

## Acridine Orange Reagent

Acridine Orange

|                              |                                                         |
|------------------------------|---------------------------------------------------------|
| CATALOG #<br><b>AAA52744</b> | APPLICATIONS<br><b>Flow Cytometry, Functional Assay</b> |
|------------------------------|---------------------------------------------------------|

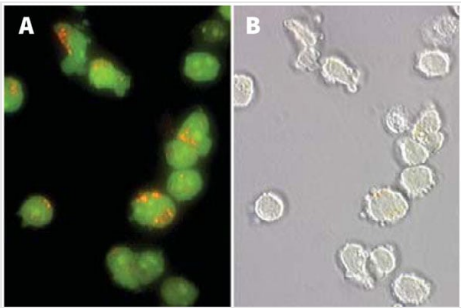
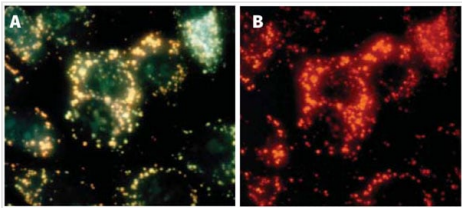
### PRODUCT SYNONYMS

Acridine Orange; Acridine Orange Staining Solution; Acridine Orange reagent

### PRODUCT SPECIFICATIONS

|                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-----------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Form/Format                                         | Supplied 0.5mL at 1 mM, 266 ug/mL.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Applicable Applications for Acridine Orange reagent | FM (Fluorescence Microscopy), FCM/FACS (Flow Cytometry)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Specifications                                      | <b>CAS number:</b> 65-61-2<br><b>Chemical name:</b> 3,6-acridinediamine, N, N, N', N'-tetramethylmonohydrochloride<br><b>Molecular formula:</b> C <sub>17</sub> H <sub>20</sub> ClN <sub>3</sub><br><b>Appearance:</b> Pale red/orange liquid<br><b>pH:</b> 5.0 ± 0.5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Safety                                              | Acridine orange may be a potent mutagen at high concentrations and probable carcinogen; knowledge about health hazards is incomplete.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| How to use                                          | <ol style="list-style-type: none"> <li>1. AO may be used neat or diluted in diH<sub>2</sub>O, PBS, or media. Is supplied as 0.5 mL liquid at 1 mM (266 ug/mL).</li> <li>2. Add AO to the cell sample media at 0.5 - 5 uM, equal to a final dilution of 1:2,000 - 1:200 in the cells (0.05-0.5% v/v). For example, if using AO at 1.0 uM in the final cell suspension, it must be diluted 1:1,000: <ol style="list-style-type: none"> <li>2a. First dilute it 1:100 in PBS or diH<sub>2</sub>O; e.g., put 10 uL AO into 990 uL PBS or diH<sub>2</sub>O.</li> <li>2b. Pipette the diluted AO into the cell suspension at approximately 1:10; e.g., put 50 uL diluted AO into 450 uL cell suspension.</li> </ol> </li> <li>3. Incubate 15-30 minutes at 37°C.</li> <li>4. Wash cells if reagent is too bright.</li> <li>5. Analyze with fluorescence: <ol style="list-style-type: none"> <li>5a. Lysosomes will appear yellowish green by illuminating cells with a blue light (488 nm) excitation filter and a green light (540-550 nm) emission/barrier filter (Figures 1A and 2A).</li> <li>5b. Alternatively, lysosomes will appear red when using an excitation filter of 550 nm (540-560 nm) and a long pass &gt;610 nm emission/barrier filter (Figure 2B).</li> </ol> </li> </ol> |
| Preparation and Storage                             | <b>Storage:</b> Store at 2-8°C.<br><b>Stability:</b> Shelf-life up to 24 months when refrigerated and protected from light.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

IMAGES



Application Data

ACRIDINE ORANGE STAINING OF MCF-7 CELLS Apoptosis was induced in human breast cancer MCF-7 cells by treating them with 0.15 uM camptothecin for 24 hours at 37°C. Cells were stained with AO in PBS for 30 minutes, then washed twice in PBS. Cells were photographed with a Nikon Microphot-FXA epifluorescence microscope at 40X using either a blue light excitation (492 nm) with a 540-550 nm emission filter (A, lysosomes appear yellowish green), or green light excitation (540 nm) with a long pass >640 nm barrier filter (B, lysosomes appear red).

Application Data

ACRIDINE ORANGE STAINING OF JURKAT CELLS Normal Jurkat cells stained with Acridine Orange (AO) show orange lysosomal staining. Jurkat cells were stained with 5uM AO in PBS for 60 minutes at 37°C. Photomicrographs were taken using a Nikon Eclipse E800 photomicroscope using a 460-500 nm excitation filter and a 505-560 nm emission / barrier filter set at 300X. Photo B shows the corresponding DIC image of the cells in A (AO appears faintly).

ADDITIONAL INFORMATION

Related Product Information for Acridine Orange reagent

Introduction:

Acridine orange (AO) (MBS258066) is an orange/red fluorescent chelating dye used to reveal lysosomes and nuclei in cultured cells. Due to its metachromatic properties, AO is commonly used in fluorescence microscopy and flow cytometry analysis of cellular physiology and cell cycle status.

AO is a slightly cationic, lipophilic, weak base capable of permeating cell and organelle membrane structure<sup>3</sup>. Although quite cell permeant in the neutral form, once protonated, this dye tends to become trapped on the low pH side of the membrane barrier leading to accumulation in acidic organelle structures. Proton pump driven lysosomal acidity generates a significant pH gradient resulting in the efficient concentration of AO within the lysosome organelles<sup>6</sup>. This is sufficient to create intra-lysosomal concentrations leading to precipitation of AO into aggregated granules. These oligomeric structures exhibit a red shift (640 nm) compared to the monomeric AO that emits at 525 nm.

As AO exhibits a very broad emission range, one of several filter pairings on the fluorescence microscope can be used to view this stain. Using an excitation filter of 550 nm (540-560 nm) with a long pass >610 nm emission/barrier filter, lysosomes appear red. When illuminating with a blue light excitation filter (488 nm) and a green light emission/barrier filter (540-550 nm), lysosomes appear yellowish green instead of red. As this filter combination is very close to the maximum emission of AO, the slide may appear too bright. Excess AO may be removed by washing cells prior to viewing.

AO can be utilized in conjunction with a number of other staining techniques and fluorogenic substrates including Magic Red®-DEVDase substrate that detects caspase-3/7 activation in apoptotic cells<sup>8</sup>. Because of the overlap in emissions, be wary of dual staining with other red stains as this will yield confusing results. Red dyes should be used separately.

AO may be used neat or diluted in diH<sub>2</sub>O, PBS, or media prior to pipetting into the cell suspension. Lysosomal structures can be visualized by staining with AO at 0.5-5.0 uM. This concentration range can be obtained by diluting AO reagent stock at 1 mM) 1:2,000-1:200 (0.05-0.5% v/v) into the final cell suspension. For example, if using AO at 1.0 uM in the final cell suspension, it must be diluted 1:1,000. First dilute it 1:100 in PBS; e.g., put 10 uL AO into 990 uL PBS. Pipette the diluted AO into the cell suspension at approximately 1:10; e.g., put 50 uL diluted AO into 450 uL cell suspension. Always protect AO from bright light.

Product Categories/Family for Acridine Orange reagent

Cellular Reagents and Cell Mountant; Cell Viability Assays; Cellular Imaging

NCBI AND UNIPROT INFORMATION

Molecular Weight 301.82

**For Research Use Only.** Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.