Hoechst 33342/PI Double Staining Kit

User Manual

Catalog # CRG1017

Used for double fluorescence staining.

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

I. INTRODUCTION

Hoechst 33342 is a cell permeable fluorescent minor groove-binding probe for DNA, and through the living cell membrane. The Hoechst 33342 and DNA complex show light blue flourescent color. Propidium iodide (PI) has been widely used as a fluorescent stain for DNA in cells; it specificity binds double-stranded nucleic acids in apoptosis, necrosis and fixed cells, but can not enter the normal living cell. PI and DNA complex show light red flourescent color. Hoechst 33342 and PI have been used for double fluorescence staining.

This product is 1mg/ml chromogen. Solute it with suitable density for applies. The recommend density is 1-5 μg/ml.

II. KIT COMPONENTS

Component	Volume	Storage
Hoechst 33342 Chromogen (1 mg/ml)	100 μl x 1	-20 °C
PI Chromogen (1 mg/ml)	100 μl x 1	-20 °C
Dilution Buffer	20 ml x 2	4 °C
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III. STORAGE AND STABILITY

Store at 2-8 °C for short time; -20 °C for long time. Each component is stable for up to 12 months.

IV. PROCEDURE

1. For double or triple fluorescence staining in immunofluorescence tests, the Hoechst 33342/PI staining is the last step after all fluorescence antibodies incubation;

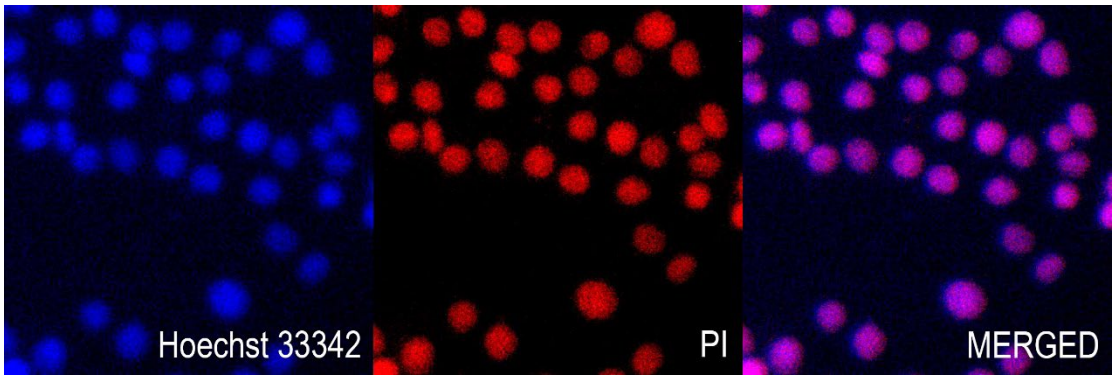
2. For culture cell, add 10 µl Hoechst 33342/PI Chromogen equally to 2 ml Dilution Buffer in the same tube and mix them (the end density is 5 µg/ml); incubate about 5 minutes in a dark incubator, at 30 °C; and then wash it with PBS/TBS 3 times for 3 minutes each time;

3. Observation with the fluorescence microscope.

Note:

1. The Hoechst 33342/PI are faded with light, all experiment process need keep away from light;
2. The Hoechst 33342/PI are suspected carcinogens, operation with gloves.

V. DATA



Immunofluorescent analysis staining in PC12 cells. Methanol-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5 - 10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with the primary antibody in 3% BSA-PBS and incubated overnight at 4 °C in a humidified chamber. Cells were washed with PBST at room temperature in the dark. Hoechst 33342 was used to stain the cell nuclei (Blue). PI was used to stain the cell nuclei (red).

V. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VI. NOTES