

Propidium iodide

Solution, 0.5 mg/ml

Cat. No. 11 348 639 001

20 ml

 **Version 05**

Content version: April 2016

Store at +2 to +8°C


1. What this Product Does

Contents

Red/orange solution containing 0.5 mg/ml propidium iodide in phosphate-buffered saline (pH approx. 7.4) with 0.09% sodium azide.

Storage and Stability

Product is stable when stored at +2 to +8°C in dark until the control date printed on the label.

 Do not freeze.

 Store protected from light.

Fluorometry

Excitation maximum ($E_{x_{max}}$) = 493 nm

Emission maximum ($E_{m_{max}}$) = 632 nm

Application

Propidium iodide is a fluorescent nucleic-acid dye. Binding of propidium iodide to double-stranded nucleic acids occurs through intercalation between base pairs with no preference to purine or pyrimidine base pairs. Propidium iodide does not bind to single-stranded nucleic acids.

2. How to Use this Product

2.1 Discrimination of Viable and Non-Viable Cells in Flow Cytometry

Dead cells may, through increased membrane permeability, exhibit nonspecific uptake of antibody during immunofluorescence labeling. This may lead to false-positive staining, particularly in flow cytometry where the traditional rim pattern of specific cell staining cannot be visualized. Propidium iodide readily enters and stains nonviable cells, but cannot cross the membrane of viable cells. In addition, the fluorescence emission spectra of propidium iodide is easily distinguished from that of fluorescein and phycoerythrin. This makes propidium iodide particularly useful as a viability stain in immunofluorescent studies (1).

Procedure

- Stain cells with the desired immunofluorescent antibody (e.g., a fluorescein or phycoerythrin conjugate).
- Immediately before flow-cytometric analysis, add 100 μ l of propidium iodide (directly from the bottle) to a 1 ml cell suspension containing 10^6 cells. This yields a final concentration of 50 μ g/ml.

Cells exhibiting fluorescence above 630 nm should be excluded from further analysis.

2.2 Determination of DNA Content in Flow Cytometry

Propidium iodide binds stoichiometrically to double-stranded nucleic acid, allowing fluorescence intensity (above 630 nm) to be used as an indicator of cellular DNA content (2-6).

Procedure

- Fix cells in cold (+2 to +8°C) 70% ethanol for 30 min.
- Wash twice in cold (+2 to +8°C) PBS.
- To remove double-stranded RNA, add 1 U of RNase, DNase-free* to the cell suspension (10^6 cells in 1 ml), and incubate for 30 min at +37°C.
- Add 100 μ l of propidium iodide (directly from the bottle) to the 1 ml cell suspension (10^6 cells).
- Store cells at +2 to +8°C protected from light until flow-cytometric analysis. Do not store overnight.

References

- 1 Sasaki, D.T., Dumas, S. E., and Engleman, E.G. (1987) *Cytometry* **8**:413.
- 2 Crissman, H.A. and Steinkamp, J.A (1973) *J. Biol.* **59**:766.
- 3 Krishan, A. (1975) *J. Cell Biol.* **68**:188.
- 4 Taylor, I.W. (1980) *J. Histochem. Cytochem.* **28**:1021.
- 5 Crissman, H.A. and Steinkamp, J.A. (1982) *Cytometry* **3**:84.
- 6 McCarthy, R.C. and Fetterhoff, T.J. (1989) *Arch. Pathos. Lab. Med.* **113**:658

Changes to Previous Version

Editorial changes

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* available from Roche Diagnostics

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