Product Information

Name：Cell Cycle and Apoptosis Analysis Kit

Catalog：C6031

Size：50 T, 100 T

Kit Contents:

Component	50 T	100 T
A. 1× Binding buffer	25 mL	50 mL
B. PI Staining Solution (20×)	1.25 mL	2.5 mL
C. RNase A (50×)	0.5 mL	1 mL

Storage and Handling

Storage at -20℃ is stable for 24 months, storage at 4℃ is stable for 12 months.PI needs to be protect from light.

Product 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.

Propidium iodide (PI) Staining Solution is used for flow cyotmetric analysis of DNA content in fixed cells., PI ,a popular red-fluorescent stain, 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. PI also binds to RNA, necessitating treatment with RNase to distinguish between RNA and DNA staining.

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. Fix cells according to your preferred protocol. We recommend using ice bath precooling 75-80% ethanol to fix cells overnight at -20℃.

3. Wash the cells. All fixative should be removed from cells before proceeding with cell staining.

4. Prepare flow cytometry samples each containing ~ 1 × 10^6 cells in suspension.

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

6. Add in turn with 0.5 mL A, 25 µL B and 10 µL C Solution stain to each flow cytometry sample, mix well.

7. Incubate the samples for 15-30 minutes at room temperature, protected from light.

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.

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