Vybrant® CFDA SE Cell Tracer Kit

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
CFDA SE (Component A) (MW = 557.47)	10 vials, 500 µg each, lyophilized powder	• ≤–20°C • Desiccate • Protect from light • Avoid repeated freezing and thawing	When stored as directed, kit will be stable for at least 6 months.
DMSO (Component B)	1 vial of 1.3 mL		
Approximate fluorescence excitation/emission maxima: 492/517 nm, after hydrolysis			

Introduction

The Vybrant® CFDA SE Cell Tracer Kit (V12883) provides a versatile and well-retained cell-tracing reagent in a convenient and easy-to-use form. The kit contains CFDA SE (carboxyfluorescein diacetate, succinimidyl ester (Figure 1); often called CFSE) in ten single-use vials. Small-scale experiments can be performed without preparing excess quantities of perishable CFDA SE stock solution. For additional convenience, we include high-quality DMSO (dimethylsulfoxide) and a detailed protocol.

CFDA SE passively diffuses into cells. It is colorless and nonfluorescent until its acetate groups are cleaved by intracellular esterases to yield highly fluorescent, amine-reactive 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 passively diffuse to the extracellular medium, where they can be washed away.

The dye–protein adducts that form in labeled cells are retained by the cells throughout development, meiosis, and *in vivo* tracing.[1] The label is inherited by daughter cells after cell division (Figure 2), or cell fusion, and is not transferred to adjacent cells in a population.[2-4] Lymphocytes labeled with CFDA SE have been detected up to eight weeks after injection into mice in lymphocyte-migration studies,[5] and viable hepatocytes that were similarly labeled were easily located by fluorescence microscopy even 20 days after intrahepatic transplantation.[6]

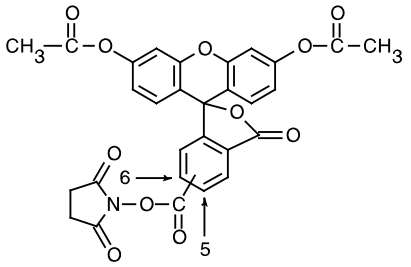

Figure 1. Structure of carboxyfluorescein diacetate, succinimidyl ester (CFDA SE). MW = 557.

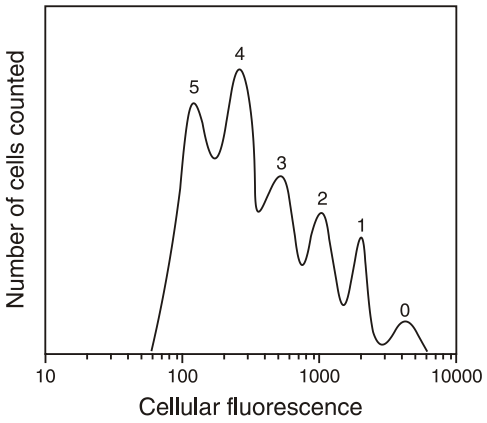

Figure 2. Tracking of asynchronous cell division using 5(6)-CFDA SE labeling and flow cytometry. Cell division results in sequential halving of CFDA SE fluorescence resulting in a cellular fluorescence histogram in which the peaks represent successive generations, labeled 0, 1, 2, 3, 4, 5.

Before You Begin

The following protocol describes culturing cells, introducing the CFDA SE reagent into the cultured cells, and imaging the stained cells by fluorescence microscopy. 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. Our suggested initial conditions may require modifications because of differences in cell types, culture conditions, etc. 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 cells will require 5–10 μM dye. Less dye (0.5–5 μM) is needed for shorter experiments, such as viability assays. To maintain normal cellular physiology and reduce potential artifacts from overloading, the concentration of dye should be kept as low as feasible.

Note: The CFDA SE dye reacts with amine groups and should not be used with amine-containing buffers or lysine-coated slides.

Materials Required but Not Provided

- PBS or other suitable buffer
- Aldehyde-containing fixative

Preparing the Reagent

Before opening the vial, allow the product to warm to room temperature.

Prepare a 10 mM CFDA SE stock solution immediately prior to use by dissolving the contents of one vial (Component A) in 90 µL of the high-quality DMSO provided (Component B). Dilute the stock solution in phosphate-buffered saline (PBS) or other suitable buffer to the desired working concentration (0.5–25 µM).

Note: Solutions of the reagent should be used promptly.

Experimental Protocol

Labeling Adherent Cells

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

1.2 When the cells have reached the desired density, remove the medium from the dish and add prewarmed (37°C) PBS containing the probe (see *Preparing the Reagent*).

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

1.4 Replace the loading solution with fresh, prewarmed medium and incubate the cultures for another 30 minutes at 37°C. During this time, CFDA SE will undergo acetate hydrolysis. If the cells are to be fixed and permeabilized, continue to *Fixing and Permeabilizing.*

Labeling Cells in Suspension

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

2.2 Resuspend the cells gently in prewarmed (37°C) PBS containing the probe (see *Preparing the Reagent*).

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

2.4 Re-pellet the cells by centrifugation and resuspend in fresh prewarmed medium.

2.5 Incubate the cells for another 30 minutes to ensure complete modification of the probe and then wash the cells again. If the cells are to be fixed and permeabilized, continue to *Fixation and Permeabilization.*

Fixing and Permeabilizing

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

3.2 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.

3.3 After fixation, the cells should be rinsed in PBS.

3.4 If needed, cells can be permeabilized by incubating them in ice-cold acetone for 10 minutes. 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.

Visualizing Stained Cells

The approximate excitation and emission peaks of this product after hydrolysis are 492 nm and 517 nm, respectively. Cells labeled with CFDA SE can be visualized by fluorescence microscopy using standard fluorescein filter sets.

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.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
V12883	Vybrant® CFDA SE Cell Tracer Kit	1 kit

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