

CFSE Fluorescent Cellular Stain Reagent

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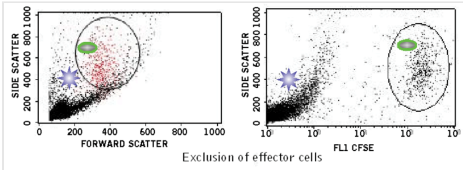
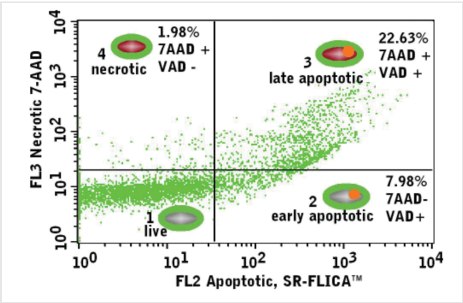
CATALOG #
AAA52739

PRODUCT SYNONYMS

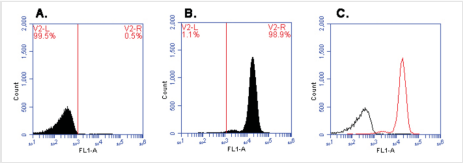
CFSE Fluorescent Cellular Stain; CFSE Fluorescent Cellular Stain reagent

PRODUCT SPECIFICATIONS

Form/Format	Lyophilized
Sequence Positions	How To Use 1. Reconstitute the vial of CFSE with 200 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 tem-peratu
How To Use	1. Reconstitute the vial of CFSE with 200 ?L 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 tem-perature (RT), the reagent should be dissolved within a few minutes. 2. If storing the stock concentrate for future use, prepare small aliquots (20 ?L) 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 ?L stock to 996 ?L PBS. Store the working solution on ice up to 2 hours protect-ed from light. Do not use media to dilute the CFSE as it will quench the fluorescent signal! 4. Prepare cells at 1-2 x 10⁷ in 1.8 mL sterile PBS. 5. Create 1 control tube of unstained cells at 1-2 x 10⁷ 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 ?g/mL (0.18 ?M) of CFSE in the cell culture. Add the 10X working solution to the cells at a dilution of 1:10. For example, add 200 ?L 10X CFSE working solution into 1.8 mL cell suspension. Mix by invert-ing or vortexing the vial. The optimal concentration of CFSE may vary among cell types. Each investigator should adjust the concentration and incubation time to accommodate the particular cell line and research conditions. 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.
Excitation/Emission	492 nm / 520-540 nm
CAS Number	150347-59-4
Molecular Formula	C ₂₉ H ₁₉ NO ₁₁
Preparation and Storage	Store at -20°C and protected from light. Avoid freeze/thaw cycles.



Application Data
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.



Application Data
Healthy Jurkat cells were stained with CFSE or were unstained and then analyzed by flow cytometry using an Accuri C6 flow cytometer equipped with a FL1 99% attenuation filter. Unstained Jurkat cells are shown in the left-most histogram below (A.), and stained Jurkat cells are shown in the middle histogram (B.). An overlay of the two populations is shown in the right-most histogram (C).

ADDITIONAL INFORMATION

Related Product Information for CFSE Fluorescent Cellular Stain reagent	5(6)-Carboxyfluorescein diacetate N-succinimidyl ester (CFSE, or CFDA SE, MBS258052) is a green fluorogenic reagent that binds to intracellular molecules. 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 molecules. 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 blue argon excitation laser. CFSE exhibits green fluorescence in the FL1 region: excitation at 492 nm and emission at 520-540 nm (Figures 1-3). As CFSE is detected in the green range, it is optimal for use in dual-staining studies with other fluorescent reagents, such as PI (MBS258060), 7-AAD (MBS258053), and SR-FLICA® with minimal spectral overlap. Use it with ICT's SR-FLICA poly caspases inhibitor reagent to identify apoptotic cells in the analysis (Figure 3).
Product Categories/Family for CFSE Fluorescent Cellular Stain reagent	Cell Viability Assays; Cellular Imaging; CFSE Fluorescent Cellular Stain

NCBI AND UNIPROT INFORMATION

Molecular Weight	557.47
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For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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Online datasheet: <https://stage.aaabiotech.com/reagent/cfse-fluorescent-cellular-stain/52739>