Calcein-AM/PI, Live/Dead Cell Double Staining Kit

Cat #: B-CHK103

Size: 500 T

Storage: Store at -20°C

Product Introduction

Calcein-AM is a cell dye that can be used for fluorescent labeling of live cells. It penetrates the cell membrane and is cleaved by intracellular esterases to produce Calcein, which is retained within the cells and emits strong green fluorescence. Compared to other similar reagents such as BCECF, AM and CFDA, Calcein-AM has low cytotoxicity. The excitation and emission wavelengths of Calcein are 490 nm and 515 nm, respectively.

Calcein-AM specifically stains live cells. On the other hand, PI, as a nuclear staining dye, cannot penetrate the cell membrane of live cells. It can pass through the disordered regions of the membrane of dead cells and reach the cell nucleus, where it intercalates into the DNA double helix, producing red fluorescence (excitation: 535 nm, emission: 617 nm). Therefore, PI specifically stains dead cells. Since both Calcein and PI-DNA can be excited at 490 nm, live cells and dead cells can be observed simultaneously using fluorescence microscopy. When excited at 545 nm, only dead cells are observed.

Due to the different optimal staining conditions for different cell lines, it is recommended to determine the appropriate concentrations of Calcein-AM and PI individually.

Kit components and Storage:

Kit component	Size	Shelf life
Calcein.AM	200ug	Store at -20°C in a dry and dark place. Shelf life is one year.
PI Stock solution(1.5 mmol/l)	200ul	
DMSO	200ul	

Note: 500T refers to the number of detections achievable when using a working solution of Calcein-AM at 2 μmol/L and a working solution of PI at 4.5 μmol/L, with each usage amounting to 100 μL.

Staining Protocol:

(1) Prepare a 1 mM Calcein-AM stock solution. Add 200 μL of DMSO to a tube containing 200 μg of Calcein-AM powder and vortex to dissolve using a pipette. Store the solution protected from light and use it as soon as possible to avoid repeated freeze-thaw cycles.
(2) Bring the Calcein-AM stock solution and PI stock solution to room temperature before use. Prepare the working solutions using PBS.

- The recommended concentration for Calcein-AM working solution is 2 μmol/L, and for PI working solution is 4.5 μmol/L.
- The working solution concentrations can be adjusted according to the specific requirements of the cells.
- It is recommended to prepare the staining working solutions immediately before use.

(3) Mix 100 μL of the staining working solution with 200 μL of cell suspension and incubate at 37°C for 15 minutes.

(4) Observe the live cells exhibiting yellow-green fluorescence and the dead cells exhibiting red fluorescence simultaneously under excitation at 490±10 nm. Additionally, observe the dead cells separately under excitation at 545 nm.

Note:

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.