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Fluo-4 Calcium Assay Kit

Cat #: KTA7010 Size: 200 T/1000 T

	Fluo-4 Calcium Assay Kit		
REF	**Cat #:** KTA7010	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells		
	Fluorescence Excitation/ Emission: Fluo-4 AM: Ex/Em=494/516 nm		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Fluo-4 Calcium Assay Kit is a kit for detecting intracellular calcium concentration based on Fluo-4 AM calcium fluorescent probe. This kit can be used for fluorescence imaging detection using a fluorescence microscopy, or quantitative fluorescence detection using florescence microplate reader or flow cytometry. Using fluorescence microscopy or fluorescence microplate reader can also detect the dynamic changes of the intracellular calcium concentration. Fluo-4 is a calcium fluorescence probe that replaces Cl in the Fluo-3 structure with F. Due to the replacement of Cl with F with stronger electronic attraction, its maximum excitation wavelength will deviate by about 10 nm towards the shorter wavelength direction. This wavelength is closer to the wavelength of an argon laser, so when excited by an argon laser, the fluorescence intensity of Fluo-4 is stronger than that of Fluo-3. Fluo-4 AM penetrates the cell membrane and enters the cell, where it is cleaved by esterases within the cell to form Fluo-4, which is then trapped within the cell. When Fluo-4 exists as a free ligand, it is almost non fluorescent, but when combined with intracellular calcium, it can produce strong fluorescence. Laser confocal microscopy can be used to detect changes in intracellular calcium concentration.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	200 T	1000 T	
Fluo-4 AM (500×)	20 μL	100 μL	-20℃, protected from light
Staining Enhancer (500×)	20 μL	100 μL	-20℃
Assay Buffer	50 mL	100 mL×2+50 mL	4℃

Materials Required but Not Supplied

- Centrifuge, cell culture plate, precision pipettes, disposable pipette tips
- Fluorescence microscopy or flow cytometer
- PBS or HBSS

Reagent Preparation

Stain Solution: Prepare before use, Mix 2 μL Fluo-4 AM (500×) in each 1 mL Assay Buffer.

Optional: If Fluo-4 AM does not enter cells well, Staining Enhancer (500×) can be added to Stain Solution until the final concentration is 1×. Staining Enhancer can prevent the Fluo-4 AM gathered in the buffer and promote Fluo-4 AM into cells.

Assay Procedure

A. Analysis by Flow Cytometry

1. For non-adherent cells, Collect cells by centrifugation (300 g, 5 min). Wash with PBS twice and discard the PBS. For adherent cells, using Trypsin (EDTA free) to digest cells firstly and then centrifugation.

Note: We recommend keeping unstained control cells (i.e. without staining) suspended in Assay Buffer for both treated and untreated samples to set up the flow cytometer instrument.

2. Add 1 mL of Stain Solution to resuspension cells to a cell density of approximately 1×10^6/mL. Incubate at 37℃ for 10-60 min, protected from light, different cells have different optimum incubation times.

Note: If the incubation temperature and time cannot be determined in the first experiment, it is recommended to try incubating at 37℃ for 30 min to observe the fluorescence effect. If there is more cell death, shorten the time. If there is more cell death, shorten the time or lower the temperature appropriately, if the fluorescence intensity is too weak, extend the time appropriately.

3. After incubation, it can be detected directly by flow cytometry (FL1/BL1 channel). It could also centrifuge at 500 g for 3 min to collect cells, remove Stain Solution and add 0.5 mL Assay Buffer to resuspension cells, then detected by flow cytometry.

B. Analysis by Fluorescence Microscopy

1. For adherent cells

(1) Grow cells directly on a coverslip in cell culture plate. Incubate in a CO_2 Incubator at 37℃ for at least 24 h before treatment.

(2) Wash cells with PBS twice.

(3) Add appropriate volumes of Stain Solution to the cells. Generally, 50 μL was added to 96-well plate per well, 200 μL to 24-well plate per well, 500 μL to 12-well plate per well, and 1 mL to 6-well plate per well. Then incubate at 37℃ for 10-60 min, protected from light, different cells have different optimum incubation times.

Note: If the incubation temperature and time cannot be determined in the first experiment, it is recommended to try incubating at 37℃ for 30 min to observe the fluorescence effect. If there is more cell death, shorten the time. If there is more cell death, shorten the time or lower the temperature appropriately, if the fluorescence intensity is too weak, extend the time appropriately.

(4) After incubation, it can be detected directly by fluorescence microscopy (Ex/Em=494/516 nm). It could also remove Stain Solution and wash cells with PBS or HBSS 2-3 times, then detected by fluorescence microscopy.

2. For non-adherent cells

(1) Follow the protocol for flow cytometry from step A.1 to step A.3.

(2) Place the cell suspension from Step A.3 on a glass slide. Cover the cells with a glass coverslip. Analyze cells by fluorescence microscopy using the appropriate filter.

Precautions

1. If a medium containing serum is used, the esterase in the serum will hydrolyze Fluo-4 AM, thus reducing the effectiveness of Fluo-4 AM in entering cells. In addition, the medium containing phenol red will make the background value slightly higher, and the residual medium should be removed as far as possible before adding the Stain Solution.

2. Ionomycin is an ionic vector with affinity for calcium. The increase in green fluorescence intensity can be observed within a few seconds after Ionomycin treatment. It is recommended to use laser confocal microscope to continuously take photos to detect the change of intracellular calcium concentration.

3. Staining Enhancer can reduce the stability of Fluo-4 AM, so it is only recommended to add it when preparing the Stain Solution.
4. This kit has optimized the Fluo-4 AM staining concentration, and can also explore the best working concentration and staining time according to the cell type, culture conditions and application direction, the working concentration of Fluo-4 AM can be adjusted at 0.2×-2×.
5. Fluo-4 AM is easy to hydrolyze when exposed to water. If it cannot be used at one time, it is recommended that Fluo-4 AM be properly packaged and stored.
6. Fluorescent dyes all have quenching problems, please try to avoid light to slow down the quenching.

Recommended Products

Catalog No.	Product Name
KTA1001	Live and Dead Cell Double Staining Kit
KTA4002	Mitochondrial Permeability Transition Pore Assay

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.