

# FxCycle™ PI/RNase Staining Solution

Catalog no. F10797

Table 1 Contents and storage

Material	Amount	Storage	Stability
FxCycle™ PI/RNase Staining Solution	100 mL	<ul style="list-style-type: none"> <li>• Room temperature</li> <li>• DO NOT FREEZE</li> <li>• Protect from light</li> </ul>	When stored as directed, product is stable for at least 1 year.
<b>Number of reactions:</b> Sufficient material is supplied for 200 assays, based on the protocol below.			
<b>Approximate fluorescence excitation and emission maxima:</b> 535/617 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 the analysis of DNA ploidy. In a given population, cells will be distributed among three major phases of cell cycle: G<sub>0</sub>/G<sub>1</sub> phase (one set of paired chromosomes per cell), S phase (DNA synthesis with variable amount of DNA), and G<sub>2</sub>/M phase (two sets of paired chromosomes per cell, prior to cell division).<sup>1-4</sup> DNA content can be measured using fluorescent DNA 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. Univariate DNA content analysis is an established assay method and is widely used for studies in oncology, cell biology, and molecular biology.

The FxCycle™ PI/RNase Staining Solution comes ready-to-use, formulated with DNase-free RNase A and a permeabilization reagent in DPBS. Propidium iodide (PI) binds to DNA by intercalating between the bases with little or no sequence preference and with a stoichiometry of one dye per 4–5 base pairs of DNA.<sup>5</sup> PI also binds to RNA, necessitating treatment with nucleases to distinguish between RNA and DNA staining. Once the dye is bound to nucleic acids, its fluorescence is enhanced 20- to 30-fold. To use the FxCycle™ PI/RNase Staining Solution, just add it to fixed cells, incubate, and acquire on a flow cytometer without washing.

## Before You Begin

---

### Materials Required but not Provided

- Reagents for fixing cells, such as alcohol or formaldehyde
- Wash buffer, such as Phosphate Buffered Saline (PBS)

### Caution

The hazards posed by the FxCycle™ PI/RNase Staining Solution have not been fully investigated. The solution contains propidium iodide, which binds to nucleic acids and should be treated as a potential mutagen; use appropriate precautions when handling this reagent.

Dispose of the reagents in compliance with all pertaining local regulations. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Always wear suitable laboratory protective clothing and gloves when handling these reagents.

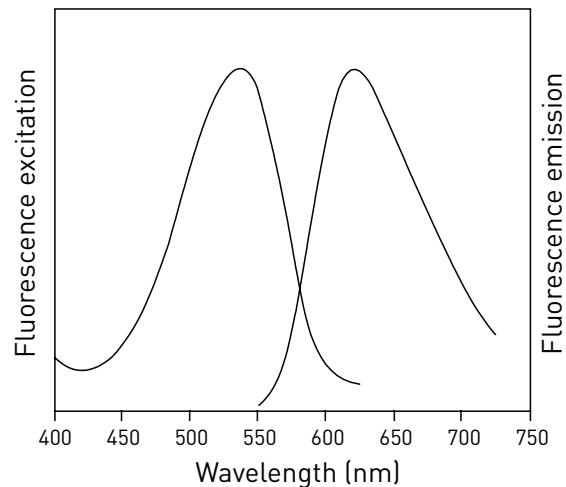
### Storage and Handling

Upon receipt, store the FxCycle™ PI/RNase Staining Solution at room temperature or below (2–25°C), protected from light. Do not freeze. When stored properly, the FxCycle™ PI/RNase Staining Solution is stable for at least 1 year.

### Spectral Characteristics

The fluorescence excitation and emission spectra of the FxCycle™ PI/RNase Staining Solution are shown in Figure 1. The spectra were obtained from samples of the dye bound to DNA with fluorescence excitation and emission maxima of 535/617 nm respectively.

**Figure 1** Fluorescence excitation and emission profiles of propidium iodide bound to DNA. Approximate fluorescence excitation and emission maxima is 535/617 nm.



The following procedure was developed using the Jurkat T-cell leukemia cell line, but can be adapted for any cell type. Fixative, cell density, cell type variations, and other factors may influence staining. All fixative should be removed from cells before proceeding with cell staining. 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™ PI/RNase Staining Solution is used in combination with other dyes for multicolor applications. It is recommended that the other stain(s) is applied to the sample first, following all manufacturers instructions, including washes. FxCycle™ PI/RNase Staining Solution should be the last stain applied to the sample, and samples should not be washed prior to flow cytometric analysis.

### General Guidelines

Follow the guidelines below for optimal DNA content cell cycle analysis.

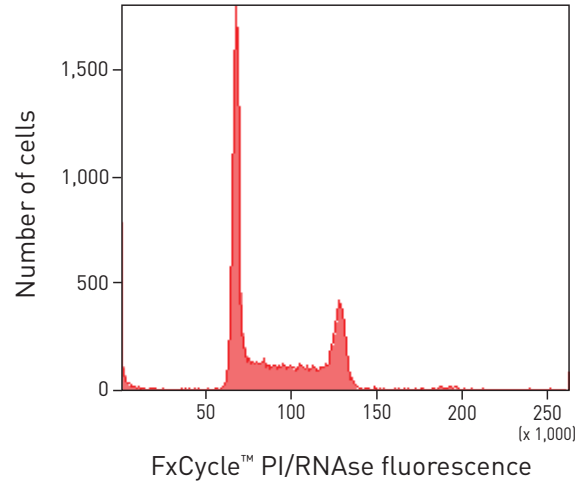
- Eliminate cell clumps and aggregates from the cell suspension before staining.
- Validate flow cytometry instrument performance on the day of use.
- Use linear amplification for DNA content.
- All collection rates using the Attune® Acoustic Focusing Cytometer for acquisition give optimal results; for systems using hydrodynamic focusing, acquisition using a low flow rate is recommended for optimal results.
- Collect adequate numbers of events for the intended application.
- Cells must be fixed before staining with the FxCycle™ PI/RNase Staining Solution for DNA content cell cycle analysis.
- Do not wash the cells after staining with FxCycle™ PI/RNase Staining Solution.
- In multicolor applications, it is recommended that the other stain(s) is applied to the sample first.

### Staining Procedure

- 1.1 Harvest the cell sample(s).
- 1.2 Fix cells according to your preferred protocol.
- 1.3 Wash the cells. All fixative should be removed from cells before proceeding with cell staining.
- 1.4 Prepare flow cytometry samples each containing  $\sim 1 \times 10^6$  cells in suspension.
- 1.5 Centrifuge the samples and decant the supernatant, leaving a pellet of cells in each sample tube.
- 1.6 Add 0.5 mL of FxCycle™ PI/RNase Staining Solution stain to each flow cytometry sample, mix well.
- 1.7 Incubate the samples for 15–30 minutes at room temperature, protected from light.
- 1.8 Analyze the samples without washing, using 488-nm, 532-nm, or similar excitation, and collect emission using a 585/42 bandpass filter or equivalent.

Example of results obtained with FxCycle™ PI/RNase Staining Solution is shown in Figure 2.

**Figure 2** Histogram of Jurkat cells stained with FxCycle™ PI/RNase stain showing DNA content distribution. Jurkat cells were fixed in 70% ethanol, washed, and then resuspended in FxCycle™ PI/RNase stain for 30 minutes at room temperature. G<sub>0</sub>/G<sub>1</sub> and G<sub>2</sub>/M phase histogram peaks are separated by the S phase distribution. Analysis was performed using 532-nm excitation with a 585/42-nm bandpass filter.



## References

1. Current Protocols in Cytometry, 7.0.1–7.27.7 (2004);
2. Practical Flow Cytometry, 4<sup>th</sup> Ed., Shapiro HM, Ed. (2003);
3. Methods Mol Biol 281, 301 (2004);
4. Cytometry A 58, 21 (2004);
5. J Mol Biol 13, 269 (1965)

## Product List

Current prices may be obtained from [www.lifetechnologies.com](http://www.lifetechnologies.com) or from our Customer Service Department.

Catalog no.	Product Name	Unit Size
F10797	FxCycle™ PI/RNase Staining Solution *200 assays*	100 mL
<b>Related Products</b>		
F10347	FxCycle™ Violet Stain *for flow cytometry* *500 assays* *DAPI*	1 set
F10348	FxCycle™ Far Red Stain *for flow cytometry* *500 assays*	1 set

# Purchaser Notification

---

## Corporate Headquarters

5791 Van Allen Way  
Carlsbad, CA 92008  
USA  
Phone: +1 760 603 7200  
Fax: +1 760 602 6500  
Email: [techsupport@lifetech.com](mailto:techsupport@lifetech.com)

## European Headquarters

Inchinnan Business Park  
3 Fountain Drive  
Paisley PA4 9RF  
UK  
Phone: +44 141 814 6100  
Toll-Free Phone: 0800 269 210  
Toll-Free Tech: 0800 838 380  
Fax: +44 141 814 6260  
Tech Fax: +44 141 814 6117  
Email: [euroinfo@invitrogen.com](mailto:euroinfo@invitrogen.com)  
Email Tech: [eurotech@invitrogen.com](mailto:eurotech@invitrogen.com)

## Japanese Headquarters

LOOP-X Bldg. 6F  
3-9-15, Kaigan  
Minato-ku, Tokyo 108-0022  
Japan  
Phone: +81 3 5730 6509  
Fax: +81 3 5730 6519  
Email: [jpinfo@invitrogen.com](mailto:jpinfo@invitrogen.com)

Additional international offices are listed at  
[www.lifetechnologies.com](http://www.lifetechnologies.com)

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

## Obtaining Support

For the latest services and support information for all locations, go to [www.lifetechnologies.com](http://www.lifetechnologies.com).

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support ([techsupport@lifetech.com](mailto:techsupport@lifetech.com))
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

## SDS

Safety Data Sheets (SDSs) are available at [www.lifetechnologies.com/sds](http://www.lifetechnologies.com/sds).

## Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support) and search for the Certificate of Analysis by product lot number, which is printed on the product packaging (tube, pouch, or box).

## For Research Use Only. Not for use in diagnostic procedures.

## Disclaimer

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

## Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at [www.lifetechnologies.com/termsandconditions](http://www.lifetechnologies.com/termsandconditions). If you have any questions, please contact Life Technologies at [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

## Limited Use Label License: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial services of any kind, including, without limitation, reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact [outlicensing@lifetech.com](mailto:outlicensing@lifetech.com) or Out Licensing, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008.

The trademarks mentioned herein are the property of Life Technologies Corporation and/or its affiliate(s) or their respective owners.

©2012 Life Technologies Corporation. All rights reserved.

