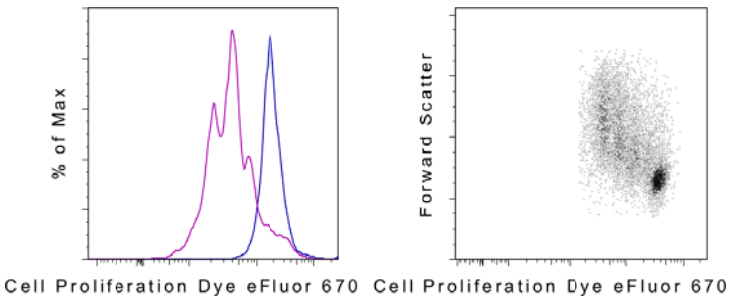
eBioscience™ Cell Proliferation Dye eFluor™ 670

Catalog Number: 65-0840

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



Left: Mouse spleen cells were labeled with 5 uM Cell Proliferation Dye eFluor® 670, then cultured for 3 days with (purple histogram) or without (blue histogram) plate-bound Anti-Mouse CD3 and soluble Anti-Mouse CD28. Cells were then stained with Anti-Mouse CD4 FITC (cat. 11-0042) and 7-AAD (cat. 00-6993). Viable CD4+ cells were used for analysis.

Right: C57Bl/6 spleen cells were labeled with 5 uM Cell Proliferation Dye eFluor® 670, then injected into B6D2F1 mice. Two days later, spleen cells from the F1 mice were stained with Anti-Mouse CD4 PE-Cyanine7 (cat. 25-0042) and Fixable Viability Dye eFluor® 450 (cat. 65-0863). Viable CD4+ cells were used for analysis.

Product Information

Contents: eBioscience™ Cell Proliferation Dye eFluor™ 670

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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® 670 is a red fluorescent dye that can be used to monitor individual cell divisions. This fluorescent dye binds to any cellular protein 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 6 generations may be visualized. Cell Proliferation Dye eFluor® 670 can also be used for long term tracking of labeled cells. Analysis using two-parameter plots may provide better resolution of each generation, especially between undivided cells and the first generation. Cells labeled with Cell Proliferation Dye eFluor® 670 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® 670 has a peak excitation of 647 nm and can be excited by the red (633 nm) laser line. It has a peak emission of 670 nm and can be detected with a 660/20 band pass filter (equivanet to APC, Alexa Fluor® 647, or eFluor® 660), making it compatible with applications that utilize GFP.

Cell Proliferation Dye eFluor® 670 has a molecular weight of 792.6 and is supplied as a lyophilized powder. Each vial may be reconstituted to a stock concentration of 5 mM with 126 µL of anhydrous DMSO; once reconstituted it should be protected from light and stored at -20°C with dessicant. It is recommended to use the reconstituted dye within 6 months and to avoid freeze-thawing.

Applications Reported

Cell Proliferation Dye eFluor® 670 has been reported for use in flow cytometric analysis and microscopy. Microscopic

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analysis of cells labeled with this dye may reveal punctate staining. If more uniform staining is desired, the use of CFSE (cat. 65-0850) is recommended.

Applications Tested
Cell Proliferation Dye eFluor® 670 has been tested by flow cytometric analysis of stimulated mouse spleen cells. It can be used to label cells at a concentration of 5 µM. It is highly recommended that the optimal concentration be determined by each investigator for optimal performance in the assay of interest.

References
Yao Y, Vent-Schmidt J, McGeough MD, Wong M, Hoffman HM, Steiner TS, Levings MK. Tr1 Cells, but Not Foxp3+ Regulatory T Cells, Suppress NLRP3 Inflammasome Activation via an IL-10-Dependent Mechanism. J Immunol. 2015 Jul 15;195(2):488-97. (**CPD eFluor 670**, FC, PubMed)

Baeke F, Korf H, Overbergh L, Verstuyf A, Thorrez L, Van Lommel L, Waer M, Schuit F, Gysemans C, Mathieu C. The vitamin D analog, TX527, promotes a human CD4+ CD25(high) CD127(low) regulatory T cell profile and induces a migratory signature specific for homing to sites of inflammation. J Immunol. 2011 Jan 1;186(1):132-42.(FC, PubMed)

Shen S, Chuck MI, Zhu M, Fuller DM, Yang CW, Zhang W. The Importance of LAT in the Activation, Homeostasis, and Regulatory Function of T Cells. J Biol Chem. 2010 Nov 12;285(46):35393-405. (FC, PubMed)

Swiecki M, Gilfillan S, Vermi W, Wang Y, Colonna M.Plasmacytoid dendritic cell ablation impacts early interferon responses and antiviral NK and CD8(+) T cell accrual. Immunity. 2010 Dec 14;33(6):955-66 (FC, PubMed)

Related Products
00-5523 eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set
00-6993 eBioscience™ 7-AAD Viability Staining Solution
11-0042 CD4 Monoclonal Antibody (RM4-5), FITC, eBioscience™ TDS DISABLED: ABMAINT SKU (RM4-5)
25-0042 CD4 Monoclonal Antibody (RM4-5), PE-Cyanine7, eBioscience™ TDS DISABLED: ABMAINT SKU (RM4-5)
65-0842 eBioscience™ Cell Proliferation Dye eFluor™ 450
65-0850 eBioscience™ CFSE
65-0863 eBioscience™ Fixable Viability Dye eFluor™ 450
88-8824 eBioscience™ Intracellular Fixation & Permeabilization Buffer Set

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invitrogen

Cell Proliferation Dye eFluor™ 670 Cell Labeling Protocol

Protocol: Cell Proliferation Dye eFluor™ 670 Cell Labeling

Materials Needed

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

Experimental Procedure

Note: Reconstitute one vial of Cell Proliferation Dye eFluor™ 670 to a stock concentration of 5 mM with 126 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 10×10^6/mL, then resuspend cells at 20×10^6/mL.
   Note: The final concentration of cells should not exceed 10×10^6/mL. If labeling fewer than 5×10^6 total cells, do not use less than 0.5 mL PBS.
4. Prepare a 10 µM solution of Cell Proliferation Dye eFluor™ 670 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 5 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. It is not recommended to use concentrations greater than 10 µM to label cells, as this has been observed to partially inhibit cell proliferation and decrease the viability of labeled cells.
5. While vortexing cells, add an equal volume of the 10 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.

Note: Analysis using two-parameter plots may provide better resolution of each generation, especially between undivided cells and the first generation.

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

Limited product warranty

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