Fluo-3 AM Calcium Assay Kit

Introduction

Fluo-3 AM is one of the most common probes for detecting Ca^{2+}. Fluo-3 AM is an acetoxymethyl (AM) ester derivative of Fluo-3 that can penetrate cell membranes and be cleaved by intracellular esterase to form Fluo-3. Fluo-3 can remain in cells and bind to intracellular Ca^{2+}, then emit bright fluorescence.

Fluo-3 AM Calcium Assay Kit is a kit for the detection of intracellular calcium concentration based on the Fluo-3 AM probe. The kit is optimized for easy to use and can be detected without washing after staining. The Assay Buffer provided in this kit can ensure the viability of the cells. The kit also provides the Solubility Enhancer and Staining Enhancer, which can be used to enhance the solubility of the probe and to reduce background fluorescence. This kit is ideal for large-scale calcium detection and screening of GPCR inhibitors or agonists.

Components and Storage

Components \ Size	200 Tests	1000 Tests	Storage
Fluo-3 AM (500×)	40 μL	200 μL	-20°C away from light
Solubility Enhancer (500×)	40 μL	200 μL	-20°C
Assay Buffer	50 mL	250 mL	-20°C
Staining Enhancer (100×)	200 μL	1 mL	-20°C
Shipping: Blue ice	**Shelf life: 1 year**		

Protocol

1. **Prepare the working solution:** For 96-well plates, refer to the table below to prepare the Fluo-3 AM working solution. Prepare a fresh Fluo-3 AM working solution every time.

Reagent	1 sample	10 samples	100 samples
Fluo-3 AM (500×)	0.2 μL	2 μL	20 μL
Solubility Enhancer (500×)	0.2 μL	2 μL	20 μL
Assay Buffer	99.6 μL	996 μL	9.96 mL
Total	100 μL	1 mL	10 mL

***Note:**

a) Staining Enhancer (100×) may affect cell function, and is unnecessary for most cells. When there is significant leakage of the fluorescence signal, dilute Staining Enhancer (100×) 1:100 into Fluo-3 AM solution and mix well. Cells should not be incubated for more than 2 h in working solution containing Staining Enhancer.

b) The concentration of Fluo-3 AM can be adjusted appropriately according to the staining effect.

2. Fluorescence microscopy:

1) Seed cells in 96-well plates, Petri dishes, or coverslips.

2) For adherent cells, remove the medium and wash the cells once with PBS. For suspension cells, centrifuge at 1,000 rpm for 5 min, discard the supernatant, and wash once with PBS. If the residual medium can be adequately aspirated with a vacuum pump, washing with PBS can be ignored. But washing can better reduce the fluorescence background.

***Note:** Esterases in serum break down AM eaters, reducing the amount of Fluo-3 AM entering cells. At the same time, phenol red can make the fluorescence background slightly high, so the residual medium should be removed as much as possible before adding the working solution.

3) For 96-well plates, add 100 μL of Fluo-3 AM working solution per well and incubate at 37°C for 30 minutes in the dark. For other plates, the amount of working fluid can be adjusted according to the plate volume.

***Note:** The incubation time can be adjusted within 15-60 minutes. For the first experiment, you can try incubating at 37°C for 30 minutes. If the cells are not in good condition, the incubation time can be reduced or the temperature can be lowered. If the signal is too weak, an extended incubation time may be considered.

4) Remove the working solution, and wash cells with PBS three times. Resuspend cells with PBS and incubate at 37°C for 20-30 minutes.

***Note:** this step is to make sure that Fluo-3 AM is completely converted to Fluo-3 intracellularly.

5) Monitoring the fluorescence intensity at 490/525 nm.

***Note:** After the incubation, the cells can be washed 1-2 times with PBS before testing, which is optional.

3. Flow Cytometry:

1) Seed cells in 96-well plates, Petri dishes, or coverslips.

2) For adherent cells, resuspend cells with medium after trypsinization, then wash the cells once with PBS. For suspension cells, centrifuge at 1,000 rpm for 5 min, discard the supernatant, and wash once with PBS.

***Note:** Esterases in serum break down AM eaters, reducing the amount of Fluo-3 AM entering cells. At the same time, phenol red will make the fluorescence background slightly high, so the residual medium should be removed as much as possible before adding the working solution.

3) For 10^6 cell pellets, resuspend the cells with 1 mL of Fluo-3 AM working solution and incubate at 37°C for 30 minutes in the dark.

***Note:** The incubation time can be adjusted within 15-60 minutes. For the first experiment, you can try incubating at 37°C for 30 minutes. If the cells are not in good condition, the incubation time can be reduced or the temperature can be lowered; If the signal is too weak, an extended incubation time may be considered.

4) Remove the working solution, and wash cells with PBS three times. Resuspend cells with PBS and incubate at 37°C for 20-30 minutes.

***Note:** this step is to make sure that Fluo-3 AM is completely converted to Fluo-3 intracellularly.

5) Monitoring the fluorescence intensity at 490/525 nm. Alternatively, centrifuge at 1000 rpm for 5 min, discard the supernatant, and resuspend the cells with 500 μLof Assay Buffer. Unstained cells containing only Assay Buffer can be used as a negative control.

***Note:** After the incubation, the cells can be washed 1-2 times with PBS before testing, which is optional.

4. **Fluorescence microplate reader:**

1) Seed cells in 96-well plates, Petri dishes, or coverslips. The number of cells per well is recommended in the range of 2,000-10,000.

2) For adherent cells, remove the medium and wash the cells once with PBS. For suspension cells, centrifuge at 1,000 rpm for 5 min, discard the supernatant, and wash once with PBS. If the residual medium can be adequately aspirated with a vacuum pump, washing with PBS can be ignored. But washing can better reduce the fluorescence background.

***Note:** Esterases in serum break down AM eaters, reducing the amount of Fluo-3 AM entering cells. At the same time, phenol red will make the fluorescence background value slightly high, so the residual medium should be removed as much as possible before adding the working solution.

3) For 96-well plates, add 100 μL of Fluo-3 AM working solution to each well and incubate at 37°C for 30 minutes in the dark. For other plates, the amount of working fluid can be adjusted according to the plate volume.

***Note:** The incubation time can be adjusted within 15-60 minutes. For the first experiment, you can try incubating at 37°C for 30 minutes. If the cells are not in good condition, the incubation time can be reduced or the temperature can be lowered; If the signal is too weak, an extended incubation time may be considered.

4) Remove the working solution, and wash cells with PBS three times. Resuspend cells with PBS and incubate at 37°C for 20-30 minutes.

***Note:** this step is to make sure that Fluo-3 AM is completely converted to Fluo-3 intracellularly.

5) Monitoring the fluorescence intensity at 490/525 nm.

***Note:** After the incubation, the cells can be washed 1-2 times with PBS before testing, which is optional.

Note

1. Fluo-3 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-3 AM to warm to room temperature before using and centrifuge for several seconds to make the probe liquid at the bottom of the tube.

2. 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-3 AM.
3. Ionomycin (B5165) can be used as a positive control for this kit. Treating cells with 10 μM Ionomycin for a few seconds can greatly increase the fluorescence signal.
4. The probe is very easy to adhere to the tube wall and the tip wall, so it needs to be heated and melted when used, and the tip also needs to be heated in the incubator before use, otherwise it is easy to solidify on the inner wall of the tip again and cause loss.
5. Fluorescent probes are easy to quench, please protect them from light when using.
6. For your safety and health, please wear lab coats and gloves during the experiment.
7. For research use only. Not to be used in clinical diagnostic or clinical trials.

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