CFSE Cell Proliferation Assay

Ordering Information	*Storage Conditions*
Product Number: 22022 (25 mg) 22028 (5 x 0.5 mg)	Keep at -20 °C and desiccated Expiration date is 12 months from the date of receipt

Introduction

It is widely recognized that fluorescent labeling of cells is an effective method for detecting the presence of viable cells in a sample. Flow cytometry combined with fluorescent staining is a powerful tool to analyze heterogeneous cell populations. Among all the existing fluorescent dyes, fluorescein diacetate (FDA) and its derivatives (such as CFSE) are non-fluorescent molecules that diffuse into cells and are hydrolyzed by intracellular non-specific esterases to give fluorescent products. The fluorescent products are generated and accumulated only in the cells that have intact cell membranes and active esterase activities while dead cells are not stained. The precise kinetics of membrane transport and intracellular hydrolysis of FDA and its analogs (such as CFSE) are related to cellular functions. For multiplexing GFP-transfected cells or using a FITC-labeled antibody we offer CytoTell™ dyes that are functionally similar to CFSE and can be used for the multicolor applications where either GFP or FITC-labeled antibody is used since those dyes have either excitation or emission spectra distinct from CFSE and its fluorescein analogs. In addition, our CytoTell™ dyes not only eliminates the dye efflux drawback associated with CFSE, but also is compatible with cell culture medium in the staining cells prior to imaging or flow cytometric analysis.

Storage and Handling Conditions

The CFSE should be stable for at least 12 months if store at -20 °C, protecting from light and moisture, avoiding freeze/thaw cycles.

Chemical and Physical Properties

Molecular Weight: 557.46
Solvent: Dimethylsulfoxide (DMSO)
Spectral Properties: Ex/Em = 494/ 521 nm

Assay Protocol

Brief Summary

Prepare cells with test compounds → Add 0.5 to 5 µM CFSEworking solution → Incubate dyes with cells at room temperature or 37 °C for 10 to 30 min → Remove the dye working solution → Analyze with a flow cytometer with Ex/Em = 490/520 nm (FL1 channel)

Note: Following is our recommended protocol for live cells. It only provides a guideline, and should be modified according to your specific needs.

1. Prepare 10 mM DMSO stock solution

For #22022: Dissolve 5.6 mg in 1 ml DMSO to make 10 mM stock solution (1 mg/ml is equivalent to 1.8 mM);
For #22028 add 90 µL DMSO to each vial to make 10 mM stock solution.
Note: The stock solution should be used promptly; any remaining solution should be aliquoted and frozen at ≤ -20 °C. Avoid repeated freeze-thaw cycles, and protect from light.

2. Prepare CFSE working solution

Prepare a CFSE working solution (0.5 to 5 μM) right before use by diluting the DMSO stock solution (20, 000 to 2,000 times) from Step 1 with Hanks and 20 mM Hepes buffer (HHBS) or the buffer of your choice, pH 7. Mix them well by vortexing.

3. Analyze cells with a flow cytometer or a fluorescence microscope:

3.1 Treat cells with test compounds for a desired period of time.

3.2 Centrifuge the cells to get 1-5 × 10^5 cells per tube.

3.3 Resuspend cells in 500 μL of the dye working solution (from Step 2).

3.4 Incubate cells with a dye solution at room temperature or 37 °C for 10 to 30 min, protected from light.

3.5 Remove the dye working solution from the cells, wash the cells with HHBS or buffer of your choice. Resuspend cells in 500 μL of pre-warmed HHBS or medium to get 1-5 × 10^5 cells per tube.

3.6 Monitor the fluorescence change at Ex/Em = 490/520 nm with a flow cytometer (FL1 channel) or a fluorescence microscope.