A Novel Non-Cytotoxic Fluorescent Dye for Cell Proliferation Analysis in Flow Cytometry

Penny Melquist and Mike O’Grady
Thermo Fisher Scientific, 29851 Willow Creek Road, Eugene, OR, USA, 97402

INTRODUCTION

Cell proliferation analyses are crucial for cell growth and differentiation studies, and are often used to evaluate compound toxicity and/or inhibition of tumor cell growth during drug development. Tools for measuring cell proliferation include probes for analyzing the average DNA content and cellular metabolism in a population, as well as single-cell indicators of DNA synthesis and cell-cycle-specific proteins, and tracking population doubling by dye dilution. Successful proliferation analysis by dye dilution requires an extremely bright dye to distinguish fluorescently labeled cells after several cell divisions. The intense fluorescent staining provided by CellTrace® Yellow dye enables the visualization of six or more generations of proliferating cells. CellTrace Yellow reagent covalently binds intracellular proteins where the stable, well-retained fluorescent dye offers a consistent signal, even after several days in cell culture. Division of a cell labeled with a CellTrace Yellow reagent results in equal partitioning of dye between daughter cells and approximately half of the fluorescence intensity in the progeny. When analyzed by flow cytometry using either a 532 nm or 561 nm laser, this partitioning of the CellTrace Yellow dye provides a direct indication of cell proliferation without compromising cell health. In this study, we demonstrate that the spectral properties of CellTrace Yellow reagent permits multiplexing with many common flow reagents (cell cycle, viability, proliferation, traceability, and resolution) excited by violet, green, yellow, and red laser lines without introducing cytotoxicity.

MATERIALS AND METHODS

Human peripheral blood mononuclear cells were isolated from whole blood using a Ficoll-Paque Plus density gradient. Cells were stained with 10 μM CellTrace Yellow reagent and resuspended in OptiM™ T-Cell Expansion media. Stained cells were stimulated to proliferate with 50 μL CO3/Di3D NA2 Dynabeads per milliliter of culture and resuspended at 37°C and 5% CO2 for 6 days. After six days, cells were harvested for two hours. Cells were then washed and stained with LIVE/DEAD® Fixable Near-IR Dead Cell Stain for 30 min, washed, and fixed for 15 minutes in 4% formaldehyde. Cells were treated with Click-IT® saponin-based perm for 20 minutes. The Click-IT Reaction Cocktail containing Alexa Fluor™ 488 azide was added for 30 minutes to complete the Click reaction. Cells were labeled with 5 μL mouse anti-human CD4 APC for 20 minutes and washed. Finally, cells were labeled with Far-Red™ dye for 30 min and analyzed on an Attune® NxT Flow Cytometer with 488 nm, 488 nm, 561 nm, and 648 nm lasers.

Figure 1. CellTrace Yellow Excitation and Emission Spectra

Figure 2. CellTrace Yellow Uniform Staining

Figure 3. CellTrace Yellow Reagent Cytotoxicity

Figure 4. Multiplexing Cell Cycle and Proliferation with CellTrace Yellow

Figure 5. DNA Content with S-phase Identification

Figure 6. Cell Cycle with Generational Analysis

Figure 7. Generational Tracing of CellTrace Yellow

The DNA content cell cycle dye Far-Red dyes easily crosses the plasma membrane of fixed and permeabilized cells, where it binds to DNA and becomes fluorescent. The fluorescent signal is proportional to the quantity of DNA in each cell, so that cells with a double complement of DNA will be twice as bright as those that have not duplicated their DNA. The Thymidine analog 5-ethylpyrimidin-2-yl-2-deoxyuridine (EdU) is incorporated into cells that are actively depleting DNA. A copper-catalyzed Click reaction is used to covalently link Alexa Fluor 488 azide to EdU, resulting in fluorescently labeled DNA. This figure combines DNA content analysis with Click-IT® EdU 5-ethylpyrimidin-2-yl-2-deoxyuridine (EdU) labeling to provide a complete cell cycle depiction of the proliferating cells in this experiment. Samples were acquired and analyzed on the Attune® NxT Acoustic Flow Cytometer at 200 mV/µm.

The DNA content information acquired from Far-Red Violet can be combined with generational information provided by CellTrace Yellow reagent to give a detailed view of the growth of a population of cells. This figure displays the progression of cell generations on the x-axis and DNA content on the y-axis. Cells which are not currently dividing (EdU+) are shown in closest to the X-axis. Cells from each generation which are actively synthesizing DNA or are about to divide are seen at the top. These samples were acquired and analyzed on the Attune® NxT Acoustic Flow Cytometer at 200 mV/µm.

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RESULTS

Human peripheral blood mononuclear cells were stained with 10 μM CellTrace Yellow reagent. Dynabeads Human T-Activator CO3/Di3D were used for T-Cell Expansion and Activation. Samples were incubated in OptiM™ T-Cell Expansion Medium at 37°C/5% CO2 for 7 days. After 7 days, cells were removed from culture and Dynabeads were removed from samples. SYTOX Green Dead Cell Stain was used to exclude dead cells and mouse anti-human CD4 APC was used to gate on live CD4+ lymphocytes. The peak in purple represents unstimulated control cells. The discrete yellow peaks represent individual generations of cells which proliferated during the course of the experiment.

CONCLUSIONS

Results indicate that CellTrace Yellow reagent can be used to successfully track multiple generations of proliferating cells. The bright, uniform staining produces minimal fluorescence variation between cells in a population, resulting in the ability to visualize resolve distinct generations by flow cytometry. The yellow excitation at 546 nm and emission at 579 nm of CellTrace Yellow dye make it ideal for multiplexing due to the limited spectral overlap with other common dyes. Results of this experiment suggest that CellTrace Yellow reagent is a useful tool to track and characterize cells as they proliferate through several generations and shows little cytotoxicity, with minimal observed effect on the proliferative ability or biology of cells.

REFERENCES


TRADEMARKS/LICENSES

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