CFSE Cell Proliferation Assay Kit

EGY0013

I. Product introduction:

CFSE (CFDA SE) is the most classic reagent for flow cytometry analysis of cell proliferation and tracking. It has a wide range of applications in studying the proliferation and tracking of natural killer (NK) cells, B cells, T cells, thymocytes, lymphocytes, fibroblasts and hematopoietic cells, liver cells, embryonic cells, etc., as well as cell aggregation and adhesion, neurite outgrowth, bacterial proliferation, etc. Among them, the two most prominent and classic applications are: labeling transplanted immune cells in vitro culture in cell therapy research, and labeling and tracking cell division generation using flow cytometry.

The full name of CFSE is 5(6)-Carboxyfluorescein diacetate succinimidyl ester; 5(6)-CFDA N-succinmidyl ester), Molecular formula: $C_{29}H1_9NO_{11}$, Molecular weight: 557.47.

CFSE itself is colorless and does not emit fluorescence. It can diffuse into cells, and its acetate group - DA SE is cleaved by cell lactonase. When excited at a wavelength of 492 nm, the released fluorescein group emits bright green fluorescence at 517 nm. Its carboxyl group reacts with the amino group of intracellular proteins to generate dye protein adducts, making the cells uniformly and stably fluorescent. Excess unbound reagents passively diffuse into the extracellular culture medium, which can be completely quenched and washed away by the culture medium.

CFSE fluorescein-protein adducts can be well retained in cells during cell division and migration, with a very uniform and stable intracellular fluorescence signal. The fluorescence intensity of the offspring cells is halved after each division, and the fluorescence remains uniform and stable in the offspring cells. Based on this feature, cell division can be tracked for several generations or weeks. CFSE flow cytometry analysis has been reported to track lymphocytes 8-10 generations, which can be used to track live bacteria in groundwater under no growth conditions for at least 28 days. The signal can still be clearly detected under a fluorescence microscope 20 days after liver transplantation.

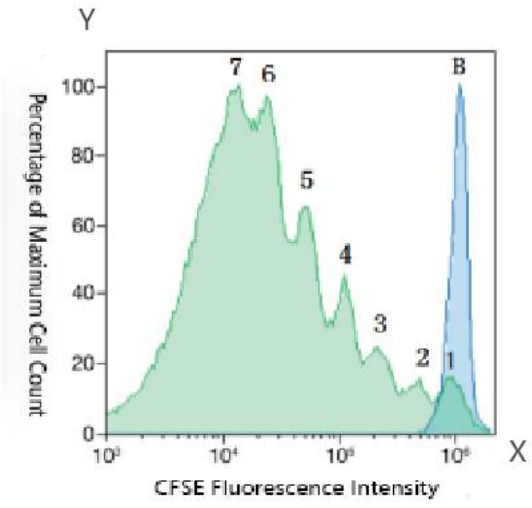


Figure: CFSE labeling and flow cytometry tracking fluorescence of monocytes undergoing asynchronous division for 7 generations after activation

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The X-axis represents fluorescence intensity in exponential form, while the Y-axis represents the percentage of maximum cell count. Peaks 1-7 represent seven consecutive generations of cell division, and B represents negative and unactivated parent cells. The fluorescence signals of B and the first generation are the strongest, with the lowest proportion of first generation cells and the weakest fluorescence signal of the seventh generation, but with the highest proportion of cells.

After labeling CFSE cells and fixing them with formaldehyde or glutaraldehyde, they still exhibit reactivity, and subsequent immunohistochemical labeling and detection can be performed using dye antibodies.

Cell labeling CFSE is suitable for imaging systems such as flow cytometry, fluorescence microscopy, and fluorescence enzyme-linked immunosorbent assay (ELISA) analysis.

CFSE is easily hygroscopic and hydrolyzed, and is not easy to store for a long time. This product (200T specification) contains one small tube of CFSE solid freeze-dried powder(500μg) and one tube of high-quality anhydrous dimethyl sulfoxide (DMSO), which can label approximately 200 flow cytometry samples per 500 μ g.

II. Product composition：

Components	EGY0011 （200 tests）	EGY0012 (1000 tests）	EGY0013 （2000 tests）	Storage Temperature
CFSE	500μg	5 x 500μg	10 x 500μg	-20℃ Sealed away from light
DMSO	0.2mL	1.0mL	2 x 1.0mL	RT Sealed away from light

III. Note:

1. CFSE is ready to use and can be easily hydrolyzed. After being prepared into 5mM stock solution, it should be used up within 24 hours.
2. Dissolve in high-quality anhydrous DMSO and wear gloves for protection during operation.
3. CFSE needs to be operated and stored away from light.
4. CFSE dyes are prone to react with amino groups and should not be used with ammonia containing buffers (such as Tris based buffers) or polylysine coated culture containers or glass slides.

IV. Instructions for use：

1. Prepare CFSE stock solution

Add 180 μL of DMSO to every 500 μg of CFSE, dissolve and prepare a 5mM stock solution. Use within 24 hours and do not store.

2. Prepare CFSE working solution

Dilute 5mM CFSE stock solution (1000X) with PBS to 5 μM working solution, that is, add 1 μL of CFSE stock solution per mL of PBS. 18 μ L of stock solution can be diluted to 18mL of labeled working solution. Preheat the working solution at 37 ℃.

(Note: Cells that undergo long-term staining or rapid division for more than about three days require a higher concentration of 5-10 μM CFSE. For shorter term experiments, such as viability assays, a lower concentration of 0.5-5 μM CFSE is required. To maintain normal cell physiology and reduce overload interference, the dye concentration should be kept as low as possible, preferably titrated according to specific cell types to determine the most suitable concentration. Microscopic observation may require a concentration five times higher than the flow cytometry cells mentioned above.)

3. Cell markers

Suspension cells: Collect the cells by centrifugation, resuspend every 10^6 cells in 1mL of labeled working solution preheated at 37 ℃, incubate in the dark at room temperature or 37℃ for 20 minutes, and shake gently several times during this period;

Adhesive cells: Discard the culture medium and add labeled working solution preheated at 37℃. Incubate in the dark at 37℃ for 20 minutes.

4.Suspension cells: Collect the cells by centrifugation, add 5 times the volume of labeled working solution to a newly prepared 37 ℃ preheated culture medium, and incubate at 37 ℃ for 5 minutes; (Removing free dyes)

Adhesive cells: Discard the labeled working solution and wash twice with newly prepared 37℃ preheated culture medium.

5. Suspension cells: Collected the cells by centrifugation, added to newly prepared complete culture medium preheated at 37 ℃, and incubated at 37 ℃ for 15-30 minutes (to hydrolyze dye acetate);

Adhesive cells: Add newly prepared complete culture medium preheated at 37 ℃ and continue incubating at 37 ℃ for 15-30 minutes.

6. **Subsequent experiments, such as cell stimulation, drug addition, culture, fixation, permeabilization, other labeling or analysis**

If flow cytometry is needed to track the division of cells, the cultivation time until the completion of the division can last for several days or even weeks.

If fixation and permeabilization are required, the cells labeled with CFSE should be washed once with PBS and fixed with aldehyde fixatives such as paraformaldehyde at room temperature in the dark for 15-20 minutes. Conventional permeabilizers can be used for permeabilization, and then washed with PBS for subsequent analysis.

If other labeling is required, resuspend the CFSE labeled cells in the buffer recommended for subsequent labeling, including immunophenotyping, DNA content, apoptosis, or other recommended dyes.

7. Analysis

Flow cytometry: Excited with a wavelength of 488nm, detected and analyzed using a FITC channel (FL1) at 530nm. For tracking cell division generations, it is recommended to use equally labeled undifferentiated parent cells as negative controls for simultaneous detection, to indicate the position and quantity of the initial cell fluorescence peak before proliferation.

Fluorescence microscopy and other imaging systems: Add a medium containing anti fluorescence quencher dropwise, excite it, and observe, photograph, and analyze it using FITC filter.

Fluorescent ELISA reader: It can quantify the number of live cells, but a standard curve for seeding cell density needs to be established to ensure that the fluorescence signal is within a linear range and proportional to the number of cells.

V. Storage conditions：

CFSE stored in the dark at -20℃, DMSO stored in the dark at RT valid for one year.