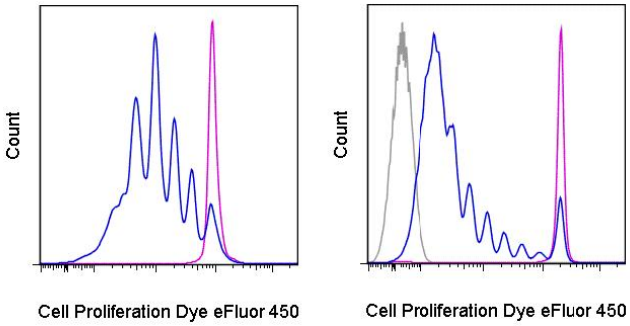
eBioscience™ Cell Proliferation Dye eFluor™ 450

Catalog Number: 65-0842

For Research Use Only. Not for use in diagnostic procedures.



Left: Mouse splenocytes were labeled with 10 uM Cell Proliferation Dye eFluor® 450 and cultured for 3 days with (blue histogram) or without (purple histogram) ConA. Cells were stained with Anti-Mouse CD4 PE (cat. 12-0042), Anti-Mouse CD25 PerCP-Cyanine5.5 (cat. 45-0251), and 7-AAD (cat. 00-6993). Viable CD4+ cells were used for analysis.

Right: Splenocytes from Thy1.1 mice were labeled with 10 uM Cell Proliferation Dye eFluor® 450 and then injected into C57Bl/6 mice (purple histogram) or B6D2F1 mice (blue histogram). Splenocytes from the C57Bl/6 and B6D2F1 mice were collected 72 hours after injection of the labeled cells. Cells were stained with Anti-Mouse CD4 APC (cat. 17-0042), Anti-Mouse/Rat CD90.1 (Thy1.1) PE (cat. 12-0900), and Fixable Viability Dye eFluor® 780 (cat. 65-0865). Viable Thy1.1+CD4+ cells were used for analysis. Thy1.1-CD4+ host cells from the B6D2F1 mice, that are unlabeled with the Cell Proliferation Dye, are shown in gray.

Product Information

Contents: eBioscience™ Cell Proliferation Dye eFluor™ 450

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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.

LOT **Batch Code:** Refer to vial

Use By: Refer to vial

Description

Cell Proliferation Dye eFluor® 450 is a violet fluorescent dye that can be used to monitor individual cell divisions. This fluorescent dye binds to any cellular proteins containing primary amines, and as cells divide, the dye is distributed equally between daughter cells that can be measured as successive halving of the fluorescence intensity of the dye. Up to 7 generations may be visualized. Cells labeled with Cell Proliferation Dye eFluor® 450 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).

Cell Proliferation Dye eFluor® 450 has a peak excitation of 409 nm and can be detected using a 450/50 band pass filter (equivalent to eFluor® 450 or Pacific Blue®), making it compatible with applications that utilize GFP.

Cell Proliferation Dye eFluor® 450 is supplied as a lyophilized powder. Each vial may be reconstituted to a stock concentration of 10 mM with 165 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

Cell Proliferation Dye eFluor® 450 has been reported for use in flow cytometric analysis.

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Applications Tested

Cell Proliferation Dye eFluor® 450 has been tested by flow cytometric analysis of stimulated mouse splenocytes. It can be used to label cells at a concentration of 10 µM. It is highly recommended that the optimal concentration be determined by each investigator for optimal performance in the assay of interest.

References

Pollizzi KN, Sun IH, Patel CH, Lo YC, Oh MH, Waickman AT, Tam AJ, Blosser RL, Wen J, Delgoffe GM, Powell JD. Asymmetric inheritance of mTORC1 kinase activity during division dictates CD8(+) T cell differentiation. Nat Immunol. 2016 Jun;17(6):704-11. (**CPD eFluor 450**, FC , PubMed)

Verhagen J, Burton BR, Britton GJ, Shepard ER, Anderton SM, Wraith DC. Modification of the FoxP3 transcription factor principally affects inducible t regulatory cells in a model of experimental autoimmune encephalomyelitis.PLoS One. 2013 Apr 8;8(4):e61334. (**CPD eFluor450**, FC, PubMed)

Related Products

00-6993 eBioscience™ 7-AAD Viability Staining Solution
12-0042 CD4 Monoclonal Antibody (RM4-5), PE, eBioscience™ TDS DISABLED: ABMAINT SKU (RM4-5)
12-0900 CD90.1 (Thy-1.1) Monoclonal Antibody (HIS51), PE, eBioscience™ TDS DISABLED: ABMAINT SKU (HIS51)
17-0042 CD4 Monoclonal Antibody (RM4-5), APC, eBioscience™ TDS DISABLED: ABMAINT SKU (RM4-5)
45-0251 CD25 Monoclonal Antibody (PC61.5), PerCP-Cyanine5.5, eBioscience™ TDS DISABLED: ABMAINT SKU (PC61.5)
65-0840 eBioscience™ Cell Proliferation Dye eFluor™ 670
65-0850 eBioscience™ CFSE
65-0865 eBioscience™ Fixable Viability Dye eFluor™ 780

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Cell Proliferation Dye eFluor™ 450 Cell Labeling Protocol

Protocol: Cell Proliferation Dye eFluor™ 450 Cell Labeling

Materials Needed

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

Experimental Procedure

Note: Reconstitute one vial of Cell Proliferation Dye eFluor™ 450 to a stock concentration of 10 mM with 165 µL of anhydrous DMSO. Once reconstituted the dye should be 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 2X the desired final concentration in PBS (pre-warmed to room temperature).
   For example, if the final concentration of cells desired is $10x10^6$/mL, then resuspend cells at $20x10^6$/mL.
   Note: The final concentration of cells should not exceed $10x10^6$/mL. If labeling fewer than 5 x 10^6 total cells, do not use less than 0.5 mL PBS.
4. Prepare a 20 µM solution of Cell Proliferation Dye eFluor™ 450 in PBS (pre-warmed to room temperature). This will be mixed 1:1 with the 2X cell suspension in Step 5.
   Note: It is recommended to use 10 µM as a starting point for labeling cells; however, it is highly recommended that each investigator determine the optimal concentration for the assay of interest.
5. While vortexing cells, add an equal volume of the 20 µM dye solution prepared in Step 4.
6. Incubate for 10 minutes at 37°C in the dark.
7. Stop labeling by adding 4-5 volumes of cold complete media (containing ≥10% serum) and incubate on ice for 5 minutes.
8. Wash cells 3 times with complete media.
9. Culture or transfer cells, as desired.

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  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

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