Carboxy-fluoresceindiacetate Succinimidyl Ester for Tracking Cell Proliferation by Flow Cytometry

Carboxy-fluoresceindiacetate succinimidyl ester (CFDA SE) is a very effective reagent to study the division progress of proliferating cells.[21] It passively crosses the cell membrane and covalently binds to free amine groups of intracellular macromolecules. Endogenous cytoplasmic esterases remove the carboxyl groups, converting non-fluorescent CFDA SE to fluorescent CFSE that remains cell associated. Upon cell division, CFSE is distributed uniformly between daughter cells. Each cell division reduces the CFSE fluorescent intensity of daughter cells by approximately half. Each successive generation can be counted by the number of discreet fluorescent frequency distributions (eg, histogram "peaks" or dot plot "clusters") that are revealed upon flow cytometric analysis. The multipeak histogram (*Figure 9A*) shows several successive divisions that human peripheral blood lymphocytes have undergone when cultured for 72 hr with phytohemaglutinin.

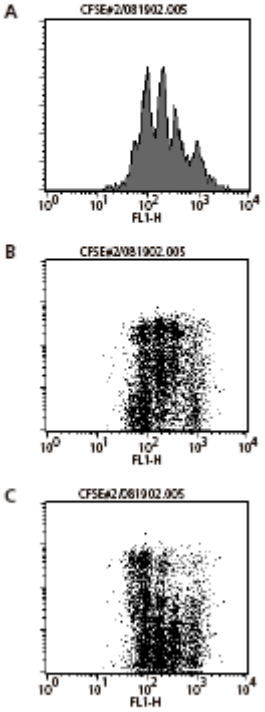

Figure 9. HPBMCs were loaded with 1 µM CFDA SE for 10 minutes at 37°C. Cells were washed twice in 1× PBS then stimulated with 1.5% PHA for 72 hrs. Cells were harvested and then stained with PE anti-human CD4 and allophycocyanin (APC)-anti-human CD8 then analyzed on a BD FACScalibur™. Panel A is the CFSE histogram for the viable cell population. Panel B is the two color dot plot generated by the flow cytometric analysis of cells stained with PE anti-human CD4 and CFSE. Panel C is the two color dot plot obtained for cells stained with allophycocyanin-anti-human CD8 and CFSE.

By using CFSE as a dye for following cell proliferation, one can select additional parameters (eg, CD markers or intracellular cytokines) and perform further flow cytometric analysis to characterize the nature of cells within any cell generation. For example, as shown in *Figure 9B and 9C*, CFSE staining can be coupled with staining for cell surface CD4 and CD8 to identify the proliferative activities of individual cells within T cell subpopulations. CFSE labeling has also been used to determine the number of divisions required for cells to express new immunoglobulin isotypes[22] or to express cytokines such as Interleukin-4.[23] In addition to its use in experimental culture systems, CFSE-labeling is very useful for determining the proliferative and migratory behavior of cells transferred to adoptive recipient animals.[1]

CFDA SE Labeling Protocol

Dilute CFDA SE in dimethysulfoxide (5 mg/ml is equivalent to 8.8 mM) and store aliquots at –80°C. The working solution of CFDA SE is between 10 nM–5 mM. Researchers should determine the optimal loading concentration for their particular cell type. Normally, a solution of 1 µM CFDA SE in 1× PBS is used to load up to 5×10^7 cells. Cells are loaded at 37°C for approximately 10 minutes. Times can vary depending on how bright or dim you wish to load the cells. CFSE is not highly toxic, but may negatively affect cell function. To stop the reaction, wash the cells twice in 1× PBS. Cells are now ready to be activated or transferred to recipient experimental animals. It is recommended that you confirm the loading of your cells on a flow cytometer prior to proceeding with an experimental protocol.

Summary

In conclusion, a brief overview of reagents and methods for BrdU and nucleic acid staining of cells and the multiparameter flow cytometric analysis of their cell cycle positions has been presented. More detailed information for performing these types of flow cytometric cell cycle analyses is provided by the references listed at the end of this chapter. Kits as well as individual reagents are available from BD Biosciences Pharmingen for staining cells that have been exposed to and incorporated BrdU. Additional reagents, including reagents that utilize propidium iodide and 7-AAD are also listed. The reagents and/or methods referred to in this chapter were presented because they are useful for performing multiparameter flow cytometric analysis of cell populations that are of particular interest in immune function studies.