Product Name: **Fluo-4 AM (2mM), Calcium Ion Fluorescent Probe**

Catalog Number: **RA20032**

Basic Information

Product Name	Fluo-4 AM (2mM), Calcium Ion Fluorescent Probe
Size	50 μL
Storage conditions	-20℃, keep away from light
Shipping	Shipped with ice pack
Validity	12 months
Ex/Em	494/516 nm

Product Introduction

Fluo-4 is a calcium fluorescent probe that replaces the Cl ions in the Fluo-3 structure with F ions. Since the Cl ions are replaced with F ions with stronger electron attraction, its maximum excitation wavelength will deviate by about 10 nm toward the shorter wavelength direction. The wavelength is closer to the wavelength of the argon laser, so when excited by the argon laser, the fluorescence intensity of Fluo-4 is stronger than that of Fluo-3.

Fluo-4, AM ester penetrates the cell membrane and enters the cell, where it is cleaved by the esterase in the cell to form Fluo-4, which is then retained in the cell. Fluo-4 is almost non-fluorescent when it exists as a free ligand, but it can produce strong fluorescence when combined with intracellular calcium ions. Laser confocal microscopy or flow cytometry can be used to detect changes in intracellular calcium ion concentrations.

Experimental procedures

1. Take out the Fluo-4, AM ester stock solution and warm it to room temperature.
2. Dilute the Fluo-4, AM ester stock solution with PBS or HBSS to prepare a 4 μM Fluo-4, AM ester working solution.

Note: The recommended working solution concentration is 4-20 μM. In order to avoid cell toxicity caused by overloading, it is recommended to use the lowest probe concentration based on obtaining valid results, and you can start from 4 μM.

3. (Optional) If the Fluo-4, AM ester does not enter the cells effectively, add an appropriate amount of 20% Pluronic F-127 solution to the Fluo-4, AM ester solution to prevent Fluo-4, AM ester from aggregating in the buffer and promote the entry of Fluo-4, AM ester into the cells. The final concentration of Pluronic F-127 should be controlled at 0.04-0.05%.

Note: (1) Preparation of 20% (w/v) DMSO stock solution of Pluronic F-127: Add 0.5 mL DMSO to 100 mg Pluronic F-127 to prepare a 20% (w/v) DMSO stock solution. Dissolution requires

heating at 40-50°C for 20-30 min. After dissolution, store at room temperature and do not refrigerate. If crystals precipitate, they can be reheated and dissolved without affecting use.
(2) Pluronic F-127 can reduce the stability of Fluo-4, AM ester, so it is only recommended to be added when preparing the working solution and is not recommended to be added to the storage solution.
4. Take out the pre-cultured cells, remove the culture medium, and wash the cells three times with PBS or HBSS solution.
5. Remove the buffer, add Fluo-4, AM ester working solution to the cells, and incubate at 37°C for 10-60 min.
Note: If the incubation temperature and time cannot be determined for the first experiment, it is recommended to try incubating at 37°C for 20 min and observe the fluorescence effect. If more cells die, shorten the time or lower the temperature appropriately; if the fluorescence intensity is too weak, extend the time appropriately.
6. Remove the Fluo-4, AM ester working solution, wash the cells three times with PBS or HBSS buffer, and then resuspend the cells with PBS or HBSS buffer to make a cell suspension of 1×10^5 cells/mL.
7. Incubate at 37℃ for 10 minutes ,complete deesterification of AM bodies in cells .
8. Perform fluorescent calcium ion detection.

Precautions

1. If a culture medium containing serum is used, the esterase in the serum will decompose the AM ester body, thereby reducing the effect of Fluo-4, AM ester entering the cells. In addition, a culture medium containing phenol red will slightly increase the background value. Before adding the working solution, the residual culture medium should be removed as much as possible.
2. All fluorescent dyes have quenching problems. Please try to avoid light to slow down fluorescence quenching.
3. Fluo-4, AM ester is easy to absorb moisture. After taking it out of the refrigerator, please make sure it is in a dry environment and put it at room temperature before opening. Since the reagent is very small, please centrifuge it briefly before opening to ensure that the powder falls

to the bottom of the tube.

4. Fluo-4, AM ester is easily decomposed when in contact with water. If it cannot be used up all at once, it is recommended to store the stock solution in small portions.

Note: This reagent is for scientific research use only!