Non-cytotoxic Near-IR DNA Stain for Cell Cycle Analysis in Living Cells

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Background: Cell cycle describes the progression of a cell through a cycle of division, a process resulting in cell growth and separation into two daughter cells. Live cell studies of cellular DNA content and cell cycle distribution are useful to detect variations of growth patterns due to a variety of physical, chemical, or biological means, to monitor apoptosis, and to study tumor behavior and suppressor gene mechanisms. In a given population, cells will be distributed among three major phases of cell cycle: G0/G1 phase (2N), S phase (DNA synthesis with variable amount of DNA), and G2/M phase (4N). These applications require dyes that bind to DNA in a stoichiometric manner.Recent advances have produced several live cell dyes useful for DNA content analysis using violet (405 nm), blue (488 nm) and green (532 nm) excitation sources with emission in visible wavelengths. We present a new dye with near-infrared emission for DNA content analysis in living cells, Vybrant® DyeCycle™ Ruby stain. This dye can be excited by the commonly available 488 nm and 633 nm excitation sources and has an emission >760 nm.

Methods: Jurkat T cells were labeled with 5µM Vybrant® DyeCycle™ Ruby stain, 5µM Hoechst 33342 or 5µM DRAQ5® (Biosstatus) to compare cell cycle post staining with unstained control cells. Cell vitality and viability were measured after 72 hours using Calcein AM and ethidium homodimer-1. HL-60 cells were labeled with 5µM Vybrant® DyeCycle™ Ruby stain and sorted based on G0 DNA content. Post-sort cell count and viability staining were performed to examine any cytotoxic effects. Finally, GFP-expressing cells were labeled with Vybrant® DyeCycle™ Ruby to observe compatibility.

Results: Vybrant® DyeCycle™ Ruby stain demonstrated equivalent cell viability and vitality to the gold standard Hoechst 33342, while DRAQ5® demonstrated a significant cytotoxic effect. After sorting, no evidence of cytotoxicity was observed with Vybrant® DyeCycle™ Ruby labeled cells; good cell recovery and cell growth were observed. Vybrant® DyeCycle™ Ruby labeling proved compatible with GFP-expressing cells.

Conclusions: Vybrant® DyeCycle™ Ruby stain is a useful stain for DNA content analysis in living cells, using near-infrared emission after either 488 nm or 633 nm excitation. It demonstrates significantly less cytotoxicity than DRAQ5® dye, allowing it to be used to determine the proliferation rate of living cells and offers the possibility of cell sorting based on DNA content. Additionally, Vybrant® DyeCycle™ Ruby stain is compatible with GFP fluorescence.