PRODUCT INFORMATION

AO/PI Cell Staining and Counting Reagent

Item No. 401158

Synonym: Acridine Orange/Propidium Iodide Mixture
Supplied as: A solution
Storage: 4°C
Stability: ≥1 year
Special Conditions: Aliquot for use at room temperature. Under sterile conditions, it will be stable for one month.

Contents

One 10 ml vial of acridine orange (AO) and propidium iodine (PI) dissolved in Hank's buffered saline solution.

Description

Acridine orange is a cell-permeable, nucleic acid-selective fluorescent cationic dye that has been used for cell cycle determination and autophagy detection.[1-3] It displays excitation/emission maxima of 502/525 nm, respectively, when bound to dsDNA, 460/650 nm, respectively, when bound to ssDNA or RNA, and 475/590 nm, respectively, under acidic conditions.[1,2] Propidium iodide is a fluorescent dye that binds to dsDNA and RNA after cell permeabilization and has been used as a nuclear label for dead cells.[4] It displays excitation/emission maxima of 488/585 nm, respectively. AO/PI Cell Staining and Counting Reagent contains optimized concentrations of AO and PI for labeling live and dead cells simultaneously. It is suitable for counting cells with a dual channel fluorescence cell counter, flow cytometer, or fluorescence microscope.

Reagent Applications

A. Fluorescence Cell Counter

Mix an equal volume of AO/PI Staining and Counting Reagent with a cell suspension at a cell density within the dynamic range of the instrument. Set the dilution factor to 2 (for a 1:1 mixture) and perform cell counting according to the protocol for the instrument.

B. Staining Cells for Fluorescence Microscopy or Flow Cytometry

Use the AO/PI Staining and Counting Reagent as a 10-20X stock solution for staining cells. Pipet the appropriate volume to the cultured cells based on the volume of medium in the vessel. Incubate cells at room temperature for 15-30 minutes before microscopy or flow cytometry.

NOTE: Make sure the instrument has the appropriate filters or settings for AO/GFP (ex/em = 480/535 nm, respectively) and PI/RFP (ex/em = 525/590 nm, respectively) fluorescence detection.

References

1. Virant-Klun, I., Tomazevic, T., and Meden-Vrtovec, H. Sperm single-stranded DNA, detected by acridine orange staining, reduces fertilization and quality of ICSI-derived embryos. *J. Assist. Reprod. Genet.* **19(7),** 319-328 (2002).
2. Han, J. and Burgess, K. Fluorescent indicators for intracellular pH. *Chem. Revs.* **110(5),** 2709-2728 (2010).
3. Thomé, M.P., Filippi-Chiela, E.C., Villodre, E.S., *et al.* Ratiometric analysis of Acridine Orange staining in the study of acidic organelles and autophagy. *J. Cell Sci.* **129(24),** 4622-4632 (2016).
4. Coder, D.M. Assessment of cell viability. *Curr. Protoc. Cytom.* **Suppl. 15,** (1997).

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