Product Information

Cell Cycle Assay Kit Plus

Catalog Number: C6078

Product Size: 50 T

Contents:

Component	50 T
A. Binding buffer (10×)	10 mL
B. RedNucleus I Staining Solution	200 μL

Storage

Store at 4℃. When stored as directed, product is stable for at least 12 months. Store at -20℃. When stored as directed, product is stable for at least 24 months. RedNucleus I needs to be protect from light.

Description

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_0/G_1 phase (one set 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 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.

RedNucleus I Staining Solution is used for flow cyotmetric analysis of DNA content in live cells. Rednucleus I is a kind of far-infrared nucleic acid dye with cell membrane permeability, which can quickly enter into living cells, specifically combine with DNA, and detect the cycle of living cells without RNase digestion. Compared with the traditional method of propidium iodide staining, the cell does not need to break the membrane or be fixed, so the operation is more simple. This reagent can also be used to detect the cell cycle of fixed cells, We recommend using ice bath precooling 75-80% ethanol to fix cells overnight at -20℃.

Protocol

The following procedure was developed using the Jurkat cell, 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.

Staining procedure

1. Harvest the cell sample(s).

2 Wash the cells with 1×Binding buffer(Diluted A with diH_2O

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for ten times). Prepare flow cytometry samples each containing ~ 1 × 10^6 cells in suspension.

5 Centrifuge the samples and decant the supernatant, leaving a pellet of cells in each sample tube.

6 Resuspend cells with 1 mL medium. Add 4 μL B Solution stain to each flow cytometry sample, mix well.

7 Incubate the samples for 20 minutes at room temperature, protected from light(or 37℃ for 5-10 min).

8 Analyze the samples without washing, using638 nm, or similar excitation, we recommend to collect emission in RL3 or FL4 channel, RL1 or RL2 channel can also collect emission.

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