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Staining Cells with Proliferation Dyes

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Introduction

The Cell Proliferation Dyes (CPDs) eFluor® 670, CPD eFluor® 450, and 5-(and 6)-Carboxyfluorescein diacetate succinimidyl ester (CFSE) are fluorescent dyes that can be used to monitor individual cell divisions. These dyes may also be used for tracking cells in vivo for up to several weeks in non-dividing cells. The dyes covalently react with cellular proteins. As cells divide, the dyes are divided equally between daughter cells, resulting in successive halving of the fluorescence intensity of the dye. Six to eight generations may be visualized, depending on which dye is used.

Cells labeled with proliferation dyes 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. no. 00-5523) or the Intracellular Fixation & Permeabilization Buffer Set (cat. no. 88-8824)

Protocol A: Cell Proliferation Dye (CPD) eFluor® 670

Materials

- CPD eFluor® 670 (cat. no. 65-0840)
- Sterile 1X PBS
- Dimethyl sulfoxide (DMSO), anhydrous
- Complete culture medium (containing ≥10% serum)

Experimental Procedure in 12 x 75 mm Tubes

1. Reconstitute one vial of CPD eFluor® 670 to a stock concentration of 5 mM with 126 µL of anhydrous DMSO.

NOTE: Once reconstituted, dye should be protected from light and stored at less than or equal to –20°C with dessicant. It is recommended to use the reconstituted dye within 6 months and to avoid freeze-thawing.

2. Prepare a single-cell suspension of cells to be labeled. (See "Cell Preparation for Flow Cytometry Protocols" in our Best Protocols).
3. Wash cells two times with PBS to remove any serum.
4. 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 1 x 10^7 cells/mL,

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then resuspend cells at 2 x 10^7 cells/mL.

NOTE: The final concentration of cells should not exceed 10 x 10^6 cells/mL. If labeling fewer than 5 x 10^6 total cells, do not use less than 0.5 mL of PBS.

5. Prepare CPD eFluor® 670 in PBS (pre-warmed to room temperature) to 10 µM. This CPD working solution will be mixed 1:1 with the 2X cell suspension in Step 6.

NOTE: It is suggested 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.

6. While vortexing the 2X cell suspension, add an equal volume of the dye solution prepared in Step 5.
7. Incubate for 10 minutes at 37°C in the dark.
8. Stop labeling by adding 4–5 volumes of cold complete media (containing ≥10% serum) and incubate on ice for 5 minutes.
9. Wash cells three times with complete media.
10. 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.

Protocol B: Cell Proliferation Dye (CPD) eFluor® 450

Materials

- CPD eFluor® 450 (cat. no. 65-0842)
- Sterile 1X PBS
- Dimethyl sulfoxide (DMSO), anhydrous
- Complete culture medium (containing ≥10% serum)

Experimental Procedure in 12 x 75 mm Tubes

1. Reconstitute one vial of CPD eFluor® 450 to a stock concentration of 10 mM with 165 µL of anhydrous DMSO.

NOTE: Once reconstituted, dye should be protected from light and stored at less than or equal to –20°C with dessicant. It is recommended to use the reconstituted dye within 6 months and to avoid freeze-thawing.

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2. Prepare a single-cell suspension of cells to be labeled. (See "Cell Preparation for Flow Cytometry Protocols" in our Best Protocols).
3. Wash cells two times with PBS to remove any serum.
4. 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 1 x 10^7 cells/mL, then resuspend cells at 2 x 10^7 cells/mL.

NOTE: The final concentration of cells should not exceed 10 x 10^6 cells/mL. If labeling fewer than 5 x 10^6 total cells, do not use less than 0.5 mL of PBS.

5. Prepare CPD eFluor® 450 in PBS (pre-warmed to room temperature) to 20 µM. This CPD working solution will be mixed 1:1 with the 2X cell suspension in Step 6.

NOTE: It is suggested 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.

6. While vortexing the 2X cell suspension, add an equal volume of the dye solution prepared in Step 5.
7. Incubate for 20 minutes at 37°C in the dark.
8. Stop labeling by adding 4–5 volumes of cold complete media (containing ≥10% serum) and incubate on ice for 5 minutes.
9. Wash cells three times with complete media.
10. 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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Protocol C: CFSE (carboxyfluorescein diacetate succinimidyl ester)

Materials

- CFSE (cat. no. 65-0850)
- Sterile 1X PBS
- Dimethyl sulfoxide (DMSO), anhydrous
- Complete culture medium (containing ≥10% serum)

Experimental Procedure in 12 x 75 mm Tubes

1. Reconstitute one vial of CFSE to a stock concentration of 10 mM with 90 µL of anhydrous DMSO.

NOTE: Once reconstituted, dye should be protected from light and stored at less than or equal to –20°C with dessicant. It is recommended to use the reconstituted dye within 6 months and to avoid freeze-thawing.

2. Prepare a single-cell suspension of cells to be labeled. (See "Cell Preparation for Flow Cytometry Protocols" in our Best Protocols).
3. Wash cells two times with PBS to remove any serum.
4. Resuspend cells at 5–10 x 10^6 cells/mL of PBS (pre-warmed to room temperature).
5. 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).
6. Mix immediately and incubate for 10 minutes at room temperature in the dark.
7. Stop labeling by adding 4–5 volumes of cold complete media and incubate on ice for 5 minutes.
8. Wash cells three times with complete media.
9. 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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