Calcein-AM

Cat. No.: HY-D0041

CAS No.: 148504-34-1

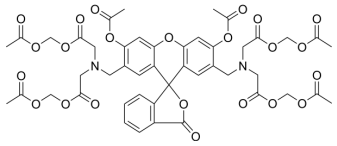
Molecular Formula: $C_{46}H_{46}N_2O_{23}$

Molecular Weight: 994.86

Target: Fluorescent Dye

Pathway: Others

Storage: -20°C, protect from light
* In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 100 mg/mL (100.52 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.0052 mL	5.0258 mL	10.0517 mL
	5 mM	0.2010 mL	1.0052 mL	2.0103 mL
	10 mM	0.1005 mL	0.5026 mL	1.0052 mL

Please refer to the solubility information to select the appropriate solvent.

BIOLOGICAL ACTIVITY

Description

Calcein AM, has cell membrane permeability and can easily enter the cell. Calcein AM has no fluorescence and is hydrolyzed by endogenous esterase in the cell to produce polar molecule Calcein (Calcein), which has strong negative charge and cannot permeate the cell membrane. Calcein can emit strong green fluorescence, so it is often used with Propidium Iodide for cell viability/virulence detection, excitation/emission wavelength: 494/515 nm[1].

In Vitro

General Protocol

1.Preparation of Calcein AM working solution
Preparation of 5 mM stock solution with DMSO.
Dilute the stock solution in serum-free cell culture medium or PBS to obtain 1-10 μM of working solution.
Note: Please adjust the concentration of Calcein AM working solution according to the actual situation.

2.Cell staining
2.1 Suspension cells（6-well plate）
a.Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Wash twice with PBS, 5 minutes each time.The cell density is 1×10^6/mL.
b.Add 1 mL of working solution, and then incubate at room temperature for 30-45 minutes.

c.Centrifuge at 400 g at 4°C for 3-4 minutes and then discard the supernatant.
d.Wash twice with PBS, 5 minutes each time.
e.Resuspend cells with serum-free cell culture medium or PBS. Observation by fluorescence microscopy or fluorescence microplate readers.
2.2 Adherent cells
a.Culture adherent cells on sterile coverslips.
b.Remove the coverslip from the medium and aspirate excess medium.
c.Add 100 µL of working solution, gently shake it to completely cover the cells,and then incubate at room temperature for 30-45 minutes.
d.Wash twice with medium, 5 minutes each time. Observation by fluorescence microscopy or fluorescence microplate readers.

Storage
-20°C
Protect from light

Precautions
1.Please adjust the concentration of Calcein AM working solution according to the actual situation.
2.This product is for R&D use only, not for drug, household, or other uses.
3.For your safety and health, please wear a lab coat and disposable gloves to operate.
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo

Calcein-AM is found to be suitable for in vivo studies, because it has no deleterious effects on cell function and is, indeed, a marker of cell viability[2].
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PROTOCOL

Cell Assay [1][2][3]

K562, Daudi, and Chang liver cells are labeled with calcein-AM. Calcein-AM's excitation and emission wavelengths are 496 nm and 520 nm, respectively. The filter/mirror combination used to detect calcein-AM's green fluorescence includes the 490-nm excitation and 520-nm emission filters with a dichroic mirror. Differences in the automatic fluorescence readings between the test and control wells determine the results[1]. A simple and sensitive cell-cell adhesion microplate assay is established using the calcein-AM. The procedure involves three steps: the labeling of lymphocytes with an adequate concentration of calcein-AM (20 µM) during a short incubation period (30 min); the adhesion of 2×10^5 labeled lymphocytes per well to confluent keratinocyte or fibroblast monolayers grown in microtiter plates for 90 min; and, finally, measurement of the fluorescent signal utilizing a new system of cold-light microfluorimetry[2]. Cells are incubated for 15 min in 1 mL of a 1% saponin solution in PBS buffer, pH 7.4, containing 0.05% sodium azide. After saponin permeabilization, 4×10^5 RBCs in suspension in PBS buffer containing 0.1% saponin and 0.05% sodium azide are incubated (37°C in the dark for 45 min) with calcein-AM to a final concentration of 5 µM, ished three times with the same PBS buffer containing 0.1% saponin and 0.05% sodium azide, and the cell viability is analyzed by flow cytometry[3].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Protein Cell. 2023 May 9;pwad027.
- Adv Funct Mater. 2024 May 1.
- Bioact Mater. 2022 Aug 11;21:20-31.
- ACS Nano. 2023 Aug 3.

- Adv Sci (Weinh). 2022 Oct;9(30):e2203031.

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REFERENCES

[1]. Holló Z, et al. Calcein accumulation as a fluorometric functional assay of the multidrug transporter. Biochim Biophys Acta. 1994 May 11;1191(2):384-8.

[2]. Wang XM, et al. A new microcellular cytotoxicity test based on calcein-AM release. Hum Immunol. 1993 Aug;37(4):264-70.

[3]. Braut-Boucher F, et al. A non-isotopic, highly sensitive, fluorimetric, cell-cell adhesion microplate assay using calceinAM-labeled lymphocytes. J Immunol Methods. 1995 Jan 13;178(1):41-51.

[4]. Bratosin D, et al. Novel fluorescence assay using calcein-AM for the determination of human erythrocyte viabilityand aging. Cytometry A. 2005 Jul;66(1):78-84.

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