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I. INTRODUCTION

Fluorescent labeling is the fundamental method for researching apoptosis. It can be used to assay the morphological and biochemical change of apoptosis for differentiation in normal, apoptotic, and dead cells, or cell cycles.

Cell Apoptosis PI Detection Kit (Cat.No.L00311) provides a rapid and convenient assay for apoptosis based upon fluorescent detection. Propidium iodide (PI), a ready-to-use and red-fluorescence dye (excitation/emission maxima ~535/617 nm) binding with DNA, is only permeant to dead cells. Observed by fluorescent microscopy, normal cells are not stained, dead cells have red strong fluorescence. The fluorescent intensity of apoptotic cells is between dead and normal cells. Observed by flow cytometry, DNA in cells is degraded by endogenous nuclease activated and diffuse out of cells with the process of apoptosis. A highly definable sub-G₁ peak occurred is easily quantified by PI. Cells should be sorted and further analyzed by microscopy to verify apoptotic cells are present. The change of DNA in apoptotic cells is assayed by flow cytometry.

II. KIT CONTENTS

The Cell Apoptosis PI Detection Kit (Cat.No.L00311) employs two dyes assaying normal and apoptotic cells. Each kit contains enough reagents for one hundred apoptosis assays.

Kit Components	100 Assays
Propidium Iodide (PI)	500 µl
10X Buffer A	10 ml

III. KEY FEATURES

- Easy to perform: simple and rapid procedure to perform.
- Versatile: directly analyze normal and apoptotic cells by flow cytometry, fluorescence microscopy, and Fluorescent photometer.
- Ready to use
- Highly competitive price

IV. STORAGE

This kit remains stable for at least one year if stored at 4°C and protected from light.

V. CELL APOPTOSIS PI DETECTION KIT PROTOCOL

Note:

1. Before the experiment, dilute 10X buffer A to 1X buffer A (add 90 ml of distilled water to 10 ml of 10X buffer A).
2. Prepare RNase A yourself, which is not supplied in this kit, if you analyze apoptotic cells using flow cytometry or fluorescent photometer.
3. Protect the Propidium Iodide reagent from light all the time. And PI is potential mutagen. It is necessary to use with appropriate precautions.

Observation by fluorescent microscope

1. Harvest the cells by centrifugation at 2000 rpm for five minutes. Then, wash them once using 1X buffer A.
2. Suspend cells and adjust the cell density to 1×10^6 cells/ml in 1X buffer A.
3. Add 5 μ l of PI to 95 μ l of cell suspension prepared as above.
4. Incubation away from light at room temperature for five minutes.
5. Place the cell suspension prepared on a glass and cover the cells with a glass cover slip.
6. Observation cells immediately under a fluorescence microscope using with appropriate filters.

Observation by flow cytometry or fluorescent photometer

1. Harvest the cells by centrifugation at 2000 rpm for five minutes and wash cells once using 1X buffer A.
2. Suspend cells and adjust the cell density to 1×10^6 cells/ml in 1X buffer A.
3. Fixation cells at -20°C for at least 12 hours by adding nine volumes of 70% ethanol to cell suspension.
4. Centrifuge cells and wash cells using 1X buffer A after fixation.
5. Suspend cells in 500 μ l of 1X buffer A.
6. Add RNase A up to 0.25 mg/ml. Incubation at 37°C for 30 minutes.
7. Add 5 μ l of PI to cell suspension and incubate away from light at room temperature for 30 minutes.

8. Observe by flow cytometry.

VI. RELATED PRODUCTS

Cell Cycle Analysis Kit:	Cat.No.L00287.
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.

VII. EXAMPLES

P388 cells are induced apoptosis by 10 μ M camptothecin for one hour at 37°C, and procedures are accomplished according with protocol as described as above. The results are as follow:

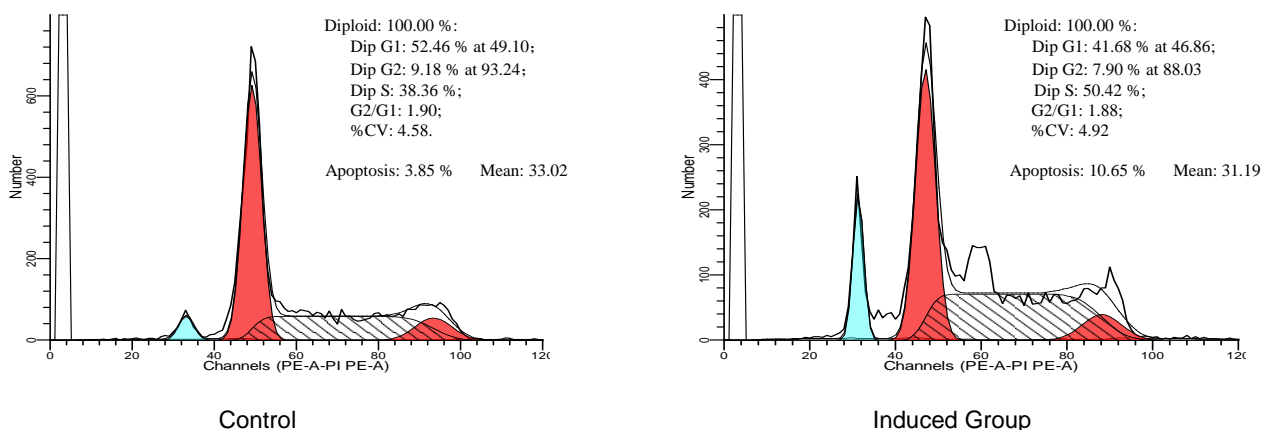


Fig.1 Observe and analyze apoptosis by flow cytometry.

VIII. ORDERING INFORMATION

Cell Apoptosis PI Detection Kit: Cat.No.L00311.

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