FT-CP7502

Fluo-8

Fluo-8 is the new generation of calcium assay dye for HTS applications

Product Information

cat.number	Kd (calcium)	MW ($g^{-1}.mol^{-1}$)	Packaging interest
Fluo-8 AM CP7501, 5 x 50 µg CP7502, 10 x 50 µg CP7503, 20 x 50 µg CP7504, 1 mg	389 nM	1000	Cell-permeable
Fluo-8, sodium salt CP7520, 10 x 50 µg	389 nM	1000	
Fluo-8H AM CP7530, 1 mg CP7531, 10 x 50 µg	232 nM	1100	Cell-permeable
Fluo-8H, sodium salt CP7540, 10 x 50 µg	232 nM	1100	
Fluo-8L AM CP7550, 1 mg CP7551, 10 x 50 µg	1,86 µM	1100	Cell-permeable
Fluo-8L, sodium salt CP7560, 10 x 50 µg	1,86 µM	1100	
Fluorescence wavelength:			$\lambda_{exc./em.}$: 490 / 514 nm

Storage: -20°C. Protect from light.

Key Features

- *Convenient Wavelengths:* maximum excitation @ ~490 nm; maximum emission @ ~514 nm.
- *Enhanced Intensity:* 2 times brighter than Fluo-4 AM; 4 times brighter than Fluo-3 AM.
- *Faster Loading:* dye loading at room temperature (rather than 37 ºC that is required for Fluo-4 AM).
- *Versatile Ca2+-Binding Kd*
- *Versatile Packing Sizes* to Meet Your Special Needs: 1 mg; 10x50 µg; 20x50 µg; HTS packages.

Introduction

Calcium acts as a universal second messenger in a variety of cells. The beginning of life, the act of fertilization, is regulated by Ca^{2+}. Numerous functions of all types of cells are regulated by Ca2+ to a greater or lesser degree. Since the 1920s, scientists have attempted to measure Ca^{2+}, but few were successful due to limited availability of Ca^{2+} probes. The first reliable measurements of Ca^{2+} were performed by Ridgway and Ashley by injecting the photoprotein aequorin into the giant muscle fiber of the barnacle. Subsequently, in the 1980s, Tsien and colleagues produced a variety of fluorescent indicators. Among them the fluorescein-based Ca^{2+} reagents (such as Fluo-3) have provided trustworthy methods for measuring Ca^{2+}. Since the development of these Ca^{2+} probes, investigations of Ca^{2+} related intracellular phenomena have skyrocketed.

Since being introduced Fluo-3 imaging and its analogs (such as Fluo-4) have revealed the spatial dynamics of many elementary processes in Ca^{2+} signaling. Fluo-3 and Fluo-4 have also been extensively used for flow cytometry and microplate-based (such as FLIPR™) calcium detections. However the weak signal and harsh dye loading conditions have limited their applications in some cellular analysis. Our **Fluo-8™** serial calcium detection reagents have been developed to address these limitations of Fluo-3 and Fluo-4.

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The most important properties of Fluo-3 and Fluo-4 in cellular applications are their absorption spectrum compatible with excitation at 488 nm by argon-ion laser sources, and a very large fluorescence intensity increase in response to Ca^{2+} binding. These two valuable properties have been retained intact with our Fluo-8™ Ca^{2+} detection reagents. The **absorption and emission peaks** of Fluo-8™ reagents are 490 nm and 514 nm, respectively. They can be well **excited with an argon ion laser at 488 nm**, and their emitted fluorescence (at wavelengths 514 nm) increases with increasing Ca^{2+}. Fluo-8™ is determined to undergo a **>200-fold increase in fluorescence upon binding Ca^{2+}**. Because the range of increase in Ca2+ in many cells after stimulation is generally 5- to10-fold, Fluo-8™ is an excellent probe to use with high sensitivity in this region. The Kd of Fluo-8™ is estimated to be **389 nM** (22°C, pH 7.0 –7.5), but this value may be significantly influenced by pH, viscosity, and binding proteins in vivo conditions.

Besides their convenient 488 nm excitation wavelength and large fluorescence enhancement by calcium, Fluo-8™ is much **brighter** in cells than Fluo-3 and Fluo-4. In addition, Fluo-8 is much **more readily loaded** into live cells than Fluo-3 and Fluo-4, both of which require 37°C for optimal cell loading. Fluo-8™ reagents have a less temperature-dependent cell loading property, giving similar results either at room temperature or 37°C. This characteristic makes Fluo-8™ more robust for HTS applications.

Lastly, Fluo-8 has been shown to have a **more photostable** signal, making it usefull to detect transient Ca2+ levels.

Directions for use

Cell loading of Fluo-8™ AM esters

AM esters are the non-polar esters that readily cross live cell membranes, and rapidly hydrolyzed by cellular esterases inside live cells. AM esters are widely used for loading a variety of polar fluorescent probes into live cell non-invasively. However, cautions must be excised when AM esters are used since they are susceptible to hydrolysis, particularly in solution. They should be reconstituted just before use in high-quality, anhydrous dimethylsulfoxide (DMSO). DMSO stock solutions may be stored desiccated at –20°C and protected from light. Under these conditions, AM esters should be stable for several months.

Following is our recommended protocol for loading Fluo-8™ AM esters into live cells. This protocol only provides a guideline, should be modified according to your specific needs.

a) Prepare a 2 to 5 mM stock solution of Fluo-8™ AM esters in high-quality, anhydrous DMSO. The nonionic detergent Pluronic® F-127 is sometimes used to increase the aqueous solubility of Fluo-8™ AM esters.
[Note: A 20% Pluronic F-127 solution can be used in replacing DMSO to prepare solutions of these calcium indicators. A variety of Pluronic F-127 solutions can be purchased from FluoProbes].
[Caution: long-term storage of AM esters in the presence of Pluronic F-127 is not recommended].

b) On the day of the experiment, either dissolve Fluo-8™ solid in DMSO or thaw an aliquot of the indicator stock solution to room temperature. Prepare a working solution of 1 to 10 µM in the buffer of your choice. For most of cell lines we recommend that 4-5 uM Fluo-8™ reagents be used. The exact concentration of indicator required for cell loading must be determined empirically. To avoid calcium buffering, toxicity and other artifacts of overloading, one should generally use the lowest probe concentration that yields sufficient signal.

c) Incubate cells with the Fluo-8™ AM esters for 20 minutes to one hour at room temperature or 37 °C.
[Note: Decreasing the loading temperature might reduce the indicator compartmentalization]

d) Wash cells to remove excess probe.

Measuring Intracellular Calcium Responses

To determine either the free calcium concentration of a solution or the Kd of a single-wavelength calcium indicator, the following equation is used:

$$[Ca]_{free} = K_d[F - F_{min}]/F_{max} - F]$$

where F is the fluorescence of the indicator at experimental calcium levels, F_{min} is the fluorescence in the absence of calcium and Fmax is the fluorescence of the calcium-saturated probe.

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The dissociation constant (K_d) is a measure of the affinity of the probe for calcium. The Ca-binding and spectroscopic properties of fluorescent indicators vary quite significantly in cellular environments compared to calibration solutions. In situ response calibrations of intracellular indicators typically yield Kd values significantly higher than in vitro determinations. In situ calibrations are performed by exposing loaded cells to controlled Ca^{2+} buffers in the presence of ionophores such as A-23187, 4-bromo A-23187 and ionomycin. Alternatively, cell permeabilization agents such as digitonin or Triton® X-100 can be used to expose the indicator to the controlled Ca^{2+} levels of the extracellular medium.

Related documents - Applications

NT-CP750m Visualization of Ca2+ wave in vascular myocyte

References

- **Norez C.** *et al*., A CF respiratory epithelial cell chronically treated by miglustat acquires a non-CF like phenotype, *Am. J. Respir. Cell Mol. Biol*., 10.1165/rcmb.2008-0285OC (2009) Abstract

Related products

- Fluo-3 AM, FP-78932C
- Fluo-8 NW for HTS, CJ2550
- Indo-1 AM, FP-42775A
- Rhod-2 AM, FP-661583
- Rhod-4 AM, CQ6060
- Fura-2 AM, FP-42776C
- Pluronic F-127, 20% solution, FP-69806A
- Coelenterazine (native), UP972333
- Coelenterazine 400a, UPBB8392
- Coelenterazine H, UPR3078B
- A23187, FP-28362A
- 4-bromo A23187, FP-37222A
- Ionomycin, FP-53989A
- Probenecid, Cell culture tested, FP-288652
- Probenecid, water soluble, FP-288653
- Calcium calibration buffer kit, FP-21527A
- DMSO anhydrous, JW7390
- Black wall, clear bottom assay plate (96-well) KT225, (384-well), BA8170

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06