Lumiprobe
Life science solutions

Live Cell Labeling and Long-Term Tracking with CytoTracer® Probes

CytoTracer® probes are cell-permeant fluorescent dyes designed for monitoring cell movement, proliferation, migration, and chemotaxis. They enter cells freely and are converted into cell membrane-impermeant, thiol-reactive products. Once inside, they are retained through several cell divisions, transfer to daughter cells, and do not leak to neighbors. At working concentrations, they show negligible cytotoxicity for at least 72 h.

CytoTracer® probes have different spectral properties. Some dyes require esterase cleavage (CMFDA, CMRA) to become fluorescent; others are intrinsically fluorescent (CMAC, CMHC, BMQC, CM-BDP®, CMTPX).

Before You Start

- Avoid buffers containing amines (e.g., Tris, glycine) or thiols (e.g., β-mercaptoethanol, DTT) – they compete with the reactive groups and reduce labeling efficiency.
- Test a concentration range before use. An optimal working concentration depends on cell type and experiment duration:
  - Long-term (>3 days) or rapidly dividing cells: 5–25 μM.
  - Short-term assays (e.g., viability): 0.5–5 μM.
- Overloading can impair cell physiology – always use the lowest effective concentration.
- For experiments lasting >72 h, re-labeling may be needed as fluorescence dilutes with each division. Seed cells at lower density to minimize overcrowding and maintain dye retention.
- Most CytoTracer® probes are well retained after fixation with paraformaldehyde (PFA) (up to 4%) but are not compatible with methanol or acetone fixation (these permeabilize membranes and leach out the dye). For immunostaining after labeling, fix with PFA and then permeabilize with mild detergent – fluorescence intensity may decrease by ~20–30%.
- Use anti-fade mounting media if imaging fixed samples; for live imaging, reduce illumination intensity and exposure time to avoid phototoxicity and bleaching.

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Solution Preparation

1. Stock Solution

- Allow the dye vial to warm to room temperature before opening (prevents moisture condensation).
- Dissolve the entire contents in high-quality anhydrous DMSO to achieve 10 mM.

2. Working Solution

- Dilute the stock in serum-free medium to the desired final concentration (e.g., 1–10 μM).
- Pre-warm the working solution to 37 °C before adding to cells.

Important! Serum and proteins can bind dye and reduce uptake – use serum-free medium during labeling; serum can be added after washing.

Cell Staining

1. Suspension Cells

1. Harvest cells by centrifugation (e.g., 5 min at 200–300 × *g*) and discard supernatant.
2. Gently resuspend the cell pellet in pre-warmed CytoTracer® Working Solution at a density of 1–5 × 10^6 cells/mL (adjust as needed).
3. Incubate for 15–45 minutes under normal growth conditions (37 °C, 5% CO_2). Longer incubation may improve loading but can increase background – 30 minutes is a good starting point.
4. Centrifuge cells and remove the dye-containing supernatant.
5. (*Optional*) Wash once with pre-warmed complete culture medium to remove extracellular dye.
6. Resuspend cells in fresh complete medium and plate onto slides or culture vessels.
7. Image using appropriate excitation/emission filters.

2. Adherent Cells

1. Grow cells on coverslips in a Petri dish with culture medium.
2. Replace medium with pre-warmed Working Solution (volume enough to cover coverslips).
3. Incubate 15–45 min at 37 °C.
4. Aspirate dye solution, wash twice with fresh medium to remove residual dye.
5. Add back complete medium and return to incubator for at least 30 min to allow esterase cleavage (if using CMFDA or CMRA) and complete conversion.
6. Mount coverslips for microscopy or continue culture for time-lapse imaging.

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Spectral Properties

Cat. No.	Probe	Ex (nm)	Em (nm)	Channel
4682-x	CMAC	345	465	Blue
4699-x	CM-BDP®	514	542	Green
4276-x	CMTMR	540	565	Orange

Troubleshooting

Issue	Likely Cause	Solution
Weak or no fluorescence	Insufficient dye loading; esterase cleavage incomplete (for CMFDA/CMRA)	Increase concentration or incubation time; ensure 37 °C incubation; allow ≥30 min post-loading before imaging
High background or extracellular staining	Insufficient washing; dye aggregates	Add a wash step with serum-containing medium after loading; use fresh working solution
Cytotoxicity or altered cell morphology	Dye concentration too high	Titrate downwards; use lowest effective concentration; check cell viability (e.g., trypan blue)
Dye not retained over generations	Cells dividing too fast or dye diluted	Use higher concentration (up to 25 µM) for rapidly dividing cells; re-stain if needed

Storage and Handling

- **Lyophilized powder**: store at ≤ -20 °C, desiccated, protected from light. Stable for 1 year.
- **DMSO stock solutions**: once dissolved, store at -20 °C in single-use aliquots; avoid repeated freeze-thaw cycles (stability >6 months if kept anhydrous).
- **Working solutions**: prepare fresh in serum-free medium on the day of use.

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