DAPI Staining Kit

Cat#: orb219885 (User Manual)

I. INTRODUCTION

DAPI (4',6-diamidino-2-phenylindole) is a cell permeable fluorescent minor groove-binding probe for DNA. It binds to the double-stranded DNA (especially to AT rich DNA), and forming a stable fluoresces complex. DAPI throughout the live and dead cell membrane that can be utilized for DAN detect for chromosome DAN, Yeast DNA, chloroplast DNA, Virus DNA and so on. DAPI-DNA complex shows light blue fluorescence color with excitation light 364 nm and emission light 454 nm. This product is 1mg/ml Chromogen. Solute it with suitable density for applies. Recommend density for tissue staining is 1-2 ug/ml (on the 0.5 ug/ml, fluorescence become light saturation). For cell culture the density is 0.1 ug/ml.

II. KIT COMPONENTS

Component	Volume	Storage
DAPI Chromogen (1mg/ml)	100 ul x 1	2 to 8 °C
Dilution Buffer	25 ml x 4	2 to 8 °C
Technical Manual	1 Manual	

III. STORAGE AND STABILITY

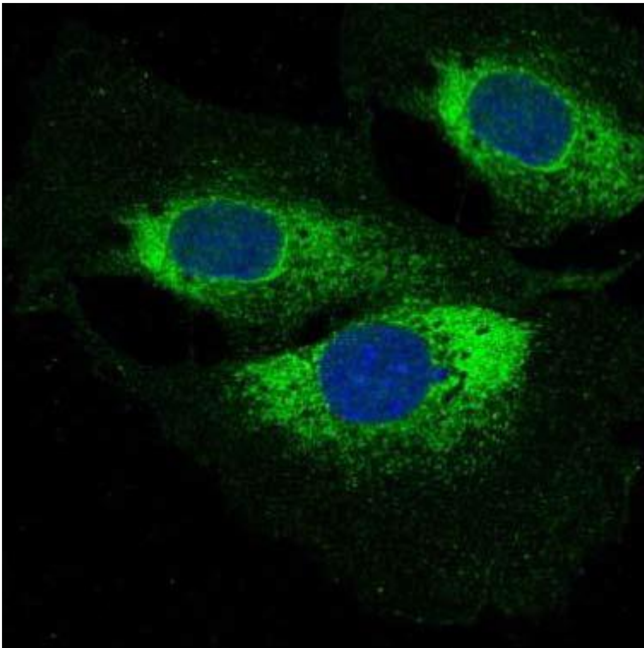
Store at 2-8 °C for short time; -20 °C for long time. Each component is stable for up to 12 months.

IV. PROCEDURE

1. For double or triple fluorescence staining in immunofluorescence tests, the DAPI staining is the last step after all fluorescence antibodies incubation; 2. For tissue cell staining, apply 10 - 20 ul Chromogen to 10 ml Dilution Buffer in tube and mix (the end density is 1 - 2 ug/ml); incubate the tissue with DAPI buffer about 15 - 30 minutes, at 30 °C; and then wash it with PBS/TBS for 3 times; 3. For culture cell, the DAPI density is 0.5 ug/ml (apply 5 ul Chromogen to 10 ml Dilution Buffer) may be suitable; incubate the cell with DAPI buffer about 15-30min, at 30 °C; 4. Cover the microscope cover glass for observation.

NOTE: It's better for observation and operation when the section is dried in no dark box before adding anti-fading buffer.

V. DATA



Immunofluorescent analysis staining in HeLa cells. Formalin-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 and incubated with a FITC-conjugated secondary antibody (green) in PBS at room temperature in the dark. DAPI was used to stain the cell nuclei (blue).

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