



CFDA SE Cell Tracing Kit manual

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CFDA SE Cell Tracing Kit manual

CFDA SE cell tracing kit is used for fluorescent labeling and long-term tracing of cells. Each kit contains single-use vials of dry dye and the required amount of high-quality anhydrous DMSO. It allows the preparation of small volumes of the working staining solution, which is convenient for conducting experiments and scaling them up without unnecessary loss of the dye.

CFDA SE ((5,6)-carboxyfluorescein diacetate succinimidyl ester) is a stable, cell-permeable diacetate precursor to CFSE. This molecule is non-fluorescent until the acetate groups are cleaved by intracellular esterases, resulting in the formation of a highly fluorescent fluorophore CFSE with emission in the green spectrum range (absorption max. at ~ 492 nm, emission max. at ~ 517 nm). CFSE interacts with cellular amines via its succinimidyl groups and covalently labels intracellular proteins. The dye-protein adducts are retained by the cells throughout development, cell division, or cell fusion and are not transferred to adjacent cells in a population.

CFDA SE is commonly used for *in vivo* and *in vitro* labeling of cells to analyze their proliferation as well as for cell tracking and motility assays. Inside the cell, CFDA SE shows little cytotoxicity, with minimal effects on the proliferative ability or biology of the cell. During proliferation, the label is partitioned approximately equally among the progeny so that cell division can be followed as a successive halving of the fluorescence intensity through multiple generational divisions.

Kit components

Kit component	Count	
	16231 3 vials	26231 15 vials
41615, CFDA SE, 500 ug	3	15
15050, DMSO (dimethyl sulfoxide), labeling grade, 1 mL	1	2

Transportation: at RT for up to 3 weeks. Store at -20 °C.

Shelf life 12 months.

Before You Begin

The following protocol describes the procedures for staining the cell cultures and their analysis by fluorescence microscopy and flow cytometry.

Adjustments to our proposed initial conditions may be required due to differences in cell types, culture conditions, and other factors. The optimal concentration of CFDA SE for staining will vary depending on the specific application; we recommend testing at least a tenfold range of concentrations. Typically, long-term staining (over three days) or working with rapidly dividing cells will require 5–10 μM dye. For shorter experiments like viability assays, a lower dye concentration (0.5–5 μM) is sufficient. Microscopy applications may need up to 25 μM CFDA SE. To preserve normal cellular function and minimize potential artifacts from excessive dye, it is recommended to use the lowest feasible concentration of dye.

Important! CFDA SE is an amine-reactive dye. Do not use it with amine-containing buffers or lysine-coated slides.

Preparing stock solution

1. Allow the product to warm to room temperature before opening the vial.
2. Prepare a 5 mM CFDA SE stock solution by dissolving the contents of the dye vial in 179 μL of the labeling-grade DMSO provided.
3. The prepared stock solution can be aliquoted and stored frozen at $-20\text{ }^{\circ}\text{C}$.

Labeling Adherent Cells

1. Grow cells on coverslips inside a petri dish using the appropriate culture medium until reaching the desired density.
2. Dilute the 5 mM CFDA SE stock solution in phosphate-buffered saline (PBS) or other suitable buffer to the desired working concentration (0.5–25 μM).
3. Replace the medium in the dish with a prewarmed to $37\text{ }^{\circ}\text{C}$ CFDA SE working solution.
4. Incubate the cells with the loading solution for 15 min at $37\text{ }^{\circ}\text{C}$.
5. Replace the loading solution with fresh, prewarmed medium and continue incubating the cells for an additional 30 min at $37\text{ }^{\circ}\text{C}$ to allow CFDA SE to deacetylate and convert to fluorescent CFSE.
6. If necessary, cells can be fixed and stained with additional markers.

Labeling Cell Suspension

1. Harvest the cells, centrifuge them for 7 min at 500 *g*, and aspirate the supernatant.
2. Resuspend cells in warm (37 °C) 0.1% BSA/PBS at a final concentration of 1×10^6 cells/mL.

Important! To achieve consistent labeling, stain a single-cell suspension without aggregates. The recommended cell quantity for in vitro labeling experiments typically ranges from 10^5 to 10^6 cells, depending on the intended duration of cell growth post-labeling. For adoptive transfers, label from $1-5 \times 10^7$ cells.

3. Add 2 μ L of 5 mM stock CFDA SE solution per milliliter of cells for a final working concentration of 10 μ M.

Important! The optimal working concentration of CFDA SE should be determined experimentally. For this purpose, dilute a portion of the CFDA SE stock solution further in DMSO before the dye loading. The recommended working concentrations are in the range of 0.5–25 μ M.

4. Incubate the cells with the loading solution for 10 min at 37 °C.
5. Stop the staining process by adding 5 volumes of ice-cold culture media to the cells.
6. *Optionally.* Incubate the cells on ice for 5 min.
7. Use centrifugation to pellet the cells.
8. Wash the cells by resuspending the pellet in fresh media. Repeat two more times for a total of three washes.
9. Prepare *in vitro* cell cultures under suitable conditions or transfer cells adoptively.
10. If necessary, harvest the cells and stain them for additional markers.

Fixation and Permeabilization

1. Wash the cells with PBS or another suitable buffer before fixation.
2. Fix the cells in aldehyde-containing fixative (for example, 3.7% formaldehyde) for 15 min at room temperature.
3. Rinse the cells three times in PBS.
4. If the cells are to be subsequently labeled with antibodies, permeabilize them by incubating them in ice-cold acetone for 10 min. After permeabilization, the cells should be rinsed in PBS.

Fluorescent microscopy

The approximate excitation and emission maxima of CFSE are 492 nm and 517 nm, respectively. Use standard fluorescein filter sets to visualize labeled cells by fluorescence microscopy.

Flow cytometry

Analyze the cells using a flow cytometer with 488 nm excitation and emission filters (fluorescein channel). Use unstained cells as a control. Labeled cells should show multiple peaks, indicating their proliferation and diluting the dye between daughter cells.

Troubleshooting

Problem	Possible causes	Recommended solutions
No signal	Esterase activity of serum in the medium prematurely cleaves the dye, preventing cell penetration	<ul style="list-style-type: none"> - Use serum-free media during the labeling step - Inactivate serum before addition to media by incubation for 40 min at 56 °C
Low signal	<ul style="list-style-type: none"> A. Cells are not healthy B. Low concentration of CFDA SE C. Insufficient duration of labeling D. Phenol Red quenching of CFSE fluorescence during the imaging 	<ul style="list-style-type: none"> A. Use only healthy cells B. Perform a titration to get an optimal concentration of CFDA SE C. Increase the time of incubation with the dye D. Use Phenol Red-free media for imaging
Cell death	<ul style="list-style-type: none"> A. Concentration of CFDA SE is too high B. Concentration of the experimental compound is too high, resulting in cytotoxicity 	<ul style="list-style-type: none"> A. Perform a titration to get an optimal concentration of the dye B. Decrease the concentration of the experimental compound
Transfer of the dye to adjacent cells	Overloading of the dye and saturation of cellular esterases resulting in leakage of uncleaved dye back out into the media	<ul style="list-style-type: none"> - Reduce the dye concentration and/or labeling time - Increase resting time and wash period after labeling
Broad peak of the dye in unstimulated control	<ul style="list-style-type: none"> A. Poor mixing of CFDA SE with cells B. Multiple cell types with different proliferation activity in the culture 	<ul style="list-style-type: none"> A. Mix cells with CFDA SE well immediately upon addition B. Phenotype cultured cells with type-specific antibodies





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