Product Information

Fluo-3, AM ester

Catalog Number: F3005-1 mg, F3015-2 mM

Product Size: 1 mg, 50 μL

Parameters

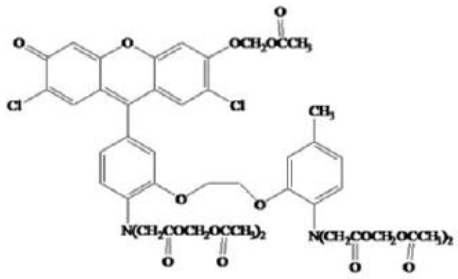
Appearance: Orange-red solid soluble in DMSO (F3005)

CAS No.: 121714-22-5

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

Molecular Weight: 1129.9

Molecular Structure:



Storage

Store at -20°C and protect from light. When stored as directed, product is stable for at least 12 months.

Description

Fluo-3, AM ester can enter into into cells via incubation. Fluo-3 AM ester itself does not bind Ca2+, but it is readily hydrolyzed to Fluo-3 by endogenous esterases once inside cells. Fluo-3 can binds to calcium ions, which can produce strong fluorescence. The maximum excitation wavelength at 506 nm and the maximum emission wavelength at 526 nm.

Protocol

1. Dissolve Fluo-3, AM in anhydrous DMSO to prepare a 2-5 mM stock solution or remove the Fluo-3, AM stock solution and return to room temperature.

2. Dilute stock solution to a final concentration of 4 μM with PBS or HBSS.

Note: In order to avoid cytotoxicity caused by overloading, it is recommended to use the lowest probe concentration to obtain effective results.

3. (Optional) If the effect of Fluo-3 entering cells is not good, you can add an appropriate volume of 20% Pluronic F127 solution to Fluo-3, AM / DMSO solution to prevent Fluo-3, AM from polymerizing in HBSS and facilitate its entry into cells. The final concentration of Pluronic F127 is controlled at 0.04-0.05%.

Note: (1) 20% (w / v) Pluronic F-127 DMSO stock solution: add 0.5 mL DMSO to 100 mg Pluronic F-127 powder, heat at 40-50 ° C for 20-30 minutes to dissolve. Store the solution at room temperature. Do not refrigerate. If crystals are precipitated, they can be dissolved after reheating without affecting the use.

(2) Pluronic F127 can reduce the stability of Fluo-3, AM, so it is only recommended to add it when preparing working solution, it is not recommended to add it to storage solution for long-term storage.

4. Remove the pre-cultured cells, remove the medium, and wash the cells 3 times with PBS or HBSS solution.

5. Add Fluo-3, AM 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

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incubate at 37 °C for 20 minutes and observe the fluorescence effect. If there are many cell deaths, shorten the time or temperature appropriately; if the fluorescence intensity is too weak, extend the time appropriately.

6. Remove Fluo-3, AM working solution, wash the cells 3 times with buffers such as PBS or HBSS, and resuspend the cells with buffers such as PBS or HBSS to make a 1×10^5 cells / mL solution.

7. Incubate at 37 °C for 10 min to ensure complete deesterification of AM in cells.

8. Perform fluorescent calcium ion detection (excitation wavelength at 506 nm, emission wavelength at 526 nm).

Notes

1. If a serum-containing medium is used, the esterase in the serum will decompose the AM , thereby reducing the effect of Fluo-3, AM entering the cells. In addition, the medium containing phenol red will slightly increase the background value, so you should try to remove the residue of the medium before adding the working solution.

2. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.

3. This Fluo-3, AM is easy to absorb moisture. After removing it from the refrigerator, please make sure to open it in a dry environment at room temperature. As the reagent is extremely small, centrifuge it briefly before opening to ensure that the powder falls to the bottom of the tube.

4. Fluo-3, AM decomposes easily in contact with water. If it cannot be used up at one time, it is recommended to store the solution in small quantities.

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