CFDA, SE Cell Proliferation and Tracer Detection Kit

Cat: CA1200

Size: 1000T/2000T

Storage: -20ºC, valid for 1 year. CFDA and SE fluorescent probes should be stored away from light.-20ºC, valid for 1 year.

Product content:

Component name	1000T	2000T
CFDA, SE fluorescent probe	1 stick dry powder	2 sticks of dry powder
CFDA, SE solvent	1mL	1mL× 2
Cell stain buffer (10×)	100mL	100mL× 2

Product introduction:

CFDA, SE cell proliferation and tracer detection kit is based on CFDA, SE cell tracing and proliferation detection kit, the main working principle of the kit is: CFDA, SE is a kind of fluorescein dye with membrane permeability, itself does not have fluorescence luminescence. When it enters the living cells through the cell membrane, it can be catalyzed by the esterase in the cytoplasm to produce carboxyfluorescein succinimidyl ester (CFSE), the latter can emit strong green fluorescence, can not penetrate the cell membrane, can be retained in the cell intact. CFSE can also spontaneously and irreversibly bind to intracellular amino groups to conjugate to cellular proteins, while excess and uncoupled CFDA, SE can be passively diffused back into extracellular culture medium and cleared by subsequent cleaning steps. The fluorescence of SE labeled non-dividing cells is stable by CFDA, and the time of stable labeling can be up to several months, so it is suitable for cell community analysis.

The fluorescence of CFDA and SE labeled cells is uniform. In the process of cell division and proliferation, the fluorescence intensity of CFSE labeled cells can be equally distributed to the two daughter cells, and the fluorescence intensity becomes half of that of the parent cells. According to the different fluorescence intensity, flow cytometry (FL1 channel) can detect the non-dividing cells, which divide once (fluorescence intensity of 1/2). Second (1/4 fluorescence intensity), third (1/8 fluorescence intensity), and more times of division of cells. CFDA, SE can detect up to eight or more times of division. With CFDA, SE labeled cells can be used for in vitro and in vivo proliferation studies, and have the function of not staining neighboring cells.

CFDA and SE are most commonly used to detect the proliferation of lymphocytes, but can also be used to detect the proliferation of fibroblasts, natural killer cells, hematopoietic progenitor cells and other cells.

CFDA, SE labeled cells showed green fluorescence, Ex=494nm, Em=521nm, FL1 detection channel could be used for flow cytometry detection. CFDA, SE labeled cells can also be observed by fluorescence microscopy. The detection can be performed with an excitation wavelength of 488nm.

This product is a CFD, SE cell proliferation and tracer detection kit. It includes the solvent

required for preparing CFDA, SE storage solution and the dye buffer for cell labeling, which simplifies the preliminary preparation of the experiment. CFDA, SE labeling cells generally can be completed in 10-15min. For different cells, it is necessary to explore the best labeling time by yourself. According to the fluorescence probe labeling volume of each sample is 1mL, the two specifications of this kit can be detected 1000 times and 2000 times respectively.

Protocols: (only for reference) :

1. Preparation of CFDA, SE storage solution (1000×)

Take 1mL of CFDA, add SE solvent to 1 tube of CFDA, SE fluorescent probe, and mix fully to obtain 1000× CFDA, SE storage solution.

Note: It is best to use the storage solution within 1 month, the longest is not more than 2 months. The remaining storage solution must be separated and dried at -20°C away from light, preferably at -70°C away from light to avoid repeated freezing and thawing, and storage at -70°C away from light can properly extend the storage cycle.

2. 2×CFDA, SE working liquid preparation

Take an appropriate volume of cell stain buffer (10×), dilute it with sterile deionized water 10 times, prepare 1×cell stain buffer, and dilute CFDA, SE (1000×) storage solution 500 times with 1× cell stain buffer to make 2×CFDA, SE working solution. For example, take 2μL CFDA, SE storage solution added to 1mL 1×cell staining buffer, after mixing can get 2×CFDA, SE dyeing working solution.

Note: Although this kit has been optimized for the CFDA, SE staining system, it is recommended that users according to their own cell type, culture conditions and different applications to gradient to find the best working concentration, the lowest working concentration to get the appropriate labeling efficiency prevail.

3. Operation steps

(1) Collect the cells by centrifugation, suspend the cells with 1× cell marker solution and adjust the density to 1-5×10^6 cells /mL, take 1mL cells and place them in a 15ml centrifugation tube.

(2) Take 1mL of CFDA preheated at 37℃, add SE working liquid (2×) into the above 15mL centrifuge tube, and gently mix.

(3) Incubation at 37℃ for 10min in the dark.

(4) Immediately add about 10mL of complete cell culture solution preheated at 37℃ (containing 10% FBS) into a 15mL centrifuge tube and mix it at reversed room temperature to terminate the labeling reaction.

(5) Centrifuge at 1000rpm at room temperature for 5 min to remove the supernatant, and then wash with 5-10ml of complete cell culture solution once.

(6) Add another 5-10mL complete cell culture solution and incubate at 37℃ for 10min to promote the retention of CFSE in cells and unreacted CFDA, and SE entered the complete cell culture solution. Centrifuge to remove the supernatant and complete the last washing.

(7) At this time, the labeled cells are ready for subsequent in vitro proliferation detection or cell tracing for specific purposes. The cells were cultured according to the normal method, and the results were analyzed by fluorescence microscope (488nm excitation light) or flow cytometry FL1

channel at the appropriate time point, showing green fluorescence. The labeled cells can also be used for transplantation in live animals and are tracered with fluorescence. (For cell fixation, use an aldehyde fixative such as 4% paraformaldehyde at room temperature for 15min).

Note: For different cells, the optimal labeling concentration and incubation time of CFDA and SE fluorescent probes are different. The initial experiment can be carried out according to the experimental procedure. If the effect is not good, it is recommended to adjust the staining concentration and incubation time to obtain the best labeling effect.

Note:

1. CFDA, SE is easily hydrolyzed and will deteriorate quickly in aqueous solution. Therefore, the powder or storage solution should be dried and preserved during the preservation process; And avoid contact with water during use. But contact with water during the tagging process is within the permissible limits
2. CFDA, SE solvent will solidify and stick to the bottom, wall or cap of the centrifuge tube at low temperatures such as 4ºC or ice bath, and can be used after being heated in water bath of 20-25ºC for a while until completely melted.
3. Different cells have different lactase activity, so the staining effect is different.
4. Although this kit has been optimized for CFDA, SE staining system, but it is recommended that users according to their own cell type, culture conditions and different applications to gradient to find the best working concentration, the lowest concentration to get the appropriate labeling efficiency prevail.
5. Fluorescent dyes have quenching problems, please pay attention to avoid light as much as possible to slow down the fluorescence quenching.
6. This product is limited to professional scientific research, shall not be used for clinical diagnosis or treatment, shall not be used for food or medicine, shall not be stored in ordinary residential.
7. For your safety and health, please wear a lab coat and wear disposable gloves.