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CellCycle™ Ruby Stain

Catalog Number: A055

Table 1. Product Package and Storage

Material	Amount	Concentration	Storage	Stability
CellCycle™ Ruby Stain	**100 µL**	**500X**	**-20 °C**	**One year when stored as directed.**

Number of assays: 100 assays.
Approximate fluorescence excitation/emission maxima, in nm: 635/685, 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: G_0/G_1 phase (one st of paired chromosomes per cell), S phase (DNA synthesis with variable amount of DNA), and G_2/M phase (two sets of paired chromosomes per cell, prior to cell division). 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 cell cycle phases. 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.

The CellCycle™ Ruby stain is DNA-selective, cell membrane-permeant, and nonfluorescent stain for DNA content analysis in living cells. The CellCycle™ Ruby stain is fluorescent upon binding to double-stranded DNA. CellCycle™ Ruby stain is excited using both 488 nm and 633 nm laser with emission ~685 nm.

The staining protocol is simple and includes incubating suspended cells in the presence of CellCycle™ 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.

Materials required but not provided

- Cells and culture medium
- Flow cytometer tubes

Experimental Protocols

Note: 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. These stains can also be used for cells suspended in Hanks' Balanced Salt Solution (HBSS) or phosphate-buffered saline (PBS).

1. Remove the CellCycle™ Ruby stain from the freezer and allow it to equilibrate to room temperature.
2. Prepare flow cytometry tubes each containing 0.5 mL of cell suspension in complete media at a concentration of 1×10^6 cells/mL.
3. To each tube add 1 µL of CellCycle™ Ruby stain.
4. Incubate at 37˚C for 30 minutes, protected from light.
5. Analyze the samples without washing, using 488 nm or 633 nm excitation, and collect emission using a 695/40 bandpass filter or equivalent.