eBioscience™ CFSE

Catalog Number: 65-0850
Also known as: 5-(and 6)-Carboxyfluorescein diacetate succinimidyl ester, CFDA SE
For Research Use Only. Not for use in diagnostic procedures.

Product Information

Contents: eBioscience™ CFSE
Catalog Number: 65-0850

Formulation: lyophilized
Temperature Limitation: Store at -20°C to -80°C. Protect from light and moisture. It is recommended to use the reconstituted dye within 6 months and to avoid freeze-thawing.
Batch Code: Refer to vial
Use By: Refer to vial

Description

CFSE is widely used for cell tracking and proliferation studies. It has also been used in CTL assays and cell motility studies. CFSE readily crosses intact cell membranes. Once inside the cells, intracellular esterases cleave the acetate groups to yield the fluorescent carboxyfluorescein molecule. The succinimidyl ester group reacts with primary amines, crosslinking the dye to intracellular proteins. Cell division can be measured as successive halving of the fluorescence intensity of CFSE. Cells labeled with CFSE may be fixed and permeabilized for analysis of intracellular targets using standard formaldehyde-containing fixatives and saponin-based permeabilization buffers, such as the Foxp3/Transcription Factor Staining Buffer Set (cat. 00-5523) or the IC Fixation Buffer (cat. 00-8222) and Permeabilization Buffer (10X) (cat. 00-8333).

CFSE has a molecular weight of 557.47. After the acetate groups are cleaved, it has a peak excitation of 494 nm and peak emission of 521 nm. Each vial of CFSE may be reconstituted to a stock concentration of 10 mM with 90 µL of anhydrous DMSO; once reconstituted it should be used within 6 months and protected from light and stored at -20°C with dessicant; avoid freeze-thawing.

Applications Reported

CFSE has been reported for use in flow cytometric analysis and fluorescent microscopy.

Applications Tested

CFSE [5-(and 6)-carboxyfluorescein diacetate succinimidyl ester] has been tested by flow cytometric analysis of stimulated mouse splenocytes. Cells can be labeled with 0.5 to 20 µM CFSE. It is recommended that the concentration used for labeling cells be carefully determined by each investigator for optimal performance in the assay of interest.

References

Budda SA, Girton A, Henderson JG, Zenewicz LA. Transcription Factor HIF-1a Controls Expression of the Cytokine IL-22 in CD4 T Cells. J Immunol. 2016 Oct 1;197(7):2646-52. (**CFSE**, FC, PubMed)

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Ingulli E. Tracing tolerance and immunity in vivo by CFSE-labeling of administered cells. Methods Mol Biol. 2007;380:365-76.

Miller MJ, Wei SH, Parker I, Cahalan MD. Two-photon imaging of lymphocyte motility and antigen response in intact lymph node. Science. 2002 Jun 7;296(5574):1869-73.

Lyons AB, Parish CR. Determination of lymphocyte division by flow cytometry. J Immunol Methods. 1994 May 2;171(1):131-7.

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Related Products
45-0900 CD90.1 (Thy-1.1) Monoclonal Antibody (HIS51), PerCP-Cyanine5.5, eBioscience™ TDS DISABLED: ABMAINT SKU (HIS51)
48-0081 CD8a Monoclonal Antibody (53-6.7), eFluor 450, eBioscience™ TDS DISABLED: ABMAINT SKU (53-6.7)
65-0840 eBioscience™ Cell Proliferation Dye eFluor™ 670
65-0842 eBioscience™ Cell Proliferation Dye eFluor™ 450

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CFSE Cell Labeling Protocol

Protocol: CFSE Cell Labeling

Materials Needed

- PBS
- Flow Cytometry Staining Buffer (cat. 00-4222)

Experimental Procedure

Note: Reconstitute one vial of CFSE to a stock concentration of 10 mM with 90 µL of anhydrous DMSO. Once reconstituted the dye should be used within 6 months, protected from light, and stored with desiccant at less than or equal to -20°C. Avoid freeze-thawing.

1. Prepare a single-cell suspension of cells to be labeled.
2. Wash cells two times with PBS to remove any serum.
3. Resuspend cells at 5-10x10^6/mL of PBS (pre-warmed to room temperature).
4. Add CFSE to the desired final concentration (e.g., for a final concentration of 1 µM, add 0.2 µL of a 5 mM stock solution per mL of cells).
5. Mix immediately and incubate for 10 minutes at room temperature in the dark.
6. Stop labeling by adding 4-5 volumes of cold complete media (containing ≥10% serum) and incubate on ice for 5 minutes.
7. Wash cells 3 times with complete media.
8. Culture or transfer cells, as desired.

Note: The concentration of CFSE, incubation time, and temperature can be modified to achieve the desired staining intensity. However, very high labeling can lead to compensation issues and may also interfere with cellular functions. Thus, it is highly recommended that each investigator determine the optimal concentration for the assay of interest.

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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