CFSE Cell Proliferation Kit, for flow cytometry

Cat. No. CTG-CP0003 Store the kit at -15 to -25°C

Product Description

The CFSE Cell Proliferation Kit contains carboxyfluorescein diacetate, succinimidyl ester (CFSE) in ten singleuse vials. Small-scale experiments can be performed without preparing excess quantities of perishable CFSE stock solution, a high-quality DMSO (dimethylsulfoxide) and a detailed protocol are also included. When CFSE passively diffuses into cells, it is colorless and nonfluorescent until the acetate groups are cleaved by intracellular esterases to yield highly fluorescent carboxyfluorescein succinimidyl ester. The succinimidyl ester group reacts with intracellular amines, forming fluorescent conjugates that are well retained and can be fixed with aldehyde fixatives. Excess unconjugated reagent and by-products can be washed away. The dye–protein adducts that form in labeled cells are retained by the cells throughout development and meiosis, and can be used for in vivo tracing. The label is inherited by daughter cells after either cell division or cell fusion, and is not transferred to adjacent cells in a population. Mouse cells labeled with CFSE have been detected up to eight weeks after injection into mice body.

Application

The approximate excitation and emission peaks of this product after hydrolysis are 492 nm and 517 nm, respectively. Cells labeled with CFSE can be visualized by fluorescence microscopy using standard fluorescein filter sets or analyzed by flow cytometry in an instrument equipped with a 488 nm excitation source.

Kit Size

0.5, 5 or 25 mg

Kit Components

- CFSE (Component A), 10 vials, each containing 50 µg of lyophilized powder
- DMSO (Component B), 1 vial, 0.5 mL.

Kit storage/stability

Upon receipt, components should be stored desiccated at ≤–20°C until required for use. AVOID REPEATED FREEZING AND THAWING. Before opening the vial, allow the product to warm to room temperature. When stored properly, both the DMSO and solid CFSE should be stable for at least six months. Solutions of the reagent should be used promptly.

Usage

Celltechgen provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please request the Safety Data Sheet (SDS) provided for the product.

Experimental Protocol

For staining cells prior to flow cytometric analysis of cell proliferation or cell division, the protocol below is appropriate. More information on this procedure can be found in reference 7.
Our suggested initial conditions may require modifications because of differences in cell types and culture conditions. For researchers who wish to image the stained cells using fluorescence microscopy, we have included alternate labeling protocols for that type of analysis. The concentration of probe for optimal staining will vary depending upon the application; we recommend testing at least a tenfold range of concentrations. In general, long-term staining (more than about three days) or the use of rapidly dividing CFSE Cell Proliferation Kit (C34554)

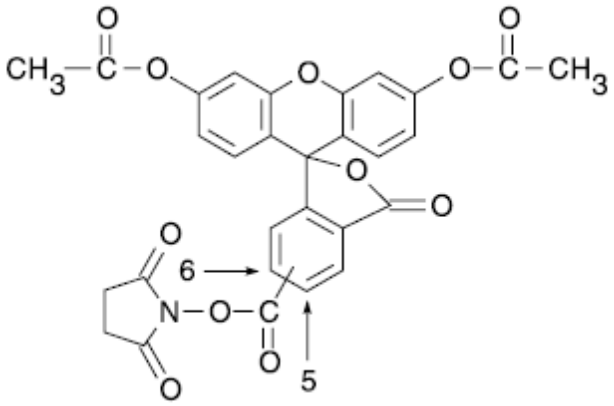

Figure 1. Structure of carboxyfluorescein diacetate, succinimidyl ester (CFSE).MW = 557.

cells will require 5–10 µM dye. Less dye (0.5–5 µM) is needed for shorter experiments, such as viability assays. Microscopy applications may require up to 25 µM CFSE. To maintain normal cellular physiology and reduce potential artifacts from overloading, the concentration of dye should be kept as low as feasible.

Note: The CFSE dye reacts with amine groups and should not be used with amine-containing buffers or lysinecoated slides.

Reagent Preparation

Prepare a 5 mM CFSE stock solution immediately prior to use by dissolving the contents of one vial (Component A) in 18 µL of the DMSO provided (Component B).

1. Labeling Cells for Analysis in Flow Cytometry

This method has been useful in determining cell division in B and T cells.

1) Resuspend cells of interest in prewarmed PBS/0.1% BSA at a final concentration of 1×10^6 cells/mL. Make sure the cells are in a single-cell suspension (no aggregates). The quantity of cells for in vitro labeling experiments is usually 10^5–10^6, depending upon how long after labeling the cells will be allowed to grow. For adoptive transfers, label from 1–5 × 10^7 cells.
2) For most applications add 2 µL of 5 mM stock CFSE solution per milliliter of cells for a final working concentration of 10 µM. Note, however, that a titration of reagent may be necessary to determine the optimal working concentration of CFSE in some applications. Working concentrations will likely be in the range of 0.5–25 µM.

3) Incubate dye at 37°C for 10 min.
4) Quench the staining by the addition of 5 volumes of ice-cold culture media to the cells.
5) Incubate 5 min on ice.
6) Pellet cells by centrifugation.
7) Wash the cells by resuspending the pellet in fresh media. Pellet and resuspend the cells in fresh media a further two times for a total of three washes.
8) Set up in vitro cell cultures under appropriate conditions or adoptively transfer cells.
9) Harvest cells and stain for other markers if appropriate.
10) Analyze using a flow cytometer with 488 nm excitation and emission filters appropriate for fluorescein.

2. Label Cells in Suspension

1) Centrifuge to obtain a cell pellet and aspirate the supernatant.
2) Dilute the 5 mM CFSE stock solution in phosphate-buffered saline (PBS) to the desired working concentration (0.5–25 µM).
3) Resuspend the cells gently in prewarmed (37°C) PBS containing the probe.
4) Incubate the cells for 15 min at 37°C.
5) Re-pellet the cells by centrifugation and resuspend in fresh prewarmed medium.
6) Incubate the cells for another 30 min to ensure complete modification of the probe and then wash the cells again.

3. Label Adherent Cells

1) Grow cells to desired density on coverslips inside a petri dish filled with the appropriate culture medium.
2) Dilute the 5 mM CFSE stock solution in phosphate-buffered saline (PBS) or other suitable buffer to the desired working concentration (0.5–25 µM).
3) Remove the medium from the dish and add prewarmed (37°C) PBS containing the probe.
4) Incubate the cells for 15 min at 37°C.
5) Replace the loading solution with fresh, prewarmed medium and incubate the cultures for another 30 min at 37°C. During this time, CFSE will undergo acetate hydrolysis.

4. Fixation and Permeabilization (Optional)

1) Before fixation, the cells must be washed with PBS or other suitable buffer.
2) Standard fixation protocols using aldehyde-containing fixatives should effectively crosslink the amines of the protein–probe conjugate. Typically, cells are fixed for 15 min at room temperature using 3.7% formaldehyde.
3) After fixation, the cells should be rinsed in PBS.
4) If needed, cells can be permeabilized by any appropriate protocol (for example, 10 minute incubation in ice-cold acetone). Following permeabilization, the cells should be rinsed in PBS.Permeabilization is required, for example, if the cells are to be subsequently labeled with an antibody.

References

1. J Cell Biol 101, 610 (1985);
2. J Cell Biol 103, 2649 (1986);
3. J Immunol Methods 171, 131 (1994);
4. J Exp Med 184, 277 (1996);
5. J Immunol Methods 133, 87 (1990);
6. Transplant Proc 24, 2820 (1992);
7. Current Protocols in Cytometry, J. P. Robinson, Ed., (1998) pp 9.11.1-9.11.9.