Ready-to-use Fluo-20 AM Calcium Ion Fluorescent Probe (2 mM)

Cat. No.: R1511495 | Pack size: 50 µL | Storage: Protected from light, Store at -20°C

Overview

Product Description Fluo-20 AM is an optimized, upgraded calcium fluorescent probe excited by visible light, specially designed for live-cell calcium imaging. It features a conveniently adaptable spectrum (Ex/Em: 495/516 nm) and can directly replace Fluo-3 AM, Fluo-4 AM, and Fluo-8 AM without adjusting instrument parameters. Fluo-20 AM exhibits low temperature dependence, making it more suitable for special cell types and highthroughput experiments. Its fluorescence brightness is 4-fold that of Fluo-4 AM, 8-fold that of Fluo-3 AM, and 2-fold that of Fluo-8 AM, 40% higher than imported Fluo-8 AM, enabling accurate detection of weak calcium signals. It maintains a high signal-to-noise ratio even in deep thick tissues or at low probe concentrations, and shows lower cytotoxicity, thus improving cell viability during long-term live-cell monitoring. In addition, Fluo-20 AM possesses excellent cell membrane permeability. After being cleaved by intracellular esterases, it can be stably retained in the cytoplasm and respond precisely to changes in Ca^{2+} concentration. Compared with UV-excited probes, it avoids cell damage caused byUV light, preserves the physiological state of cells, and offers stronger tissue penetration, easier operation, and good compatibility with other fluorescent labels, making it ideal for multicolor co-staining experiments.

With its key advantages including visible light excitation, high brightness, flexible loading, low toxicity, strong tissue penetration and multicolor compatibility, Fluo-20 AM is widely suited for diverse research needs. Its applications cover cellular physiological mechanism research, signaling pathway analysis, drug activity screening, preclinical in vivo imaging and other fields. It is the preferred probe for calcium-related studies in biomedicine, pharmacology and other disciplines, especially suitable for special experimental scenarios with weak signals such as primary cells and low-calcium-response cells.

Application: Calcium ion detection

Product Features

1. Convenient spectral compatibility: Ex/Em: 495/516 nm; directly interchangeable with Fluo-3/4/8 AM without instrument adjustment.

2. Flexible loading conditions: Low temperature dependence; efficient staining at room temperature or 37 °C, ideal for high-throughput screening.

3. Ultra-strong fluorescence brightness: 4× brighter than Fluo-4 AM, 8× brighter than Fluo-3 AM, and 40% brighter than imported Fluo-8 AM.

4. Low cytotoxicity: Visible light excitation preserves cell physiology and improves survival in long-term live-cell imaging.

5. Broad applicability: Excellent membrane permeability and tissue penetration; compatible with multicolor staining; suitable for live cells, thick tissues, in vivo detection, and drug screening.

Product Parameters

1. Ex/Em: 495/516 nm.

2. Spectrum:

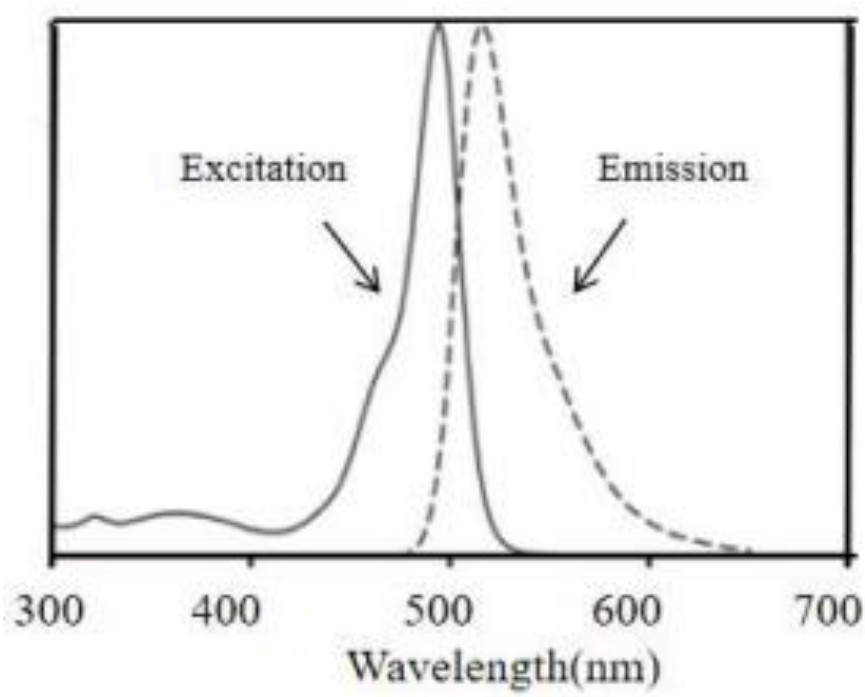

Figure 1. Spectrum of Ready-to-use Fluo-20 AM Calcium Ion Fluorescent Probe (2 mM).

Product Components

Component	250 T
Ready-to-use Fluo-20 AM Calcium Probe (2 mM)	50 µL

Note: *Calculated based on 100 µL staining solution per well in a 96-well plate, working concentration of 4 µM, and 0.2 µL probe per well.*

Precautions

1. Fluo-20 AM is highly unstable in water. If not used entirely at once, aliquot the stock solution into small portions for storage.

2. All fluorescent dyes are prone to photobleaching; protect from light during use and storage.

3. This product is for research use only and must not be stored in residential areas.

4. For your safety and health, please follow the general laboratory safety regulations of your institution.

Instructions for Use

I. Pre-Experiment Preparation

1. Working Solution Preparation

(1) Remove the reagent and equilibrate to room temperature before use.

(2) Taking a 96-well plate as an example, prepare 2-20 μM Fluo-20 AM working solution by diluting the Fluo-20 AM stock solution with PBS or HBSS, using a system of 100 μL Fluo-20 AM staining working solution per well. (Volumes of staining working solution for other plate formats are as follows: 150 μL/well for 48-well plates, 250 μL/well for 24-well plates, 500 μL/well for 12-well plates, and 1 mL/well for 6-well plates.)

Table 1. Volume of Fluo-20 AM Staining Solution

Reagent	1 well	10 wells	100 wells
Fluo-20 AM	0.2 μL	2 μL	20 μL

Note: *Prepare working solution freshly, protect from light, and do not freeze-store. The volume can be adjusted between 0.2-2 μL per well based on staining performance. The above volumes are for a final concentration of 4 μM.*

(3) (Optional) If cellular uptake is inefficient, add 20% Pluronic F-127 solution to a final concentration of 0.04-0.05% to prevent aggregation and enhance loading. Note: Preparation of 20% (w/v) Pluronic F-127 stock solution in DMSO: Add 0.5 mL DMSO to 100 mg Pluronic F-127 to prepare a 20% (w/v) stock solution. Dissolve by heating at 40-50 °C for 20-30 min. Store at room temperature after dissolution; do not refrigerate. If crystals precipitate, reheat to redissolve before use, which does not affect performance.

Note: Pluronic F-127 may reduce probe stability and is only recommended in working solution, not stock solution.

Note: For cells expressing organic anion transporters, add probenecid (1-2 mM stock) to a final concentration of 0.5-1 mM to reduce indicator leakage.

2. Instrument Preparation

(1) Fluorescence microscope: Ex/Em 495/516 nm.

(2) Flow cytometer: Ex/Em 495/516 nm.

(3) Fluorescence microplate reader: Ex/Em 495/516 nm.

3. Control Group Setup

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

(1) Negative Control: Cells without any treatment, used as baseline for calcium signal comparison.

(2) Experimental Group: Used to validate probe activity and cellular loading efficiency.

II. Protocols (96-well plate)

Protocol 1: Fluorescence Microscopy (Suspension Cells)

1. Cell Collection & Washing

(1) Collect suspension cells into a centrifuge tube.

(2) Centrifuge at 1000 rpm for 5 min at room temperature; carefully remove supernatant.

Note: Washing reduces background fluorescence. A vacuum aspirator is recommended. Phenol red and serum may interfere.

2. Cell Resuspension and Counting

(1) Resuspend cells in PBS and perform cell counting.

(2) Take 5×10^4-1×10^5 cells, centrifuge at 1000 rpm for 5 min at low speed, and discard PBS.

(3) Resuspend in 100 µL of standard buffer such as PBS or HBSS. Centrifuge at 1000 rpm for 5 min at low speed, then remove the supernatant.

Note: The microscopy method is not strict with cell density, as long as the density is consistent between the experimental group and the control group. It can be flexibly adjusted according to the sample type and experimental conditions. The optimal cell distribution in the microscope field is about 70%-85%.

3. Cell Staining:

(1) Prepare the Fluo-20 AM staining working solution according to Table 1.

(2) Add 100 µL of Fluo-20 AM staining working solution to each well of the 96-well plate, and incubate at 37 °C for 30 min in the dark. The incubation time can be adjusted from 10 to 60 min.

Rapid staining can be completed in as fast as 10 min, maximizing cell viability and simplifying the experimental procedure.

Note: If the incubation temperature and time are uncertain for the first experiment, it is recommended to start with incubation at 37 °C for 30 min and evaluate the fluorescence signal. Shorten the incubation time accordingly if excessive cell death occurs, or prolong it accordingly if the fluorescence intensity is insufficient.

4. Washing

(1) Centrifuge at 1000 rpm for 5 min.

(2) Discard dye-containing supernatant.

(3) Wash once with PBS or HBS to remove residual probe and reduce background.

5. Observation & Imaging

Add cell suspension to 96-well plate; allow to settle, then observe under fluorescence microscope.

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

Protocol 2: Fluorescence Microscopy (For Adherent Cells)

1. Cell Preparation

Seed cells in the culture plate one day in advance to achieve 70%-85% confluency. After the cells have adhered completely, treat them according to the experimental design.

2. Cell Washing

(1) Carefully aspirate the cell culture medium.

(2) Add the corresponding volume of regular buffer (e.g., PBS or HBS) to gently wash the cells once, according to the plate format (e.g., 100 µL/well for 96-well plates, 150 µL/well for 48-well plates, 250 µL/well for 24-well plates, 500 µL/well for 12-well plates, 1 mL/well for 6-well plates). Aspirate the buffer completely after washing.

3. Cell Staining

(1) Prepare the Fluo-20 AM staining working solution according to Table 1.

(2) Add 100 µL of Fluo-20 AM staining working solution to each well and incubate at 37 °C for 30 min in the dark. The incubation time can be adjusted between 10-60 min. Rapid staining is achievable in as little as 10 min to maximize cell viability and simplify the workflow.

Note: For the first experiment, it is recommended to incubate at 37 °C for 30 min as a starting point. If excessive cell death is observed, shorten the incubation time appropriately. If the fluorescence intensity is too weak, extend the incubation time appropriately.

4. Washing

(1) After incubation, aspirate the staining working solution from each well.

(2) Add PBS or HBS buffer and wash the cells once to thoroughly remove residual Fluo-20 AM probe and reduce background noise.

5. Microscopic Observation and Imaging

(1) After washing, add the corresponding volume of PBS or HBS buffer to each well to keep the cells moist.

(2) Place the culture plate directly under a fluorescence microscope for observation and imaging. Use the same filter settings as described in Protocol 1.

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

1. Cell Preparation

(1) Suspension cells: Follow the steps in Protocol 1 and use 5×10^4-1×10^5 cells.

(2) Adherent cells:

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

b. Add an appropriate amount of trypsin 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 serum-containing complete medium to terminate the digestion.

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

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

f. Aliquot 5×10^4-1×10^5 cells, centrifuge at 1000 rpm for 5 min at room temperature, and aspirate the supernatant.

2. Cell Staining

(1) Prepare the Fluo-20 AM staining working solution according to Table 1.

(2) Add 1 mL of Fluo-20 AM staining working solution to the cell pellet and incubate at 37 °C for 30 min in the dark. The incubation time can be adjusted between 10-60 min. Rapid staining is achievable in as little as 10 min to maximize cell viability and simplify the workflow.

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

3. Washing

(1) After incubation, centrifuge at 1000 rpm for 5 min at room temperature to collect the cells.

(2) Carefully aspirate the dye-containing supernatant (treat as waste and dispose of properly).

(3) Gently resuspend the cells in PBS or HBS buffer and wash once to thoroughly remove residual Fluo-20 AM probe and reduce background noise.

(4) After the final wash, aspirate the supernatant completely and resuspend the cells in 500 µL of PBS or HBSS buffer to prepare the sample for flow cytometry.

4. Instrument Detection

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

(2) Analyze the samples on a flow cytometer using the preset detection channel.

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

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

(5) Finally, detect the experimental samples and record the mean fluorescence intensity (MFI) for analysis. Note: All stained samples must be protected from light, kept on ice, and analyzed immediately. For quantitative experiments (e.g., flow cytometry), strict completion within 1 hour is mandatory to ensure accurate and reproducible data.

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

1. Cell Seeding and Culture

Seed cells in a black 96-well plate. The number of cells per well should be controlled between 100-10,000, with a recommended range of 20005000 cells per well.

2. Washing

Collect the test suspension cell suspension into a centrifuge tube.

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

(2) Adherent cells: Aspirate the culture medium and wash the cells once with PBS or HBSS buffer. Note: Washing generally helps reduce background fluorescence. A vacuum pump is recommended for aspirating culture medium, PBS, or HBSS. Phenol red or serum may interfere with the detection of this kit.

3. Cell Staining

(1) Prepare the Fluo-20 AM staining working solution according to Table 1.

(2) Add 100 µL of Fluo-20 AM staining working solution and incubate at 37 °C for 30 min in the dark. The incubation time can be adjusted between 10-60 min. Rapid staining is achievable in as little as 10 min to maximize cell viability and simplify the workflow. Note: If the incubation temperature and duration are not determined in the first experiment, it is recommended to incubate at 37 °C for 30 min and observe the fluorescence signal. Shorten the incubation time accordingly if excessive cell death is observed, or prolong the incubation time accordingly if the fluorescence intensity is insufficient.

4. Washing

(1) After incubation, centrifuge at 1000 rpm for 5 min at room temperature to collect the cells.

(2) Carefully aspirate the dye-containing supernatant (treat as waste and dispose of properly).

(3) Add PBS or HBSS buffer and wash the cells once to reduce background noise.

(4) After the final wash, aspirate the supernatant completely and resuspend the cells in 100 µL of PBS or HBSS buffer to prepare the test samples.

5. Fluorescence Microplate Reader Detection Detect the fluorescence intensity under the preset conditions of the microplate reader. The effect of drug stimulation can be determined by comparing the Relative Fluorescence Units (RFU) between the control and treatment groups.

III. Result Interpretation

1. Qualitative Analysis (Microscopy)

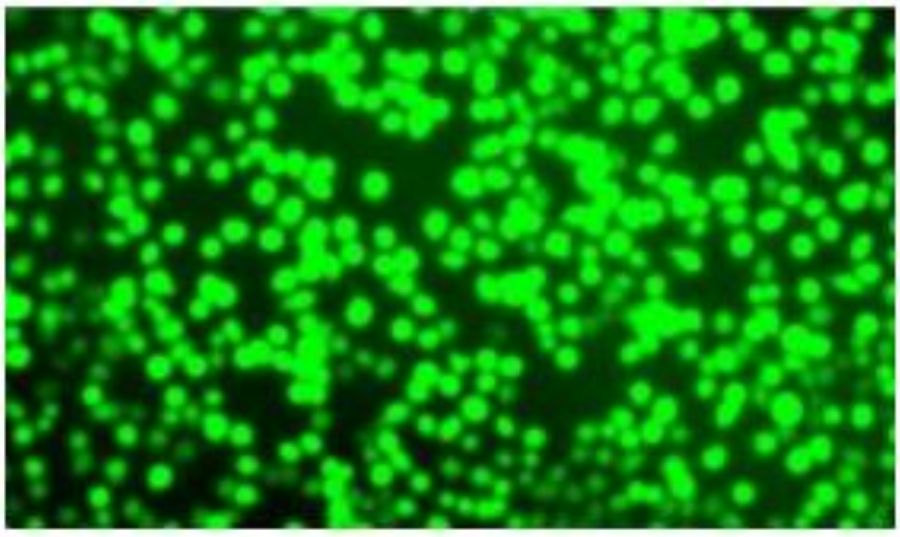

Figure 2. Fluorescence Detection of Intracellular Calcium Concentration ([Ca^{2+}]i) in HeLa Cells 15 Seconds After Ionomycin Treatment

Under the fluorescence microscope:

Figure 2 shows the distribution and intensity of green fluorescence in HeLa cells after ionomycin treatment. When the calcium ion concentration rises upon drug treatment, the fluorescence intensity of Fluo-20 AM-labeled cells increases significantly.

2. Quantitative Analysis (Flow Cytometry)

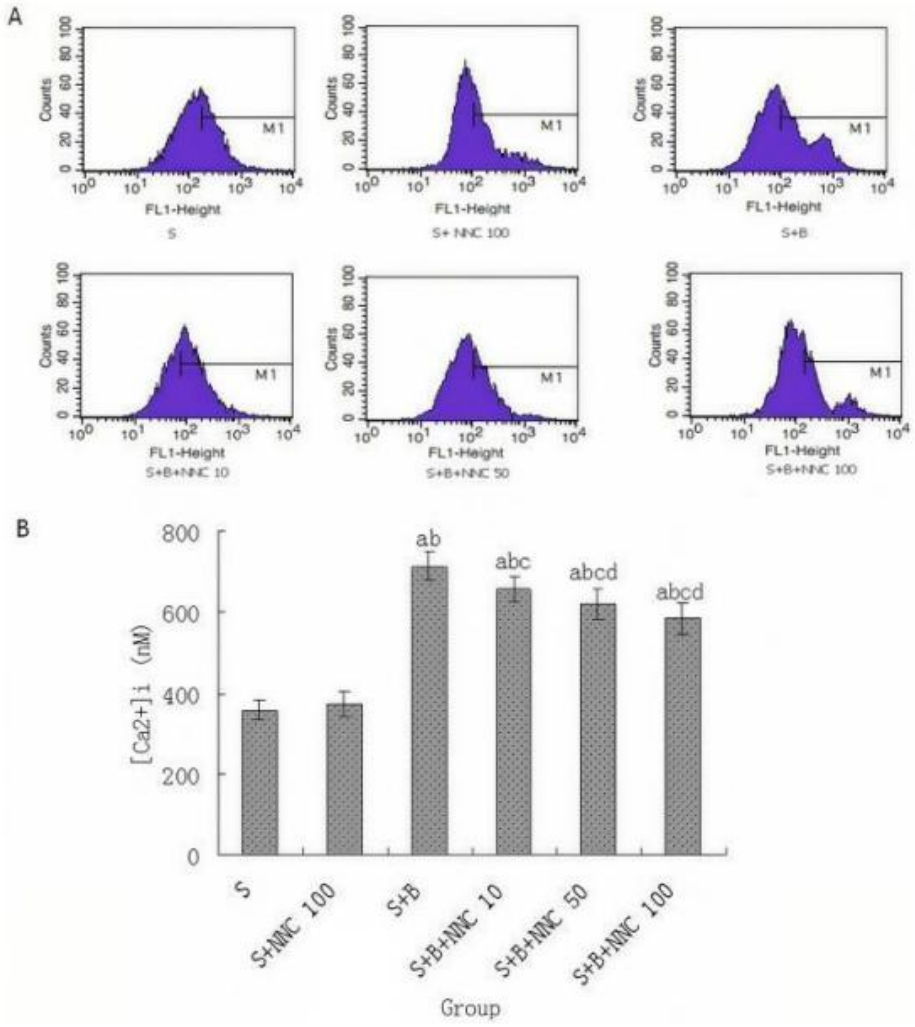

Figure 3. Flow Cytometric Analysis of Cytosolic Calcium Concentration ([Ca^{2+}]i) Changes in SH-SY5Y Cells During Bupivacaine-Induced Injury Note: Image adapted from the article Neurotoxicity Induced by Bupivacaine via T-Type Calcium Channels in SH-SY5Y Cells (doi: 10.1371/journal.pone.0062942).

Flow Cytometry Results:

In Figure 3, the [Ca^{2+}]i levels in the control group (Group S) and the NNC 100 mM pre-treatment group (Group S+NNC 100) were 358±25 nM and 372±32 nM, respectively, showing no significant difference. The bupivacaine-treated group (Group S+B, 1 mM bupivacaine) exhibited a significant increase in [Ca^{2+}]i to 715±35 nM, which was much higher than that of the control group. In the NNC pre-treatment plus bupivacaine groups, [Ca^{2+}]i gradually decreased with increasing NNC concentrations: 657±29 nM (10 mM NNC), 619±37 nM (50 mM NNC), and 585±39 nM (100 mM NNC). There was no significant difference between the 50 mM and 100 mM NNC groups, indicating that the protective effect had reached a plateau.

Frequently Asked Questions (FAQ)

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

A: This may be due to insufficient cell density. You can increase the cell density or consider extending the incubation time.

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

A: First, evaluate 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 the cell status during the experiment.

Specifications

Attribute	Value
Synonyms	Ready-to-use Fluo-20 AM Calcium Probe | Ready-to-use Fluo-20 AM Calcium Ion Fluorescent Probe
Specifications & Purity	BioReagent,sterile,for microscopy,Biological Stain,for fluorescence analysis,ready-to-use
Stability And Storage	Store at -20 ℃ 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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