# FxCycle™ Violet Stain

## Catalog no. F10347

**Table 1.** Contents and storage information.

| Material | Amount | Storage* | Stability |
|---|---|---|---|
| FxCycle™ Violet stain | 5 × 100 µg | • 2–6˚C<br>• Desiccate<br>• Protect from light | When stored as directed the product is stable for at least 1 year. |
| **Number of assays:** Sufficient material is supplied for 500 assays based on the protocol below. | | | |
| **Approximate fluorescence excitation/emission maxima:** FxCycle™ Violet stain**:** 358/461 nm, bound to DNA. | | | |

## Introduction

Analysis of nucleic acids is a common application of flow cytometry. Measurement of DNA content allows the study of cell populations in various phases of the cell cycle as well as analysis of DNA ploidy. 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 stains that exhibit emission signals proportional to the DNA mass. Flow cytometric analysis of these stained populations is then used to produce a frequency histogram that reveals the various cell cycle phases.

Univariate DNA content analysis is an established assay method and is widely used for studies in oncology, cell biology, and molecular biology. Using flow cytometry, multicolor cell cycle studies are possible, and it is advantageous to analyze DNA content on alternative lasers to preserve the common 488 nm laser for other markers. Well suited for the popular violet laser line, FxCycle™ Violet stain (4', 6-diamidino-2-phenylindole, dihydrochloride) can also be used with UV excitation. With DNA content measurement on the violet laser, other parameters such as cyclins, cyclin-dependent kinases, cell cycle checkpoints, nuclear proteins, and proliferation markers can be measured on the familiar 488 nm laser. FxCycle™ Violet stain preferentially stains dsDNA; it appears to associate with AT clusters in the minor groove.[5] Binding of FxCycle™ Violet stain to dsDNA produces a ~20-fold fluorescence enhancement.[6]

For long-term storage, the dye is supplied as five vials of 100 µg solid dye and stored at 2–6˚C; individual vial stock solution in deionized water is also stored at 2–6˚C for convenience.

## Before Starting

**Materials Required but Not Provided**

- Deionized water
- Reagents for fixing cells such as alcohol or formaldehyde
- Reagents for permeabilizing cells such as Triton® X-100
- Buffer such as phosphate buffered saline (PBS)

**Caution**

The hazards posed by FxCycle™ Violet stain has not been fully investigated. The stain is a known mutagen and may cause sensitization by inhalation and skin contact, and is irritating to eyes, respiratory system, and skin. Do not breath dust. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, safety glasses, and gloves. Avoid contact with skin and eyes. Dispose of the reagents in compliance with all pertaining local regulations.

**Preparing Stock Solution**

To make a 1 mg/mL stock solution of FxCycle™ Violet stain, add 100 µL deionized water to one vial of the stain. Mix well. Store this solution at 2–6˚C, **protected from light**. When stored as directed, this FxCycle™ Violet stock solution is stable for at least six months.

**Spectral Characteristics**

The fluorescence excitation and emission spectra of the FxCycle™ Violet stain are shown in Figure 1. The spectra were obtained from samples of the stain bound to DNA with fluorescence excitation and emission maxima of 358/461 nm respectively. FxCycle™ Violet stain may be used with the violet 405 nm excitation laser commonly found on flow cytometers, as well as UV excitation.

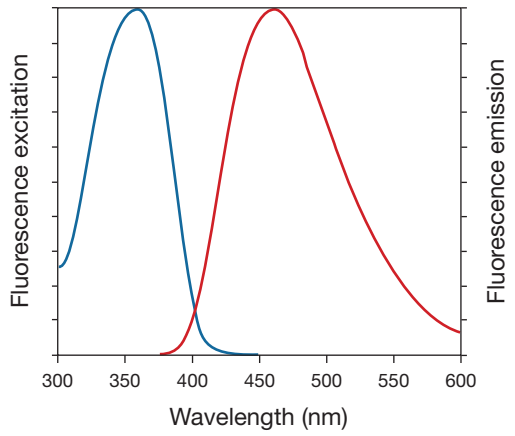


**Figure 1.** Fluorescence excitation and emission spectra of FxCycle™ Violet stain bound to dsDNA.

## Experimental Protocol

The following staining procedure was developed using the Jurkat T-cell leukemia cell line, but can be adapted for any cell type. Fixative, permeabilization reagent, cell density, cell type variations, and other factors may influence staining.

In initial experiments, try a range of stain concentrations to determine the concentration that yields optimal staining for the given cell type and experimental conditions. All fixative should be removed from cells before proceeding with cell staining, however staining with FxCycle™ Violet stain may be done concurrent with the addition of a permeabilization reagent if desired. 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 FxCycle™ 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. FxCycle™ Violet stain should be the last stain applied to the sample, and do not wash samples prior to flow cytometric analysis.

### General Guidelines

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

- Eliminate cell clumps and aggregates from the cell suspension before staining
- Validate flow cytometry instrument performance on the day of use
- Use linear amplification for DNA content
- Use low flow rate for acquisition
- Collect adequate number of events for the intended application
- Cells must be fixed before staining with FxCycle™ Violet stain for DNA content cell cycle
- **Do not wash cells after staining with FxCycle™ Violet stain**

### Staining Procedure

**1.1** Harvest the cell sample(s).

**1.2** Fix cells according to your preferred protocol.

**1.3** Wash cells. Remove all fixative from cells before proceeding with the cell staining.

**1.4** Using an appropriate buffer, adjust the sample cell concentration to be $1 \times 10^6$ cells/mL.

**1.5** Prepare flow cytometry samples each containing 1 mL cell suspension. Optional permeabilization reagent may be added.

**1.6** Add 1 µL of FxCycle™ Violet stain to each flow cytometry sample and mix well.

**1.7** Incubate flow cytometry tubes for 30 minutes at room temperature or 2–6°C, **protected from light**.

**1.8** Analyze samples **without washing** in a flow cytometer, using 405 nm excitation and emission collected in a 450/50 bandpass or equivalent.

Example of results obtained using FxCycle™ Violet stain for DNA content cell cycle analysis is shown in Figure 2.

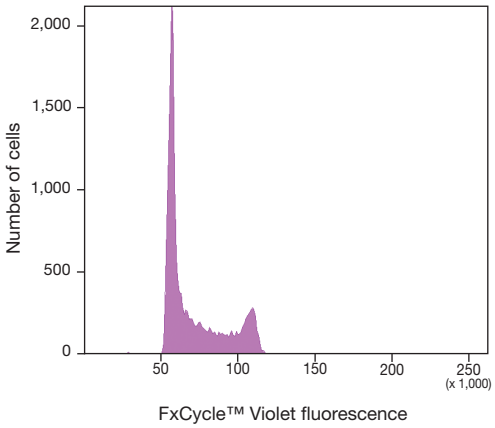


**Figure 2.** Histogram of HL-60 promyeloblast cells stained with FxCycle™ Violet stain showing DNA content distribution. HL-60 cells were fixed overnight with alcohol, washed, and then resuspended in 0.1% Triton® X-100/PBS/1% BSA before staining with FxCycle™ Violet stain for 30 minutes at room temperature. G0/G1 and G2/M phase histogram peaks are separated by the S-phase distribution. Analysis was performed at 405 nm excitation with a 450/50 bandpass filter.

## 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); **5.** Biochemistry 26, 4545 (1987); **6.** Biochem Biophys Res Commun 170, 270 (1990).

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

| Cat. no. | Product Name | Unit Size |
|---|---|---|
| F10347 | FxCycle™ Violet Stain *for flow cytometry* *500 assays* *DAPI* | 1 set |
| F10348 | FxCycle™ Far Red Stain *for flow cytometry* *500 assays* | 1 set |
| *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 |
| GAS-003 | Fixation and Permeabilization, 1 × 5 ml | 50 tests |
| GAS-004 | Fixation and Permeabilization, 4 × 5 ml | 200 tests |
| GAS001S-100 | Fixation Medium - Bulk, (MEDIUM A), 1 × 100 ml Fixation Medium | 1000 tests |
| GAS002S-100 | Permeabilization Medium - Bulk, (MEDIUM B), 1 × 100 ml | 1000 tests |
| 10010-049 | Phosphate Buffered Saline (PBS) 7.2 (1X), liquid | 10 × 500 mL |
| 20012-050 | Phosphate Buffered Saline (PBS) 7.4 (1X), liquid | 10 × 500 mL |
| 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 |
| 12091-039 | RNAse A (20 mg/ml) | 25 mL |

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