Fluo-3AM

Cat No. IF0150

Storage: Powder: -20℃, 2 years; Insolvent (mother liquid): -20℃, 6 months; -80℃, 1 year (protect from light)

Introduction

The calcium probe Fluo-3 AM is one of the most commonly used fluorescent probes for detecting intracellular calcium ion concentration. It penetrates the cell membrane, enters the cell and is sheared by the esterase enzyme to form Fluo-3, which is retained in the cell. Fluo-3 is almost non-fluorescent when it exists in the form of free ligand, but when it binds with intracellular calcium ions, it can produce strong fluorescence, with the maximum excitation wavelength of 506 nm and the maximum emission wavelength of 526 nm. The recommended excitation wavelength is about 488 nm and the emission wavelength is 525-530 nm, and changes in intracellular calcium concentration can be detected by laser confocal microscopy or flow cytometry.

Parameter:

Ex/Em: 506/526nm

CAS No: 121714-22-5

Molecular Formula: $C_{51}H_{50}Cl_2N_2O_{23}$

Molecular Weight: 1129.85

Purity: HPLC≥93%

Appearance: Orange Solid

Solubility: Soluble in DMSO

Protocols *(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: 10mM 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-3 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 1-5 mM stock solution in DMSO. For example, 1 mg of Fluo-3 AM powder was dissolved in 0.8851 mL of DMSO to obtain 1 mM of Fluo-3 AM stock solution.

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 reservoir solution with a suitable buffer (e.g., HBSS, etc.) and prepare a 1-5 μM Fluo-3 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. Please adjust the concentration of the working fluid according to the actual situation, and use immediately after dissolution

Dyeing

***Labeling conditions vary depending on the cell type; determine the optimal conditions before each experiment.**

1. Remove the pre-cultured cells, remove the medium and wash the cells with HBSS three times.
2. Remove the buffer and add Fluo-3 AM working solution to the cells, the amount of solution should be enough to cover the cells. Incubate at 37℃ for 10-60 min.
3. Remove the Fluo-3 AM working solution and wash the cells three times with HEPES buffer saline solution.
4. Add HBSS solution containing 1% fetal bovine serum to cover the cells, and incubate at 37℃ in a cell culture incubator for about 20-30 min to ensure complete de-esterification of AM bodies in the cells.
5. Detect the cells by laser confocal or fluorescence microscope, excitation wavelength 480-500 nm, emission wavelength 525-530 nm. If fluorescence zymography or flow detection is required, the cells need to be resuspended with HEPES buffer saline to make a solution of 1×10^5 cells/mL.

Note

1. Regarding the cell incubation time, it is recommended to incubate for 30 min first to see the fluorescence effect. If the cells die more, the time should be shortened; if the fluorescence intensity is too weak, extend the time appropriately.
2. If serum-containing medium is used, lipases in the serum break down the AM bodies, thus reducing the effectiveness of Fluo-3 AM in entering the cells.
3. Media containing phenol red will cause the background value to be slightly higher, so you should try to remove as much of the media residue as possible before adding the working solution.
4. Pluronic F127 prevents Fluo-3,AM from polymerizing in HBSS and helps it enter the cell. Therefore, an appropriate amount of Pluronic F127 can be added to the Fluo-3 AM solution. It is not recommended to keep the Fluo-3 AM solution in Pluronic F127 for a long time.

5. Fluorescent dyes have quenching problems, please pay attention to avoid light as much as possible to slow down the fluorescence quenching.
6. For your safety and health, please wear lab coat and disposable gloves.
7. 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].Wang T, Liu Y, Zhou Y, Liu Q, Zhang Q, Sun M, Sun M, Li H, Xu A, Liu Y. Astaxanthin protected against the adverse effects induced by diesel exhaust particulate matter via improving membrane stability and anti-oxidative property. J Hazard Mater. 2023 Aug 15;456:131684. doi: 10.1016/j.jhazmat.2023.131684. Epub 2023 May 22. Erratum in: J Hazard Mater. 2023 Oct 5;459:132092. doi: 10.1016/j.jhazmat.2023.132092. PMID: 37236114.（IF：13.6）

Note: More literature is available on the Solarbio website