Vybrant® DyeCycle™ Green and Orange Stains

Catalog nos. V35004, V35005

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage*	Stability
Vybrant® DyeCycle™ Green stain	400 µL	5 mM solution in dimethyl sulfoxide (DMSO)	• ≤–20˚C • Desiccate • Protect from light	When stored as directed this product is stable for at least 6 months.
Vybrant® DyeCycle™ Orange stain	400 µL			
Number of assays: Sufficient material is supplied for approximately 200 flow cytometry assays based on a 1 mL test volume.				
Approximate fluorescence excitation/emission maxima: Vybrant® DyeCycle™ Green stain**:** 506/534 nm, bound to DNA; Vybrant® DyeCycle™ Orange stain: 519/563 nm, bound to DNA.				

Introduction

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 are distributed among three major phases of cell cycle: G0/G1 phase (one set of paired chromosomes per cell), S phase (DNA synthesis with variable amount of DNA), and G2/M phase (two sets of paired chromosomes per cell, prior to cell division).[1–4] DNA content can be measured using fluorescent, DNA-selective stains that exhibit emission signals proportional to DNA mass. Flow cytometric analysis of these stained populations is then used to produce a frequency histogram that reveals the various cell cycle phases. This analysis is typically performed on permeabilized or fixed cells using a cell-impermeant nucleic acid stain, but is also possible using live cells and a cell-permeant nucleic acid stain. While the choices for fixed cell staining are varied, there are only a few examples of useful cell-permeant nucleic acid stains.

The Vybrant® DyeCycle™ Green and Orange stains are DNA-selective, cell membrane-permeant, and nonfluorescent stains for DNA content analysis in living cells. The Vybrant® DyeCycle™ Green and Orange stains are fluorescent upon binding to double-stranded DNA. These stains take advantage of the commonly available 488 nm excitation source, placing cell cycle studies on live cells within reach of all flow cytometrists. Vybrant® DyeCycle™ Green stain is excited at 488 nm with emission ~520 nm (Figure 1). Vybrant® DyeCycle™ Orange stain is excited using both 488 nm and 532 nm laser lines with emission ~570 nm (Figure 2).

The staining protocol is simple and includes incubating suspended cells in the presence of Vybrant® DyeCycle™ stain and directly measuring the fluorescence without the need for any additional treatment or centrifugation steps. This live cell stain allows the simultaneous

co-staining of the cell population for other parameters, and allows for the possibility of cell sorting based on DNA content.

Spectral Characteristics

The fluorescence excitation and emission spectra for the stains are shown in Figures 1 and 2. The spectra were obtained from samples of the Vybrant® DyeCycle™ stain bound to DNA. The Vybrant® DyeCycle™ Green stain/DNA complex has fluorescence excitation and emission maxima of 506/534 nm, respectively. The Vybrant® DyeCycle™ Orange stain/DNA complex has fluorescence excitation and emission maxima of 519/563 nm, respectively.

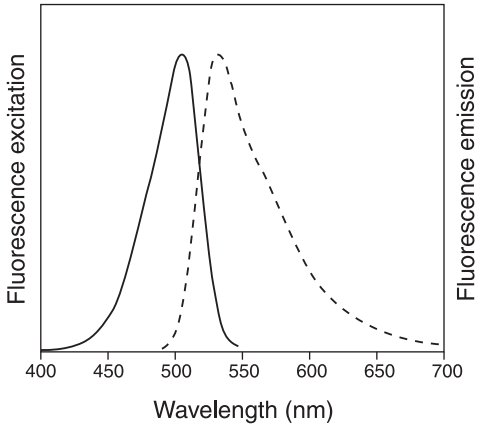

Figure 2. Fluorescence excitation and emission spectra for the Vybrant® DyeCycle™ Green stain bound to DNA in TBE, pH 8.3.

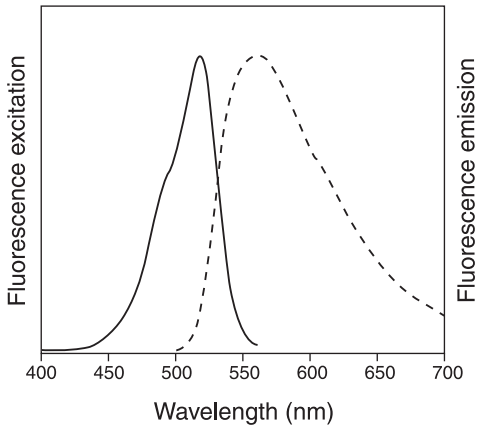

Figure 3. Fluorescence excitation and emission spectra for the Vybrant® DyeCycle™ Orange stain bound to DNA in TBE, pH 8.3.

Before Starting

Materials Required but Not Provided

- Cells and culture medium
- Flow cytometer tubes

Caution

The hazards posed by these stains have not been fully investigated. Since Vybrant® DyeCycle™ Green and Orange stains are known to bind to nucleic acids, treat the stain as a potential mutagen and use with appropriate care. The stains are supplied as a solution in DMSO, which is known to facilitate the entry of organic molecules into tissues. Use the stain using equipment and practices appropriate for the hazards posed by such materials. Dispose of the reagents in compliance with all pertaining local regulations.

Experimental Protocol

The following staining protocol was optimized using Jurkat cells, a human T-cell leukemia line, in complete RPMI medium containing 10% fetal bovine serum with staining at 37˚C, but can be adapted to most cell types. These stains can also be used for cells suspended in Hanks' Balanced Salt Solution (HBSS) or phosphate-buffered saline (PBS). Growth medium or buffer used, cell density, cell type variations, and other factors may influence staining. In initial experiments, try a range of dye concentrations to determine the one that yields optimal staining for the given cell type, buffer, and experimental condition. For a given experiment, each flow cytometry sample should contain the same number of cells, as sample-to-sample variation in cell number leads to significant differences in fluorescence signal.

If a Vybrant® DyeCycle™ stain is used in combination with other stains for multicolor applications, apply the other stain(s) to the sample first, following all manufacturers' instructions, including wash steps. The Vybrant® DyeCycle™ stain should be the last stain applied to the sample, and do not wash or fix samples prior to flow cytometric analysis.

General Guidelines

For optimal DNA content cell cycle analysis, follow these guidelines:

- Eliminate cell clumps and aggregates from the cell suspension before staining
- Use 37˚C for incubation with the Vybrant® DyeCycle™ stains, keep cells at 37˚C until acquisition
- Staining may be performed at room temperature, with the staining time about twice as long as the 37˚C incubation time
- Do not use glass containers with this stain
- **Do not wash or fix cells after staining cells with Vybrant® DyeCycle™ stains**
- Validate flow cytometry instrument performance on the day of use
- Use linear amplification for DNA content
- Use low flow rate for acquisition
- Collect adequate numbers of events for the intended application
- Eliminate dead cells from the DNA content analysis of living cells using a dead cell discriminating stains such as SYTOX® Green, SYTOX® Red or SYTOX® AADvanced™ dead cell stains or LIVE/DEAD Fixable Dead cell stains such as Green, Red, Far Red, or Near-IR kits
- Eliminate or correct for cell aggregates during data analysis using gating or modeling software

Vybrant ® DyeCycle™ Staining Protocol

This basic protocol is optimized using Jurkat cells suspended in complete medium (RPMI/10% fetal bovine serum) and stained with Vybrant® DyeCycle™ stain at 37˚C.

1.1 Remove the Vybrant® DyeCycle™ Green stain or Vybrant® DyeCycle™ Orange stain from the freezer and allow it to equilibrate to room temperature.

1.2 Prepare flow cytometry tubes each containing 1 mL of cell suspension in complete media at a concentration of 1×10^6 cells/mL.

1.3 To each tube add 2 µL of Vybrant® DyeCycle™ Green stain or Vybrant® DyeCycle™ Orange stain. Final stain concentration is 10 µM.

After use, seal the stain vial tightly. The stain in DMSO solution may be subjected to many freeze-thaw cycles without reagent degradation.

1.4 Incubate at 37˚C for 30 minutes, **protected from light**.

1.5 For cells stained with Vybrant® DyeCycle™ Green stain, analyze samples on flow cytometer using 488 nm excitation and green emission (Figure 3).

For cells stained with Vybrant® DyeCycle™ Orange stain, analyze samples on a flow cytometer using 488 nm excitation or 532 nm excitation and orange emission (Figure 4).

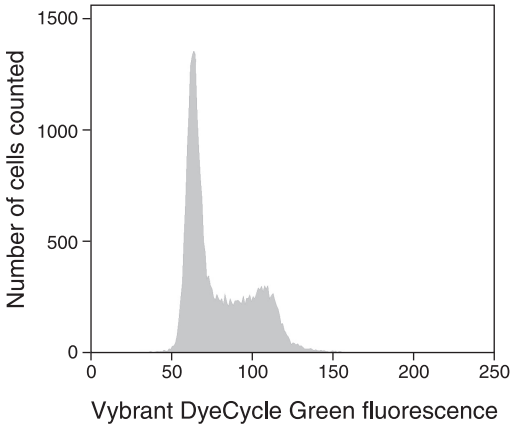

Figure 3. Histogram of live Jurkat cells stained with Vybrant® DyeCycle™ Green stain showing DNA content distribution. G0/G1 and G2/M phase histogram peaks are separated by the S-phase distribution. Excitation at 488 nm was used with a 530/30 bandpass filter.

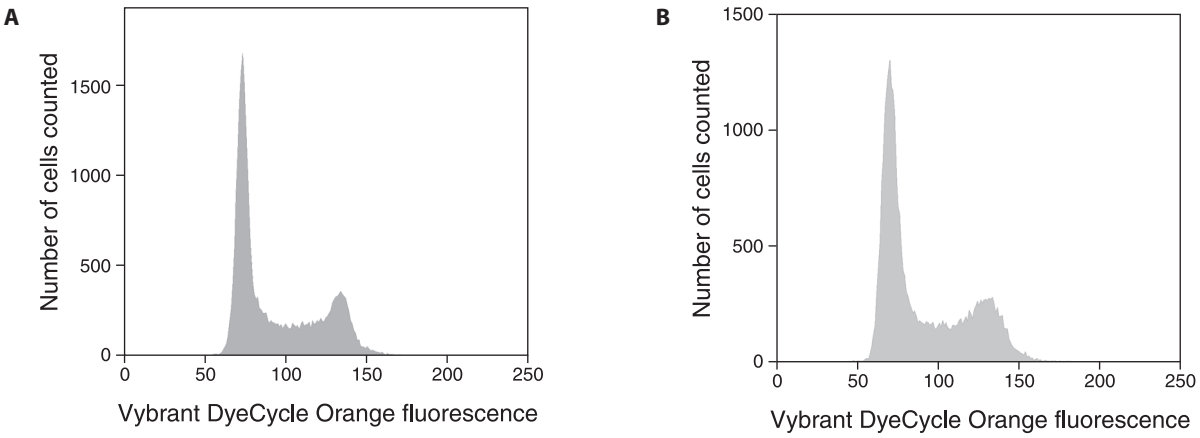

Figure 4. Histogram of live Jurkat cells stained with Vybrant® DyeCycle™ Orange stain showing DNA content distribution. G0/G1 and G2/M phase histogram peaks are separated by the S-phase distribution. Panel A shows the distribution of this population of cells when 488 nm excitation was used with a 585/42 bandpass filter. Panel B shows the same population when 532 nm excitation was used with a 585/42 bandpass filter.

References

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

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
V35004	Vybrant® DyeCycle™ Green stain *5 mM solution in DMSO* *200 assays*	400 µL
V35005	Vybrant® DyeCycle™ Orange stain *5 mM solution in DMSO* *200 assays*	400 µL
Related Products		
V35003	Vybrant® DyeCycle™ Violet stain *5 mM solution in DMSO* *200 assays*	200 µL
V10273	Vybrant® DyeCycle™ Ruby stain *2.5 mM solution in DMSO* *400 assays*	400 µL
V10309	Vybrant® DyeCycle™ Ruby stain *2.5 mM solution in DMSO* *100 assays*	100 µL
L10119	LIVE/DEAD® Fixable Near-IR Dead Cell Stain Kit *for 633 or 635 nm excitation* *200 assays*	1 kit
L10120	LIVE/DEAD® Fixable Far Red Dead Cell Stain Kit *for 633 or 635 nm excitation* *200 assays*	1 kit
L23101	LIVE/DEAD® Fixable Green Dead Cell Stain Kit *for 488 nm excitation* *200 assays*	1 kit
L23102	LIVE/DEAD® Fixable Red Dead Cell Stain Kit *for 488 nm excitation* *200 assays*	1 kit
L23105	LIVE/DEAD® Fixable Blue Dead Cell Stain Kit *for UV excitation* *200 assays*	1 kit
L34955	LIVE/DEAD® Fixable Violet Dead Cell Stain Kit *for 405 nm excitation* *200 assays*	1 kit
L34957	LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit *for 405 nm excitation* *200 assays*	1 kit
S34859	SYTOX® Red dead cell stain *for 633 or 635 nm excitation* *5 µM solution in DMSO*	1 mL
S7020	SYTOX® Green nucleic acid stain *5 mM solution in DMSO*	250 µL
V10309	SYTOX® AADvanced™ dead cell stain *for 488 nm excitation* *100 assays*	100 assays
V10274	SYTOX® AADvanced™ dead cell stain *for 488 nm excitation* *500 assays*	500 assays
14025-092	Hanks' Balanced Salt Solution (HBSS) (1X), liquid, contains calcium and magnesium, but no phenol red	500 mL
14170-112	Hanks' Balanced Salt Solution (HBSS) (1X), liquid contains no calcium chloride, magnesium chloride, or magnesium sulfate.	500 mL
14175-095	Hanks' Balanced Salt Solution (HBSS) (1X), liquid, contains no calcium chloride, magnesium chloride, magnesium sulfate, or phenol red	500 mL
24020-117	Hanks' Balanced Salt Solution (HBSS) (1X), liquid, contains calcium and magnesium	500 mL

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