# Propidium Iodide

Cat. No. CTG-AP0013 Store the kit at -15 to -25°C

## Experimental Protocol

### Making a Stock Solution

from Solid PI To make a stock solution from the solid form, dissolve PI (MW = 668.4) in deionized water ($dH_2O$) 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.

### Counterstaining Adherent Cells for Fluorescence Microscopy

1. Preparing the Sample

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

2. RNase Treatment

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

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.

3. Counterstaining

1) Equilibrate the sample in 2X SSC.
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.
3) Rinse the sample several times in 2X SSC. Drain excess buffer from the coverslip and mount in a medium with an anti-fade reagent.
4) View sample using a fluorescence microscope with appropriate filters.

### Counterstaining Cells in Suspension for Flow Cytometry

1. Preparing the Sample

1) Use the fixation protocol appropriate for your sample, or use the following protocol.
2) Collect a volume of cell suspension corresponding to $2 \times 10^5$ to $1 \times 10^6$ 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) 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.
4) 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.

2. Counter-staining

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.

2) Centrifuge the cell suspension, discard the superna-tant, 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.

**Counterstaining the Specimen by Chromosome FISH**

1. Preparing the Sample

Prepare the specimen according to standard procedures.Briefly rinse the final preparations in $dH_2O$ before counter-staining to remove residual buffer salts from the slide. Air dry. This final rinse will help reduce nonspecific background staining on the glass.

2. Counterstaining

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.
2) Incubate the specimen in the dark for 30 minutes at room tempera-ture, or at 37°C if RNase is included.
3) Remove the coverslip and rinse briefly with PBS or dH2O to remove unbound dye.
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. Alter-natively, the preparation can be mounted in an antifade reagent accor-ding to the manufacturer's directions.
5) View sample using a fluorescence microscope with appropriate filters.

**References**

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