Acridine Orange Staining Solution ab270791

Overview

Product name: Acridine Orange Staining Solution

Tested applications: Suitable for: Flow Cyt, FM

General notes:

Acridine Orange (AO) is a slightly cationic, lipophilic, fluorochrome stain capable of permeating cell and organelle membrane structure. Although quite cell permeant in the neutral form, once protonated, these dyes tend to become trapped on the low pH side of the membrane barrier leading to their accumulation in acidic organelle structures, such as lysosomes.

Due to its metachromatic properties, AO is commonly used in fluorescence microscopy and flow cytometry analysis of cellular physiology and cell cycle status, including the fluorescent microscopic examination of microorganisms. Proton pump-driven lysosomal acidity generates a significant pH gradient resulting in the efficient concentration of AO within the lysosome organelles. The effectiveness of this AO concentration process is sufficient to create intr lysosomal concentrations leading to precipitation of the AO into aggregated granules. These oligomeric structures exhibit a red shift (640 nm) compared to the monomeric AO that emits at 525 nm.

Acridine Orange (AO) can be utilized in conjunction with a number of other staining techniques and fluorogenic substrates, including the Magic Red substrate in the Magic Red Caspase 3/7 Assay Kit (ab270771) that detects caspase 3/7 activation in apoptotic cells.

⚠️ Note: 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.

HOW TO USE

1. AO may be used neat or diluted in diH2O, PBS, or media. Supplied as 0.5 mL liquid at 1 mM (266 μg/mL).

2. Add AO to the cell sample media at 0.5 - 5 μM, 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 μM in the final cell suspension, it must be diluted 1/1,000:

   2a. First dilute it 1/100 in PBS or diH2O; e.g., put 10 μL AO into 990 μL PBS or diH2O.

   2b. Pipette the diluted AO into the cell suspension at approximately 1/10; e.g., put 50 μL diluted AO into 450 μL cell suspension.

3. Incubate 15-30 mins at 37°C.

4. Wash cells if reagent is too bright.

5. Analyze with fluorescence:

   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.

   5b. Alternatively, lysosomes will appear red when using an excitation filter of 540-560 nm.
nm) and a long pass >610 nm emission/barrier filter.

**FLUORESCENCE**
- Monomeric form (0.01M phosphate buffer - 0.15M NaCl pH 7.0): Ex 492 nm/ Em 525 nm.
- Aggregated or DNA complexed form: Ex 502 nm/ Em 520-524 nm.
- Aggregated or RNA complexed form: Ex 457 nm/ Em 630-644 nm.

**Properties**

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<tr>
<th>Form</th>
<th>Liquid</th>
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<tr>
<td>Storage instructions</td>
<td>Shipped at 4°C. Store at +4°C. Store In the Dark.</td>
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<tr>
<td>Storage buffer</td>
<td>Constituent: 0.027% Acridine Orange</td>
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**Applications**

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab270791 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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