B25011-25

Baran Biotech

Cell Cycle Assay Kit (25 Tests)

Overview

The cell cycle is a fundamental and complex series of events that leads to cell growth and division. This cycle describes a cell's progression through various phases of division, encompassing both cytoplasmic and nuclear activities. In most eukaryotic cells, the cell cycle is categorized into four distinct phases: M (Mitosis), G1 (Growth 1 Phase), S (Synthesis Phase), and G2 (Growth 2 Phase). During interphase, which encompasses G1, S, and G2, the cell continues to grow. The S phase is specifically when DNA replication takes place.

Cells at various stages of the cell cycle can be identified based on their DNA content. During the S phase, the DNA content doubles from 2n to 4n, and this 4n level is maintained in the G2 and M phases before returning to 2n following cytokinesis.

G1 Phase: 2N cells

S Phase: Variable DNA amounts within cells (2 to 4N cells)

G2 Phase: 4N cells

After mitosis Cells may exit the cycle to a quiescent, non-dividing stage (G0), and with proper activation, they can re-enter the cycle.

To experimentally determine cellular DNA content, cells can be treated with a fluorescent dye that binds to DNA. This is followed by analyzing the fluorescence intensity of individual cells using a flow cytometer, where the fluorescence signal correlates with the amount of DNA present in the cells.

Baran Cell Cycle Assay Kit is an assay kit designed for the determination of cell cycle progression using a Flow Cytometer. A red fluorescent dye (Propidium Iodide) binds DNA, and the relative DNA content of cells can be determined by flow cytometry. Propidium iodide (PI) is a fluorescent compound that attaches to nucleic acids without a strong preference for specific sequences. Since PI can bind to both RNA and DNA, RNase A is included in this kit to degrade cellular RNA, thereby reducing background staining from RNA during the experiment.

Sample Type: Living cells (suspension and adherent)

Kit Components Item Quantity

Item	Quantity
Propidium Iodide (1mg/mL)	130 µL
RNase A Solution (1mg/mL)	130 µL
Fixation Solution	100 mL
10X Phosphate Buffered Saline	12.5 mL
Permeabilization Buffer	15 mL

Storage

Upon receipt, store RNase A solution at -20°C. Store the PI Reagent and other solutions at +4°C. Protect PI solution from the light.

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Working solutions

- For a 1X PBS, Dilute 10X Phosphate Buffered Saline by 10-fold with distilled water.

Remarks

- PI is a suspected carcinogen; contact with eyes, skin, and mucous membranes should be avoided. Always wear proper protective clothing and gloves when handling the solution.
- Store the fixation solution at -20°C overnight before use.

Assay Protocol

1. Harvest the cells and centrifuge them at 600 x g for 5 min. Aspirate and discard the supernatant.

2. Wash the cells in 1 ml of 1X PBS. Centrifuge at 600 x g for 5 min. Aspirate and discard the supernatant.

Note: After adding PBS, transfer the suspension to a flow cytometry tube.

3. Fix the cells by adding 4 ml Fixation Buffer (precooled at -20 °C overnight). Store fixed cells on ice at least 1 h and for up to several days.

4. Spin down the cells for 5 min at 600 x g and discard the supernatant.

5. Wash the cells as described in step 2.

6. Resuspend cell pellet in 0.5 mL Permeabilization Buffer and incubate on ice for 15 min.

7. Spin down cells for 5 min at 600 x g and discard the supernatant.

8. Resuspend cell pellet in 0.5 ml 1X PBS. Add 5 µl RNase A solution and 5 µl PI dye. Gently mix and incubate at room temperature in the dark for 30 min.

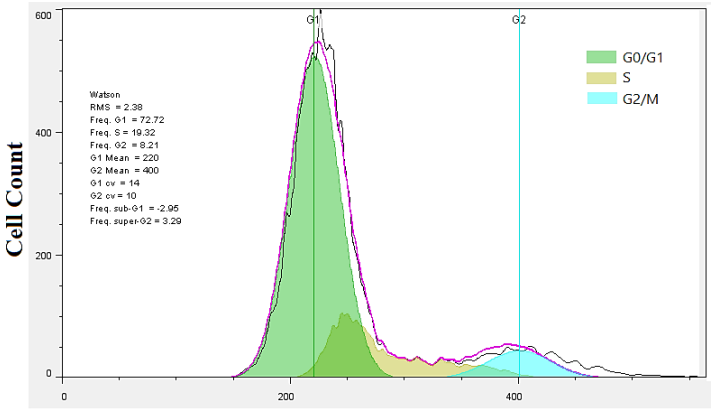
Note: Gently tap the bottom of the RNase A vial. Then briefly spin down both RNaseA and PI vials before opening

9. Analyze by flow cytometry.

Data Analysis

1- Choose the main cell population in the FSC versus SSC plot to filter out debris and cell aggregates.

2- Capture PI fluorescent signals in FL2 channel.



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