CFDA SE Cell Tracer Kit

1. Introduction

The CFDA SE Cell Tracer Kit provides a versatile and well-retained cell tracing method. The kit is simple and convenient for users which containing CFDA SE dye and DMSO.

CFDA SE can passively diffuse into cells. It is colorless and nonfluorescent until entering a live cell, the acetate groups of CFDA-SE are cleaved by intracellular esterase to create amine-reactive and highly fluorescent carboxyfluorescein succinimidyl ester (CFSE). The succinimidyl ester group would react with intracellular amines when entering into cells, forming fluorescent conjugates that are well-retained and can be fixed with aldehyde fixatives. Redundant unconjugated reagent and by-products passively diffuse to the extracellular medium, where they will be washed away.

The dye–protein adducts that formed in labeled cells will be retained by the cells throughout development, meiosis, and in vivo tracing. The label can be inherited by daughter cells after cell division or cell fusion. It isn't transferred to the adjacent cells in one population. Cell division will result in sequential halving of CFDA SE fluorescence appeared in a cellular fluorescence histogram in which the peaks represent successive generations. In previous literature, viable hepatocytes labeled were easily located by fluorescence microscopy even 20 days after intrahepatic transplantation.

CFDA SE Cell Tracer Kit contains dry dye and DMSO. Users should be careful to extend the shelf life of the dye by not dissolved in solution. A stock solution is prepared by dissolving the dye in anhydrous DMSO until prior to use.

2. Experimental Protocol

2.1. Guideline

The following protocols describe introducing the CFDA SE into the cultured cells and imaging the stained cells by fluorescence microscopy. Our recommended initial conditions might require modifications because of differences in cell types, culturing conditions, desired effect, and so on. The optimal concentration of dye for staining is variable depending upon the application; we recommend preliminary experiment in a tenfold range concentration gradient. In general, long-term staining (more than about three days) or the use of rapidly dividing cells requires 5–10 μM concentration. Lower concentration (0.5–5 μM) is needed for shorter experiments, such as viability assays.

To maintain normal cellular physiology and reduce potential artifacts from overdose, the concentration of dye should be kept as low possible.

Note: The CFDA SE dye would react with amine groups, therefore, don't use with amine containing buffers or lysine-coated slides.

2.2. Preparing the Reagent

Before opening the vial, allow it warm to room temperature. Prepare an approximately 10 mM CFDA SE stock solution immediately prior to use by dissolving 1 mg CFDA SE dye in 179 μL high-quality DMSO provided. Dilute the stock solution in phosphate-buffered saline (PBS) or other suitable buffer to the desired working concentration (0.5–25 μM). Be careful that solutions of the reagent should be used immediately.

2.3. Labeling Adherent Cells

a. Grow cells on coverslips inside a petri dish filled with the appropriate culture medium.

b. When the cells have reached the desired density, remove the medium from the dish and add preheated (37°C) PBS containing the dye(prepared in step 2.2)..

c. Incubate the cells for 15 min at 37°C.

d. Replace the loading solution with fresh, preheated medium and incubate the cultures for another 30 min at 37°C.

During this time, CFDA SE will undergo acetate hydrolysis. If the cells are to be fixed and permeabilized, continue to step2.5.

2.4. Labeling Cells in Suspension

a. Centrifuge to obtain a cell pellet and aspirate the supernatant.

b. Resuspend the cells gently in preheated (37°C) PBS containing the dye (prepared in step 2.2).

c. Incubate the cells for 15 minutes at 37°C.

d. Re-pellet the cells by centrifugation and resuspend in fresh preheated medium.

e. Incubate the cells for another 30 minutes at 37°C to ensure complete modification of the dye and then wash the cells again. If the cells are to be fixed and permeabilized, continue step 2.5.

2.5. Fixing and Permeabilizing

a. Before fixation, the cells must be washed with PBS or other suitable buffer.

b. Standard fixation protocols using aldehyde-containing fixatives should effectively crosslink the amines of the protein–probe conjugate. Typically, we fix the cells for 15 minutes at room temperature using 3.7% formaldehyde.

2.6. Flow cytometry

For researchers who wish to analyze labeled cells and/or study cell division via flow cytometry, we recommend the excellent protocol described in reference [7], [8].

3. References

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