Cell Cycle Assay Kit

Cat #: orb545646 (manual)

Detection cell cycle and cell proliferation using flow cytometry.

For research use only. Not for diagnostic or therapeutic procedures.

INTRODUCTION

For normal cells, the content of DNA is changed with the process of cell cycle. Observed DNA stained by dyes using flow cytometry to calculate percentage of G0 /G1, S, and G2/M. It will be clear known that how about the distribution of cell cycle and the activity of proliferation. For apoptotic cells, DNAs in cells is degraded by endogenous nuclease activated and diffuse out of cells with the process of apoptosis.

PRINCIPLES

A highly definable sub-G1 peak occurs and is easily quantified by dyes. The change of DNA in apoptotic cells is also assayed for sorting and further analyzing apoptotic cells. After RNA is degraded by RNase, the nucleic acid dye in this kit bind with DNA composed of chromatin in the nucleus. And the results can be analyzed by flow cytometry.

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KIT COMPONENTS

Component	50 Assays	100 Assays	Storage
Propidium Iodide (25X)	0.75 ml	1.5 ml	4 °C
Staining Buffer	20 ml	20 ml x 2	4 °C
RNase A (2.5 mg/ml)	0.2 ml	0.4 ml	-20 °C
Manual	1	1	

MATERIALS REQUIRED BUT NOT PROVIDED

1. 5 ml and 10 ml graduated pipettes
2. 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips
3. Beakers, flasks, cylinders necessary for preparation of reagents
4. Glass-distilled or deionized water, 95% ethanol
5. Bench top centrifuge
6. Flow Cytometer
7. PBS

ASSAY PROCEDURE

1．Induce cell apoptosis using proper method and set a negative control. Harvest cells.

2．Add PBS to wash cells once. Then, centrifugate cells at 2000 rpm for five minutes.

3．Add PBS to resuspend cell and adjust cell concentration to 1×10^6/ml.

4．Centrifugate cells at 2000 rpm for five minutes and discard the supernatant.

5．Fix cells using 95% ethanol (cold) at 4 °C for two hours or overnight.

6．Use PBS to wash cells twice for removing fixing solution.

7．Staining Working Solution preparation: for one sample, add 4 µl RNase A and 16 µl Propidium Iodide (25X) into 380 µl Staining Buffer, mix.

8．Add 400 µl of Staining Working Solution to stain. Incubate cells at 37 °C for 30 minutes and protect from light.

9. Add 2 ml PBS, centrifugate cells at 2000 rpm for five minutes and discard the supernatant, then add 500 µl PBS.

9．Observe at 488 nm of excitation wavelength by flow cytometry.

Note:

*Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.

*Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergent before use.

STORAGE/STABILITY

The kit ships on wet ice and storage at 2-8 °C is recommended.

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