Product Name: **5(6)-CFDA SE Live Cell Fluorescent Probe**

Catalog Number: **RA20016**

Basic Information

Product Name	5(6)-CFDA SE Live Cell Fluorescent Probe
Size	5mg
Storage	-20 ℃, protected from light
Shipping	Shipped with ice pack
Validity	12 months
Ex/Em	490-500 / 517-520 nm

Product Introduction

5(6)-CFDA, SE is a fluorescent cell tracer dye that can label live cells. It is not only used for in vitro cell proliferation experiments but also for tracking cell division and proliferation in vivo.

5(6)-CFDA, SE is a derivative of fluorescein diacetate (FDA) and is cell membrane-permeable. It does not emit fluorescence by itself. Once it passes through the cell membrane into live cells via passive diffusion, it is catalyzed by intracellular esterases to generate carboxyfluorescein succinimidyl ester (CFSE), which emits intense green fluorescence. CFSE cannot penetrate the cell membrane and remains within the cell. CFSE can also spontaneously and irreversibly bind to intracellular amines, thereby conjugating to cellular proteins. Excess 5(6)-CFDA, SE that is not conjugated diffuses back into the extracellular medium through passive diffusion and is removed during subsequent washing steps. The fluorescence of non-dividing cells labeled with 5(6)-CFDA, SE is highly stable, with stable labeling lasting for several months, making it highly suitable for cell population analysis.

The fluorescence of cells labeled with 5(6)-CFDA, SE is highly uniform, superior to other cell-tracking fluorescent probes previously used, such as PKH26, and the fluorescence distribution in daughter cells after division is also very uniform. During cell division and proliferation, the CFSE-labeled fluorescence is evenly distributed between the two daughter cells, with the fluorescence intensity being halved compared to the parent cell. Flow cytometry (FL1 channel) can be used to detect cells that have not divided, divided once (1/2 fluorescence intensity), twice (1/4 fluorescence intensity), three times (1/8 fluorescence intensity), and cells that have divided more times based on the differences in fluorescence intensity. 5(6)-CFDA, SE can detect up to eight or even more cell divisions. Cells labeled with 5(6)-CFDA, SE can be used for both in vitro and in vivo proliferation studies and have the advantage of not staining neighboring cells. 5(6)-CFDA, SE is most commonly used for the detection of lymphocyte proliferation but can also be used for the proliferation detection of other cells such as fibroblasts, natural killer cells, and

hematopoietic progenitor cells.

Cells labeled with 5(6)-CFDA, SE exhibit green fluorescence. In addition to detecting cell proliferation using flow cytometry, the number of live cells can also be quantified using a fluorescent plate reader, or uniform staining of cells can be observed for cell tracking under a fluorescence microscope.

Experimental steps (recommended steps for live cell staining, which can be adjusted appropriately according to actual conditions)

Note: 5(6)-CFDA, SE reacts with amine groups, so amine-containing buffers should not be used during the experiment.

(1) Allow the solution to return to room temperature before opening the lid, and then use DMSO to prepare a 10 mM 5(6)-CFDA, SE stock solution. Use PBS or an appropriate buffer to dilute the solution to a 0.5-25 μM 5(6)-CFDA, SE working solution (the diluted working solution should be used promptly). Note: If staining is performed for a longer period of time or the cells divide rapidly, a working concentration of 5-10 μM is recommended, otherwise a working concentration of 0.5-5 μM is recommended. The optimal working concentration varies from cell to cell, and it is recommended to explore within a range.

(2) The cells were collected by centrifugation and resuspended in 5(6)-CFDA, SE working solution preheated at 37°C.

(3) Incubate the cells at 37°C for 15-30 min.

(4) Wash the cells twice with PBS or appropriate buffer and observe the cells using flow cytometry (FL1/BL1 channel) or fluorescence microscopy. The following steps are optional (fixation and permeabilization can be performed if antibody labeling is required later):

(5) Fixation: Use 3.7% paraformaldehyde for 15 min at room temperature.

(6) Permeabilization. Permeabilize in ice-cold acetone for 10 min. After fixation and permeabilization, cells need to be washed with PBS.

Note: This reagent is for scientific research use only!