Fluo-4AM

Cat :No. IF1500

Storage: Powder: -20℃, 1 year; Insolvent (mother liquid): -20℃, 6 months; -80℃, 1 year（protect from light）

Introduction

Fluo-4 is a calcium fluorescent probe that replaces Cl with F in the Fluo-3 structure. Since the Cl is replaced by the more electronically attractive F, its maximum excitation wavelength is shifted by about 10 nm to the short wavelength. This wavelength is closer to the wavelength of an argon laser, so the fluorescence intensity of Fluo-4 is stronger than that of Fluo-3 when excited by an argon laser.

Fluo-4 AM penetrates the cell membrane and enters the cell, then is sheared by intracellular esterases to form Fluo-4, which is retained in the cell. Fluo-4 is almost non-fluorescent when it exists in the form of free ligand, but when it binds to intracellular calcium ions, it can produce strong fluorescence, which can be detected by using a confocal laser microscope or flow cytometer to detect changes in the concentration of intracellular calcium ions.

Parameter

Ex/Em: 494/516 nm

CAS No: 273221-67-3

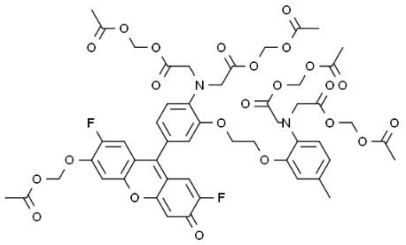
Molecular Formula: $C_{51}H_{50}F_2N_2O_{23}$

Molecular Weight: 1096.94

Purity: ≥95%

Appearance: Orange Solid

Solubility: Soluble in DMSO



Protocols *(For Human T cells. only for reference)*

Self-prepared reagents (optional)

1. Hanks balanced salt solution (HBSS): Calcium and magnesium free, phenol red free HBSS (Cat No: H1045) is recommended.
2. HEPES buffer saline: 10 mM HEPES, 1 mM Na_2HPO_4, 137 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM Glucose, 0.1% BSA, pH 7.4 (Cat No: H1070)
3. 20% Pluronic F127 stock solution: 100mg Pluronic F127 powder dissolved in 0.5mL DMSO.

Note:a. Dissolution requires heating at 40-50°C for 20-30 min. Dissolve and store at room temperature, do not refrigerate. If crystals precipitate out, it can be reheated and dissolved without affecting the use.

b. Pluronic F-127 reduces the stability of Fluo-4, AM, so it is only recommended to add it when preparing the working solution, and is not recommended to add it to the storage solution.

Preparation of storage solution

Prepare a 2 mM stock solution in DMSO. For example, 1 mg Fluo-4, AM powder was dissolved in 455.8 μL DMSO.

Note:a. Unused storage solution is recommended to be stored in portions at -20℃ to avoid repeated freezing and thawing.

b. Moisture-absorbing DMSO has a significant effect on the solubility of the product, use freshly opened DMSO.

Preparation of working fluid

Dilute the storage solution with a suitable buffer (e.g., HBSS or PBS, etc.) to make a 4-20 μM Fluo-4 AM working solution.

Note:a. The final concentration of the working solution is recommended to be optimized according to different cell lines and experimental systems.

b. If it is found to be difficult to dissolve, it can be sonicated appropriately to promote dissolution.

c. To avoid cytotoxicity caused by overloading, it is recommended to use the lowest probe concentration based on effective results, starting from 4 μM.

Dyeing

1) Remove the pre-cultured cells, remove the medium and wash the cells 3 times using PBS or HBSS solution.

2) Remove the buffer, add Fluo-4 AM working solution into the cells and incubate at 37℃ for 10-30 min.

Note:a. If the incubation temperature and time cannot be determined in the first experiment, it is recommended to try to incubate at 37℃ for 20 min to observe the fluorescence effect.

b. If more cells die, shorten the time or lower the temperature appropriately; if the fluorescence intensity is too weak, extend the time appropriately.

3) Add 5 times volume of HBSS containing 1% fetal bovine serum and continue incubation for another 40 min.

4) Wash the cells 3 times with buffers such as HEPES buffer saline (or PBS, HBSS), and then resuspend the cells with buffers such as HEPES buffer saline to make a cell suspension of 1×10^5 cells/mL.

5) Incubate at 37°C for 10 min to ensure complete de-esterification of AM bodies in the cell.

6) Perform fluorescent calcium ion detection.

Note

1. This product is in the form of lyophilized powder and may adhere to the wall of the tube, which is a normal phenomenon and can be used without worry.
2. Labeling conditions vary depending on the cell type; determine the optimal conditions before each experiment.
3. Fluo-4 AM absorbs moisture readily, so after removing it from the refrigerator, make sure it is at room temperature in a dry environment before opening. Due to the extremely small amount of

reagent, please centrifuge the reagent briefly before opening to ensure that the powder falls to the bottom of the tube.

4. If serum-containing medium is used, lipases in the serum will break down the AM bodies, thus reducing the effectiveness of Fluo-4 AM in entering the cells.

5. Media containing phenol red will slightly increase the background value, so you should try to remove as much of the media residue as possible before adding the working solution.

6. If the entry of Fluo-4 AM ester into the cell is not effective, an appropriate amount of 20% Pluronic F-127 solution can be added to the Fluo-4 AM solution to prevent the Fluo-4 AM ester from accumulating in the buffer and to promote the entry of Fluo-4 AM into the cell, and the final concentration of Pluronic F-127 can be controlled to be in the range of 0.04- 0.05%.

7. Fluorescent dyes have the extraction problem, please pay attention to avoid light to slow down the fluorescence extraction.

8. For your safety and health, please wear lab coat and disposable gloves.

9. This product is for scientific research use only. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in a residential area.

Related Literature

[1]. Zheng J, Lu T, Zhou C, Cai J, Zhang X, Liang J, Sui X, Chen X, Chen L, Sun Y, Zhang J, Chen W, Zhang Y, Yao J, Chen G, Yang Y. Extracellular Vesicles Derived from Human Umbilical Cord Mesenchymal Stem Cells Protect Liver Ischemia/Reperfusion Injury by Reducing CD154 Expression on CD4+ T Cells via CCT2. Adv Sci (Weinh). 2020 Aug 20;7(18):1903746. doi: 10.1002/advs.201903746. PMID: 32999825.（IF:15.4）

[2]. Zhao P, Gong L, Chang L, Du H, Geng M, Meng S, Dai L. Multifunctional Fe-based coordination polymer nano-bomb modified with β-lapachone and CaO2 for targeted tumor dual chemodynamic therapy with enhanced ferroptosis and H2O2 self-supply. J Nanobiotechnology. 2024 Jan 3;22(1):3. doi: 10.1186/s12951-023-02287-2. PMID: 38166978.（IF:10.2）

Note: More literature is available on the Solarbio website