A Novel Ultraviolet Dye for Cell Proliferation Analysis in Flow Cytometry

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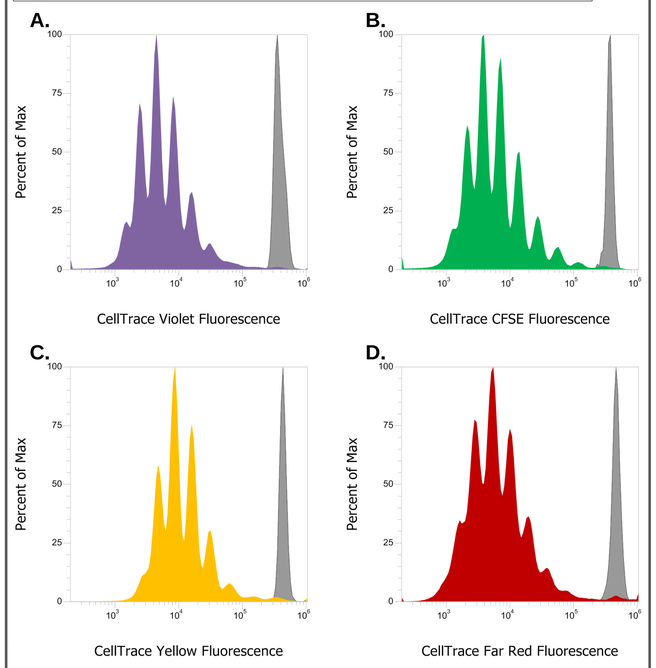
INTRODUCTION

Cell proliferation reagents are crucial for determining cell growth and differentiation and are often used to evaluate compound toxicity and/or inhibition of tumor cell growth. 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, however many of the existing reagents require compensation. The CellTrace™ Blue ultraviolet dye frees up channels for other reagents that can be designed into a multi-color panel. The fluorescent staining provided by CellTrace Blue enables the visualization of five generations of proliferating cells. CellTrace Blue 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 Blue results in partitioning of dye between daughter cells and approximately half of the fluorescence intensity in the progeny. When analyzed by flow cytometry using either a 355 nm or 375 nm laser, this partitioning of the CellTrace Blue dye provides a direct indication of cell proliferation without compromising cell health. In this experiment, we will look at how CellTrace Blue complements the existing CellTrace portfolio and demonstrate that the spectral characteristics permit multiplexing with many common flow reagents without compensation, distinct peak determination upon cell proliferation, and performance with tissue culture cells.

MATERIALS AND METHODS

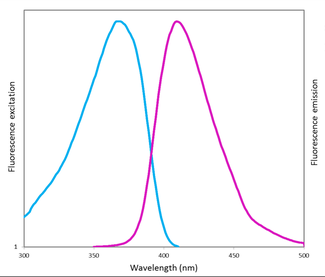
Human peripheral blood mononuclear cells were isolated from whole blood using a Ficoll-Paque Plus density gradient. Cells were stained with 10 µM CellTrace Blue reagent and resuspended in CTS™ OpTmizer™ T-Cell Expansion media. Stained cells were stimulated to proliferate with 50 µL CD3/CD28 Dynabeads™ per milliliter cells and incubated at 37° C and 5% CO_2 for 5 days. Cells were removed from culture, washed, and labeled with 5 µL mouse anti-human CD4 FITC for 20 minutes. Finally, cells were washed and labeled with SYTOX™ Red Dead Cell Stain and data was acquired on the BD LSR II using 355 nm 450/50. Software for the Invitrogen Attune™ NxT was used to analyze the data.

Figure 1. CellTrace Violet, CFSE, Yellow, and Far Red



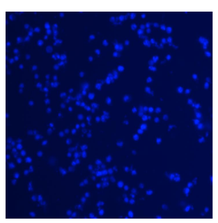
PBMC's were stained with (A) 5 µM CellTrace Violet, (B) 2 µM CellTrace CFSE, (C) 10 µM CellTrace Yellow, and (D) 2 µM CellTrace Far Red. Dynabeads Human T-Activator CD3/CD28 were used for T cell expansion and activation. Samples were incubated in OpTmizer T-cell Expansion Medium at 37° C / 5% CO_2 for 7 days. Samples were analyzed using SYTOX Green or SYTOX Red dead cell stains to gate on live cells and mouse anti-human CD4 Pacific Blue or CD4 FITC were used to gate on proliferating cells. The gray peaks represents unstimulated control cells (parent generation) and the peaks to the left of each gray peak represent individual generations of cells that proliferated during the course of the experiment.

Figure 2. CellTrace Blue Excitation and Emission spectra



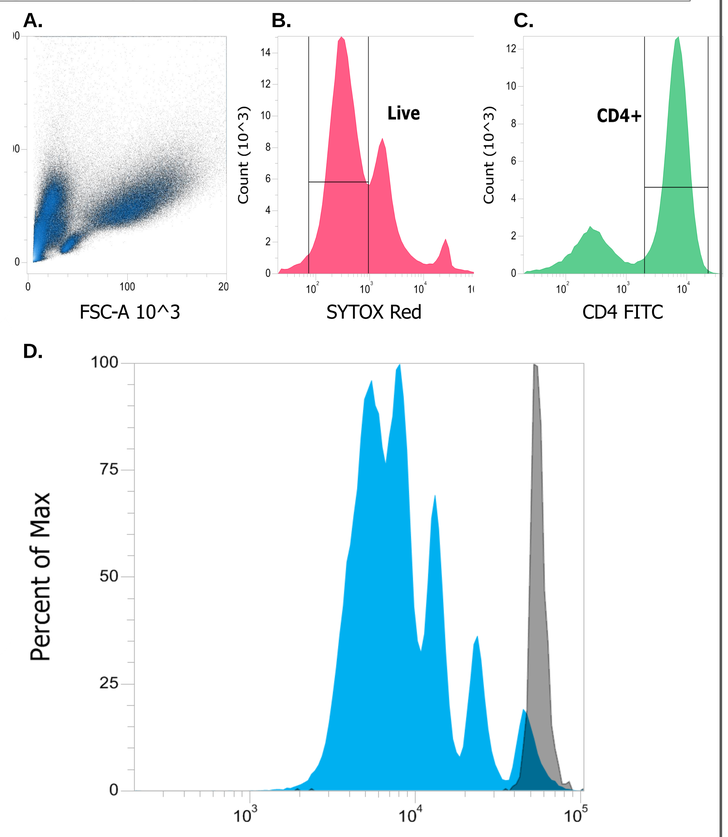
CellTrace Blue reagent is excited by 355 nm and 375 nm lasers

Figure 3. CellTrace Blue Stain



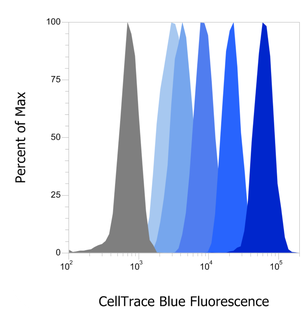
Unstimulated human peripheral blood mononucleated cells were labeled with 10 µM CellTrace Blue reagent. After 5 days at 37° C / 5% CO_2 cells were labeled with ReadyProbes™ BackDrop Background Suppressor and imaged using the EVOS™ FL imaging system .

Figure 4. Multiplexing with CellTrace Blue



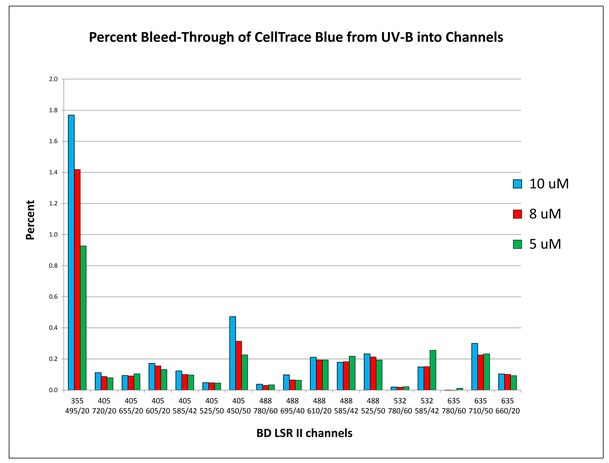
PBMC's were stained with 10 µM CellTrace Blue reagent, stimulated, and kept in culture for 5 days. (A) all events are depicted in the scatter plot (B) SYTOX Red Dead Cell Stain was used to exclude dead cells and (C) mouse anti-human CD4 FITC was used to gate on live $CD4^+$ lymphocytes. (D) Proliferation using CellTrace Blue reagent represented as gray peak (unstimulated control cells). The discrete blue peaks represent individual generations of cells that proliferated during the course of the experiment.

Figure 5. CellTrace Blue Reagent in Tissue Culture cells



Ramos cells were stained with 8 uM CellTrace Blue and incubated in RPMI + 10% FBS at 37° C / 5% CO_2 and analyzed daily for 5 days. SYTOX Red Dead Cell Stain was used to exclude dead cells. In the overlay of all of the generations, the dark blue peak represents the parent generation and the subsequent shades of blue peaks represent individual generations of cells that proliferated during the course of the experiment. The gray peak represents the unstained control cells.

Figure 6. CellTrace Blue Bleed-Through



PBMC's were stained with either 10 µM, 8 µM, or 5 µM CellTrace Blue reagent. Voltages were adjusted in all non-target channels using unstained control. Voltage for target channel 355 nm 450/50 was adjusted for optimized signal using stained control. Percent bleed-through is a calculation of the median value of the channel measured divided by the median value of the reference channel 355 nm 450/50.

CONCLUSIONS

Results indicate that CellTrace Blue reagent can be used to successfully track multiple generations of proliferating cells, including tissue culture cells. The bright, staining produces minimal fluorescence variation between cells in a population, resulting in the ability to visualize resolve distinct generations by flow cytometry. The ultraviolet excitation at 355 nm or 375 nm make it ideal for multiplexing due to the limited spectral overlap with other common dyes. Results of this experiment suggest that CellTrace Blue reagent is a useful tool to track and characterize cells as they proliferate through several generations.

REFERENCES

1. Wallace, et al. Cytometry Part A 73A: 1019-1034, 2008.
2. Lyons AB, Parish CR. *Journal of Immunological Methods* 1994; 171:131-37.
3. Givan AL, et al. *Journal of Immunological Methods* 1999; 230:99-112.

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