

## Calcein, AM

**Cat:** C8950

**Storage:** Store at -20°C in a dry and dark place, and it is valid for 1 year.

### Product Information

**CAS:** 148504-34-1

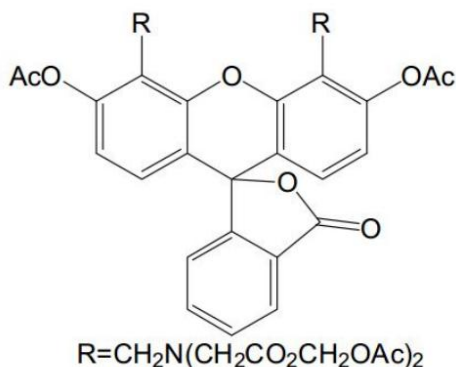
**English Name:** 3',6'-Di(O-acetyl)-4',5'-bis[N,N-bis(carboxymethyl)aminomethyl]fluorescein, tetraacetoxymethylester

**Molecular Formula:** C<sub>46</sub>H<sub>46</sub>N<sub>2</sub>O<sub>23</sub>

**Molecular Weight:** 994.86

**Appearance (Character):** White and light crystal

**Purity:** ≥90% (HPLC)



yellow

### Introduction

Calcein, AM is a cell staining reagent that can fluorescently label living cells. It penetrates the cell membrane and enters the cell, where it is cleaved by intracellular esterases to form Calcein, which is then retained within the cell and emits strong green fluorescence. Compared with other similar reagents such as BCECF, AM and CFDA, Calcein, AM has very low cytotoxicity. The excitation and emission wavelengths of Calcein are 490 nm and 515 nm, respectively.

Calcein, AM only stains living cells. As a nuclear stain dye, PI cannot pass through the cell membrane of living cells. It passes through the disordered regions of the cell membrane of dead cells to reach the nucleus and embeds into the DNA double helix of the cell, producing red fluorescence excitation: 535 nm, emission: 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 fluorescence microscopy. Using 545 nm excitation, only dead cells can be observed. Based on the above characteristics, Calcein, AM and PI are often combined to be used as a dual staining of live and dead cells.

Due to the different optimal staining conditions for different cell lines, we recommend determining the appropriate concentrations of Calcein, AM, and PI individually.

### Usage Instructions:

- (1) Prepare a 1 mM solution of Calcein, AM in DMSO and dilute it with PBS to obtain a 1-50 $\mu$ M solution of Calcein, AM [1].
- (2) Add an amount of Calcein, AM solution equal to 1/10 of the volume of the cell culture medium to the cell culture [2].
- (3) Incubate the cells at 37°C for 15-30 minutes.
- (4) Wash the cells twice with PBS or an appropriate buffer solution.
- (5) Observe the cells using a fluorescence microscope equipped with a filter set for an excitation wavelength of 490 nm and an emission wavelength of 515 nm.

**Note:**

[1].If Calcein, AM has difficulty entering the cells, a surfactant such as Pluronic F127 can be used to facilitate its entry..

[2].Alternatively, the culture medium can be replaced with a 1/10 concentration of the Calcein, AM solution for the duration of the staining process.