**For Research Use Only. Not For Use In Diagnostic Procedures**

USER GUIDE **MesGen Biotechnology**

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| **碘化丙啶染液** |

**Propidium iodide solution 1.0mg/ml**

**Catalog Number** MG5015

**Size :** 1 ml□ 10ml□ (1.0mg/ml, 1.0mg per ml in water )

**CAS :**  25535-16-4 **Purity** ≥ 94% (HPLC)

**Molecular Formula :** C27H34I2N4

**Molecular Weight :** 668.39

**Technical literature is available at:** [**www.mesgenbio.com**](http://www.mesgenbio.com)**. E-mail MesGen Technical Services if you have questions on use of this system:** **tech@mesgenbio.com**

**Introduction**

Propidium iodide (PI) is a fluorescent dye that intercalates into double-stranded nucleic acid. It is excluded from viable cells, but can penetrate cell membranes of dead or dying cells. Therefore, it is widely used for evaluation of cell death and apoptosis or for determination of DNA content in cell cycle analysis. The fluorescence emission maximum for DNA-bound PI is about 615–620 nm. When excited by a 488 nm laser, PI can therefore be detected in both, the red fluorescence channel commonly used for R-phycoerythrin (PE)-Cy5 tandem dye detection as well as the yellow fluorescence channel commonly used for PE detection. PI can be used in combination with other fluorochromes excited at 488 nm such as fluorescein isothiocyanate (FITC).

**Fluorescence Microscopy Protocols for Adherent Cells**

**1.** Equilibrate the sample briefly in 2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0).

**2.** Incubate the sample in 100 μg/mL DNase-free RNase in 2X SSC for 20 minutes at 37°C.

**3.** Rinse the sample three times, 1 minute each, in 2X SSC.

**4.** Equilibrate the sample in 2X SSC.

**5.** Make a 500 nM solution of PI by diluting the 1 mg/mL (1.5 mM) stock solution 1:3000 in 2X SSC. About 300 μL is usually enough stain for one coverslip preparation. Incubate the cells, covered with the dilute stain, for 1–5 minutes.

**Version 2.0**

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**Do not eat**

**Flow Cytometry Protocols for Cells**

**1.** Collect a volume of cell suspension corresponding to 2 × 105 to 1 × 106 cells. Pellet the cells by centrifugation. Discard the supernatant, tap the tube to resuspend the pellet in the residual liquid and add 1 mL of phosphate buffered saline (PBS) at room temperature.

**2.** Transfer the full volume of resuspended cells to 4 mL of absolute ethanol at –20°C by pipetting the cell suspension slowly into the ethanol while vortexing at top speed. Leave the cells in ethanol at –20°C for 5–15 minutes.

**3.** Pellet the cells by centrifugation, discard the ethanol, tap the tube to loosen the pellet, and add 5 mL of PBS at room temperature. Allow the cells to rehydrate for 15 minutes.

**4.** Make a 3 μM solution of PI by diluting the 1 mg/mL (1.5 mM) stock solution 1:500 in staining buffer (100 mM Tris, pH 7.4, 150 mM NaCl, 1 mM CaCl2, 0.5 mM MgCl2, 0.1% Nonidet P-40). A 1 mL volume will be required for each cell sample.

**5.** Centrifuge the cell suspension from step 3, discard the supernatant, tap to loosen the pellet, and add 1 mL of PI diluted in staining buffer. Incubate for 15 minutes at room temperature and analyze by flow cytometry in the presence of the dye. If the cells are to be viewed by fluorescence microscopy, centrifuge the sample, remove the supernatant, and resuspend the cells in fresh buffer. Apply a drop of the suspension to a microscope slide, cover with a coverslip, and view using appropriate filters.

**Caution**

Propidium iodide is a suspected carcinogen; contact with eyes, skin, and mucous membranes should be avoided. Always wear proper protective clothing and gloves when handling the solution.

**Storage Temperature**

2-8°C and Protect from light

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