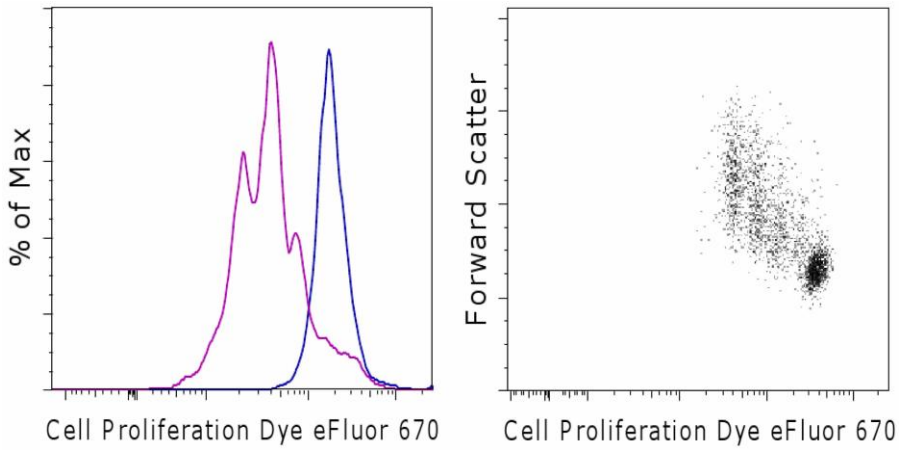
Cell Proliferation Dye eFluor® 670

Catalog Number: 65-0840
GPR: General Purpose Reagents. For Laboratory Use.



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-Cy7 (cat. 25-0042) and Fixable Viability Dye eFluor® 450 (cat. 65-0863). Viable CD4+ cells were used for analysis.

Product Information

Contents: Cell Proliferation Dye eFluor® 670
REF **Catalog Number:** 65-0840

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 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.

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Cell Proliferation Dye eFluor® 670

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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 uL 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.

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
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 Foxp3 / Transcription Factor Staining Buffer Set
00-6993 7-AAD Viability Staining Solution
00-8222 IC Fixation Buffer
00-8333 Permeabilization Buffer (10X)
11-0042 Anti-Mouse CD4 FITC (RM4-5)
25-0042 Anti-Mouse CD4 PE-Cy7 (RM4-5)
65-0842 Cell Proliferation Dye eFluor® 450
65-0850 CFSE
65-0863 Fixable Viability Dye eFluor® 450
88-8823 Intracellular Fixation & Permeabilization Buffer (plus Brefeldin A) (previously named IC Fixation & Permeabilization Buffer)

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full spectrum cell analysis
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Cell Proliferation Dye eFluor® 670 Cell Labeling Protocol

Research Use Only

Protocol: Cell Proliferation Dye eFluor® 670 Cell Labeling

Experimental Procedure

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 at -20°C with dessicant. 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 $5x10^6$ 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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