Propidium Iodide (PI) is a red-fluorescent cell viability dye which is excluded from live cells with intact membranes, but penetrates dead or damaged cells and binds to DNA and RNA by intercalating between the bases. It is widely used as a counterstain to differentiate and exclude non-viable cells in flow cytometric analyses, and can be excited using blue (488 nm), green (532 nm), or yellow-green (561 nm) laser lines, with detection in the FL2 or FL3 channels. PI is used in DNA fluorescence imaging applications to discriminate early and late stages of apoptosis, to study cell-mediated cytotoxicity, and for chromosome analysis. It is also commonly used in quantitative DNA assays.

**Chemical Name:** 3,8-diamino-5-[3-(diethylmethylammonio)propyl]-6-phenylphenanthridinium diiodide
**Alternative Names:** 3,8-Diamino-5-[3-(diethyl(methyl)ammonio)propyl]-6-phenylphenanthridinium diiodide; PI; Propidium diiodide

**CAS Number:** 25535-16-4
**Chemical Formula:** C_{27}H_{34}N_{4} · 2I
**Molecular Weight:** 668.4 g/mol
**Excitation Wavelength:** 488 - 535 nm (DNA or RNA complex)
**Emission Wavelength:** 617 nm (DNA or RNA complex)

**Properties**

- **Storage:** Store at -20°C.
- **Shelf Life:** Product stable until expiry date (EXP) on label. Protect product from prolonged exposure to light.
- **Format/Formulation:** A crystalline solid

**Applications**

- **Verified:** FC
- **Reported:** FC, FISH, Fluorescence microscopy, Fluorometry, ICC
- **Special Applications:** This product has been verified for viability assessments of cells isolated with EasySep™ and RosetteSep™ kits.

Abbreviations: CellSep: Cell separation; ChIP: Chromatin immunoprecipitation; FA: Functional assay; FC: Flow cytometry; ICC: Immunocytochemistry; IF: Immunofluorescence microscopy; IHC: Immunohistochemistry; IP: Immunoprecipitation; RIA: Radioimmunoassay; WB: Western blotting
Handling/Directions for Use

For preparing a stock solution, PI is soluble in aqueous buffers and organic solvents, as follows:
- Phosphate-buffered saline (PBS), pH 7.2 ≤ 2 mg/mL
- Ethanol ≤ 0.2 mg/mL
- Dimethyl sulfoxide (DMSO) ≤ 2.5 mg/mL
- Dimethyl formamide (DMF) ≤ 3.3 mg/mL

NOTE: If preparing stock solution using an organic solvent, further dilute into aqueous buffer or isotonic saline before performing biological experiments. Ensure that the residual amount of organic solvent is insignificant, as it may have physiological effects at low concentrations.

Whenever possible, prepare and use stock solution on the same day. Protect stock solution from prolonged exposure to light. If stock solution must be made in advance, aliquot and store in tightly sealed vials at -20°C and protect from prolonged exposure to light. Generally these will be stable for up to 1 month.

FLOW CYTOMETRY
1. Prepare a 1 mg/mL (1.5 mM) stock solution by dissolving solid PI in PBS.
2. Add to cells at a final concentration of ≤ 1 µg/mL.
3. Incubate for 5 - 10 minutes in the dark, then analyze immediately.

Titrade the dye for optimal performance in each application.

Notes and Tips

For flow cytometric analysis, PI can be detected in the FL2 (DNA content) or FL3 (viability) channels. Use FL2 to analyze PI staining if it is being used as a counterstain with fluorescein-conjugated Annexin V.

For microscopy analysis, PI can be viewed using a rhodamine (red) filter. Cells will be stained with PI if the membrane has been permeated, e.g. as a result of natural cell death or detergent treatment.

Data/Structure

(A) Flow cytometry analysis of human peripheral blood mononuclear cells (PBMCs) labeled with PI.
(B) Chemical structure of PI.

References

Related Products

For a complete list of related products available from STEMCELL Technologies, visit www.stemcell.com/dyesandstains or contact us at techsupport@stemcell.com.

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