
C2927	CellTracker™ Orange CMTMR [5-(and-6)-[[[4-chloromethyl]benzoyl]amino]tetramethylrhodamine] *mixed isomers*. . . . .	1 mg
C34552	CellTracker™ Red CMTMX *special packaging*. . . . .	20 × 50 µg
C34565	CellTracker™ Deep Red . . . . .	20 × 15 µg

## Purchaser Notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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