Product Instruction Manual

Cat	Name	SIze	Ex/Em
BGT-CHM-05134	Calcein AM	1mg	494/517 nm (pH = 8)

Storage and Shipping Conditions

Store at -20°C protected from light. Ship with ice packs.

Product Specifications

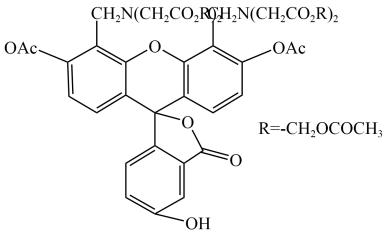
Appearance: Off-white solid soluble in DMSO

CAS Number: 148504-34-1

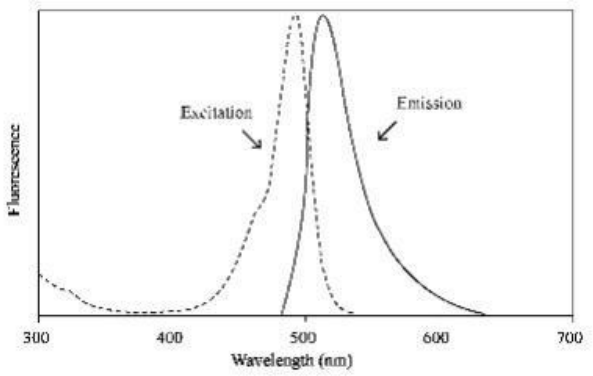
Molecular Formula: $C_{46}H_{46}N_2O_{23}$

Molecular Weight: 994.9

Molecular Structure Diagram:



Spectrum:



Product Introduction

Calcein-AM is a cell staining reagent for green fluorescent labeling of live cells (Ex: 490 nm, Em: 515 nm). Based on traditional Calcein, an acetoxymethyl ester (AM) group is introduced to increase hydrophobicity, allowing it to easily penetrate the cell membrane of live cells. Calcein-AM itself is non-fluorescent. After entering cells, it is cleaved by intracellular esterases to form Calcein, a membrane-impermeable polar molecule that is retained within cells and emits strong green fluorescence. Since dead cells lack esterases, Calcein-AM is only used for cell viability assays and short-term labeling of live cells.

Compared with other similar reagents (such as BCECF-AM and CFDA), Calcein-AM has extremely low cytotoxicity and does not inhibit any

cellular functions, such as cell proliferation and lymphocyte chemotaxis, making it the most suitable fluorescent probe for live cell staining.Propidium iodide (PI), a nuclear staining dye, cannot penetrate the cell membrane of live cells. It crosses the disordered regions of dead cell membranes to reach the nucleus and intercalates into the DNA double helix of cells, producing red fluorescence (Ex: 535 nm, Em: 617 nm). Therefore, PI only stains dead cells.Since both Calcein and PI-DNA can be excited at 490 nm, live and dead cells can be observed simultaneously using a fluorescence microscope. When excited at 545 nm, only dead cells can be observed.Based on the above characteristics, Calcein-AM can be used in combination with Propidium Iodide (PI) for simultaneous detection of live and dead cells.

Product Features

High Stability: Strong fluorescence brightness, long luminescence duration, and resistance to quenching.

Easy to Use: Compatible with other reagents from our company, providing convenience and flexibility.

Protocol

1. Prepare a 1 mM Calcein-AM solution using DMSO, and dilute it with PBS to make a 1–50 μM Calcein-AM working solution.

Note:The optimal Calcein-AM concentration varies for different cell types. A concentration of 2 μM is suitable for cells such as NIH3T3, PtK2, HeLa, and MDCK.

2. For staining adherent cells such as HeLa, first digest the cells with Trypsin-EDTA to prepare a cell suspension.
3. Centrifuge the cell suspension for 3 minutes at 1000 rpm.
4. Remove the supernatant, add PBS buffer to adjust the cell density to 10^5–10^6 cells/mL, and mix thoroughly with a pipette.

Note: Since serum and other components in the culture medium contain esterases that can degrade Calcein-AM and increase background signal, it is necessary to centrifuge and wash the cells with PBS several times until completely cleaned.

5. Add 50 μL of the diluted Calcein-AM solution to 200 μL of the cell suspension, and incubate at 37°C for 15 minutes.
6. Place an appropriate amount of the stained cell solution on a coverslip. Observe the cells using a fluorescence microscope equipped with a 490 nm excitation filter and a 515 nm emission filter.

Note: If Calcein-AM has difficulty entering cells, a surfactant such as Pluronic F127 can be used for treatment.

Precautions

1. The ester bond of Calcein-AM is susceptible to hydrolysis upon exposure to moisture. After use, please store it tightly sealed at -20°C to prevent moisture ingress.
2. Diluted Calcein-AM stock solutions should be used promptly and preferably prepared immediately before use.
3. Since Calcein-AM is unstable in aqueous solutions such as PBS, prepared working solutions must be used as soon as possible.
4. Serum and phenol red in the culture medium have certain effects on Calcein-AM staining. It is recommended to wash the cells thoroughly before adding the Calcein-AM working solution.
5. Cells labeled with Calcein-AM exhibit uniform fluorescence and perform very well for short-term cell migration tracing. However, the duration of fluorescence is related to factors such as cell type and culture conditions, generally lasting only a few hours. Additionally, it may be rapidly effluxed by certain cell types
6. This product is for research use only and must not be stored in residential premises.
7. For your safety and health, please follow the standard laboratory safety regulations of your institution.