BD Horizon™ CFSE (carboxyfluorescein diacetate succinimidyl ester) is a blue laser excitable dye that can be used for flow cytometric monitoring of cell divisions. The non-fluorescent dye passively diffuses across cell membranes and is cleaved by intracellular esterases within viable cells. The cleaved dye becomes highly fluorescent and covalently binds to protein amine groups within the cells via its succinimidyl ester group. Nonviable cells remain nonfluorescent. As viable cells divide, CFSE dye is distributed uniformly between daughter cells; each daughter cell retains approximately half of the CFSE fluorescence intensity of its parent cell. The covalently bound dye is well retained within the cells allowing for applications involving live or aldehyde-fixed and permeabilized cells.

BD Horizon™ CFSE has an excitation maximum of 490 nm and an emission maximum of 520 nm.

Flow cytometric analysis of proliferating peripheral blood lymphocytes stained with BD Horizon™ CFSE. Human PBMC were labeled (10 min, 37°C) with BD Horizon™ CFSE (Cat. No. 565082; 2.5 μM). Cells were washed twice and cultured (4 days) alone (dashed line histogram) or with (solid line histogram) phytohemagglutinin. The cells were stained with 0.1 μg/mL DAPI (Cat. No. 564907) to exclude nonviable cells. The histogram shows CFSE fluorescence peaks of gated events with the light-scatter characteristics of viable lymphocytes (DAPI-negative). Unstimulated cells show a single, bright CFSE fluorescence peak, indicating no proliferation. Stimulated cells show multiple CFSE fluorescence peaks, indicating multiple generations of proliferating cells. Analysis was performed using a BD LSRFortessa™ Cell Analyzer System.

Preparation and Storage

Store at -20°C, protected from exposure to light.

Application Notes

<table>
<thead>
<tr>
<th>Application</th>
<th>Routinely Tested</th>
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<tbody>
<tr>
<td>Intracellular staining (flow cytometry)</td>
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Recommended Assay Procedure:

Materials Provided
CFSE dye (2 vials, 500 μg dye per vial)

Materials needed but not provided
• Viable single-cell suspensions of primary lymphoid cells of interest
• Fresh cell culture-grade Dimethyl Sulfoxide (DMSO; eg, Sigma D2650)
• Sterile Dulbecco’s Phosphate Buffered Saline (1× DPBS)
• Cell culture medium, eg, with 10% Fetal Bovine Serum (FBS)
• Sterile polypropylene 15-mL or 50-mL conical tubes
• Microcentrifuge tubes or 2-mL cryovials
• Fluorescent antibodies for immunophenotypic or functional analysis (optional)

Required Equipment
• Blue laser-equipped cytometer; eg, BD FACSCanto™ II, BD LSRFortessa™, BD LSRII Flow Cytometer, or BD Accuri™ C6
• Centrifuge
• 37°C CO2 Incubator and 37ºC Water Bath

Storage
Upon arrival, store the dye powder with desiccant and protected from light at ≤ -20°C until use. After reconstitution with DMSO, store the stock solution with desiccant at ≤ -20°C in small aliquots. The stock solution is stable for 3 months if stored as indicated.

Procedure
CFSE has a maximum absorption of 490 nm and a maximum emission of 520 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the emitted fluorescence.

Preparation of CFSE dye solution in DMSO
1. Prepare a 10 mM stock solution of CFSE dye by adding 90 μL DMSO to one vial of CFSE. Mix thoroughly by vortexing.
2. Prepare single-use aliquots of the CFSE stock solution (user-defined volume) using appropriately labeled microcentrifuge tubes or 2-mL cryovials.
3. Freeze all aliquots at ≤ -20°C with desiccant and protected from light.

CFSE labeling of cells
This procedure has been optimized for labeling mouse and human lymphocytes at cell concentrations of 10-30 × 10^6 cells/mL. Assay conditions may need to be optimized for other cell types or cell densities.
1. Thaw the 10 mM stock solution of CFSE, if previously frozen.
2. Transfer cells to 15- or 50-mL polypropylene centrifuge tubes.
3. Wash cells in 1× DPBS to remove any residual serum proteins.
4. Repeat step 3.
5. Resuspend the cells thoroughly into a single cell suspension at a concentration of 10-30 × 10^6 cells/mL in 1× DPBS.
   a. Note: Avoid staining in azide-, serum-, or protein-containing buffers, as these moieties bind free dye and may interfere with staining of the cells.
6. Add stock CFSE to the single cell suspension to achieve a final concentration of 1-10 μM. If necessary, the stock solution may be diluted further with DMSO before addition to the single cell suspension. Vortex immediately.
7. Incubate the dye-cell suspension in a 37°C water bath for 10-15 minutes.
8. Add 9× the original volume of 1× DPBS to the cells and pellet cells by centrifugation.
9. Decant the supernatant and gently mix to disrupt the cell pellet.
10. Add 10 mL of complete medium with 10% FBS and repeat centrifugation step.
11. Decant supernatant and gently mix to disrupt the cell pellet.
12. Resuspend the cells in complete medium and proceed to cell culture or analysis by flow cytometry.

Notes:
1. Confirm brightness of the cell labeling by comparing labeled cells with unlabeled control cells.
2. BD Horizon™ CFSE can be used in intracellular staining assays that require fixation with formaldehyde and permeabilization with methanol and detergents such as those used for BD Phosflow™ staining (eg, Cat. No. 558050, BD Phosflow™ Perm Buffer III), intracellular cytokine staining (eg, Cat. No. 554714, BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit), or transcription factor staining (eg, Cat. No. 562574/562725, BD Pharmingen™ Transcription Factor Buffer Set).
3. Higher initial staining intensities can be achieved by increasing the initial CFSE dye loading concentration. However, as with other succinimidyl ester containing tracking dyes, higher loading concentrations may lead to increased cell toxicity or cell death.
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**Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
3. Cy is a trademark of GE Healthcare.
4. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.

**References**


