Propidium Iodide (PI) Solution(750 μM)

Cat. No: E-CK-A165 **Size: 100 Tests/500 Tests/500 Tests×10**

Cat.	Products	100 Tests	500 Tests	500 Tests×10	Storage
E-CK-A165	Propidium Iodide (PI) Solution(750 μM)	100 μL	500 μL	500 μL× 10	2-8℃/-20℃, shading light
Manual		One Copy			

Storage

Store at -20°C for 12 months.

Introduction

PI is a kind of nucleic acid dye. Due to the loss of cell membrane selective permeability of dead cells, Propidium Iodide (PI) can enter the cell and specifically bind with double-stranded DNA and produce red fluorescence (Ex/Em=535nm/617nm), thus marking dead cells.

Experimental Procedure

1 Flow cytometry detection

1.1 Preparation of working solution

1.1.1 Reagent preparation: Take out the frozen PI Solution (750 μM), after thawing at room temperature, vortex mixing each reagent.

1.1.2 Preparation of PI staining working solution: After thawing at room temperature, the vortex-mixed PI solution were prepared into the staining working solution at a ratio of 1~5×10^5 cells/200 μL. Prepare the staining working solution according the number of samples. Please refer to the table below.

Component	PI staining working solution		
	1 mL	5 mL	10 mL
PI Solution (750 μM)	1 μL	5 μL	10 μL
PBS	1 mL	5 mL	10 mL

1.2 Staining procedure

1.2.1 Collect the cells, centrifuge at 300×g for 5 min, discard the supernatant. Add 1 mL of PBS to resuspend the cells, centrifuge at 300×g for 5 min, discard the supernatant. Wash repeatedly 1 time, discard the supernatant.

1.2.2 Add 200 μL of PI staining working solution to resuspend 1~5×10^5 cells in each group and incubate for 5~15 min at room temperature in the dark.

1.2.3 After incubation, flow cytometry can be performed directly. If it cannot be detected in time, it is recommended to avoid light and place in a 4°C refrigerator for detection within 2 hours.

Note: PI can be detected in PE or Percp/Cy5.5 channel. Calcein AM Solution (100 μM) (E-CK-A164) can be selected for co-staining when it is necessary to distinguish living cells.

2 Fluorescence microscope detection

2.1 Preparation of working solution

2.1.1 Reagent preparation: Take out the frozen PI Solution (750 μM), after thawing at room temperature, vortex mixing each reagent.

2.1.2 Preparation of PI staining working solution: After thawing at room temperature, the vortex-mixed PI solution were prepared into the staining working solution according to 100 μL per well of 96-well plate or 200 μL per well in a 24-well plate. Prepare the staining working solution according the number of samples. Please refer to the table below.

Component	Volume of PI staining working solution		
	1 mL	5 mL	10 mL
PI Solution (750 μM)	10 μL	50 μL	100 μL
PBS	1 mL	5 mL	10 mL

2.2 Staining process

2.2.1 Carefully remove the culture medium of adherent cells, add an appropriate amount of PBS to each well to wash cells, repeat wash the slides and remove PBS.

2.2.2 Add PI staining working solution in a ratio of 100 μL per well in a 96-well plate or 200 μL per well in a 24-well plate and incubate at 37°C for 10~30 min.

2.2.3 Observe under fluorescence microscope after incubation (Calcein is green fluorescent, Ex/Em=494nm/517nm; PI is red fluorescent, Ex/Em=535nm/617nm).

Note1: For suspended cells, after collecting cell precipitation, add Calcein AM/PI staining working solution at a ratio of 1~5×10^5 cells/200 μL and incubate at room temperature for 15~20 min. Add the cell suspension to the glass slide, cover the cover glass gently, and then observe under the microscope.

Note 2: Calcein AM Solution (100 μM) (E-CK-A164) can be selected for co-staining when it is necessary to distinguish living cells (Calcein is green fluorescent, Ex/Em=494nm/517nm).

Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. Please store the product at the appropriate temperature to avoid failure.
4. Mn^{2+} has fluorescence quenching effect, so pay attention not to contain metal ions such as Mn^{2+} in the washing buffer
5. Excessive acceleration and deceleration of centrifuge may cause cell loss. It is suggested to adjust the acceleration no more than 3 and deceleration no more than 2, that is, Acc≦3, Dec≦2.

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