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: G_0/G_1 phase (2N), S phase (DNA synthesis with variable amount of DNA), and G_2/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 >670 nm.

Methods: Jurkat T cells were labeled with 5µM Vybrant® DyeCycle™ Ruby stain, 5µM Hoechst 33342 or 5µM DRAQ5® (Biostatus) to compare cell health 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 DyeCycle™ Ruby stain and sorted based on G_1 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.

Figure 1: Spectral Characteristics

The fluorescence excitation and emission spectra the Vybrant® DyeCycle™ Ruby stain are shown. These spectra were obtained from samples of the dye bound to DNA. The Vybrant® DyeCycle™ Ruby stain/DNA complex has fluorescence excitation and emission maxima of 638/686nm respectively. This is compatible with excitation lasers from 488 - 640 nm.

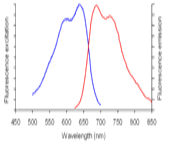
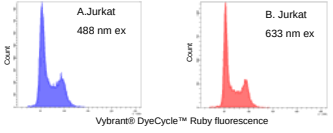


Figure 2: DNA content analysis with various configurations

Plots A and B: Histogram of live Jurkat cells stained with Vybrant® DyeCycle™ Ruby stain showing DNA content distribution. G_0/G_1 and G_2/M phase histogram peaks are separated by the S-phase distribution. **Panel A** shows the distribution of this population of cells when 488 nm excitation was used with a 695/40 bandpass filter. **Panel B** shows the same population when 633 nm excitation was used with a 695/40 bandpass filter.



Plots C-G: Live HL-60 cells labelled with 5uM DyeCycle™ Ruby where dead cells are excluded using DAPI, run on a BD™ LSRII looking at fluorescence in several detectors: **Panel C** uses 488 nm excitation with 660/20 bandpass emission, **Panel D** uses 488 nm excitation with 695/50 bandpass emission, **Panel E** uses 635 nm excitation with 660/20 bandpass emission, **Panel F** uses 635 nm excitation with 735/45 bandpass emission, and **Panel G** uses 635 nm excitation with 780/60 bandpass emission.

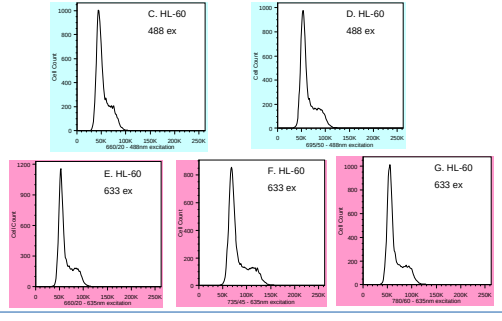
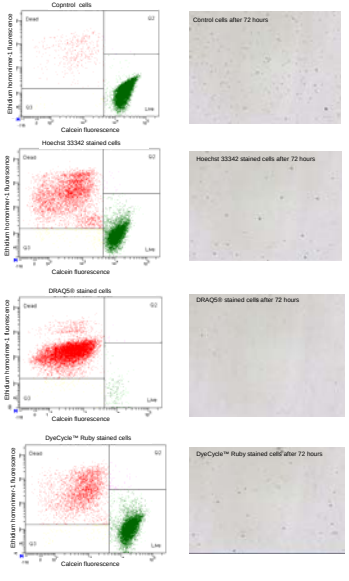


Figure 3: Viability and Vitality of cells 72 hours after labeling

Unlabeled control Jurkat T cells (A) were compared to cells labeled with either 5µM Hoechst 33342 (B) , 5µM DRAQ5™ (C), or 5µM Vybrant® DyeCycle™ Ruby stain (D) and allowed to grow for 72 hours in RPMI media + 10% FBS at 37˚C with 5% CO_2. After 72 hours, cell health was measured using Calcein AM as a measure of cell vitality and ethidium homodimer-1 as a measure of cell viability. Samples were acquired using an BD™ LSRII flow cytometer using 488 nm excitation with 530/30 bandpass for Calcein fluorescence and 610/20 bandpass for ethidium homodimer-1 fluorescence. Dual parameter plots are displayed on the left, and a brightfield image of each cell culture is displayed on the right. Cells labeled with DyeCycle™ Ruby stain have viability and vitality comparable to cells labeled with the gold standard Hoechst 33342 while cells labeled with DRAQ5® shows considerable cell death.



A) Control cells
Live cells = 96.4%
Dead cells = 3.2%

B) Cells labeled with Hoechst 33342
Live cells = 57.7 %
Dead cells = 39.3 %

C) Cells labeled with DRAQ5®
Live cells = 2.1 %
Dead cells = 95.7 %

C) Cells labeled with DyeCycle™ Ruby
Live cells = 71.2 %
Dead cells = 26.4 %

Figure 4: Post sort recovery after Vybrant® DyeCycle™ Ruby stain labeling

Both Jurkat and HL-60 cells were labeled using 5 X10^6 total cells, suspended in 10mL media and added 10µl DyeCycle™ Ruby stain, incubated for 15 minutes at 37˚C. Cells were pelleted and resuspended in 1mL media, adding 5µl DAPI at 200µg/ml. Live cells were gated on DAPI-negative cells, followed by a singlet gate based on area and height before sorting the G_1/G_0 population and collecting 1.6 million cells of each . Cells were then cultured and evaluated for cell count (left graph) and viability (right graph). No evidence of cytotoxicity was seen and good recovery and growth post sort was observed.

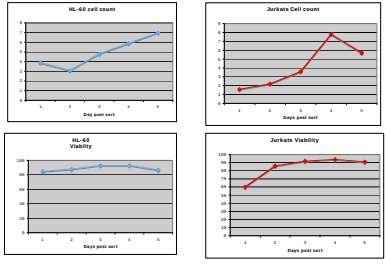
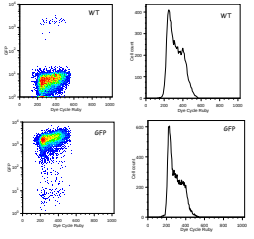


Figure 5: Compatibility with GFP

DT40 chicken B cells, either wild-type or stably transfected with a GFP reporter construct, were labelled with DyeCycle™ Ruby stain. Data was acquired on a BD™ FACSCalibur™ instrument using 488nm excitation and >670 nm emission. The staining is compatible with GFP.



References:

1.Current Protocols in Cytometry, 7.0.1–7.27.7 (2004); **2.** Practical Flow Cytometry, 4th Ed., Shapiro HM, Ed. (2003); **3.** Methods Mol Biol 281, 301 (2004); **4.** Cytometry A 58, 21 (2004).