CFSE Cell Division Tracker Kit

Cat. No: E-CK-A345 **Size: 500 Assays/2000 Assays**

Cat.	Products	500 Assays	2000 Assays	Storage
E-CK-A345A	CFDA SE Reagent Powder	750 μg×2 vials	750 μg×8 vials	-20 ℃, shading light
E-CK-A345B	CFDA SE Solvent	600 μL×1 vial	600 μL×4 vials	-20 ℃, shading light
	Manual		One Copy	

Storage

Store at -20 ℃ with shading light for 12 months. CFDA SE Solvent has hygroscopicity and should be sealed and store in the dark.

Introduction

Elabscience® CFSE Cell Division Tracker Kit is a kit that use fluorescent probe, CFDA SE, for fluorescence tracing detection of cell proliferation. Compared with other detection methods such as MTT method or [3H]-thymidine incorporation, this kit has the advantages of being more stable, more sensitive and less cytotoxic. It can be used for proliferation detection of primary cells and cell lines, and can be co-stain with other phenotypic antibodies after cell proliferation, and detected and analyzed by flow cytometry.

CFDA SE is a membrane permeable fluorescein dye, which has no fluorescence itself. When CFDA SE enters living cells through the cell membrane, it can be catalyzed by esterase in the cytoplasm to produce carboxyfluorescein succinimide ester (CFSE), which can emit strong green fluorescence and can not penetrate the cell membrane, remaining inside the cell. CFSE can also spontaneously and irreversibly combine with amino groups of intracellular proteins to couple to these proteins. At the same time, excessive and uncoupled CFDA SE is passively diffused back into extracellular medium and removed by subsequent washing steps.

The fluorescence of CFDA SE-labeled cells is uniform and stable. In the process of cell division and proliferation, the fluorescence of CFDA SE-labeled cells could be evenly distributed to two progeny cells, and the fluorescence intensity becomes half of the parental cells. According to the difference of fluorescence intensity, the detection results can be divided into undivided cells, cells that have split once (1/2 fluorescence intensity), cells that have split twice (1/4 fluorescence intensity), cells that have split three times (1/8 fluorescence intensity), and cells that have split more times. CFDA SE-labeled cells can be used for in vitro and in vivo proliferation studies without staining of neighboring cells. CFDA SE-labeled cells showed green fluorescence, Ex=494 nm, Em=521 nm, which can be detected by flow cytometry or fluorescence microscope.

Reagent and Instrument Not Supplied

PBS buffer (pH7.2~7.4), centrifuge, carbon dioxide incubator, ultra-clean bench, flow cytometry or fluorescence microscope.

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Reagent Preparation

Preparation of CFDA SE stock solution (5 mM): CFDA SE Regent Powder, centrifuge at 10000rpm for 1 min to settle the powder to the bottom of the tube, add 270 μL of CFDA SE solvent to dissolve a vial of CFDA SE Regent Powder to prepare 5 mM CFDA SE stock solution.

Note: The preparation process should be perform under ultra-clean bench in the dark. The prepared solution can be packaged according to the amount required for experiment, and then sealed store at -20 ℃ in the dark, and used within 1 month.

Experimental Procedure

Note: Except for the detection by flow cytometry or fluorescence microscope, the following experimental operations were performed under sterile conditions.

1. Cell line detection

1) Collect the cells, centrifuge at room temperature of 250×g for 3~5 min, discard the supernatant.
2) Add 5 mL of PBS to resuspend the cells, centrifuge at room temperature of 250×g for 3~5 min, discard the supernatant.
3) Resuspend the cells with an appropriate amount of PBS buffer, count the cells, and adjust the cell density to 1×10^7 cells /mL (the best staining density). Add CFDA SE stock solution (5 mM) according to the following table, mix fully, and incubate in a cell incubator at 37 ℃ for 10 min in the dark.

Cell Number	Cell Volume (PBS resuspended)	Volume of CFDA SE stock solution (5 mM)
1×10^6	100 μL	0.1 μL
5×10^6	500 μL	0.5 μL
1×10^7	1000 μL	1 μL
2×10^7	2000 μL	2 μL

4) Immediately add 5~10 mL of 37 ℃ preheated cell complete medium, mix fullly at room temperature, and terminate the labeling reaction.
5) Centrifuge at room temperature of 250×g for 3~5 min, discard the supernatant, wash the cells with 5~10 mL cell complete medium, centrifuge at room temperature of 250×g for 3~5 min and discard the supernatant.
6) Add 5 mL of cell complete medium the resuspend cells, and incubate in a cell incubator at 37 ℃ for 5~ 10 min in the dark.
7) Centrifuge at room temperature of 250×g for 3~5 min, discard the supernatant.
8) Add an appropriate amount of cell complete medium to resuspend the cells and culture in a 37 ℃ incubator.
9) According to the experimental requirements (design), choose the appropriate time point to detect cell proliferation by flow cytometry (FITC detection channel) or fluorescence microscope.

2. Primary cell detection

1） Collect the prepared primary cells, centrifuge at room temperature of 250×g for 3~5 min, discard the supernatant.
2） Add 5 mL of PBS to resuspend the cells, centrifuge at room temperature of 250×g for 3~5 min, discard the supernatant.
3） Resuspend the cells with an appropriate amount of PBS buffer, count the cells, and adjust the cell density to 1×10^7 cells /mL (the best staining density). Add CFDA SE stock solution (5 mM) according to the following table, mix fully, and incubate in a cell incubator at 37 ℃ for 5 min in the dark.

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Cell Number	Cell Volume (PBS resuspended)	Volume of CFDA SE stock solution (5 mM)	Cell culture time
5×10^6	500 μL	0.25 μL	24~72 h
1×10^7	1000 μL	0.5 μL	
2×10^7	2000 μL	1 μL	
5×10^6	500 μL	0.5 μL	More than 72 h
1×10^7	1000 μL	1 μL	
2×10^7	2000 μL	2 μL	

Note: The optimal concentration of primary cell staining is related to the culture time, and the cell concentration and staining concentration in the table can be refereed for staining.

4） Immediately add 5~10 mL of 37 ℃ preheated cell complete medium, mix fullly at room temperature, and terminate the labeling reaction.

5） Centrifuge at room temperature of 250×g for 3~5 min, discard the supernatant.

6） Add 5 mL of cell complete medium the resuspend cells, and incubate in a cell incubator at 37 ℃ for 5~ 10 min in the dark.

7） Centrifuge at room temperature of 250×g for 3~5 min, discard the supernatant.

8） Add an appropriate amount of cell complete medium to resuspend the cells and culture in a 37 ℃ incubator.

9） According to the experimental requirements (design), choose the appropriate time point to detect cell proliferation by flow cytometry (FITC detection channel) or fluorescence microscope.

Note: After the end of culture, it also could add phenotypic flow antibody for incubation, and after washing, it can be detected by flow cytometry to analyze the proliferation of specific cell groups.

Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. CFDA SE is easy to be hydrolyzed and deteriorated in aqueous solution, so pay attention to sealed preservation.
4. CFDA SE Solvent will solidify at a lower temperature and stick to the bottom, wall or cap of the centrifuge tube. After rewarming and dissolving, centrifuge appropriately, blow and mix thoroughly before use
5. Esterase activity varies depending on cell type, so the staining effect is different. In the case of too strong or too weak of fluorescence, it is recommended to explore the best working concentration according to the cell type, culture conditions and application.
6. The higher the concentration of the dye, the longer the staining time, the stronger the fluorescence intensity of the cell, but if the concentration of the dye is too high, it will also reduce the activity and function of the cell.
7. Fluorescent substances are prone to quenching. When observing fluorescence, try to shorten the observation time and avoid light during operation and storage.
8. Excessive acceleration and deceleration of centrifuge may cause cell loss. It is suggested to adjust the acceleration no more than 3 and deceleration no more than 1, that is, Acc≦3, Dec≦1.

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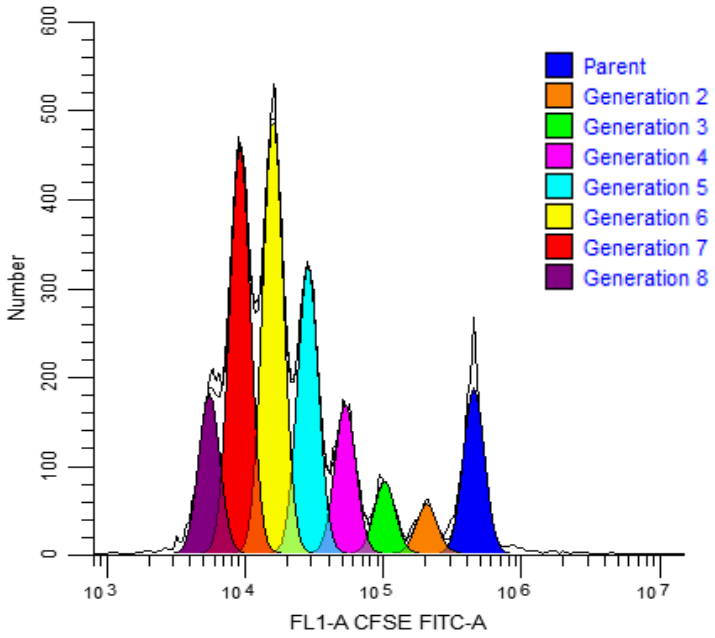
Troubleshooting

Symptoms	Causes	Comments
FITC signal out of bounds	High staining concentration of CFDA SE.	Reduce the staining concentration of CFDA SE.
	The number of stained cells is small.	Increase the amount of stained cells.
	The voltage setting of the flow cytometer is too high.	Reduce the voltage.
The cell proliferation was not detected	The viability of isolated cells are poor.	Optimize the cell separation process.
	Cell viability are poor after staining.	Increase cell density and reduce CFDA SE staining concentration.
	Poor effect of serum and cytokines.	Select excellent quality serum and cytokines.
	Cell staining time is too long.	The cell staining time is controlled within 10 min, and the primary cells are less than 5 min.
	The effect of stimulant is poor.	Select the stimulator with better stimulation effect.

Typical Result



Parent: 9.57 % at 452658.97

Generation 2: 2.85 % at 205603.65

Generation 3: 4.17% at 101682.20

Generation 4: 8.66% at 52717.45

Generation 5: 16.70% at 28261.91

Generation 6: 25.20% at 15764.93

Generation 7: 23.60% at 9066.95

Generation 8: 9.25% at 5423.63

The mouse bone marrow-derived dendritic cells cultured in vitro were matured by LPS, and co-cultured with the spleen of 4T1 tumor-bearing mice (E-CK-A345 staining). After co-staining with Elab Fluor® Red 780 Anti-Mouse CD8a Antibody (E-AB-F1104S) and APC Anti-Mouse CD4 (E-AB-F1097E), detect the proliferation of CD8+T cells.

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