5(6)-CFDA, SE

Cat #: BMD0066 Size: 5 mg/50 mg

	5(6)-CFDA, SE		
REF	**Cat #:** BMD0066	LOT	**Lot #:** Refer to product label
	Application range: Nuclear staining reagents for DNA staining		**Recommended working concentrations:** 0.5-25 μM
	Excitation/Emission wavelengths: Ex/Em=494/521 nm (pH=7)		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

5(6)-CFDA, SE has the molecular formula $C_{29}H_{19}NO_{11}$, molecular weight 557.47 and CAS number 150347-59-4. It is a cell tracer dye that can be used for fluorescent labeling of living cells, not only for in vitro experiments on cell proliferation, but also for tracking the process of cell division and proliferation in vivo. 5(6)-CFDA, SE is a derivative of fluorescein diacetate (FDA) and has cell membrane permeability without fluorescein luminescence itself. After penetrating the cell membrane through passive transportation and entering living cells, 5(6)-CFDA, SE is catalysed by esterases in the cytoplasm to generate carboxyfluorescein succinimide ester (CFSE), which can generate strong green fluorescence and cannot penetrate the cell membrane, and can be well retained in the cell. CFSE can also spontaneously and irreversibly bind to intracellular amino acids to couple to cellular proteins. At the same time, excessive and uncoupled 5(6)-CFDA is passively diffused back into the extracellular medium and cleared by subsequent washing steps. The fluorescence of non-dividing cells labeled with 5(6)-CFDA, SE is very stable and stable for several months, so it is very suitable for cell community analysis. The fluorescence of 5(6)-CFDA, SE labeled cells is very uniform, which is better than other previously used cell tracer fluorescence probes such as PKH26, and the fluorescence distribution of the daughter cells after division is also very uniform. During the process of cell division and proliferation, CFSE labeled fluorescence can be evenly distributed between the two offspring cells, and the fluorescence intensity becomes half of that of the parent cells. According to the different fluorescence intensities, undivided cells can be detected by flow cytometry (FL1 channel). Cells that divide once (1/2 fluorescence intensity), twice (1/4 fluorescence intensity), three times (1/8 fluorescence intensity), and more times of division can be detected. 5(6)-CFDA, SE can detect cell division times up to eight or more times. Cells labeled with 5(6)-CFDA, SE can be used in vitro and in vivo proliferation studies without staining adjacent cells. 5 (6) - CFDA, SE are most commonly used for the detection of lymphocyte proliferation, as well as for the detection of proliferation of fibroblasts, NK cells, hematopoietic progenitor cells, and other cells. 5 (6) - CFDA, SE labeled cells exhibit green fluorescence. In addition to flow cytometry for cell proliferation detection, florescence microplate reader can also be used to quantify the number of living cells, or fluorescence microscopy can be used for uniform staining of cell tracing observation.

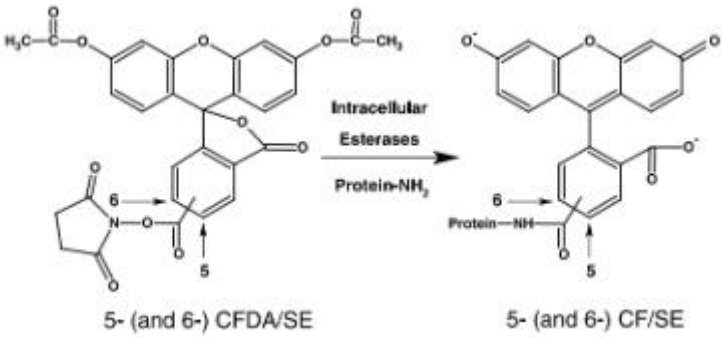

Figure 1. Cell labeling diagram

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
5(6)-CFDA, SE	5 mg	50 mg	-20℃, protected from light

Materials Required but Not Supplied

• Fluorescent microscope or flow cytometer, precision pipettes, disposable pipette tips, DMSO, PBS

Assay Procedure

1. Preparation of 5(6)-CFDA, SE staining solution

(1) Equilibrium to room temperature before capping, and then add DMSO to prepare 10 mM of 5(6)-CFDA, SE storage solution. 5(6)-CFDA, SE storage solutions should be used as soon as possible, and the maximum storage time should not exceed 2 months.

(2) Dilute with PBS or other suitable buffer to 0.5-25 μM 5(6)-CFDA, SE staining solution. The optimal working concentration varies with different cells, so it is recommended to explore within a range.

Note: Diluted staining solution should be used in time.

2. Labeling and detection

(1) Collect cells by centrifugation, and 1-5×10^6 cells were suspended with 1 mL of 5(6)-CFDA, SE staining solution preheated at 37℃.

(2) Incubate at 37℃ 15-30 min.

(3) Wash the cells twice with PBS or other appropriate buffers, and analysis by flow cytometry (FL1/BL1 channel) or fluorescence microscopy.

Precautions

1. 5(6)-CFDA , SE react with amine groups, so buffers containing amine should not be used during the experiment.
2. Fluorescent dyes all have quenching problems, please try to avoid light to slow down the quenching.
3. If antibody labeling is required in subsequent experiments, fixation and permeation steps can be performed.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.