Product Name: Fluo-3 AM (2mM)

Product number: RA20031

Basic Information

Product name	Fluo-3 AM (2mM)
Size	50 μL
Storage	-20℃, protected from light
Shipping	Shipped with ice pack
Validity	12 months

Product Introduction

Fluo-3, AM ester is a fluorescent dye that can penetrate the cell membrane. After entering the cell, Fluo-3, AM ester can be cleaved by endogenous esterase to form Fluo-3, which is then retained in the cell. Fluo-3 can bind to calcium ions and produce strong fluorescence after binding to calcium ions.

Experimental procedures

1. Take out the Fluo-3, AM ester stock solution in solution form and warm it to room temperature.
2. Dilute the Fluo-3, AM ester stock solution with a buffer such as PBS or HBSS to prepare a 4 μM Fluo-3, AM ester working solution.

Note: To avoid cytotoxicity due to overloading, it is recommended to use the lowest probe concentration that achieves valid results.

3. (Optional) If the Fluo-3, AM ester does not enter the cells effectively, add an appropriate amount of 20% Pluronic F-127 solution to the Fluo-3, AM ester solution to prevent Fluo-3, AM ester from aggregating in the buffer and promote the entry of Fluo-3, 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) Pluronic F-127 DMSO stock solution: 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.

Store at room temperature, do not refrigerate. If crystals are precipitated, they can be reheated and dissolved without affecting the use.

(2) Pluronic F-127 can reduce the stability of Fluo-3, 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 solution, add Fluo-3, AM ester working solution to the cells, and incubate at 37℃ 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-3, 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°C for 10 minutes to ensure complete de-esterification of the AM ester within the cells.
8. Perform fluorescent calcium ion detection.

Fluo-3 AM excitation/emission wavelength: 506/ 526 nm

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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-3, 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-3, 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-3, 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!