

Fluo-4 AM

Introduction

Fluo-4 AM is one of the most common probes for detecting Ca^{2+} . Fluo-4 AM is an acetoxymethyl (AM) ester derivative of Fluo-4 that can penetrate cell membranes and be cleaved by intracellular esterase to form Fluo-4. Fluo-4 can remain in cells and bind to intracellular Ca^{2+} , then emit bright fluorescence. Fluo-4 AM is an analog of Fluo-3 AM with two chlorine substituents replaced by fluorines. This modification results in increased fluorescence excitation at 488 nm and a greater signal level for the image. In addition, the fluorescence produced by Fluo-4 binding Ca^{2+} is at least 100 folds stronger than UV-excited probes (Fura-2 and Indo-1). This reagent is provided as a 2 mM stock solution in DMSO.

Components and Storage

Components	B8807-20 μL
Fluo-4 AM	20 μL (2 mM)
This product should be stored at -20°C away from light and moisture. Avoid repeated freeze/thaw cycles, stable for 6 months.	

Properties

Physical Appearance	Liquid
M.Wt	1096.95
Cas No.	273221-67-3
Formula	$\text{C}_{51}\text{H}_{50}\text{F}_2\text{N}_2\text{O}_{23}$
Ex/Em	494/516 (after binding Ca^{2+})

Protocol

- Preparation of the working solution:** Dilute appropriate Fluo-4 AM (2 mM) in a suitable buffer (for example, PBS or HBSS with Calcium and Magnesium) to make a working solution. The recommended concentration of the working solution is 1-5 μM . To reduce potential labeling artifacts, keep the concentration of working solution as low as possible. It is a must to dilute Fluo-4 AM when using it.

***Note:** Fluo-4 AM is susceptible to hydrolysis (particularly in solution). It is suggested to aliquot the stock solution into small volumes and avoid repeated freeze/thaw cycles. In addition, allow the stock solution to warm to room temperature before using and centrifuge for several seconds to make the probe liquid at the bottom of the tube. The optimal concentration of working solution varies depending

on the type of cells.

- 2. Add Pluronic F-127 (optional step):** If the staining result is not ideal, add 20% Pluronic F-127 into the Fluo-4 AM working solution to assist in dispersion of the Fluo-4 AM. The recommended, final concentration of Pluronic F-127 is 0.02%.

***Note:** Pluronic F-127 can reduce the stability of Fluo-4 AM and is only recommended to add in working solution, not in stock solution.

- 3. Detection:** For adherent cells, grow cells to reach the desired density. Remove the growth medium and wash with a suitable buffer (for example, PBS or HBSS with Calcium and Magnesium) 3 times. Add a working solution to cover the cells. Incubate at 20-37°C away from light for 10-60 min. After incubation, wash cells in a fresh, suitable buffer 3 times. Then incubate in a fresh, suitable buffer at 37°C away from light for 10-30 min to allow complete de-esterification of AM esters. Then detect the fluorescence signal of cells (Ex/Em: 494/516 nm).

***Note:** Serum can prematurely cleave probes with AM esters and bind probes non-specifically. Phenol red can increase the fluorescence background. So, make sure to remove the growth medium clearly before the loading step with Fluo-4 AM. The optimal time for incubation varies depending on the type of cells. For suspension cells, harvest cells and perform similarly to the adherent cells.

Note

- Fluo-4 AM is susceptible to hydrolysis (particularly in solution). It is suggested to aliquot the stock solution into small volumes and avoid repeated freeze/thaw cycles. In addition, allow Fluo-4 AM to warm to room temperature before using and centrifuge for several seconds to make the probe liquid at the bottom of the tube.
- Serum can prematurely cleave probes with AM esters and bind probes non-specifically. Phenol red can increase the fluorescence background. So, make sure to remove the growth medium clearly before the loading step with Fluo-4 AM.
- Fluorescent probes are easy to quench, please protect them from light when using.
- For your safety and health, please wear lab coats and gloves during the experiment.
- For research use only. Not to be used in clinical diagnostic or clinical trials.

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