

Product Data Sheet

Product Name: Acridine Orange

Cat. No.: GC41242

Chemical Properties

Cas. No. 494-38-2

Chemical Name N3,N3,N6,N6-tetramethyl-3,6-acridinediamine

SMILES CN(C)C1=CC(N=C(C=C(N(C)C)C=C2)C2=C3)=C3C=C1

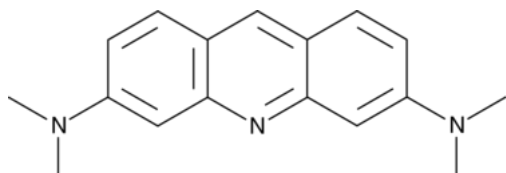
Formula C₁₇H₁₉N₃ M.Wt 265.4

Solubility 0.3mg/mL in ethanol, 20mg/mL in DMSO, 2mg/mL in DMF Storage Store at -20°C, protect from light

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

This plan only provides a guide, please modify it to meet your specific needs.

1 Preparation of Acridine Orange

(1) Staining solutions: Dissolve Acridine Orange powder in DMSO to 1mg/mL. Aliquot and store unused stock at -20°C or -4°C in the dark (preferably under nitrogen); avoid repeated freeze-thaw cycles.

(2) Working solution: Dilute the stock in an appropriate buffer (serum-free medium, HBSS, or PBS) to 0.5–20µg/mL. Prepare fresh for each experiment and titrate to optimal concentration.

2 DNA/RNA staining of suspended cells

(1) Harvest cells; place ~0.2mL of original suspension into 2–5mL tubes on ice.

(2) Slowly add 0.4mL ice-cold permeabilization buffer; keep on ice for ~15s.

Caution: Product has not been fully validated for medical applications. For research use only.

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(3) Slowly add 1.2mL ice-cold Acridine Orange working solution; incubate on ice in the dark for 2–15min.

(4) Analyze immediately by flow cytometry: excite at 488nm; collect green emission for dsDNA at 530nm and red emission for RNA/ssDNA at ≥ 640 nm (AO–nucleic-acid complex peaks at 488/650nm).

3 Adherent-cell staining (on ice)

(1) Culture cells on sterile coverslips.

(2) Remove medium, blot excess, and keep coverslips in a humid chamber.

(3) Add 100 μ L Acridine Orange working solution to one edge; gently tilt to cover all cells. Incubate on ice in the dark for 2–15min.

(4) Aspirate stain, rinse coverslip 2–3 times with fresh medium, and examine under a fluorescence microscope.

4 Detection

(1) Fluorescence microscope or flow cytometer: excite at 488nm; acquire green fluorescence at 530nm (dsDNA) and orange fluorescence at 640nm (RNA/ssDNA).

Precautions:

(1) If the cells need to be stained after fixation, it is recommended to use 70% ethanol for fixation, fix on ice for ≥ 2 hours, rinse with pre-cooled PBS 1-2 times after fixation, remove all ethanol, and then follow the above steps for staining;

(2) There is a quenching problem in fluorescent dyes, please try to avoid light to slow down fluorescence quenching;

(3) For your safety and health, please wear lab coats and disposable gloves when operating.

References:

[1] Hu P, Wang J, Qing Y, et al. FV-429 induces autophagy blockage and lysosome-dependent cell death of T-cell malignancies via lysosomal dysregulation. Cell Death Dis. 2021 Jan 13;12(1):80.

Background

Acridine Orange is a cell-permeable, nucleic-acid-selective fluorescent dye. When bound to nucleic acids, Acridine Orange emits fluorescence: upon intercalation into double-stranded DNA produces green fluorescence (excitation 488nm, emission 530nm). Because Acridine Orange associates with DNA and RNA to different extents, Acridine Orange binding to single-stranded DNA or RNA yields orange to red fluorescence (excitation 488nm, emission 640nm). Consequently, Acridine Orange is commonly employed to distinguish normal, apoptotic, and necrotic cells^[1].

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References :

[1] Ma T, Zhao Y, Cheng X. Detection of dsRNA by Acridine Orange Staining. Methods Mol Biol. 2024;2771:7-12.

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