

Vybrant® DyeCycle™ Violet Stain

Catalog no. V35003

Table 1. Contents and storage information.

| Material | Amount | Concentration | Storage | Stability |
|---|--------|----------------------------------|--|---|
| Vybrant® DyeCycle™ Violet stain | 200 µL | 5 mM solution in deionized water | <ul style="list-style-type: none"> • 2–6°C • Protect from light • DO NOT FREEZE | When stored as directed this product is stable for at least 6 months. |
| 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™ Violet stain: 369/437 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 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 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™ Violet stain is a DNA-selective, cell membrane-permeant, and nonfluorescent stain that uses the violet laser for DNA content analysis in living cells. The Vybrant® DyeCycle™ Violet stain is fluorescent upon binding to double-stranded DNA. Well suited for the popular violet laser line (Figure 1), Vybrant® DyeCycle™ Violet stain can also be used with UV excitation, having emission at ~440 nm.

The staining protocol is simple and includes incubating suspended cells in the presence of Vybrant® DyeCycle™ Violet 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. Vybrant® DyeCycle™ Violet stain does efflux in rodent and human stem cells, making the Side Population (SP) technique now available with violet excitation.⁵

Spectral Characteristics

The fluorescence excitation and emission spectra of the stain are shown in Figure 1. The spectra were obtained from samples of the Vybrant® DyeCycle™ Violet stain bound to DNA. The Vybrant® DyeCycle™ Violet stain /DNA complex has fluorescence excitation and emission maxima of 369/437 nm, respectively.

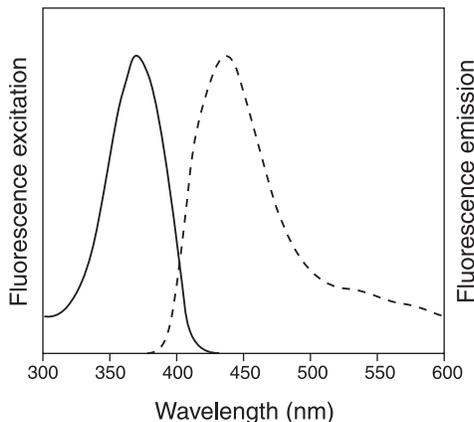


Figure 1. Fluorescence excitation and emission spectra for the Vybrant® DyeCycle™ Violet stain bound to DNA in TBE, pH 8.3.

Before Starting

Materials Required but Not Provided

- Cells and culture medium
- Flow cytometer tubes

Caution

The hazards posed by this stain have not been fully investigated. Since Vybrant® DyeCycle™ Violet stain is known to bind to nucleic acids, treat the stain as a potential mutagen and use with appropriate care. The stain is 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. Test samples comprise of 1×10^6 cells per 1 mL. 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 Vybrant® DyeCycle™ Violet 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. Vybrant® DyeCycle™ Violet 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™ Violet stain
- Hanks' Balanced Salt Solution (HBSS) is recommended if media is not desired, however phosphate buffers are **not recommended**
- Do not use glass containers with this stain
- **Do not wash or fix cells after staining cells with Vybrant® DyeCycle™ Violet stain**
- 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
- Compensation may be required for Alexa Fluor® 488 and PE channels
- Eliminate or correct for cell aggregates during data analysis using gating or modeling software
- Human and rodent stem cells efflux Vybrant® DyeCycle™ Violet stain, and this is the basis for the Side Population (SP) technique; the efflux can be blocked with verapamil, fumitermogin C, or other such blocking agents, to prevent dye efflux for accurate DNA content analysis in these stem cells

Vybrant® DyeCycle™ Violet Staining Protocol

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

- 1.1 Remove the Vybrant® DyeCycle™ Violet stain from the refrigerator and allow the vial to equilibrate to room temperature.
- 1.2 Prepare flow cytometry tubes each containing 1 mL of cell suspension in complete media at a cell concentration of 1×10^6 cells/mL.
- 1.3 To each tube, add 1 µL of Vybrant® DyeCycle™ Violet stain and mix well. Final stain concentration is 5 µM.
- 1.4 Incubate at 37°C for 30 minutes, **protected from light**. Keep cells at 37°C until acquisition.
- 1.5 Analyze samples **without washing or fixing** on a flow cytometer using ~405 nm excitation and ~440 nm emission (Figure 2). Vybrant® DyeCycle™ Violet stain may also be excited with a UV light source.

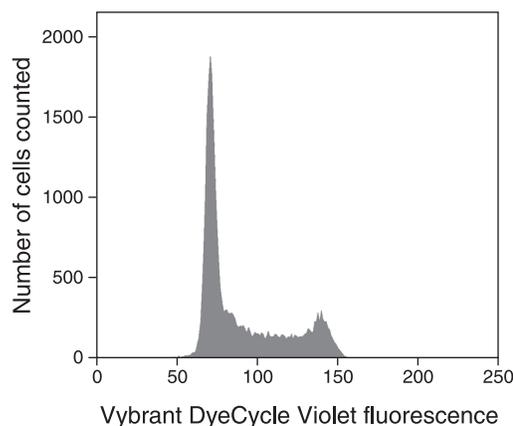


Figure 2. Histogram of live Jurkat cells stained with Vybrant® DyeCycle™ Violet stain showing DNA content distribution. G0/G1 and G2/M phase histogram peaks are separated by the S-phase distribution. Violet 405 nm excitation was used with a 440/40 nm bandpass filter.

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); 5. Stem Cells 25, 1029 (2007).

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

| Cat. no. | Product Name | Unit Size |
|-------------------------|---|------------|
| V35003 | Vybrant® DyeCycle™ Violet stain *5 mM solution in DMSO* *200 assays* | 200 µL |
| Related Products | | |
| 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 |
| 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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