Propidium Iodide
Catalog #638

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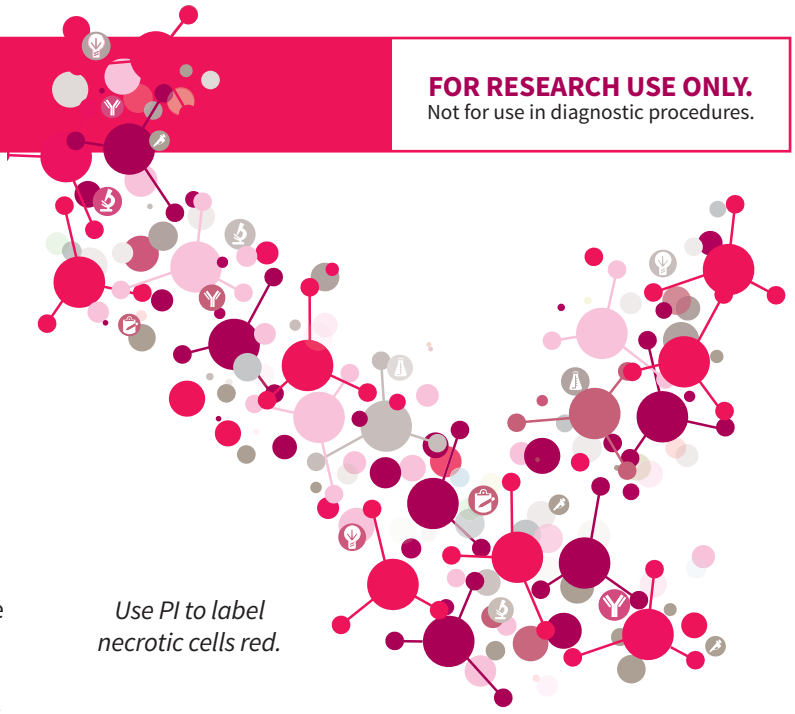
Not for use in diagnostic procedures.



Use PI to label necrotic cells red.

INTRODUCTION

Propidium iodide (PI, catalog #638) is an intercalating red fluorescent reagent that binds between the base pairs of DNA in membrane-compromised cells and is used to identify necrotic and apoptotic cells. As PI is membrane impermeant, it cannot reach the DNA in viable cells, thus allowing the identification of cells with permeabilized membranes in a population. PI distinguishes between living and dead cells by counterstaining nucleic acids red in necrotic, dead, apoptotic and membrane-compromised cells, while the DNA in healthy cells remains unstained.

Propidium iodide can be used with ICT's FAM-FLICA® caspase inhibitor reagents (such as catalog #92) to identify four populations of cells: living; early apoptotic; late apoptotic; and necrotic (Figures 2 and 3).

Propidium iodide is provided ready-to-use at 250 µg/mL. Just add it to the cell culture media, incubate for a few minutes, and analyze. One molecule of PI stoichiometrically binds every four to five base pairs of DNA. Unbound PI excites at 488-492 nm and exhibits an emission maximum at 635 nm. Upon binding to DNA, the fluorescence of PI is enhanced 20-30 fold. When bound to nucleic acids, the maximum absorption is 535 nm and the maximum emission is 617 nm (Figure 1). Cells may be viewed through a fluorescence microscope or analyzed with a flow cytometer. PI is for research use only. Not for use in diagnostic procedures.

HOW TO USE

Propidium iodide is supplied ready-to-use at 250 µg/mL.
To stain nucleic acids in membrane-compromised cells (necrotic, dead, apoptotic cells):

1. Add PI to the cell media at 0.5% v/v. For example, add 1.5 µL PI to 300 µL of cells.
2. Incubate 5-10 minutes at room temperature.
3. Analyze with a fluorescence microscope or flow cytometer. When bound to DNA, PI has a maximum absorption at 535 nm and a maximum emission at 617 nm (Figure 1).
4. If visualizing with a fluorescence microscope, PI can be detected with an excitation between 490-550 nm and emission at 635 nm.
5. If analyzing with a flow cytometer, use a blue laser at 488 nm or a green laser at 525 nm. Read the fluorescent output in FL-2 or FL-3.

SPECIFICATIONS

- 1 mL at 250 µg/mL
- Pale pink liquid
- pH 4.5 - 6.5

STORAGE

- 2-8°C
- Shelf-life up to 24 months when refrigerated and protected from light.

SAFETY

- See Safety Data Sheet (SDS) for any warnings.
- SDS available at www.immunochemistry.com and by calling ICT at 952-888-8788 or 800-829-3194.
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FIGURE 1: PROPIDIUM IODIDE (NUCLEIC ACID-BOUND) EXCITATION AND EMISSION SPECTRA

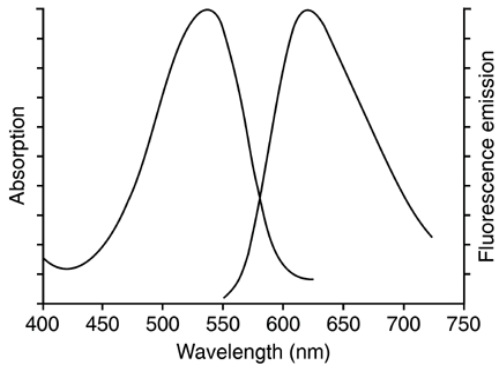

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FIGURE 2: FOUR POPULATIONS OF HL-60 CELLS

HL-60 cells (human promyelocytic leukemia) were treated with a drug, then stained with ICT's FLICA® FAM-VAD-FMK poly-caspase inhibitor reagent (catalog #92) and propidium iodide (PI), a red vital stain. Cells were analyzed on a scanning laser cytometer to detect red on the X-axis (PI, necrosis) and green on the Y-axis (FLICA®, apoptosis). Four populations of cells were detected:

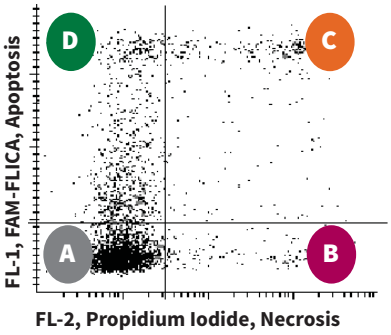
(A) Unstained live cells do not fluoresce

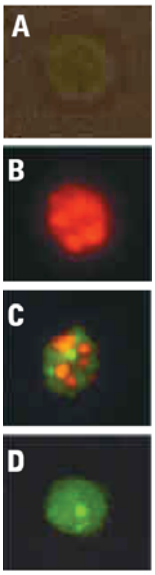
(B) Necrotic cells fluoresce red with PI

(C) Cells in late apoptosis are dual stained with green FAM-FLICA® caspase probe and red PI

(D) Cells in early apoptosis fluoresce green with FAM-FLICA®

In this experiment, the drug triggered the caspase cascade rather than being overtly toxic to the cells. Data courtesy of Dr. Zbigniew Darzynkiewicz, Brander Cancer Center, NY.





A: Live FLICA- PI-

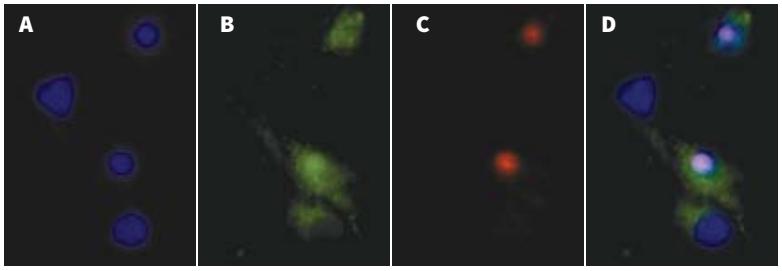
B: Necrotic FLICA- PI+

C: Late apoptotic FLICA+ PI+

D: Early apoptotic FLICA+ PI-

FIGURE 3: CELL DEATH IN PRIMARY RAT HIPPOCAMPAL NEURONS

Propidium iodide (PI, catalog #638), ICT's FAM-FLICA® caspase-3/7 inhibitor reagent (catalog #94), and Hoechst stain (catalog #639) were used to assess cell death in primary rat hippocampal neurons. Subjects were first-generation descendants of Sprague–Dawley albino rats. Hippocampi from postnatal day 0 male pups were used for primary cultures of hippocampal neurons. Cells were plated on 25-mm poly-l-lysine-coated coverslips at 300,000 cells per coverslip and were analyzed at 4 or 8 days *in vitro*. (A) Four cells are revealed by labeling the DNA blue with Hoechst. (B) Three apoptotic cells fluoresce green with FLICA® FAM-DEVD-FMK. (C) Two of the apoptotic cells have progressed to secondary necrosis and are in the late stages of apoptosis. They have compromised membranes (PI-positive, C) and also have active caspases (FLICA®-positive, B). In the composite image (D), only one cell out of four is healthy (blue) and the other three cells are apoptotic (green). Data courtesy of Dr. Z. Kahraman Akozer, University of Maryland.



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Thank you for using propidium iodide!
If you have any questions, or would like to share your data, please contact us at help@immunochemistry.com.

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ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.

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