BioTracker™ 488 Green CSFE Cell Proliferation Kit

Live Cell Dye

Cat. # SCT110

pack size: Kit

Store at -20ºC

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

BioTracker™ SE Cell Proliferation Kits diffuse passively into live cells and are used for long-term cell labeling. They are initially non-fluorescent esters of amine-reactive dyes, but are converted to fluorescent dyes by intracellular esterases. The dyes then covalently react with amine groups on intracellular proteins, forming fluorescent conjugates that are retained in the cell. Immediately after staining a single, bright fluorescent population will be detected by flow cytometry. Each cell division that occurs after labeling is revealed by the appearance of a successively dimmer fluorescent peak on a flow cytometry histogram (Fig. 1). Cell proliferation dyes can be used to track cell divisions in vivo or in vitro. The staining can withstand fixation and permeabilization for subsequent immunostaining. Alternative applications of cell proliferation dyes include uniform labeling of cell cytoplasm for microscopy, or labeling cells for quantitation of cell number by microplate reader.

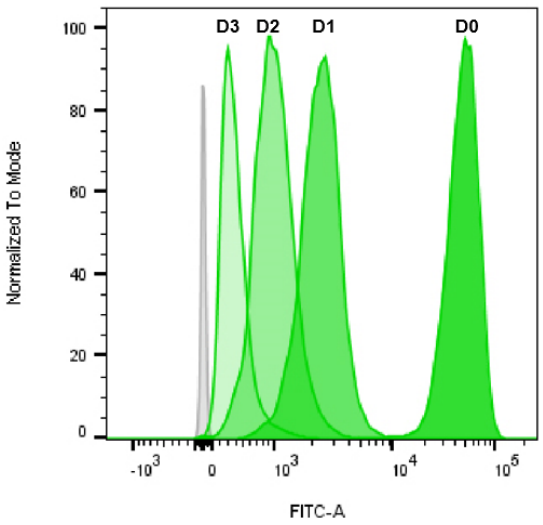

Figure 1. Principle of cell division tracking with BioTracker™ SE Cell Proliferation Dyes. When a stained cell divides, each daughter cell receive half the dye in the parent cell, with each cell division represented as a successively dimmer population on a flow cytometry histogram. Data shown using 5 uM BioTracker™ 488 Green CSFE Cell Proliferation Kit to stain Jurkat cells.

Kit Components

1. 10 vials of BioTracker™ 488 Green CFSE Dye (50µg) (CS224588)

2. 1 vials of Anhydrous DMSO (500µL) (CS224578)

Note: When used at a dye concentration of 1 uM in 1 mL of cells at 1x10^6 cells/mL, each kit can be used to label 1000 samples

Storage

Store BioTracker™ 488 Green CSFE Cell Proliferation Kit -20ºC.
Protect From Light.

Note: Centrifuge vial briefly to collect contents at bottom of vial before opening. Ideally the 5 mM DMSO stock solution should be prepared on the day of use. Aliquots may be stored for later use, but activity may be reduced over time. The dyes should only be added to aqueous buffer immediately before staining.

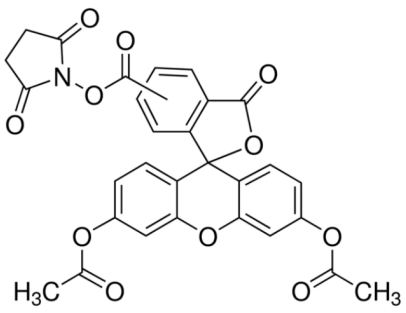

Figure 2. Carboxyfluorescein succinimidyl ester (CFSE) is a fluorescent cell staining dye. CFSE is cell permeable and covalently couples, via its succinimidyl group, to intracellular molecules. Due to this covalent coupling reaction fluorescent CFSE can be retained within cells for extremely long periods.

Spectral Properties

Absorbance: 495nm
Emission: 519nm

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Assay Protocol

The following protocol is a general labeling procedure. Because of differences in cell types and variations in culture conditions, optimization of the dye concentration, staining time, and/or staining temperature may be necessary. Higher dye concentrations may be required to track more cell generations, while lower concentrations may be sufficient to track fewer divisions. We recommend using the lowest dye concentration that yields sufficient signal for your assay, because cell proliferation dyes can be toxic to cells at high concentrations.

Cell Proliferation Dye Preparation

Prepare a cell proliferation dye stock solution by dissolving one vial of dye in 18µL of anhydrous DMSO to make a 5mM stock solution. Recommended staining concentration is 1-5µM. Protect dye stock solutions from light. The DMSO stock solution should only be prepared on the day of use, and not subjected to freeze/thaw cycles. The dyes should only be added to aqueous buffer immediately before staining. Do not use buffers containing Tris or other free amines.

Labeling Cells in Suspension

1. Pellet cells by centrifugation and aspirate the supernatant.
2. Resuspend the cells at 10^6 cells/mL in pre-warmed (37°C) PBS (or similar buffer) containing 1µM cell proliferation dye. Protect cells from light for this and all subsequent steps.

Note: staining can be performed in cell culture medium containing serum, however, this results in 5-10 fold lower fluorescent signal compared to labeling in buffer without serum or other proteins.

3. Incubate the cells for 10-15 minutes at room temperature or 37°C, to allow dye uptake.
4. Add an equal volume of cell culture medium and incubate for 5 minutes at room temperature or 37°C to hydrolyze free dye.
5. Pellet the labeled cells by centrifugation and resuspend in an equal volume of fresh pre-warmed cell culture medium.
6. Incubate the cells for 15-30 minutes at 37°C to allow the dye to react with intracellular proteins.
7. Pellet the labeled cells by centrifugation and resuspend in an equal volume of fresh pre-warmed cell culture medium. Proceed to flow cytometry analysis (step 9). Alternatively, return cells to incubator and culture for the desired period of time to allow cells to divide.
8. Optional: perform formaldehyde fixation, permeabilization, and/or immunostaining.
9. Analyze by flow cytometry in the appropriate channel (see Spectral Properties).

Labeling of Adherent Cells

1. Grow cells to desired density on coverslips or chamber slides.
2. Remove the medium and add a sufficient volume of pre-warmed PBS containing cell proliferation dye to completely cover cells. Protect cells from light at this and all subsequent steps.

Note: staining can be performed in cell culture medium containing serum, however, this results in 5-10 fold lower fluorescent signal compared to labeling in buffer without serum or other proteins.

3. Incubate the cells for 10-15 minutes at room temperature or 37°C to allow dye uptake.
4. Replace the staining solution with fresh, pre-warmed cell culture medium and incubate for 5 minutes at 37°C to hydrolyze free dye.
5. Replace that media with fresh, pre-warmed cell culture medium and incubate for 15-30 minutes at 37°C to allow the dye to react with intracellular proteins.
6. Replace with fresh, pre-warmed cell culture medium and proceed to analysis (step 8). Alternatively, culture cells for desired period of time to allow cells to divide.
7. Optional: perform formaldehyde fixation, permeabilization, and/or immunostaining.
8.Analyze by microscopy, or harvest cells by trypsinization or other cell dissociation method for flow cytometry analysis. Analyze fluorescence in the appropriate channel (see Spectral Properties).