# **Product Information**

## Calcein AM

Catalog Number: C4041

Product Size:1 mg

Application Scope: Live cell staining, cell imaging, cell proliferation and function, cell tracking, cell counting

#### **Parameters**

Appearance: Off-white solid soluble in DMSO

Ex/Em: 494/517 nm (pH = 8)

CAS No.: 148504-34-1

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

Molecular Weight: 995

Molecular Structure:

$$AcO \qquad O \qquad CH_2N(CH_2CO_2R)_2 \qquad CH_2N(CH_2CO_2R)_2 \qquad OAc \qquad OAc \qquad OAc \qquad R = -CH_2OCOCH_3$$

## **Storage**

Store at -20°C and protect from light. Expiration date marked on the outer packing.

## **Description**

Calcein AM is a widely used green fluorescent cell marker. Calcein AM is a cell-permeant dye that can be used to determine cell viability in most eukaryotic cells. In live cells the nonfluorescent calcein AM is converted to greenfluorescent calcein, after acetoxymethyl ester hydrolysis by intracellular esterases. Calcein AM is an excellent tool for the studies of cell membrane integrity and for long term cell tracing.

Calcein-AM has low toxicity to cells and can be used in combination with PI to distinguish between live and dead cells.

#### **Protocol**

1. The solid dye may be dissolved in DMSO to make concentrated stock solutions up to 1 mM. Dilute the storage solution with PBS to prepare a working solution with a concentration of  $1\sim50~\mu M$ .

Note: Different types of cells have different calcein-AM concentrations.  $2\mu M$  calcein-AM is suitable for NIH3T3, PtK2, HeLa, and MDCK cells.

- When staining adherent cells, the cells are first digested with Trypsin-EDTA to make a cell suspension.
- 3. Centrifuge the cell suspension at 1000-1500 rpm for 3 minutes.
- 4. Remove the supernatant, add PBS buffer to adjust the number of cells to  $10^5$ - $10^6$  / mL, and mix thoroughly with a pipette.

Note: The esterase in the serum will decompose Calcein-AM, leading to an increase in the blank background value, so it needs to be centrifuged several times and washed with PBS several times until completely washed.

- 5. Add 50  $\mu L$  Calcein-AM working solution to 200  $\mu L$  cell suspension and incubate at 37  $^{\circ}$  C for 15 min.
- 6. An appropriate amount of stained cell solution was added dropwise to the coverslip. Cells were observed using a



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fluorescence microscope with an excitation wavelength of 490 nm and an emission wavelength of 515 nm.

Note: If Calcein AM is difficult to enter the cells, you can use a surfactant such as Pluronic F127.

## **Notes**

1. The ester bond of Calcein AM will decompose when it encounters moisture. Please keep it frozen at -20 °C to prevent

moisture from entering.

- 2. Please use the Calcein AM stock solution after dilution, and try to use it immediately.
- 3. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.
- 4. For your safety and health, please wear lab coats and disposable gloves.

