CFDA SE Cell Proliferation and Cell Tracking Kit

Cat #: KTA6010 Size: 200 T×5/200 T×10

	CFDA SE Cell Proliferation and Cell Tracking Kit		
REF	**Cat #:** KTA6010	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells		
	Fluorescence Excitation/ Emission: CFDA SE: Ex/Em=494/521 nm		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

5(6)-CFDA, SE 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.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	200 T×5	200 T×10	

CFDA SE	5	10	-20℃, protected from light
DMSO	1 mL	1 mL×2	RT
Assay Buffer (10×)	50 mL×2	100 mL×2	4℃

Materials Required but Not Supplied

- Cell culture plate, precision pipettes, disposable pipette tips
- Centrifuge
- Fluorescence microscopy or flow cytometer
- PBS

Reagent Preparation

CFDA SE Storage Solution: Prepare before use, add 200 μL DMSO to each CFDA SE to prepare CFDA SE Storage Solution, stored at -20℃, protected from light. CFDA SE Storage Solution should be used as soon as possible, the maximum storage time at 20℃ should not exceed 2 months.

Assay Buffer: Dilute Assay Buffer (10×) 10 times with sterile cell culture grade pure water to obtain Assay Buffer, Store at 4℃.

Stain Solution: Prepare before use, Mix 1 μL CFDA SE Storage Solution in each 1 mL Assay Buffer, preheat to 37℃ before use, protected from light.

Assay Procedure

A. Analysis by flow cytometry

1. For non-adherent cells, Collect cells by centrifugation (300 g, 5 min). Wash with PBS twice and discard the PBS. For adherent cells, using Trypsin (EDTA free) to digest cells firstly and then centrifugation, wash cells with PBS 2-3 times, then remove PBS.

Note: We recommend keeping unstained control cells (i.e. without staining) suspended in Assay Buffer for both treated and untreated samples to set up the flow cytometer instrument.

2. Add 1 mL of 37℃ preheated Stain Solution to resuspension cells to a cell density of approximately 1-5×10^6/mL. Incubate at 37℃ for 10-30 min, protected from light, different cells have different optimum incubation times.

3. Cells were collected by centrifugation at 300 g for 5 min. Add 1 mL preheated fresh culture medium, then gently resuspend the cells. Incubate at 37℃ for 5-10 min, protected from light. Then centrifugate at 300 g for 5 min to collect cells.

4. Wash cells with PBS twice, cells can be cultured using normal culture methods or directly detected using flow cytometry (FL1/BL1 channel).

B. Analysis by fluorescence microscopy

1. For adherent cells

(1) Grow cells directly on a coverslip in cell culture plate. Incubate in a CO_2 Incubator at 37℃ for at least 24 h before treatment.

(2) Wash cells with PBS twice.

(3) Add appropriate volumes of 37℃ preheated Stain Solution to the cells. Generally, 100 μL was added to 96-well plate per well, 250 μL to 24-well plate per well, 500 μL to 12-well plate per well, and 1 mL to 6-well plate per well. Then incubate at 37℃ for 10-30 min, protected from light, different cells have different optimum incubation times.

(4) After incubation, replace staining solution with fresh culture medium preheated at 37℃, and incubated at 37℃ for 5-10 min, protected from light.

(5) Wash cells with PBS 2-3 times, then observe the samples under the fluorescence microscopy (CFDA SE is green fluorescence, Ex/Em=494/521 nm).

2. For non-adherent cells

(1) Follow the protocol for flow cytometry from step A.1 to step A.4.

(2) Place the cell suspension from Step A.4 on a glass slide. Cover the cells with a glass coverslip. Analyze cells by fluorescence microscopy using the appropriate filter.

Precautions

1. Please immediately centrifugal CFDA SE to the bottom of the tube before use, and then conduct the subsequent experiments.
2. This kit has optimized the CFDA SE staining concentration, and can also explore the best working concentration and staining time according to the cell type, culture conditions and application direction.
3. CFDA SE is is easily hydrolyzed and will deteriorate quickly in aqueous solution. Please avoid contact with water during use. Contact with water during the process of labeling cells is within the permitted range.
4. Fluorescent dyes all have quenching problems, please try to avoid light to slow down the quenching.
5. If antibody labeling is required in subsequent experiments, fixation and permeation steps can be performed.

Strawberry moment: In addition to CFDA SE Cell Proliferation and Cell Tracking Kit, Abbkine also offers Maximum Sensitivity Cell Counting Kit-8 (BMU106-EN), EdU Cell Proliferation Image Kit (KTA2030、KTA2031) and other cell state assay kits, such as Apoptosis Detection kit (KTA0002), One-step TUNEL Apoptosis Assay Kit (KTA2010/KTA2011), etc. Scan the QR code on the right and follow the Abbkine official account to learn more about Abbkine products.

FAQ

Question	Answer
When living cells are stained with the kit, if they go to apoptosis, are they going to lose its original fluorescence?	Normally the signal may decline, but it doesn't go away.
Can the kit detect the proliferation of single cells in co-cultured cells?	The kit does not detect co-cultured single cells and may stain all of them.
Can the kit be used for bacterial staining? How do you do it?	This kit has not been verified with bacterial samples at present, so the effect is uncertain.
Can the kit be used to detect the proliferation of mammalian stem cells?	Yes, it could.
If you use this kit for cell tracing, how long does the fluorescence last?	The fluorescence intensity and maintenance time varied with cell division rate and lactase activity.

Recommended Products

Catalog No.	Product Name
BMU106-EN	SuperKine™ Maximum Sensitivity Cell Counting Kit-8 (CCK-8)
KTA2030	EdU Cell Proliferation Image Kit (Green Fluorescence)
KTA2010	One-step TUNEL Apoptosis Assay Kit (Green Fluorescence)
KTA0002	Annexin V-AbFluor™ 488/PI Apoptosis Detection kit

Disclaimer

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