Acridine Orange Staining Solution (1mg/mL)

Cat #: A-CSH394

Size: 10mL

Storage: 2-8°C, avoid light (6 months)

Product Description

Acridine Orange (AO) belongs to the group of tricyclic heteroaromatic dyes and is commonly referred to as AO. It is a metachromatic fluorescent dye that can penetrate cell membranes and stain nuclear DNA and RNA. Therefore, AO is often used for the detection of intracellular DNA and RNA. AO primarily binds to nucleic acids in two ways: 1) intercalative binding, where AO inserts between the base pairs of double-stranded DNA, resulting in green fluorescence with an emission peak at 530nm upon excitation; 2) electrostatic attraction, where positively charged AO binds to the phosphate groups of single-stranded RNA (which carries a negative charge) through electrostatic interactions, resulting in red fluorescence with an emission peak at 640nm upon excitation. A small amount of binding may exhibit orange or reddish-orange fluorescence. Therefore, when acridine orange intercalates into double-stranded DNA, it appears green, while binding to single-stranded DNA or RNA results in orange or reddish-orange fluorescence.

AO staining solution (1mg/ml) is a stock solution and should be diluted to the appropriate concentration before use. AO staining is often combined with ethidium bromide (EB) staining for dual staining, as EB only stains dead cells, producing orange fluorescence, allowing differentiation of normal cells, apoptotic cells, and necrotic cells.

Usage

1. Collect cells (if using flow cytometry, cells should be fixed first), wash the cells once with PBS, count and adjust the cell density to 106/ml.

2. Take an appropriate amount of cell suspension and add AO staining solution (1mg/ml) to achieve a final AO concentration of 8.5-17μg/ml. Gently mix.

3. Incubate the cells at room temperature in the dark for 15-20 minutes, then drop the stained cells onto a glass slide,

cover with a coverslip, or analyze using a flow cytometer.

4. Observe under a fluorescence microscope (excitation filter wavelength: 488nm, emission filter wavelength: 515nm), count and capture images.

Notes

1. AO staining solution (1mg/ml) does not contain a membrane permeabilizing agent and is less frequently used alone.

2. AO staining is often combined with EB staining to differentiate normal cells, apoptotic cells, and necrotic cells.

3. If available, centrifugation using a low-temperature centrifuge yields better results.

4. Minimize exposure of the reagent to strong light during the procedure.

5. For your safety and health, please wear a lab coat and disposable gloves while performing the operation.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.