Vybrant® DyeCycle™ Ruby stain

Catalog nos. V10273, V10309

Table 1. Contents and storage information.

Material	Amount		Concentration	Storage	Stability
	V10273	V10309			
Vybrant® DyeCycle™ Ruby stain	400 µL	100 µL	2.5 mM solution in DMSO	• ≤–20˚C • Desiccate • Protect from light	When stored as directed this product is stable for at least 1 year.
Number of assays: Sufficient material is supplied for 400 assays (Cat. no. V10273) or 100 assays (Cat. no. V10309) based on the protocol below.					
Approximate fluorescence excitation/emission maxima: Vybrant® DyeCycle™ Ruby stain**:** 638/686 in 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 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 cell populations is then used to produce a frequency histogram that reveals various phases of the cell cycle. 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™ Ruby stain from Invitrogen offers near-infrared emission for DNA content analysis in living cells. The Vybrant® DyeCycle™ Ruby stain is cell membrane-permeant, DNA-selective, and essentially nonfluorescent until bound to double-stranded DNA. This stain takes advantage of the commonly available 488 nm and 633/5 nm excitation sources with emission >670 nm, placing cell cycle studies within reach of all flow cytometrists. Vybrant® DyeCycle™ Ruby stain may also be used with any excitation source from 488 nm through to 690 nm wavelengths. Staining protocol is simple; the suspended cells are incubated in the presence of Vybrant® DyeCycle™ Ruby stain and fluorescence is measured directly—no additional treatment or centrifugation is required. The Vybrant® DyeCycle™ Ruby stain allows simultaneous co-staining of the cell population for other parameters and offers the possibility of cell sorting based on DNA content.

Spectral Characteristics

The fluorescence excitation and emission spectra of the stain are shown in Figure 1 and 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 nm and 686 nm, respectively.

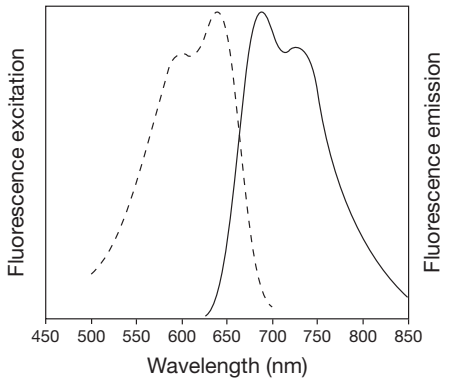

Figure 1. Fluorescence excitation and emission spectra of Vybrant® DyeCycle™ Ruby stain bound to DNA.

Before Starting

Materials Required but Not Provided

- Cells and culture medium or Hanks' Balanced Salt Solution (HBSS)
- Flow cytometry tubes

Caution

No data are available addressing the mutagenicity or toxicity of Vybrant® DyeCycle™ Ruby stain. Since Vybrant® DyeCycle™ Ruby stain binds to nucleic acids, treat the stain as a potential mutagen and use with appropriate care. Handle the DMSO dye solution with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. Always wear suitable protective clothing, gloves, and eye/face protection when handling this reagent. Dispose of the reagent 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. Growth medium or buffer used, cell density, cell type variations, and other factors may influence staining. In initial experiments, try a range of stain concentrations to determine the optimal stain concentration for the given cell type, buffer, and experimental conditions. 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 Vybrant® DyeCycle™ Ruby stain is used in combination with other dyes for multicolor applications, apply other dyes to the sample first following manufacturers' instructions, including washes. Apply the Vybrant® DyeCycle™ Ruby stain as the last stain 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, and eliminate or correct for cell aggregates during analysis using gating or modeling software
- Staining with Vybrant® DyeCycle™ Ruby stain may be performed at room temperature with the staining time about twice as long
- Hanks' Balanced Salt Solution (HBSS) is recommended if media is not used, **do not** use phosphate buffers
- Do not use glass containers with Vybrant® DyeCycle™ Ruby stain
- Do not wash or fix cells after staining with Vybrant® DyeCycle™ Ruby stain
- Eliminate dead cells from the DNA content analysis of living cells by using a dead cell discriminating stain such as SYTOX® Green, SYTOX® Blue, SYTOX® Red, or SYTOX® AADvanced™ dead cell stains, or LIVE/DEAD® Fixable Dead cell stains such as Blue, Violet, Aqua, Green, Red, or Far Red kits
- 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 intended application

Vybrant® DyeCycle™ Ruby Cell Staining Protocol

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

1.1 Remove the Vybrant® DyeCycle™ Ruby stain from the freezer and allow the stain to equilibrate to room temperature.

1.2 Prepare flow cytometry tubes each containing 0.5 mL of cell suspension in complete media at a concentration of 5×10^5 cells/mL.

1.3 To each tube, add 1 µL of Vybrant® DyeCycle™ Ruby stain and mix well. Final stain concentration is 5 µM.

1.4 Incubate at 37˚C for 15–30 minutes, **protected from light**.

1.5 Analyze without washing cells on a flow cytometer using 488 nm or 633/5 nm excitation and >670 nm emission (Figure 2). The Vybrant® DyeCycle™ Ruby stain may be excited with any excitation source from 488 nm through 690 nm wavelengths.

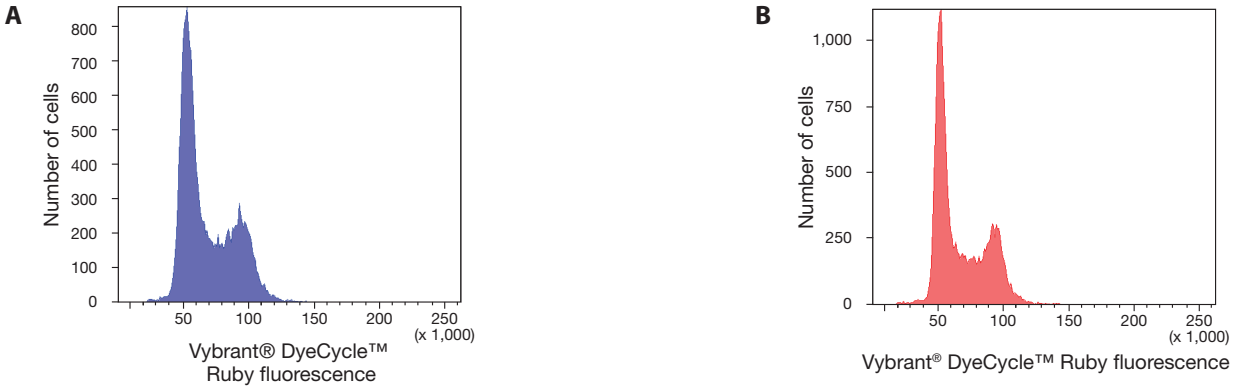

Figure 2. Histogram of live Jurkat cells stained with Vybrant® DyeCycle™ Ruby stain showing DNA content distribution. G0/G1 and G2/M phase histogram peaks are separated by the S-phase distribution. Panel A shows the distribution of this population of cells when 488 nm excitation was used with a 695/40 bandpass filter. Panel B shows the same population when 633 nm excitation was used with a 695/40 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
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
Related Products		
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
S7020	SYTOX® Green nucleic acid stain *5 mM solution in DMSO*	250 µL
S34857	SYTOX® Blue dead cell stain *for flow cytometry* *1000 assays* *1 mM solution in DMSO*	1 mL
S34859	SYTOX® Red dead cell stain *for 633 or 635 nm excitation* *5 µM solution in DMSO*	1 mL
V10274	SYTOX® AADvanced™ dead cell stain *for 488 nm excitation* *500 assays*	1 kit
V10309	SYTOX® AADvanced™ dead cell stain *for 488 nm excitation* *100 assays*	1 kit
V35003	Vybrant® DyeCycle™ Violet stain *5 mM in water* *200 assays*	200 µL
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
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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