
Fluo-8 AM Calcium Ion Fluorescent Probe

Cat. No.: F1511493 | Pack size: 50 µg, 1 mg | Storage: Protected from light, Store at -20°C

Overview

Fluo-8 AM Calcium Probe is a professional tool for the accurate detection of intracellular calcium ion concentration changes. Centered on Fluo-8 AM calcium ion fluorescent probe as the core detection component, its detection principle is as follows: Fluo-8 AM is an acetoxymethyl ester derivative of Fluo-8 with cell membrane permeability. It can easily enter cells through simple incubation. Once inside the cell, it is cleaved by intracellular esterases to form Fluo-8, which lacks membrane permeability and thus accumulates in the cytoplasm to exert its corresponding physiological functions. This product is compatible with multiple detection platforms such as fluorescence microscopes, fluorescent microplate readers, and flow cytometers for measuring calcium ion concentration changes, providing critical technical support for cell biology research, disease mechanism exploration, and drug development.

As an upgraded product of the classic calcium indicators Fluo-4 AM and Fluo-3 AM, Fluo-8 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 studies, but they only exhibit moderate fluorescence intensity in live cells and require relatively stringent cell loading conditions to achieve optimal calcium responses. In contrast, Fluo-8 AM allows cell staining at room temperature, whereas Fluo-3 AM and Fluo-4 AM must be loaded at 37 °C. Fluo-8 AM significantly improves cell loading efficiency and calcium response performance. Its fluorescence brightness is twice that of Fluo-4 AM and four times that of Fluo-3 AM, making it an ideal replacement for both.

Note: Equivalent in efficacy to AAT Fluo-8[®], AM.

Note: Fluo-8[®] in this document is a trademark and registered trademark of AAT Bioquest.

Application

Calcium Ion Detection

Product Features

1. 10-Minute Rapid Staining: Add the mixed reagent directly to samples; staining can be completed in as little as 10 minutes after incubation, greatly reducing experimental time and operational steps.
2. Flexible Loading Conditions: Achieves excellent staining at either room temperature or 37 °C incubation, eliminating the need for stringent cell loading conditions.

3. Higher Fluorescence Intensity: Fluorescence brightness is twice that of Fluo-4 AM and four times that of Fluo-3 AM, significantly enhancing the distinguishability of detection signals.
4. Ultra-High Sensitivity: Low background signal interference and high detection sensitivity enable easy capture of low amplitude calcium signal fluctuations.

Product Specifications

1. Appearance: Solid powder soluble in DMSO
2. CAS No.: 1345980-40-6
3. Ex/Em: 494/516 nm
4. Molecular Formula: $C_{50}H_{50}N_2O_{23}$
5. Molecular Weight: 1046.93
6. Spectrum:

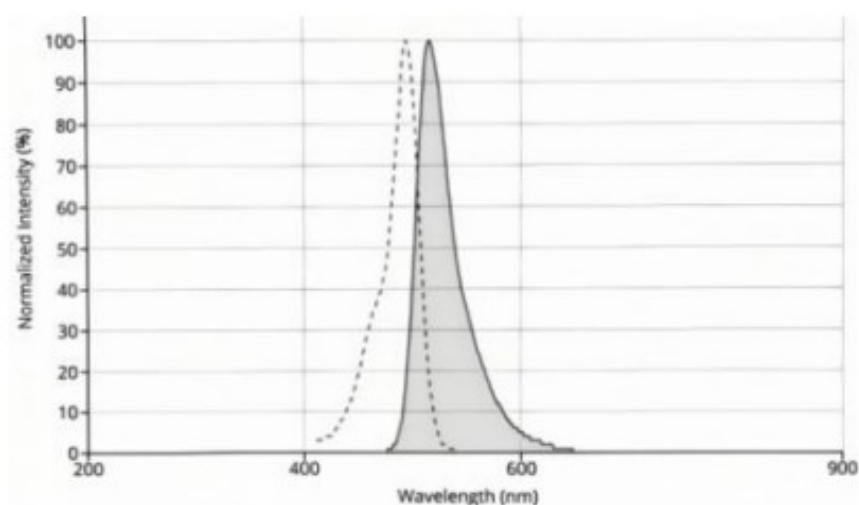


Figure 1. Spectrum of Fluo-8 AM Calcium Ion Fluorescent Probe.

Product Components

Component	2300 T
Fluo-8 AM Calcium Probe	1 mg

Note: Calculated based on using 100 μ L of staining working solution at a concentration of 4 μ M per assay.

Precautions

1. AM esters are hygroscopic. Ensure the product reaches room temperature before opening after removal from the refrigerator. Fluo-8 AM is present in trace amounts and may be difficult to observe; briefly centrifuge after warming to ensure the powder collects at the bottom of the tube.
2. Fluo-8 AM decomposes rapidly upon contact with water. If not used in one batch, aliquot the stock solution for storage.
3. All fluorescent dyes are subject to photobleaching; avoid light exposure as much as possible.
4. This product is for research use only and shall not be stored in ordinary residential premises.
5. For your safety and health, comply with the general laboratory safety regulations of your institution.

Instructions for Use

I. Pre-Experiment Preparation

1. Stock Solution Preparation

Remove Fluo-8 AM from the refrigerator, allow it to reach room temperature, and briefly centrifuge to collect the powder at the tube bottom. Prepare a 2-5 mM Fluo-8 AM stock solution using anhydrous DMSO. Example: To prepare a 5 mM stock solution, add 191 μ L of anhydrous DMSO to a 1 mg vial.

Note: Aliquot the prepared stock solution and store at -20 °C; tightly seal the tube caps with parafilm for optimal preservation.

2. Working Solution Preparation

(1) Dilute the Fluo-8 AM stock solution with PBS or HBSS to prepare a 4 μ M working solution. A final concentration of 4-5 μ M is recommended for most cell lines. Note: Optimal loading concentrations vary by cell type. Perform a concentration optimization assay before formal experiments. To avoid cytotoxicity from overloading, use the lowest effective probe concentration, starting with 4 μ M.

(2) (Optional) If Fluo-8 AM uptake is inefficient, add an appropriate amount of 20% Pluronic F-127 solution to the Fluo-8 AM working solution to prevent aggregation in the buffer and enhance cellular uptake. Maintain the final concentration of Pluronic F-127 at 0.04-0.05%.

Notes: (1) Preparation of 20% (w/v) Pluronic F-127 DMSO Stock Solution: Dissolve 100 mg of Pluronic F-127 in 0.5 mL of DMSO. Heat at 40-50 °C for 20-30 minutes to dissolve completely. Store at room temperature (do not refrigerate). If crystallization occurs, reheat to redissolve; this does not affect performance.

(2) Pluronic F-127 may reduce the stability of Fluo-8 AM. Add it only to the working solution, not the stock solution.

(3) If your cells express organic anion transporters, add probenecid (1-2 mM stock) 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) Fluorescent microplate reader (Ex/Em: 494/516 nm)

4. Control Group Setup

Group	Fluo-8 AM Calcium Probe	Sample Cells
Negative Control	+	Untreated cells
Experimental Group	+	Experimentally treated cells

(1) Negative Control Group: Cells without any treatment, serving as a reference for calcium signal changes in the experimental group after treatment.

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

II. Operating Procedures (96-Well Plate Format)

Protocol 1: Fluorescence Microscopy (for Suspension Cells)

1. Cell Harvesting and Washing

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

(2) Wash the cells once with PBS or HBSS (or other standard buffers).

Note: Washing typically reduces background fluorescence. Use a vacuum pump for aspirating culture medium, PBS, or HBSS. Phenol red or serum may interfere with the assay.

2. Cell Resuspension and Counting

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

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

Note: Cell density is not critical for microscopy, but must be consistent between the experimental and control groups. Adjust flexibly based on sample type and experimental conditions; optimal confluency is 70-85% in the microscopic field.

3. Cell Staining

(1) Add 100 μ L of Fluo-8 staining working solution to each well of the 96-well plate. Incubate at 37 °C for 30 minutes in the dark. Incubation time can be adjusted between 10-60 minutes; rapid staining is achievable in 10 minutes to maximize cell viability and simplify the workflow. Note: For first-time use, incubate at 37 °C for 30 minutes to evaluate fluorescence performance. If excessive cell death occurs, shorten the incubation time; if fluorescence intensity is too low, extend the incubation time.

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

4. Microscopic Observation and Imaging Transfer the cell suspension to the 96-well plate, allow the cells to settle and adhere to the bottom, and observe under a fluorescence microscope.

Note: Adjust the volume of the cell suspension based on cell count. Optimal cell distribution in the microscopic field is 70-85%.

Protocol 2: Fluorescence Microscopy (for Adherent Cells)

1. Cell Seeding

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

2. Cell Washing

(1) Carefully aspirate the cell culture medium.

(2) Add 100 μ L of PBS or HBSS to gently wash the cells once; completely aspirate the PBS/HBSS after washing.

3. Cell Staining

(1) Add 100 μ L of Fluo-8 staining working solution to each well. Incubate at 37 °C for 30 minutes in the dark. Incubation time can be adjusted between 10-60 minutes; rapid staining is achievable in 10 minutes to maximize cell viability and simplify the workflow.

Note: For first-time use, incubate at 37 °C for 30 minutes to evaluate fluorescence performance. If excessive cell death occurs, shorten the incubation time; if fluorescence intensity is too low, extend the incubation time.

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

4. Microscopic Observation and Imaging

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

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

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

1. Cell Preparation

(1) Suspension Cells: Follow the steps in Protocol 1.

(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 (covering the cells) and incubate at room temperature. Observe under a microscope; once cells round up and intercellular spaces increase, gently pipette to detach cells completely.
- c. Critical Step: Immediately add serum-containing complete medium 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 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-8 staining working solution to the cells. Incubate at 37 °C for 30 minutes in the dark. Incubation time can be adjusted between 10-60 minutes; rapid staining is achievable in 10 minutes to maximize cell viability and simplify the workflow. Note: For first-time use, incubate at 37 °C for 30 minutes to evaluate fluorescence performance. If excessive cell death occurs, shorten the incubation time; if fluorescence intensity is too low, extend the incubation time.

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

3. Flow Cytometry Analysis

- (1) Important: Perform flow cytometry analysis within 1 hour of staining to ensure stable fluorescence signals.
- (2) Analyze samples on a flow cytometer using the pre-set detection channel.
- (3) First, adjust the voltage using blank controls (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/analyze the mean fluorescence intensity (MFI) of the cells.

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

Protocol 4: Fluorescent Microplate Reader (for Suspension and Adherent Cells)

1. Cell Seeding

Seed cells in a black 96-well microplate. The number of cells per well should be 100–10,000; a range of 2,000–5,000 cells is typically recommended.

2. Cell Washing

Collect the target cell suspension into centrifuge tubes.

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

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

Note: Washing typically reduces background fluorescence. Use a vacuum pump for aspirating culture medium and PBS/HBSS. Phenol red or serum may interfere with the assay.

3. Cell Staining

(1) Add 100 µL of Fluo-8 staining working solution to each well. Incubate at 37 °C for 30 minutes in the dark. Incubation time can be adjusted between 10–60 minutes; rapid staining is achievable in 10 minutes to maximize cell viability and simplify the workflow. Note: For first-time use, incubate at 37 °C for 30 minutes to evaluate fluorescence performance. If excessive cell death occurs, shorten the incubation time; if fluorescence intensity is too low, extend the incubation time.

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

4. Fluorescent Microplate Reader Detection

Detect samples using the pre-set parameters on the fluorescent microplate reader. The effect of drug stimulation can be determined by comparing the Relative Fluorescence Units (RFU) of the control and treatment groups.

III. Result Interpretation

1. Qualitative Analysis (Microscopy)

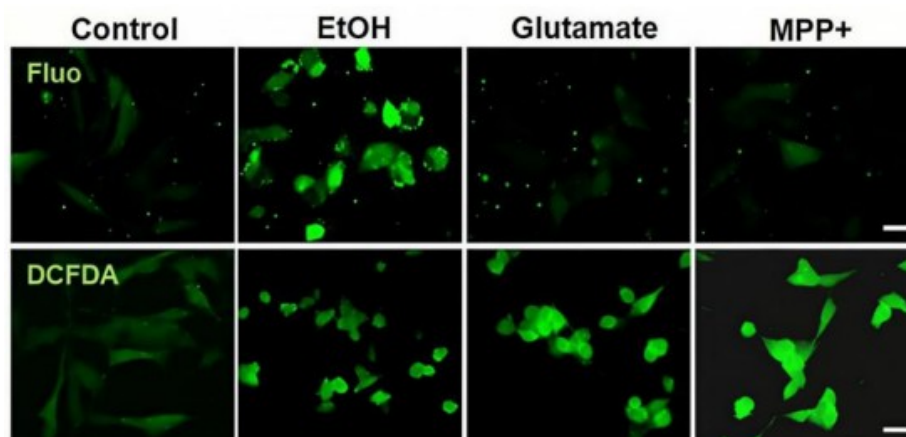


Figure 2. Dynamic Changes in Intracellular Calcium Ion Concentration in SH-SY5Y Cells

Under a fluorescence microscope:

Figure 2 compares the fluorescence intensities of Fluo (calcium signal) and DCFDA (ROS signal) in different treatment groups, enabling systematic analysis of the synergistic or independent effects of various stimuli on cellular calcium signaling pathways and oxidative stress.

Note: Image adapted from *Stressed neuronal cells can recover from profound membrane blebbing, nuclear condensation and mitochondrial fragmentation, but not from cytochrome c release* (doi:10.1038/s41598-023-38210-w).

Frequently Asked Questions (FAQs)

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

A: This may be due to insufficient cell density; increase the cell density and consider extending the incubation time.

2. Q: What should I do if excessive cell death is observed after Fluo-8 incubation?

A: First, evaluate whether the dye concentration and treatment time are appropriate. Although Fluo-8 AM is relatively safe, excessively high concentrations or prolonged staining may cause cell damage. Reduce the dye concentration or shorten the staining time, and closely monitor cell status during the experiment.

Specifications

Attribute	Value
Synonyms	Fluo-8 AM Calcium Probe
Specifications & Purity	BioReagent,Biological Stain,for microscopy,for fluorescence analysis

Attribute	Value
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.

Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, our regional teams are ready to assist. Please contact the office for your region; for general inquiries, the North American office is the corporate primary.

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