

## Hoechst 33342 Staining Kit

### Cat#: orb219886 (Product Manual)

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

#### INTRODUCTION

Hoechst 33342 is a cell permeable fluorescent minor groove-binding probe for DNA, and specific stain for AT-rich regions of double-stranded DNA. This fluorescent dye has been used in sorting living cells, used in flow cytometry for the determination of DNA and used for visualization of chromatin distribution in living cells. Hoechst 33342 and DNA complex show light blue fluorescent color with excitation light 355 nm and emission light 465 nm.

This product is 5 mg/ml chromogen. Solute it with suitable density for applies. Recommend density is 5 µg/ml.

#### KIT COMPONENTS

Component	Volume	Storage
Hoechst 33342 Chromogen (5mg/ml)	100 µl x 1	-20 °C
Dilution Buffer	25 ml x 4	4 °C
Technical Manual	1 Manual	

#### STORAGE AND STABILITY

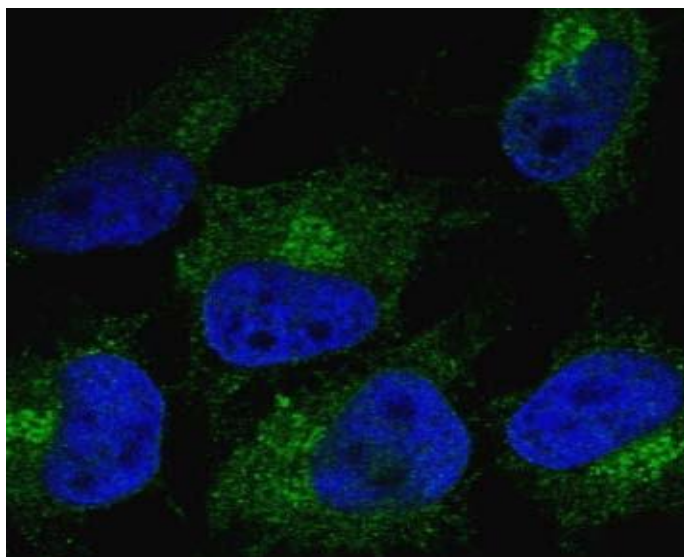
Store at 2-8 °C for short time; -20 °C for long time. Each component is stable for up to 12 months.

#### PROCEDURE

1. For double or triple fluorescence staining in immunofluorescence tests, the Hoechst 33342 staining is the last step after all fluorescence antibodies incubation;
2. For culture cell, add 10 ul Chromogen to 10 ml Dilution Buffer in tube and mix (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 is faded with light, all experiment process need keep away from light;
2. The Hoechst 33342 is suspected carcinogens, operation with gloves.

**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. Hoechst 33342 was used to stain the cell nuclei (blue).