LIFESCT
Life Scientists' Service Center

Email: support@lifesct.com
Tel: +1-301-890-6420 Fax: +1-240-252-7376
9610 Medical Center Drive, Rockville, MD 20850

LiFluor™ CFSE Cell Proliferation Kit

Cat. #: C0014 Size: 1000 rxns

Introduction

LiFluor™ CFSE Cell Proliferation Kit provides convenient single-use vials for cell labeling. CFSE [5-(and 6)- carboxyfluorescein diacetate, succinimidyl ester] is a fluorescent tracer that diffuses passively into cells and covalently labels intracellular proteins, resulting in long term cell labeling. It is non-fluorescent but becomes brightly green fluorescent once it is hydrolyzed by intracellular esterases in viable cells. After hydrolysis, the succinimidyl ester group reacts with intracellular amines forming fluorescent conjugates that are retained in the cells. The excess unconjugated CFSE diffuses passively back to the extracelluar medium and can be rinsed away. After labeling, cells can be fixed with formaldehyde or gluteraldehyde.

CFSE label is inherited by daughter cells through successive cell divisions. With every cell division, each of the two daughter cells receives half of the label contained in the parent cell. Therefore, for asynchronously diving cells, the number of cell divisions that have occurred since the cells were labeled can be tracked by flow cytometry. On a histogram of fluorescence versus counts, each cell generation will appear as a successively dimmer peak.

CFSE labeling also can be used to quantitate viable cell numbers by fluorescence microplate reader, or for uniform intracellular staining of cells for fluorescence microscopy.

Package Information

Components	C0014
CFSE	10× 500 µg
Anhydrous DMSO	1 ml

Storage

Store at -20°C and protect from light.

Protocols

The following protocols are for use as general guidelines. Because of differences in cell types and culture conditions, optimization of the protocols is required. We recommend testing CFSE at a starting concentration 1-10 µM. A dye concentration of 5–10 µM is recommended for tracking five or more generations, while 1–2 µM may be sufficient for tracking less than five generations. Microscopy experiments may require up to five–fold higher concentration than that used for flow cytometry. Use the lowest concentration of dye that yields good fluorescence signal to minimize cellular toxicity.

For cell number quantitation, we recommend plating a standard curve of cell densities to ensure that fluorescence signal is in the linear range and is proportional to cell number.

For cell division tracking, we recommend analyzing a sample of freshly labeled cells that have not allowed to divide after labeling to observe the location and density of the fluorescent peak representing the undivided cell population.

Note: CFSE dye reacts with amine groups and should not be used with amine-containing buffers such as Tris-based buffers, or with poly-lysine coated culture vessels or slides.

CFSE Stock Solution Preparation

Prepare a 10 mM CFSE stock solution by adding 90 µl of anhydrous DMSO to one 500 µg vial of CFSE. Vortex briefly to mix. To prepare the working solution, dilute the stock solution to final working concentration in PBS or other non-amine containing buffer just before use. Store the remaining stock solution at -80°C.

Labeling of Cells in Suspension

1.1 Pellet cells by centrifugation and aspirate the supernatant.

1.2 Resuspend the cells in pre-warmed (37°C) PBS containing CFSE at the appropriate concentration (1-10 µM).

1.3 Incubate the cells for 20 minutes at 37°C to label the cells.

1.4 Add five times the original staining volume of culture medium (containing at least 1% protein) to the cells and incubate for 5 minutes. This step removes any free dye remaining in the solution.

1.5 Pellet the labeled cells by centrifugation and resuspend in fresh pre-warmed cell culture medium.

1.6 Incubate the cells for at least 10-15 minutes at 37°C to ensure sufficient hydrolysis of CFSE.

1.7 For microplate quantitation of viable cells, proceed to step

1.8. For flow cytometry analysis of cell division, proceed with cell stimulation, incubation, or analysis.

1.8 Wash the cells in PBS or other similar buffer.

1.9 Transfer cells to multiwell plate and measure fluorescence by microplate reader, or mount cells on a slide and analyze by fluorescence microscopy. For cell division tracking, analyzed by flow cytometry.

Labeling of Adherent Cells

2.1 Grow cells to desired density on coverslips or chamber slides.

2.2 Remove the medium and add pre-warmed (37°C) PBS containing CFSE at the appropriate concentration (1-10 µM). Use sufficient working solution to completely submerge the cells.

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2.3 Incubate the cells for 20 minutes at 37°C to label the cells.

2.4 Replace the labeling solution with fresh pre-warmed cell culture medium.

2.5 Incubate for at least 10-15 minutes at 37°C to ensure sufficient hydrolysis of CFSE.

2.6 For microplate quantitation or fluorescence microscopy, proceed to step 2.7. For flow cytometry tracking of cell division, proceed with cell stimulation, incubation, or analysis.

2.7 Wash the cells in PBS or other similar buffer.

2.8 Analyze by fluorescent microplate reader or fluorescence microscopy. For cell division tracking, detach cells from the substrate by trypsinization or other cell dissociation method and analyzed by flow cytometry.

Fixation and Permeabilization (Optional)

3.1 Before fixation, wash and resuspend the cells in PBS or other protein-free buffer.

3.2 Fix the cells for 15–20 minutes at room temperature using an aldehyde-based fixative such as paraformaldehyde, protected from light.

3.3 Wash the cells with PBS.

3.4 If desired, permeabilize the cells using any appropriate protocol.

3.5 Following permeabilization, wash the cells with PBS.

3.6 Resuspend the cells in PBS prior to analysis.

Combining with other Fluorescent Markers (Optional)

4.1 Resuspend the cells in a buffer appropriate for the subsequent staining applications.

4.2 Apply stains for immunophenotyping, DNA content, apoptosis, or other markers as recommended for each stain.