5(6)-Carboxyfluorescein Diacetate N-Succinimidyl Ester (CFSE)

CATALOG #6162
RESEARCH USE ONLY

INTRODUCTION

5(6)-Carboxyfluorescein diacetate n-succinimidyl ester (CFSE, or CFDA SE, catalog #6162) is a green fluorogenic reagent that binds to intracellular membranes. It is often used for cell proliferation and cytotoxicity studies.

CFSE diffuses into the cell and covalently binds to primary amino groups present on intracellular membrane structures. Intracellular esterases quickly cleave the acetate groups from the dye thus converting it to the fluorescent form. Any unbound reagent diffuses back out of the cell. Because CFSE forms a strong bond inside the cell, it is retained within the cell indefinitely and is inherited by daughter cells. It will not be incorporated into adjacent cells.

CFSE is supplied as a concentrated lyophilized powder at 0.05 mg. Reconstitute it with 200 µL DMSO to yield a stock concentrate at 2500X (0.25 mg/mL). Dilute it 1:250 in PBS to form the 10X working solution, and then add it to cells at 1:10 (a final concentration of 0.1 µg/mL). Analyze with a fluorescence microscope or flow cytometer with a 488 nm argon excitation laser. CFSE exhibits green fluorescence in the FL1 region: excitation at 492 nm and emission at 520-540 nm (Figures 1, 2, and 3).

As CFSE is detected in the green range, it is optimal for use in dual-staining studies with other fluorescent reagents, such as PI (catalog #638), 7-AAD (catalog #6163), and SR-FLICA® (catalog #917) with minimal spectral overlap. Use it with ICT's SR-FLICA® poly caspases inhibitor reagent to identify apoptotic cells in the analysis (Figure 3).

CFSE is for research use only. Not for use in diagnostic procedures.

SPECIFICATIONS

- Quantity per vial: 0.05 mg
- Form: lyophilized powder
- CAS number: 150347-59-4
- Chemical name: 5(6)-carboxyfluorescein diacetate n-succinimidyl ester
- Molecular formula: $C_{29}H_{19}NO_{11}$
- Molecular weight: 557.47

FLUORESCENCE

- Excitation: 492 nm
- Emission: 520-540 nm

STORAGE

- Temperature: -20°C
- Conditions: protect from light and avoid freeze-thaw cycles
- Shelf-life: 24 months when frozen and protected from light

WARNING

- For research use only.
- Not for human or drug use.
- MSDS available at www.immunochemistry.com.

Thank you for using CFSE! If you have any questions, or would like to share your data, please contact us at 1-800-829-3194 or help@immunochemistry.com.

ImmunoChemistry TECHNOLOGIES LLC

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- **Catalog#:** 6162
- **Size:** 0.05 mg/vial
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FIGURE 1: CFSE STAINING FL1 VS. FL3

In this experiment, K562 target cells (human lymphocytes derived from a patient with chronic myelogenous leukemia) were stained green with CFSE (catalog #6162) to distinguish them from effector cells (Figure 2), or left unstained. Cells stained green with CFSE (right) migrate along the X-axis (FL1) of the plot compared with unstained cells (left).

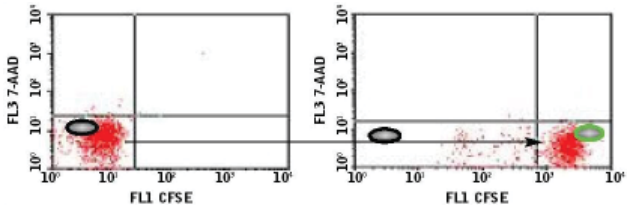


FIGURE 2: IDENTIFICATION OF CFSE STAINED CELLS

In this experiment, K562 target cells were stained green with CFSE and then subjected to effector cells. Green stained target cells are easily identifiable when analyzed by FL1 vs. SSC on a flow cytometer. Target cells stained green with CFSE move to the right along the X-axis (FL1) of the plot (right) compared with unstained effector cells. This plot becomes particularly important when gating on target cells that are the same size as effector cells.

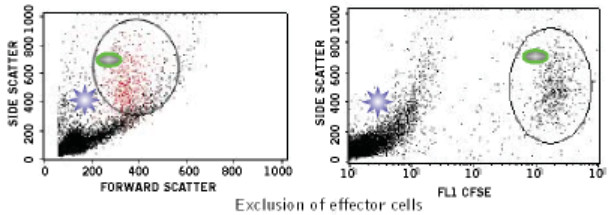
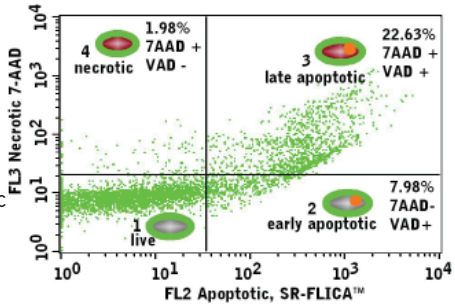


FIGURE 3: USE WITH OTHER FLUORESCENT REAGENTS

As CFSE is detected in the green range, it is optimal for use in dual-staining studies with other fluorescent reagents with minimal spectral overlap. In this experiment, K562 target cells were stained green with CFSE to distinguish them from effector cells in FL1 (Figure 2). The target cells were stained with 7-AAD (catalog #6163) to identify membrane-compromised necrotic target cells (red in FL3), and with SR-FLICA® (catalog #917) to identify caspase-positive apoptotic target cells (orangy-red in FL2). When used together (catalog #972), these reagents identified four populations of cells: live; early apoptotic; late apoptotic; and necrotic leading to more accurate results when assessing cell death.



HOW TO USE

1. Reconstitute the vial of CFSE with 200 µL DMSO to create a 2500X stock concentrate at 0.25 mg/mL. Mix by swirling or tilting the vial, allowing the DMSO to travel around the base of the amber vial until completely dissolved. At room temperature (RT), the reagent should be dissolved within a few minutes.
2. If storing the stock concentrate for future use, prepare small aliquots (20 µL) to avoid freeze-thaw cycles. The stock concentrate will be stable for 6 months when protected from light and stored at or below -20°C.
3. Create the 10X working solution by diluting the 2500X stock solution 1:250 in sterile PBS; e.g., add 4 µL stock to 996 µL PBS. Store the working solution on ice up to 2 hours protected from light. Do not use media to dilute the CFSE as it will quench the fluorescent signal!
4. Prepare cells at 1-2 x 10^7 in 1.8 mL sterile PBS.
5. Create 1 control tube of unstained cells at 1-2 x 10^7 in 2 mL sterile PBS. These cells will be used to compensate the flow cytometer to ensure that stained cells shift along the FL1 axis. In Figure 1, FL1 is shown on the X-axis and stained cells shift to the right compared with unstained cells.
6. Stain cells at a final concentration of 0.1 µg/mL (0.18 µM) of CFSE in the cell culture. Add the 10X working solution to the cells at a dilution of 1:10. For example, add 200 µL 10X CFSE working solution into 1.8 mL cell suspension. Mix by inverting or vortexing the vial. The optimal concentration of CFSE may vary among cell types. The concentration of CFSE and the incubation time should be adjusted for cell line to adequately stain them. Excessive staining may cause problems when compensating the instrument. Do not add CFSE to the control tube.
7. Incubate 15 minutes at room temperature.
8. Add 1 mL cell culture media to stop the reaction.
9. Incubate 5 minutes.
10. Wash the cells once or twice by centrifugation and discard the supernatant.
11. Resuspend in cell culture media such that each tube contains the desired level of target cells, or resuspend in PBS and fix cells with formaldehyde.
12. Analyze cells, or incubate at 37°C up to 1 hour until ready for additional staining or further experimentation.
13. Analyze with a flow cytometer equipped with a 15 mW 488 nm argon laser: excitation at 492 nm; emission at 520-540 nm in FL1. Stained cells appear green (Figures 1, 2, and 3).

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