Cell Cycle Analysis Kit **Cat. No. L00287**
Technical Manual No. 0360 **Version 11172008**

I. INTRODUCTION

Cell Cycle Analysis Kit (Cat.No.L00287) provides a rapid and convenient assay for cell cycle and cell proliferation. 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 G_0/G_1, S, and G_2/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. 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.

II. KIT CONTENTS

Cell Cycle Analysis Kit is used for detecting the different stages of cell cycle. Each kit contains enough reagents for fifty assays.

Kit Components	50 Assays
RNase A	5.0 ml
Propidium Iodide (PI)	20.0 ml

III. KEY FEATURES

- Easy to perform: simple and rapid procedure to perform.
- Versatile: this kit is used for detecting cell cycle not only suspension cells but also adherence cells
- Direct quantitation for normal, apoptotic, and dead cells by flow cytometry.
- Ready to use
- Highly competitive price

IV **STORAGE**

This kit remains stable for at least one year if stored at -20^{o}C and protected from light.

V. CELL CYCLE ANALYSIS KIT PROTOCOL

Note:

Propidium Iodide (PI) contained in this kit is a mutagen. Gloves, protective clothing, and eyewear should be worn and safe laboratory practices followed.

Protocol:

1. Induce cell apoptosis using proper method and set a negative control. Harvest cells.
2. Add PBS to wash cells once. Then, centifugate cells at 2000 rpm for five minutes.
3. Add PBS to resuspend cell and adjust cell concentration to 1×10^{6}/ml.
4. Centifugate cells at 2000 rpm for five minutes and discard the suspernatant.
5. Fix cells using 70% ethanol at 4°C for two hours or overnight.
6. Use PBS to wash cells for removing fixing solution. If necessary, filter cell suspension once using sieve with 200 meshes.
7. Add 100 µl of RNase A to cells suspension and incubate cells at 37 °C for 30 minutes.
8. Add 400 µl of PI to stain. Incubate cells at 4 °C for 30minutes and protect from light.
9. Observe at 488 nm of excitation wavelength by flow cytometry.

VI. EXAMPLES

P388 cells are induced apoptosis by 10 µM camptothecin for 1 hours at 37°C, and procedures are accomplished as describe as above. The result is as follow:

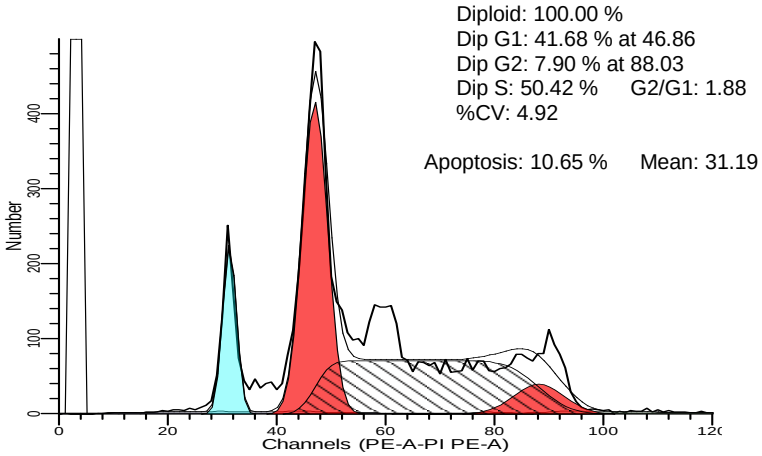

Fig.1 Observe and analyze the distribution of cell cycle by flow cytometry.

VII. RELATED PRODUCTS

Annexin V-EGFP Apoptosis Detection Kit: Cat.No.L00288
Caspase-3 Colorimetric Assay Kit: Cat.No.L00289
Cell Apoptosis DAPI Detection Kit: Cat.No.L00312.
Double Stain Apoptosis Detection Kit (Hoechst 33342/PI): Cat.No.L00309.
Cell Apoptosis PI Detection Kit: Cat.No.L00311.

VIII. TROUBLESHOOTING

Problem	Possible cause	Solution
G_O/G_1 drifts or changes suddenly	The channels in flow cytometry have air bubble or have been blocked.	Check the channels to make them go on wheels.
	The concentration of PI is too low.	Improve the concentration of PI
	It is not enough to incubate cells and PI.	Lengthen incubation time between cells and PI.
Peak width	The flow rate of flow cytometry is too fast.	Adjust the flow rate.
	There are air bubbles in the samples.	Check and removal air bubbles in the samples.
	There are dead cells in the samples.	Choose new samples to replace the old one.
No peak for samples	Necrotic cells are too much.	Observe samples using microscopy, or choose new samples to replace the old one.

	There is no nucleus in the samples.	
	There is too much cell fragment.	

IX. ORDERING INFORMATION

Cell Cycle Analysis Kit: Cat.No.L00287

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