Cell Cycle Analysis Kit

(Catalog #BN01094; 100 assays; Store at -20°C)

I. Introduction:

Cell cycle is a ubiquitous, complex sequence of events leading to growth and proliferation of cells. Cell Cycle progression is tightly regulated due to its involvement in development, DNA damage and repair, etc. Anomalies in cell cycle progression can lead to tissue hyperplasia and diseases such as cancer. Cell cycle can be subdivided into interphase (G_0/G_1, S and G_2) and mitotic (M) phase (prophase, metaphase, anaphase and telophase). Assay Genie's Cell Cycle Analysis Kit provides a quick and easy method to detect the number of cells in a cell population, which are at a specific stage of the cell cycle. Our kit utilizes a nuclear dye, the binding of which to nucleic acids in the cell results in fluorescence signal, which is proportional to cellular DNA content. The percentages of cells in different phases of the cell cycle (G_0/G_1, S, and G_2/M) can be quantified by flow cytometry. Our method is non-radioactive, rapid and accurate and can be used for high throughput cell cycle analysis with contemporary flow cytometer instruments.

II. Application:

- Analysis of cell cycle regulation in response to growth factors, cytokines, mitogens, and nutrients, etc.
- Monitoring of cell cycle progression
- Study effects of drugs which affect cell growth and division

III. Sample Type:

- Adherent or Suspension cells

IV. Kit Contents:

Components	BN01094	Cap Code	Part Number
10X Cell Cycle Assay Buffer	50 ml	NM	BN01094-1
Enzyme A Solution	2 x 250 µl	Blue	BN01094-2
Nuclear Dye	2 x 1 ml	Red	BN01094-3

V. User Supplied Reagents and Equipment:

- 6-well tissue culture plate
- Cell Culture Media and Fetal Bovine Serum
- 70% Ethanol (Pre-chilled on ice)
- Flow Cytometer with excitation filter at 488 nm wavelength

VI. Reagent Preparation and Storage:

Store the kit at -20°C, protected from light. Warm all reagents to room temperature (RT) before use. Read the entire protocol before performing the assay.

- **10X Cell Cycle Assay Buffer:** Dilute10X Cell Cycle Assay Buffer with ddH_2O to prepare 1X working solution. Pre-chill 1X Cell Cycle Assay Buffer on ice before use.
- **Nuclear Dye:** For long-term storage, aliquot and store at -20°C to avoid repeated freeze/thaw.
- **Staining Solution:** Before performing the analysis, prepare Staining Solution - for every 20 samples (based on 6-well plate sample size): add 100 µl of Enzyme A Solution and 400 µl of Nuclear Dye into 10 ml of 1X Cell Cycle Assay Buffer, mix well and protect from light. Stable for one week at 4°C.

VII. Cell Cycle Analysis Protocol:

1. **Sample Preparation:** Grow cells of interest (2-5 x 10^5 cells/well) in desired medium and culture conditions preferably in 6-well plates for 24 hr prior to the experiment. Synchronize cells with culture medium containing 0.1% FBS for 24 hr. Treat cells with test compounds in culture medium containing 10% FBS for 4-24 hr. As controls, incubate cells of interest in culture medium with 10% FBS without any test compound. Harvest cells and centrifuge at 400 x g for 5 min. Remove the supernatant and wash cells in 2 ml ice cold 1X Cell Cycle Assay Buffer, centrifuge cells at 400 x g for 5 min., remove the supernatant and save the cell pellet.

   Notes:
   a. Cell density depends on the cell type, and it may be necessary to adjust the cell numbers for optimal cell density.
   b. For longer incubation times, change culture medium containing 10 % FBS with test compounds every 24 hr.

2. **Nucleic Acid Labeling:** Fix the cells by adding 2 ml ice cold 70% ethanol (add drop by drop while vortexing) to the cell pellet, put on ice for at least 30 min. Centrifuge cells at 400 x g for 5 min. and carefully remove the supernatant. Wash cells in 2 ml of 1X Cell Cycle Assay Buffer, centrifuge cells at 400 x g for 5 min. and carefully remove the supernatant. Resuspend cells completely with 500 µl of Staining Solution, protect from light exposure. Incubate at RT for 30 min.

   Notes:
   a. After fixing in ethanol, cells are harder to pellet. We recommend removing supernatant carefully to avoid cell loss.
   b. After fixing, cells can be stored at -20°C for several weeks in 70% ethanol.

3. **Data Analysis:** During flow cytometry data analysis, select the main cell population in the FSC vs SSC plot. Within the main cell population, exclude the cell debris and cell aggregates by gating on single cells in the FL2-A vs FL2-W plot. Cell aggregates should have higher value

of FL2-W than the main single cell population. Cell cycle analysis is performed with FL2-A histogram of single cells. Cell cycle status can be quantified by programs within the flow cytometer software or gating on the FL2-A histogram.

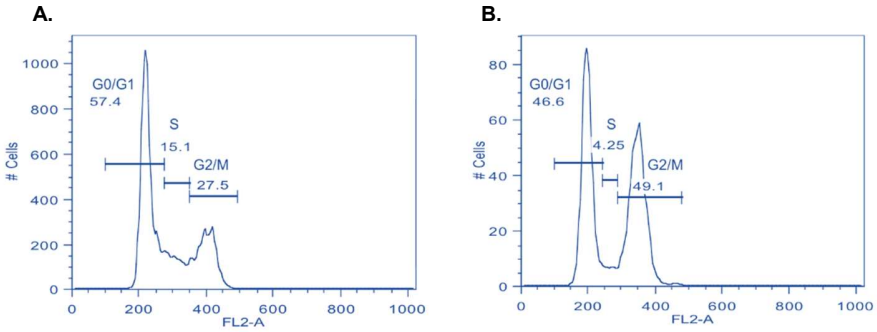

Figure: 3T3 Cell Cycle Analysis: 3T3 cells were seeded at 2 x 10^5 cells/well into a 6-well tissue culture plate with 10% FBS culture medium on day 1. On day 2, medium was removed and cells synchronized with 0.1% FBS culture medium for 24 hr. On day 3, 3T3 cells were incubated in culture medium with either 0.1% FBS, 10% FBS, or 10% FBS containing 20 µM of SKPin C1 (G1 or G2/M phase blocker) for an additional 24 hr. On day 4, cells were harvested, fixed and stained with the Cell Cycle Analysis Kit according to the protocol. Fluorescence intensity was detected and recorded on a BD Flow Cytometer in FL-2 channel. **A.** Cell cycle analysis of 3T3 cells in 10% FBS culture medium; **B.** Cell cycle analysis of 3T3 cells in 10% FBS culture medium containing 20 µM of SKPin C1.

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