MediRes Corp.

USER GUIDE **G1021**

Propidium Iodide (PI) Stain (100 µg/mL)

Product #: G1021
Quantity: 5 x 1mL

Product Description

- PI (propidium iodide) is an analog of ethidium bromide.
- Binds strongly to DNA and releases red fluorescence upon embedding in double-stranded DNA, staining DNA or nuclei.
- Cannot penetrate intact cell membranes, but can penetrate broken membranes of late apoptotic and dead cells.
- Commonly used with fluorescent probes like Calcein-AM or FDA to stain and observe dead cells.
- Used in flow cytometry for relative quantitative detection of apoptosis and cell cycle.
- PI-double-stranded DNA complex has a maximum excitation wavelength of 535 nm and a maximum emission wavelength of 615 nm.
- PI staining solution is a ready-to-use, cell-impermeable fluorescent solution at a concentration of 100 µg/mL.
- Can be directly used to stain nuclei of necrotic cells or tissues.
- Cell suspensions can be used to detect cell cycle by flow cytometry after staining.

Storage

- Store at 2-8°C and protect from light.

Important Notes (PLEASE READ CAREFULLY)

1. All fluorescent dyes are quenched over time; therefore, it is recommended to complete detection on the same day as staining.
2. Prepare the working solution with a 10-fold dilution and add 0.2 mL dropwise per sample. This product is sufficient for approximately 500 stains.
3. For safety and health, wear a lab coat and disposable gloves during use.

Protocol

Flow cytometry assay for cell cycle detection:

1. Digest cells, wash with PBS, pellet by low-speed centrifugation, and remove the supernatant.
2. Slowly add 1-3 mL of 90% ethanol precooled to -20°C, resuspend cells, and incubate in an ice bath overnight.
3. Collect cells by centrifugation at 1,500 rpm for 5 minutes, resuspend with PBS, and centrifuge again to remove the supernatant.
4. Resuspend cells in 250 µL of PBS.
5. Add 2 µL of 1 mg/mL RNase A (recommended: **Bi2M-RNaseA**) and incubate the mixture for 40 minutes in a 37°C water bath.
6. Add 50 µL of **PI Staining Solution** and incubate for 20 minutes at room temperature, protected from light (adjust incubation time based on staining results).
7. Analyze using flow cytometry.

Fluorescence microscopy assay 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 achieve a final concentration of 5-10 µg/mL.
3. Add an appropriate amount of the PI staining working solution per well and incubate for 5-10 minutes at room temperature, protected from light.
4. Remove the PI staining working solution, add an appropriate amount of PBS to each well, and observe using a fluorescence microscope.

Note: *The nuclei of dead or late apoptotic cells will appear red under the fluorescence microscope.*