

Propidium Iodide Nucleic Acid Stain

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
Propidium iodide solid (including FluoroPure™ PI *) (P1304MP, P21493)	100 mg	<ul style="list-style-type: none"> • Room temperature • Protect from light 	When stored as directed, product is stable at least 1 year.
Propidium iodide solution (P3566)	1 mg/mL in water	<ul style="list-style-type: none"> • 2–6°C • Protect from light 	When stored as directed, solution is stable at least 6 months.

* ≥98% purity.

Approximate fluorescence excitation/emission maxima: 535/617 nm, bound to nucleic acids

Introduction

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.¹ 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.² 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.

Before You Begin

Materials Required but Not Provided

See the protocols below to determine materials required for your particular use of PI.

Fluorescence Microscopy

- 2X SSC
- DNase-free RNase
- Antifade reagent, such as *SlowFade*[®] Gold (S36936) or ProLong[®] Gold (P36930) antifade reagents

OR

Flow Cytometry

- PBS
- Ethanol
- Tris staining buffer (see step 4.1)

OR

Chromosome FISH

- dH₂O
- PBS
- RNase A
- Antifade reagent, *optional*

Making a Stock Solution from Solid PI

To make a stock solution from the solid form, dissolve PI (MW = 668.4) in deionized water (dH₂O) at 1 mg/mL (1.5 mM) and store at 2–6°C, protected from light. When stored properly, solutions are stable for at least six months.

Caution

PI is a potential mutagen and should be handled with care. The dye must be disposed of safely and in accordance with applicable local regulations.

Fluorescence Spectral Characteristics

When bound to nucleic acids, the fluorescence excitation maximum for PI is 535 nm and the emission maximum is 617 nm (Figure 1). PI can be excited with a xenon or mercury-arc lamp or with the 488 line of an argon-ion laser. Generally, PI fluorescence is detected in the FL2 channel of flow cytometers.

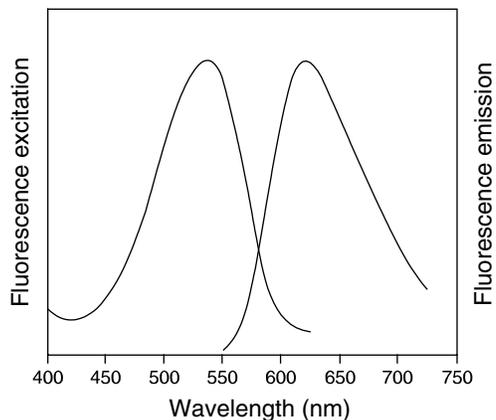


Figure 1. Fluorescence excitation and emission profiles of propidium iodide bound to dsDNA.

Experimental Protocols

Counterstaining Adherent Cells for Fluorescence Microscopy

Preparing the Sample

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

- 2.1 Equilibrate the sample in 2X SSC.
- 2.2 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.

- 2.3 Rinse the sample several times in 2X SSC. Drain excess buffer from the coverslip and mount in a medium with an antifade reagent such as Molecular Probes *SlowFade*[®] Gold antifade reagent (S36936) or ProLong[®] Gold antifade reagent (P36930).
- 2.4 View sample using a fluorescence microscope with appropriate filters (see *Fluorescence Spectral Characteristics*).

Counterstaining Cells in Suspension for Flow Cytometry

Preparing the Sample

Use the fixation protocol appropriate for your sample, or use the following protocol.

- 3.1 Collect a volume of cell suspension corresponding to 2×10^5 to 1×10^6 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.
- 3.2 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.
- 3.3 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.

Counterstaining

- 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 3.3, 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 (see *Fluorescence Spectral Characteristics*).

Counterstaining the Specimen by Chromosome FISH

Preparing the Sample

Prepare the specimen according to standard procedures.^{3,4} Briefly rinse the final preparations in dH_2O before counterstaining to remove residual buffer salts from the slide. Air dry. This final rinse will help reduce nonspecific background staining on the glass.

Counterstaining

- 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 $\mu\text{g}/\text{mL}$. 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 dH₂O 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 (see *Fluorescence Spectral Characteristics*).

References

1. J Mol Biol 13, 269 (1965); 2. Methods Cell Biol 30, 417 (1989); 3. Methods Enzymol 168, 741 (1989); 4. Pardue, M.L. in *Nucleic Acid Hybridization, A Practical Approach*, B.D. Hames and S.J. Higgins, Eds., IRL Press, Oxford, England (1985).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat #	Product Name	Unit Size
P1304MP	propidium iodide	100 mg
P21493	propidium iodide *FluoroPure™ grade*	100 mg
P3566	propidium iodide *1.0 mg/mL solution in water*	10 mL

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