Cell Cycle Assay Kit

Introduction

Measurement the amount of DNA is usually used to monitoring cell cycle progression. The cell cycle consists of G1, S, G2, and M phases. In the G1 phase, the DNA content of the cells is the lowest (2N), and in the S phase, the DNA begins to replicate, the DNA content gradually increases, and by the G2 and M phases, the DNA content doubles (4N).

Cell Cycle Assay Kit is a kit for cell cycle detection by propidium iodide PI. PI is a nuclear dye that is able to cross the cell membrane to stain dead or fixed cells, but not the membrane of living cells. When stained with propidium iodide, cells at different cell cycle stages exhibit different fluorescence intensities. Cells in the G0/G1 phase have a fluorescence intensity of 1 because they contain a copy of genomic DNA. Cells in the G2/M phase, on the other hand, have a fluorescence intensity of 2 because they contain double genomic DNA. The fluorescence intensity of cells in the S phase is between 1 and 2. Apoptotic cells, exhibit weaker fluorescence intensities during staining, typically less than 1. This is because the concentration of the nucleus during apoptosis is accompanied by DNA fragmentation, resulting in the loss of some genomic DNA fragments. On the fluorescence detection map of the flow cytometer, this phenomenon appears as the so-called sub-G1 peak, which marks the presence of apoptotic cells.

Components and Storage

Components	K2263-50 T
PI (20X)	1.25 mL
RNase A (50X)	0.5 mL
Staining Buffer	25 mL
Store the kit at -20°C, stable for 1 year. PI (20X) should be stored at -20°C away from light.	

Protocol

1. **Cell sample preparation:** Collect approximately 5x10^5 cells as follows.

   A. For adherent cells

   1) Collect the culture medium in the tested cells into a centrifuge tube for later use.

   2) Trypsinize the cells until the cells can be gently pipetted down, add the previously collected medium,

thoroughly blow down all adherent cells, and gently blow them away.

3) Centrifugation at 1000 g for 3-5 minutes, discard the supernatant, leaving approximately 50 μL of supernatant to avoid aspiration of cells.

4) Resuspend cells with 1 mL of pre-chilled PBS and transfer to a new centrifuge tube. Then centrifugation at 1000 g for 3-5 minutes, discard the supernatant, leaving approximately 50 μL of supernatant to avoid aspiration of cells. Gently flick the bottom of the tube to disperse the cells, avoiding clumping of cells.

B. For suspension cells

1) Centrifugation at 1000 g for 3-5 minutes, discard the supernatant, leaving approximately 50 μL of supernatant to avoid aspiration of cells.

2) Resuspend cells with 1 mL of pre-chilled PBS and transfer to a new centrifuge tube. Then centrifugation at 1000 g for 3-5 minutes, discard the supernatant, leaving approximately 50 μL of supernatant to avoid aspiration of cells. Gently flick the bottom of the tube to disperse the cells, avoiding clumping of cells.

2. **Cell fixation:**

1) Add 1 mL of pre-chilled 70% ethanol to the cells, mix well, and fix at 4ºC for 30 minutes or longer.

***Note:** In order to ensure the detection effect, it is usually necessary to fix for more than 2 hours, and 12-24 hours may be better.

2) Discard the supernatant after centrifugation at 1000 g for 3-5 minutes, leaving about 50 μL of supernatant to avoid aspiration of cells.

3) Resuspend the cells with 1 mL of pre-chilled PBS and transfer to a new centrifuge tube. Then centrifugation at 1000 g for 3-5 minutes, discard the supernatant, leaving approximately 50 μL of supernatant to avoid aspiration of cells. Gently flick the bottom of the tube to disperse the cells, avoiding clumping of cells.

3. **Preparation of staining solution:** Refer to the following table to prepare the staining solution according to the experimental needs.

Sample	1	6	12
Staining Buffer	0.5 mL	3 mL	6 mL
PI (20X)	25 μL	150 μL	300 μL
RNase A (50X)	10 μL	60 μL	120 μL
Final Volume	0.535 mL	3.21 mL	6.42 mL

***Note:** The prepared staining solution can be stored briefly at 4ºC, but it is recommended to be used on the day it is prepared.

4. **Staining:** Add 0.5 mL of staining solution to each sample, slowly and adequately resuspend the cell pellet, and then incubate at 37ºC water bath protect from light for 30 minutes.

***Note:** Detection should be performed promptly after staining. If detection is not immediate, samples can be stored at 4ºC or in an ice bath temporarily protected from light, but the detection needs to be completed within 24 hours.

5. **Flow cytometry:** Detect the samples using a flow cytometer (usually the 488 nm excitation laser, 650 nm emission filter), and analyze the cell cycle using appropriate analysis software.

Note

1. The 70% ethanol is required but not provided in this kit.
2. Fluorescent probes are easy to quench, please pay attention to avoid light as much as possible during storage and use.
3. PI is toxic to humans, please take precautions when using it to avoid direct contact with the human body or inhalation into the body.
4. For your safety and health, please wear lab coats and gloves during the experiment.
5. For research use only. Not to be used in clinical diagnostic or clinical trials.

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