



Propidium Iodide

Product Information

Product Name	Cat. No	Spec.
PI Staining Solution	AO-03-G1021	3x10 mL

Product Description/Introduction

PI, or propidium iodide, is an analog of ethidium bromide that binds strongly to DNA, and it releases red fluorescence upon embedding in double-stranded DNA to achieve staining of the DNA or nucleus. PI cannot penetrate intact cell membranes but can penetrate the broken cell membranes of late apoptotic cells and dead cells, and utilizing this feature, PI is usually used in combination with fluorescent probes such as Calcein-AM or FDA to stain and observe dead cells, or flow cytometry is used for relative quantitative detection of apoptosis and cell cycle. The PI-double-stranded DNA complex has a maximum excitation wavelength of 535 nm and a maximum emission wavelength of 615 nm.

The PI staining solution is a ready-to-use cell impermeable fluorescent solution with a concentration of $100 \mu g/mL$. It can be directly used to stain the nuclei of necrotic cells or tissues, and the cell suspension can be used to detect cell cycle by flow cytometry after staining.

Storage and Shipping Conditions

Ship with wet ice; store at 2-8°C protecting from light, valid for 6 months.

ProductContents

Component	AO-03-G1021
PI Staining Solution	3x10 mL
Manual	One copy

Assay Protocol / Procedures

I Flow cytometry assays for detection of cell cycle:

- 1. After digesting cells, wash cells with PBS, pellet cells by centrifugation at low-speed, and remove supernatant.
- 2. Slowly add 1-3 mL of 90% ethanol precooled at 20°C, resuspend cells. and incubate in ice bath for overnight.
- 3. Collect cells by centrifugation at 1,500 rpm for 5min, resuspended with PBS, and centrifuged again to remove the supernatant.
- 4. Add 250 µL PBS to resuspend the cells.
- 5. Add 2 μ L of 1 mg/mL RNase A **(recommend G3405)**, and then incubate the mixture for 40 min in water bath at 37°C.





- $6. Add 50 \, \mu L$ PI Staining Solution and incubate for 20 min at room temperature, protected from light. (the length of time can be adjusted according to the staining results of experimental materials).
- 7. Detected by flow cytometry.

Il Fluorescence Microscopy assays for identification of dead cells:

- 1. Remove the culture medium, and wash the cells twice with PBS.
- 2. Dilute the PI Staining Solution 1:20-1:10 in PBS to obtain a final concentration of 5-10 μ g/mL PI staining working solution.
- 3. Add appropriate amount of PI staining working solution per well. Incubate for 5-10 minutes at room temperature, protected from light.
- 4. Remove the PI staining working solution. Add appropriate amount of PBS to each well and observed by fluorescence microscope.

Note: The nucleus of dead or late apoptotic cells show red observed by fluorescence microscope.

Note

- 1. All fluorescent dyes are quenched, and it is recommended to complete the detection on the same day after stained.
- 2. Prepare the working solution according to a 10-fold dilution, and add 0.2 mL of working solution dropwise per sample. This product can be used for approximately 500 staining.
- 3. For your safety and health, please wear a lab coat and disposable gloves during operation.

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