GeneCopoeia™
Expressway to Discovery

9620 Medical Center Drive, Suite 101
Rockville, MD20850, USA
Web: www.genecopoeia.com

Product Information

Propidium Iodide

Catalog Number	Packaging Size
C007	10 mg
C008	1 mL

Storage upon receipt:
- -20°C
- Protect from light

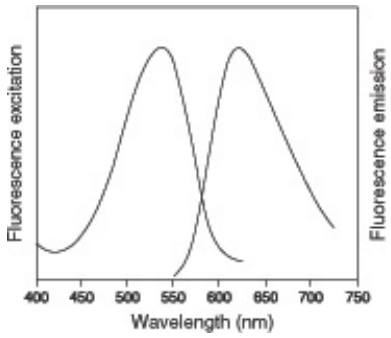
Ex/Em: 535/617 nm, bound to DNA

Product Description

Propidium iodide (PI) binds to DNA by intercalating between the bases with little or no sequence preference and with a stoichiometry of one dye per 4–5 base pairs of DNA. PI also binds to RNA, necessitating treatment with nucleases to distinguish between RNA and DNA staining. Once the dye is bound to nucleic acids, its fluorescence is enhanced 20- to 30-fold, the fluorescence excitation maximum is shifted ~30–40 nm to the red and the fluorescence emission maximum is shifted ~15 nm to the blue. Although its molar absorptivity (extinction coefficient) is relatively low, PI exhibits a sufficiently large Stokes shift to allow simultaneous detection of nuclear DNA and fluorescein-labeled antibodies, provided the proper optical filters are used. PI is suitable for fluorescence microscopy, confocal laser scanning microscopy, flow cytometry, and fluorometry.

PI is membrane impermeant and generally excluded from viable cells. PI is commonly used for identifying dead cells in a population and as a counterstain in multicolor fluorescent techniques. The counterstaining protocols below are compatible with a wide range of cytological labeling techniques—direct or indirect antibody-based detection methods, mRNA *in situ* hybridization, or staining with fluorescent reagents specific for cellular structures. These protocols can be modified for tissue staining.

Spectral Characteristics



Fluorescence excitation and emission profiles of propidium iodide bound to dsDNA.

Experimental Protocols

Counterstaining Adherent Cells for Fluorescence Microscopy

Sample Preparation

Use the fixation protocol appropriate for your sample. PI staining is normally performed after all other staining. Note that permeabilization of the cells is required for counterstaining with PI.

RNase Treatment

RNase treatment is required if the sample is fixed in paraformaldehyde, formaldehyde, or glutaraldehyde. If the sample is fixed with methanol/acetic acid or acetone, RNase treatment is usually not required.

1.1 Equilibrate the sample briefly in 2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0).
1.2 Incubate the sample in 100 µg/mL DNase-free RNase in 2X SSC for 20 minutes at 37°C.
1.3 Rinse the sample three times, 1 minute each, in 2X SSC.

Counterstaining Protocol

2.1 Equilibrate the sample in 2X SSC.
2.2 Prepare the PI stock solution by dissolving PI solid in dH_2O at 1 mg/mL (1.5 mM).
2.3 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.
2.4 Rinse the sample several times in 2X SSC. Drain excess buffer from the coverslip and mount in a medium with an antifade reagent.
2.5 View sample using a fluorescence microscope with appropriate filters.

Counterstaining Cells in Suspension for Flow Cytometry

Sample Preparation

Use the fixation protocol appropriate for your sample, or use the following protocol.
3.1 Collect a cell suspension of 2×10^5 to 1×10^6 cells.
3.2 Pellet the cells by centrifugation and discard the supernatant.
3.3 Tap the tube to resuspend the pellet in the residual liquid and add 1 mL of PBS at room temperature.
3.4 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.
3.5 Pellet the cells by centrifugation and discard the ethanol.
3.6 Tap the tube to loosen the pellet and add 5 mL of PBS at room temperature. Allow the cells to rehydrate for 15 minutes.

Counterstaining Protocol

4.1 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 $CaCl_2$, 0.5 mM $MgCl_2$, 0.1% Nonidet P-40). A 1 mL volume will be required for each cell sample.
4.2 Centrifuge the cell suspension from step 3.6, 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.

Chromosome FISH Counterstaining

Sample Preparation

Prepare the specimen according to standard procedures.[1,2] Briefly rinse the final preparations in dH_2O before counterstaining to remove residual buffer salts from the slide. This final rinse will help reduce nonspecific background staining on the glass. Allow the preparation to air dry.

Counterstaining Protocol

5.1 Make a 1.5 µM PI staining solution by diluting the 1 mg/mL (1.5 mM) stock solution 1:1000 in PBS. Pipet 300 µL of this staining solution directly onto the specimen. If necessary, RNase A (freshly made) may be added to a final concentration of 10 µg/mL. A plastic coverslip can be used to distribute the dye evenly on the slide.
5.2 Incubate the specimen in the dark for 30 minutes at room temperature, or at 37°C if RNase is included.
5.3 Remove the coverslip and rinse briefly with PBS or dH_2O to remove unbound dye.
5.4 Remove excess liquid from the slide by gently blotting around the sample with an absorbent tissue. Place a glass coverslip on the slide, and seal the edges with wax or nail polish. Alternatively, the preparation can be mounted in an antifade reagent according to the manufacturer's directions.
5.5 View sample using a fluorescence microscope with appropriate filters.

Reference

1. Methods Enzymol 168, 741 (1989);
2. Pardue, M.L. in *Nucleic Acid Hybridization, A Practical Approach*,. B.D. Hames and S.J. Higgins, Eds., IRL Press, Oxford, England (1985).