Fluo-20 AM Calcium Ion Fluorescent Probe

Cat. No.: F1511494 | Pack size: 50 μg, 1 mg | Storage: Protected from light, Store at -20°C

Overview

Fluo-20 AM Calcium Probe is a professional tool for the precise detection of intracellular calcium ion concentration changes. This product uses the Fluo-20 AM calcium ion fluorescent probe as its core detection component, and its detection principle is as follows: Fluo-20 AM is cell membrane-permeable and can easily enter cells with simple incubation. Once inside the cell, it is cleaved by intracellular esterases to generate membrane-impermeable Fluo-20, which is retained in the cell to exert its corresponding physiological functions. This product enables the detection of calcium ion concentration changes on multiple platforms, including fluorescence microscopes, fluorescence microplate readers, and flow cytometers, providing key technical support for cell biology research, disease mechanism exploration, and drug development.

As an upgraded version of the classic calcium indicators Fluo-8 AM, Fluo-4 AM, and Fluo-3 AM, Fluo-20 AM achieves comprehensive performance optimization. Fluo-3 AM and Fluo-4 AM are the most commonly used visible-light-excitable calcium indicators in live-cell calcium imaging research; however, they only exhibit moderate fluorescence intensity in live cells, and relatively harsh cell loading conditions are required to achieve optimal cellular calcium responses. In contrast, Fluo-20 AM allows cell staining at room temperature, whereas Fluo-3 AM and Fluo-4 AM must be loaded onto cells at 37 °C. Fluo-20 AM significantly improves cell loading and calcium response outcomes. Its fluorescence brightness is twice that of Fluo-8 AM, four times that of Fluo-4 AM, and eight times that of Fluo-3 AM, far exceeding the fluorescence brightness of Fluo-8 AM, Fluo-4 AM, and Fluo-3 AM.

Application: Calcium ion detection

Product Features

1. 10-minute rapid staining: Directly add the reagent after mixing, and staining can be completed in as little as 10 minutes after incubation, greatly reducing experimental time and operational steps.

2. Flexible loading conditions: Excellent staining can be achieved at either room temperature or 37 °C, eliminating the need for harsh cell loading conditions.

3. Higher fluorescence intensity: Fluorescence brightness is 4 times that of Fluo-4 AM, 8 times that of Fluo-3 AM, and 2 times that of Fluo-8 AM, significantly improving the distinguishability of detection signals.

4. Ultra-high sensitivity: Low background signal interference, high detection sensitivity, and easy capture of low-amplitude calcium signal fluctuations.

Product Parameters

1. Appearance: Solid powder soluble in DMSO.

2. Ex/Em: 494/516 nm.

3. Spectrum:

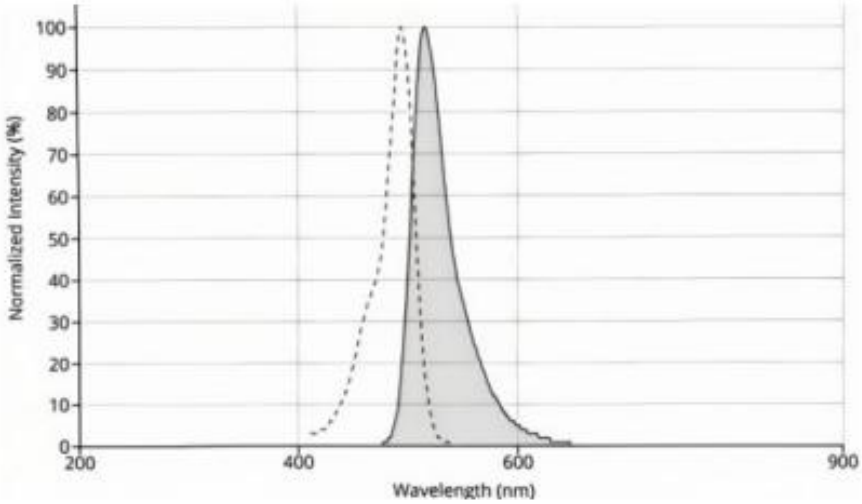

Figure 1. Spectrum of Fluo-20 AM Calcium Ion Fluorescent Probe.

Product Components

Component	2300 T
Fluo-20 AM Calcium Probe	5 mg

Note: *Calculated based on 100 μL of staining working solution per use, with a working solution concentration of 4 μM.*

Precautions

1. AM ester groups are hygroscopic. Ensure the product is equilibrated to room temperature before opening. Fluo-20 AM is a trace amount and difficult to observe; after equilibration to room temperature, perform a quick spin to ensure the powder is centrifuged to the bottom of the tube.

2. Fluo-20 AM is extremely sensitive to hydrolysis in aqueous solutions. If not used entirely at once, it is recommended to aliquot the stock solution into small portions for storage.

3. All fluorescent dyes are subject to photobleaching; minimize exposure to light.

4. This product is for research use only and must not be stored in ordinary residential premises.

5. For your safety and health, please comply with the standard laboratory safety regulations of your institution.

Instructions for Use

I. Pre-Experiment Preparation

1. Stock Solution Preparation:

Remove Fluo-20 AM from the refrigerator, equilibrate to room temperature, and perform a quick spin to pellet the powder at the bottom of the tube. Prepare a 2-5 mM Fluo-20 AM stock solution using anhydrous DMSO. For example: To prepare a 5 mM stock solution, add 191 µL of anhydrous DMSO to a tube containing 1 mg of Fluo-20 AM.

Note: *The prepared stock solution should be aliquoted and stored at -20 °C, preferably with Parafilm tightly wrapped around the tube cap.*

2. Working Solution Preparation:

(1) Dilute the Fluo-20 AM stock solution with PBS or HBSS to prepare a 4 µM Fluo-20 AM working solution. For most cell lines, a final working concentration of 4-5 µM is recommended.

Note: *The optimal loading concentration varies by cell type. It is recommended to determine the optimal concentration before formal experiments. To avoid cytotoxicity from overloading, use the lowest probe concentration that yields valid results, starting with 4 µM for optimization.*

(2) (Optional) If Fluo-20 AM uptake into cells is poor, add an appropriate amount of 20% Pluronic F-127 solution to the Fluo20 AM solution to prevent aggregation in the buffer and promote cellular uptake. The final concentration of Pluronic F-127 should be controlled between 0.04–0.05%.

Note:

a) To prepare a 20% (w/v) Pluronic F-127 DMSO stock solution: Add 0.5 mL of DMSO to 100 mg of Pluronic F-127. Dissolution requires heating at 40-50 °C for 20-30 minutes. Store at room temperature after dissolution; do not refrigerate. If crystals precipitate, reheat to dissolve before use, which does not affect performance.
b) The use of Pluronic F-127 reduces the stability of Fluo-20 AM. Therefore, it is only recommended to add it when preparing the working solution, not the stock solution.
c) If your cells express organic anion transporters, add probenecid (1-2 mM) to the dye working solution (final concentration 0.5-1 mM) to reduce leakage of the de-esterified indicator.

3. Instrument Preparation:

(1) Fluorescence microscope, Ex/Em: 494/516 nm.

(2) Flow cytometer, Ex/Em: 494/516 nm.

(3) Fluorescence microplate reader, Ex/Em: 494/516 nm.

4. Control Setup:

Group	Fluo-20 AM Calcium Probe	Sample Cells
Negative Control	+	Untreated cells
Experimental Group	+	Experimentally treated cells

(1) Negative control: Cells are not subjected to any treatment, providing a reference for calcium signal changes after experimental treatment in the test group.

(2) Experimental group: To verify probe activity and cell loading efficiency.

II. Operating Procedures (Using a 96-well Plate as an Example)

Protocol 1: Fluorescence Microscopy Detection (Suspension Cells)

1. Cell Collection and Washing:

(1) Collect the test suspension cells into a centrifuge tube, centrifuge at 1000 rpm for 5 minutes at room temperature, and carefully aspirate the supernatant.

(2) Wash the cells once with a conventional buffer such as PBS or HBSS.

Note: *Washing generally reduces background fluorescence. When aspirating culture medium, PBS, HBSS, or other conventional buffers, a vacuum pump is preferred. Phenol red or serum may interfere with the detection of this kit.*

2. Cell Resuspension and Counting:

(1) Resuspend the cell pellet in PBS or HBSS and perform cell counting.

(2) Take 5×10^4-1×10^5 cells, centrifuge at 1000 rpm for 5 minutes at low speed, and remove PBS or HBSS.

Note: *The microscopy method is not strict on cell density, as long as the density is consistent between the experimental and control groups. It can be flexibly adjusted according to sample type and experimental conditions, with 70–85% cell distribution in the microscope field of view being optimal.*

3. Cell Staining:

(1) Add 100 µL of Fluo-20 staining working solution to the 96-well plate and incubate at 37 °C in the dark for 30 minutes. The incubation time can be adjusted between 10-60 minutes, with rapid staining achievable in as little as 10 minutes to maximize cell viability and simplify the experimental workflow.

Note: *If the incubation temperature and time are uncertain for the first experiment, it is recommended to incubate at 37 °C for 30 minutes initially and observe the fluorescence effect. If excessive cell death occurs, shorten the time appropriately; if the fluorescence intensity is too weak, extend the time appropriately.*

(2) After incubation, wash the cells 1-3 times with PBS or HBSS.

4. Microscopic Observation and Photography:

Add the cell suspension to the 96-well plate, let it stand briefly to allow the cells to settle naturally to the bottom, and observe under a fluorescence microscope.

Note: *The volume of cell suspension can be adjusted according to the cell count, with 70-85% cell distribution in the microscope field of view being optimal.*

Protocol 2: Fluorescence Microscopy Detection (Adherent Cells)

1. Cell Treatment:

Seed cells in a 96-well plate one day in advance to achieve 70-85% confluency. After the cells adhere, treat them according to the experimental design.

2. Cell Washing:

(1) Carefully aspirate the cell culture medium.

(2) Add 100 µL of PBS or HBSS to gently wash the cells once, then aspirate the PBS or HBSS completely after washing.

3. Cell Staining:

(1) Add 100 µL of Fluo-20 staining working solution to the 96-well plate and incubate at 37 °C in the dark for 30 minutes. The incubation time can be adjusted between 10-60 minutes, with rapid staining achievable in as little as 10 minutes to maximize cell viability and simplify the experimental workflow.

Note: *If the incubation temperature and time are uncertain for the first experiment, it is recommended to incubate at 37 °C for 30 minutes initially and observe the fluorescence effect. If excessive cell death occurs, shorten the time appropriately; if the fluorescence intensity is too weak, extend the time appropriately.*

(2) After incubation, wash the cells 1-3 times with PBS or HBSS.

4. Microscopic Observation and Photography:

(1) After washing, add an appropriate volume of PBS or HBSS to each well to keep the cells moist.

(2) Place the culture plate directly under the fluorescence microscope for observation and photography. Filter settings are the same as in Protocol 1.

Protocol 3: Flow Cytometry Detection (Suspension and Adherent Cells)

1. Cell Preparation:

(1) Suspension cells: Same as Protocol 1; take 5×10^4-1×10^5 cells.

(2) Adherent cells:

a. Aspirate the culture medium and gently wash the cells once with PBS or HBSS.

b. Add an appropriate amount of trypsin digestion solution (just enough to cover the cells), observe under a microscope at room temperature until the cells round up and intercellular spaces increase, then gently pipette to detach the cells completely.

c. Critical step: Immediately add complete medium containing serum to terminate digestion.

d. Transfer the cell suspension to a centrifuge tube, centrifuge at 1000 rpm for 5 minutes at room temperature, and aspirate the supernatant.

e. Resuspend the cells in PBS or HBSS and perform cell counting.

f. Take 1×10^6 cells, centrifuge at 1000 rpm for 5 minutes at low speed at room temperature, and aspirate the supernatant.

2. Cell Staining:

(1) Add 1 mL of Fluo-20 staining working solution to the cells and incubate at 37 °C in the dark for 30 minutes. The incubation time can be adjusted between 10-60 minutes, with rapid staining achievable in as little as 10 minutes to maximize cell viability and simplify the experimental workflow.

Note: *If the incubation temperature and time are uncertain for the first experiment, it is recommended to incubate at 37 °C for 30 minutes initially and observe the fluorescence effect. If excessive cell death occurs, shorten the time appropriately; if the fluorescence intensity is too weak, extend the time appropriately.*

(2) After incubation, wash the cells 1-3 times with PBS or HBSS.

3. Instrument Detection:

(1) Important: Perform flow cytometry detection within 1 hour after staining to ensure stable fluorescence signals.

(2) Analyze using a flow cytometer under preset detection channels.

(3) First, adjust the voltage using a blank control (unstained cells) to position the cell population in the lower-left corner of the coordinate system.

(4) Next, detect the control and experimental groups to confirm the validity of the experimental system.

(5) Finally, detect the experimental samples and record and analyze the mean fluorescence intensity of the cells.

Note: *All samples must be kept in the dark, on ice, and detected immediately after staining. Quantitative experiments (e.g., flow cytometry) must be completed strictly within 1 hour to ensure accurate and reproducible data.*

Protocol 4: Fluorescence Microplate Reader Detection (Suspension and Adherent Cells)

1. Seeding and Culture:

Seed cells in a black 96-well plate, controlling the number of cells per well between 100-10,000, typically 2000-5000.

2. Washing:

Collect the test suspension cell suspension into a centrifuge tube.

(1) Suspension cells: Centrifuge at 250-1000× g for 5 minutes at room temperature, aspirate the supernatant, and wash once with PBS or HBSS.

(2) Adherent cells: Aspirate the culture medium and wash the cells once with PBS or HBSS.

Note: *Washing generally reduces background fluorescence. When aspirating culture medium, PBS, or HBSS, a vacuum pump is preferred. Phenol red or serum may interfere with the detection of this kit.*

3. Cell Staining:

(1) Add 100 µL of Fluo-20 staining working solution to the 96-well plate and incubate at 37 °C in the dark for 30 minutes. The incubation time can be adjusted between 10-60 minutes, with rapid staining achievable in as little as 10 minutes to maximize cell viability and simplify the experimental workflow.

Note: *If the incubation temperature and time are uncertain for the first experiment, it is recommended to incubate at 37 °C for 30 minutes initially and observe the fluorescence effect. If excessive cell death occurs, shorten the time appropriately; if the fluorescence intensity is too weak, extend the time appropriately.*

(2) After incubation, wash the cells 1-3 times with PBS or HBSS.

4. Fluorescence Microplate Reader Detection:

Detect under the preset conditions of the fluorescence microplate reader. By comparing the RFU (Relative Fluorescence Units) of the control and treatment groups, the effect of drug stimulation can be determined.

III. Result Interpretation:

Qualitative Analysis (Microscopy):

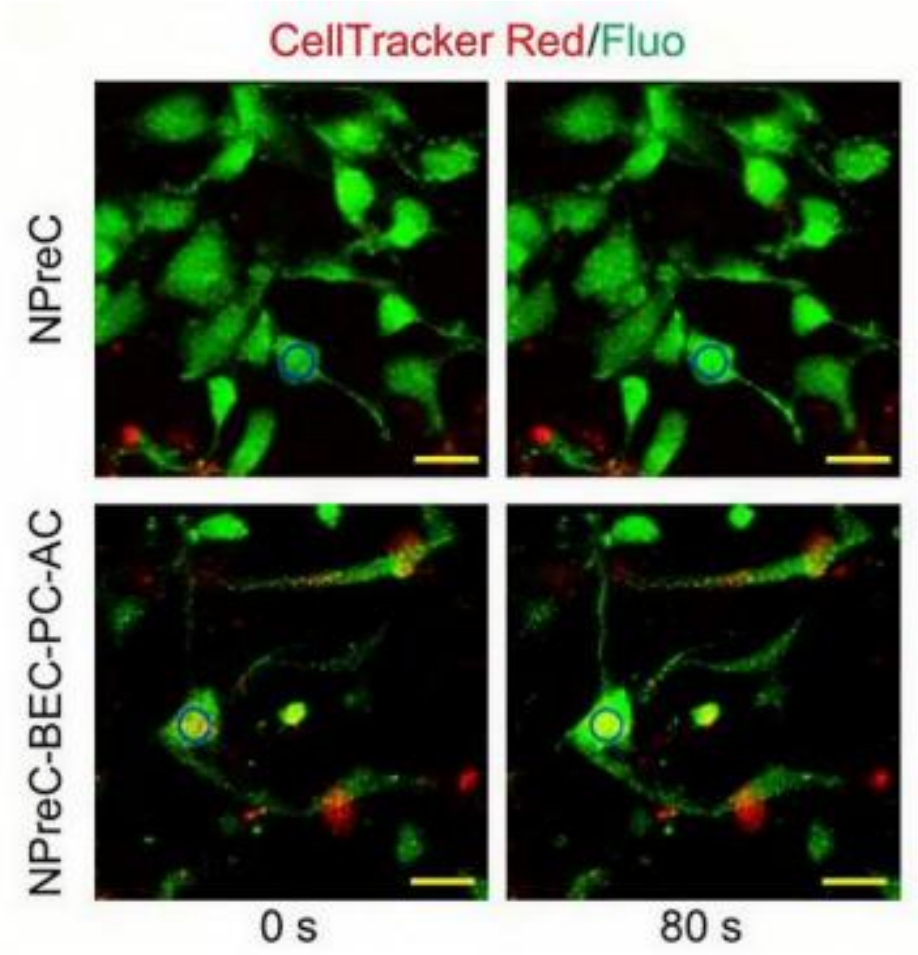

Figure 2. Dynamic changes in intracellular calcium concentration in neuronal cells

Under a fluorescence microscope:

Figure 2 shows the distribution and intensity of green fluorescence within cells at different time points, analyzed to study the dynamic calcium response of neurons under stimulation and the mechanism of calcium signaling in neuronal function.

Note: *Image adapted from "Bioengineered perfused human brain microvascular networks enhance neural progenitor cell survival, neurogenesis, and maturation" (doi:10.1126/sciadv.aaz9499).*

Frequently Asked Questions and Answers

1. Q: Why is no fluorescence value detected or the fluorescence value is low?

A: This may be due to insufficient cell density; you can increase the cell density. Alternatively, consider extending the incubation time.

2. Q: What should I do if excessive cell death is observed after Fluo-20 incubation?

A: First, assess whether the dye concentration and treatment time are appropriate. Although Fluo-20 AM is relatively safe, excessively high concentrations or prolonged staining times may still cause cell damage. It is recommended to reduce the dye concentration or shorten the staining time, and closely monitor cell status during the experiment. Note 2:

Specifications

Attribute	Value
Synonyms	Fluo-20 AM Calcium Probe
Specifications & Purity	BioReagent,Biological Stain,for microscopy,sterile,for fluorescence analysis
Stability And Storage	Store at -20 °C long term (60 months). Store in the dark. Storage Conditions Protected from light,Store at -20°C
Shipped In	Ice chest + Ice pads This product requires cold chain shipping. Ground and other economy services are not available.

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