FxCycle™ Far Red Stain

Catalog no. F10348

Table 1. Contents and storage information.

Material	Amount	Storage*	Stability
FxCycle™ Far Red stain	5 vials	• ≤–20˚C • Desiccate • 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™ Far Red stain**:** 640/658 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 red laser line, FxCycle™ Far Red is collected in the 660 nm bandpass common on most flow cytometers. With DNA content measurement on the red 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™ Far Red stains RNA as well as dsDNA, so addition of RNase A is required for DNA content analysis.

For long-term storage, the dye is supplied as five vials of solid dye and stored at ≤–20˚C; individual vials of stock solution in DMSO are also stored at ≤–20˚C for convenience.

Before Starting

Materials Required but Not Provided

- High quality, anhydrous DMSO (dimethyl sulfoxide)
- RNAse A (20 mg/mL, Cat. no. 12091-039)
- 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 the FxCycle™ Far Red stain have not been fully investigated. Since the stain is known to bind to nucleic acids, treat the stain as a potential mutagen and use with appropriate care. The FxCycle™ Far Red stain is used as a solution in DMSO, which is known to facilitate the entry of organic molecules into tissues. Handle the stain in DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of the reagents in compliance with all pertaining local regulations.

Preparing Stock Solution

To make a 200 µM stock solution of FxCycle™ Far Red stain, add 100 µL high quality, anhydrous DMSO to one vial of the stain. Mix well. Store this solution at ≤–20°C, **protected from light**. Before refreezing, seal the vial tightly. The DMSO solution may be subjected to many freeze-thaw cycles without reagent degradation. When stored as directed, the FxCycle™ Far Red stain stock solution is stable for at least six months.

Spectral Characteristics

The fluorescence excitation and emission spectra of the FxCycle™ Far Red stain are shown in Figure 1. The spectra were obtained from samples of the dye bound to DNA with fluorescence excitation and emission maxima of 640/658 nm, respectively.

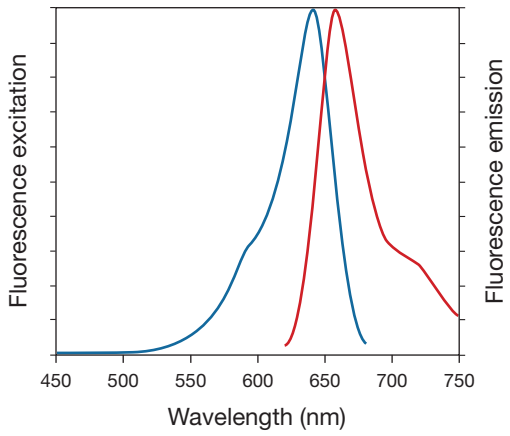

Figure 1. Fluorescence excitation and emission spectra of FxCycle™ Far Red stain bound to dsDNA.

Experimental Protocol

The following 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. Remove all fixative from cells before proceeding with cell staining, however staining with FxCycle™ Far Red 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™ Far Red stain is used in combination with other dyes for multicolor applications, apply the other stain(s) to the sample first, following all manufacturers instructions, including wash steps. FxCycle™ Far Red 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 the flow cytometry instrument performance on 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™ Far Red stain for DNA content cell cycle
- **Do not wash cells after staining with FxCycle™ Far Red 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 cell staining.

1.4 Using an appropriate buffer, adjust the sample cell concentration to be 1×10^6 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™ Far Red stain to each flow cytometry sample and mix well. The final stain concentration is 200 nM.

1.7 Add 5 µL of RNase A (20 mg/mL) to each flow cytometry sample and mix well.

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

1.9 Analyze samples in a flow cytometer **without washing**, using 633/5 nm excitation and emission collected in a 660/20 bandpass or equivalent.

Example of results obtained with FxCycle™ Far Red stain is shown in Figure 2.

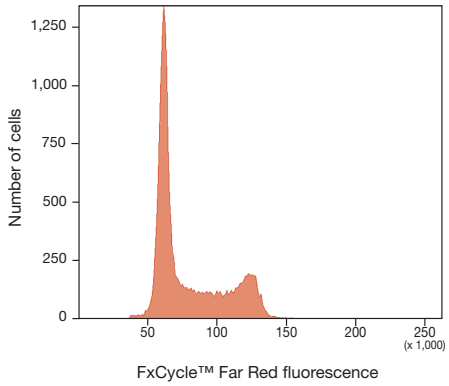

Figure 2. Histogram of TF-1 erythroblast cells stained with FxCycle™ Far Red stain showing DNA content distribution. TF-1 cells were fixed overnight with alcohol, washed, and then resuspended in 0.1% Triton® X-100/PBS/1% BSA before staining with FxCycle™ Far Red stain plus RNase A for 30 minutes at room temperature. G0/G1 and G2/M phase histogram peaks are separated by the S-phase distribution. Analysis was performed using 633 nm excitation with a 660/20 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
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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