## Acridine Orange base

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Cat. No.:	HY-D0952
CAS No.:	494-38-2
Molecular Formula:	C <sub>17</sub> H <sub>19</sub> N <sub>3</sub>
Molecular Weight:	265.35
Target:	Parasite
Pathway:	Anti-infection
Storage:	4°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)

N<sup>^</sup>

BIOLOGICAL ACTIVITY		
Description	Acridine Orange base is a cell-permeable fluorescent dye that stains organisms (bacteria, parasites, viruses, etc.) bright orange and, when used under appropriate conditions (pH=3.5, Ex=460 nm), distinguishes human cells in green for detection by fluorescence microscopy. Acridine Orange base fluoresces green when bound to dsDNA (Ex=488, Em=520-524) and red when bound to ssDNA (Ex=457, Em=630-644) or ssRNA (Ex=457, Em=630-644), also can be used in cell cycle assays <sup>[1][2][3]</sup> .	
In Vitro	<ul> <li>Guidelines (Following is our recommended protocol. This protocol only provides a guideline, and should be modified according to your specific needs).</li> <li>1. Differential staining of DNA and RNA of unfixed cells<sup>[11]</sup>:</li> <li>(1) Set up the flow cytometer with excitation at 488 nm, using emission filters and a dichroic mirror that discriminate green fluorescence (measured at 515-545 nm) and red luminescence (measured preferably above 640 or 650 nm).</li> <li>(2) Transfer a 0.2-mL aliquot of the original cell suspension to a small glass or plastic tube (e.g., 2- or 5-mL volume). Chill on ice.</li> <li>(3) Gently add 0.4 mL ice-cold cell permeabilizing solution. Wait 15 s, keeping cells on ice.</li> <li>(4) Gently add 1.2 mL ice-cold Acridine Orange base staining solution. Keep cells on ice in the dark.</li> <li>(5) Measure and record cell fluorescence in the flow cytometer during the 2 to 10 min after addition of Acridine Orange base staining solution.</li> <li>2. Differential staining of fixed cells<sup>[1]</sup>:</li> <li>(1a) For cells in suspension culture or hematologic samples: Rinse cells once with ice-cold PBS and suspend in ice-cold PBS at -10<sup>6</sup> cells/mL.</li> <li>(1b) For cells insuspension culture plates: Collect cells from flasks or petri plates by trypsinization, pool the trypsinized cells with cells floating in the medium (mostly detached mitotic and dead cells), and rinse once with medium containing serum to inactivate the trypsin. Suspend cells in ce-cold PBS at -10<sup>6</sup> cells/mL.</li> <li>(2) With a Pasteur pipet transfer 1 mL cell suspension to a 15-mL conical glass tube containing 10 mL ice-cold 70% ethanol. Fix cells ≥ 2 ho ice.</li> <li>(3) Centrifuge tubes 5 min at 300 × g, 48. Remove all ethanol, rinse cells once with ice-cold PBS, and suspend in ice-cold PBS at a density of &lt; 2 × 10<sup>6</sup> cells/mL.</li> <li>(4) Withdraw 0.2 mL cell suspension (≤ 2 × 10<sup>5</sup> cells) and transfer to a small tube (e.g., 2 or 5 mL volume). Chill on ice.</li> <li>(5) Add 0.4 mL ice-cold permeabilizing solution.</li></ul>	

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## **CUSTOMER VALIDATION**

- Adv Funct Mater. 2023 Apr 14.
- Nat Commun. 2023 Jun 30;14(1):3877.
- Acta Pharm Sin B. 2021 Feb 11.
- Clin Transl Med. 2023 Mar;13(3):e1229.
- Cell Death Dis. 2021 Jan 13;12(1):80.

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## REFERENCES

[1]. Darzynkiewicz Z, et al. Differential staining of DNA and RNA. Curr Protoc Cytom. 2004 Nov; Chapter 7: Unit 7.3.

[2]. Mirrett S. Acridine orange stain. Infect Control. 1982 May-Jun;3(3):250-2.

[3]. Yektaeian N, et al. Lipophilic tracer Dil and fluorescence labeling of acridine orange used for Leishmania major tracing in the fibroblast cells. Heliyon. 2019 Dec 18;5(12):e03073.

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

Tel: 609-228-6898Fax: 609-228-5909E-mail: tech@MedChemExpress.comAddress: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA