Double Stain Apoptosis Detection Kit (Hoechst 33342/PI) **Cat. No. L00309**

Technical Manual No. 0361 **Version 11182008**

I. INTRODUCTION

The **Double Stain Apoptosis Detection Kit (Hoechst 33342/PI)** provides a rapid and convenient assay for apoptosis based upon fluorescent detection for the compacted state of the chromatin in apoptotic cells. This kit contains two ready-to-use dyes bound to DNA. Hoechst 33342, a kind of blue-fluorescence dye (excitation/ emission maxima ~350/461 nm，when bound to DNA), stains the condensed chromatin in apoptotic cells more brightly than the chromatin in normal cells. Propidium iodide (PI), a red-fluorescence dye (excitation/emission maxima ~535/617 nm，when bound to DNA), is only permeant to dead cells. The staining pattern resulted from the simultaneous use of these dyes makes it possible to distinguish normal, apoptotic, and dead cell populations by flow cytometer and fluorescence microscopy.

II. KIT CONTENTS

Double Stain Apoptosis Detection Kit (Hoechst 33342/PI) employs two dyes assaying normal, apoptotic, and dead cells. Each kit contains enough reagents for one hundred apoptosis assays.

Kit Components	100 Assays
Hoechst 33342	1.0 ml
Propidium Iodide (PI)	500 µl
10X Buffer A	10 ml

III. KEY FEATURES

- Easy to perform: simple and rapid procedure to perform.
- Fast and quick: all of the procedure is in 20 minutes.
- Versatile: directly analyze normal, apoptotic, and dead cells by flow cytometry and fluorescence microscopy.
- 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. DOUBLE STAIN APOPTOSIS DETECTION KIT PROTOCOL

Before the experiment, dilute 10X buffer A to 1X buffer A (add 90 ml of distilled water to 10 ml of 10X buffer A).

1. Induce apoptosis in cells using the desired method. A negative control should be prepared without inducing reagent.
2. Harvest the cells after the incubation period, wash by cold phosphate-buffered saline or culture medium and adjust the cell density to 1×10^6 cells/ml or less in PBS. For each assay, 1 ml of cell suspension should be used.

   Note: Directly collect suspension cells by centrifugation. Otherwise, inhered cells should be digested firstly to use.
3. Add 10 µl of Hoechst 33342 to each 1 ml of the cell suspension and mix thoroughly. Incubate the cells at 37°C for 5-15 minutes.
4. Centrifuge the cells at 1,000 rpm for five minutes at 4°C and discard the supernatant.
5. Resuspend cells in 1000 µl of 1X buffer A.
6. Add 5 µl of PI to each 1 ml of cell suspension and mix thoroughly. Incubate the cells at room temperature for 5-15 minutes and avoid exposing to light.
7. After the incubation, analyze the stained cells by flow cytometry immediately, using UV/488 nm dual excitaiton and measuring the fluorescence emission at ~460 nm emission of Hoechst 33342 dye and >575 nm emission of Propidium Iodide. The population should be separated into three groups: live cells will show only a low level of fluorescence; apoptotic cells will show a higher level of blue fluorescence, and dead cells will show low-blue and high-red fluorescence. Confirm the flow cytometer results by viewing the cells under a fluorescence microscope.

Note:

1. Propidium iodide (PI) has toxicity. Gloves, protective clothing, and eyewear should be worn and safe laboratory practices followed.
2. Control the incubated time for cells and Hoechst 33342 seriously. It should be less than 20 minutes.

VI. RELATED PRODUCTS

Cell Apoptosis PI Detection Kit: Cat.No.L00311

Cell Apoptosis DAPI Detection Kit: Cat.No.L00312

Cell Cycle Analysis Kit: Cat.No.L00287

VIII. ORDERING INFORMATION

Double Stain Apoptosis Detection Kit (Hoechst 33342/PI): Cat.No. L00309.

For Research Use Only.

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