CFDA SE Cell Proliferation Assay and Tracking Kit User Manual

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(Version 1.1A)

Cell proliferation and tracking for cell line and primary cells

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

CFDA SE Cell Proliferation Assay and Tracking Kit utilizes the fluorescent probe CFDA SE for the sensitive tracing and detection of cell proliferation. Compared to conventional methods such as the MTT assay or [3H]-thymidine incorporation, this kit offers superior stability, higher sensitivity, and significantly lower cytotoxicity. It is broadly applicable to both primary cells and established cell lines. Furthermore, the labeled cells can be co-stained with phenotypic antibodies post-proliferation, enabling multi-parameter analysis via flow cytometry.

II. PRINCIPLES

CFDA SE is a cell-membrane-permeable fluorescein derivative that is intrinsically non-fluorescent. Upon entering living cells, it is cleaved by intracellular esterases to yield carboxyfluorescein succinimidyl ester (CFSE). CFSE exhibits strong green fluorescence, is membrane-impermeable, and thus remains trapped within the cytoplasm. It spontaneously and irreversibly couples to intracellular proteins via their amine groups. Excess, unreacted CFDA SE passively diffuses out of the cells and is removed during subsequent washing steps.

Cells labeled with CFDA SE display uniform and stable fluorescence. During cell division, the fluorescent dye is equally distributed to daughter cells, resulting in a sequential halving of fluorescence intensity with each generation (e.g., 1/2, 1/4, and 1/8 of the original intensity). This allows for the precise discrimination of undivided cells from those that have undergone one, two, three, or multiple rounds of division. CFDA SE-labeled cells are suitable for both in vitro and in vivo proliferation studies and do not transfer label to neighboring cells. The labeled cells emit green fluorescence (Ex = 494 nm, Em = 521 nm), which can be readily detected and analyzed using flow cytometry or fluorescence microscopy.

III. KIT COMPONENTS

Component	500 Assays	2000 Assays	Storage
CFDA SE	Powder x 1	Powder x 4	-20 °C
Diluent	1 ml x 1	1 ml x 4	RT
Manual	1	1	

IV. MATERIALS REQUIRED BUT NOT PROVIDED

1. Standard fluorescence microscope
2. Calibrated, adjustable precision pipets, preferably with disposable plastic tips
3. Sterile PBS (pH 7.2-7.4)
4. Centrifuge
5. CO_2 incubator
6. Laminar flow hood
7. Flow cytometer

V. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use.

CFDA SE

Briefly centrifuge the vial prior to opening. Add 1 mL of Diluent to a vial of CFDA SE powder to prepare a CFDA SE (1000X) stock solution, and mix thoroughly until completely dissolved. Seal tightly and store at -20°C for 1-2 months in the dark. Aliquot to avoid repeated freezing and thawing. Then dilute CFDA SE (1000X) stock solution 500-fold with sterile PBS to generate CFDA SE (2X) working solution (eg. 2 µl to 1 ml sterile PBS for one assay)**.** Adjust the volume according to experimental requirements.

Note: All preparation steps should be performed in a laminar flow hood, protected from light.

VI. ASSAY 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 250 × g for 3-5 min at room temperature, and discard the supernatant.

2) Resuspend the cells in 5 mL of PBS, centrifuge at 250 × g for 3-5 min at room temperature, and discard the supernatant.

3) Resuspend the cells in an appropriate volume of PBS, count the cells, and adjust the cell density to 1 × 10^7 cells/mL (optimal staining density). Add 1 mL CFDA SE (2X) working solution to 1 mL cell suspension, mix thoroughly, and incubate at 37°C in the dark for 10 min.

4) Immediately add 10 mL of pre-warmed (37°C) complete medium, mix thoroughly at room temperature, and terminate the labeling reaction. Centrifuge at 250 × g for 3-5 min at room temperature, discard the supernatant.

5)Wash the cells with 5-10 mL of complete medium, centrifuge at 250 × g for 3-5 min, and discard the supernatant.

6) Resuspend the cells in 5-10 mL of complete medium and incubate at 37°C in the dark for 5 min.

7) Centrifuge at 250 × g for 3-5 min at room temperature and discard the supernatant.

8) Resuspend the cells in an appropriate volume of complete medium and culture in a 37°C incubator.

9) According to the experimental design, select an appropriate time point to detect cell proliferation by flow cytometry (using the FITC channel) or fluorescence microscopy.

2. Primary Cell Detection

1) Collect the cells, centrifuge at 250 × g for 3-5 min at room temperature, and discard the supernatant.

2) Resuspend the cells in 5 mL of PBS, centrifuge at 250 × g for 3-5 min at room temperature, and discard the supernatant.

3) Resuspend the cells in an appropriate volume of PBS, count the cells, and adjust the cell density to 1×10^7 cells/mL (optimal staining density). Add 1 mL CFDA SE (2X) working solution to 1 mL cell suspension; mix thoroughly, and incubate at 37°C in the dark for 5 min.

4) Immediately add 10 mL of pre-warmed (37°C) complete medium, mix thoroughly at room temperature, and terminate the labeling reaction. Centrifuge at 250 × g for 3-5 min at room temperature, discard the supernatant.

5)Wash the cells with 5-10 mL of complete medium, centrifuge at 250 × g for 3-5 min, and discard the supernatant.

6) Resuspend the cells in 5-10 mL of complete medium and incubate at 37°C in the dark for 5 min.

7) Centrifuge at 250 × g for 3-5 min at room temperature and discard the supernatant.

8) Resuspend the cells in an appropriate volume of complete medium and culture in a 37°C incubator.

9) According to the experimental design, select an appropriate time point to detect cell proliferation by flow cytometry (using the FITC channel) or fluorescence microscopy.

VII. CAUTIONS

1. Please observe standard laboratory safety protocols and follow the recommended procedures for reagent handling.
2. CFDA SE is prone to hydrolysis and degradation in aqueous solutions; ensure proper sealed storage to maintain stability.
3. The CFDA SE solvent may solidify at low temperatures and adhere to the tube walls or cap. If this occurs, warm to room temperature, centrifuge briefly, and mix thoroughly by pipetting before use.
4. Esterase activity varies among cell types, which may affect staining efficiency. If fluorescence is too strong or too weak, optimize the working concentration based on your specific cell type, culture conditions, and experimental application.
5. While higher dye concentrations and longer staining times generally increase fluorescence intensity, excessive dye concentration may compromise cell viability and function.
6. Fluorescent compounds are susceptible to photobleaching. Minimize light exposure during handling and storage, and keep observation times as short as possible.

VIII. EXAMPLE DATA ANALYSIS AND FIGURES

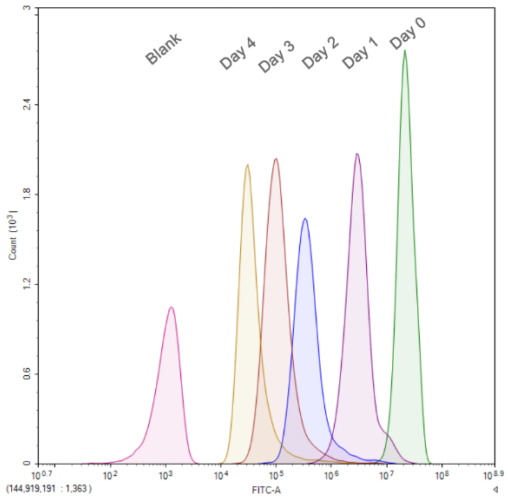


Fig. 1 Flow cytometry analysis of Hela labeled with CFDA-SE and analyzed by flow cytometry after being cultured for 0, 1, 2, 3 and 4 days

IX. STORAGE/STABILITY

The kit ships on wet ice and storage at 2-8 °C is recommended. Stored at -20°C for 1 year, avoid multiple freeze-thaw cycles.