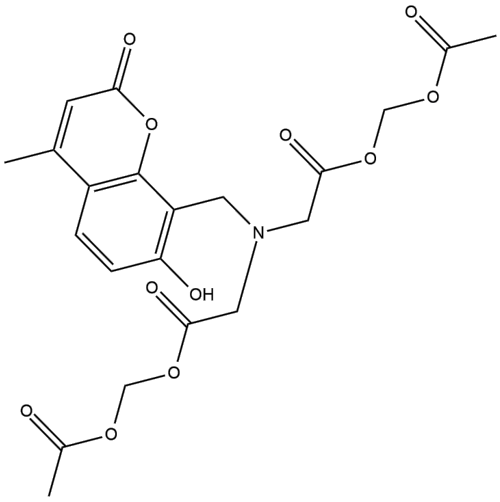
Product Data Sheet

Product Name: Calcein Blue AM
Cat. No.: GC18604

Chemical Properties

Cas. No.	168482-84-6		
Chemical Name	N-[2-[(acetyloxy)methoxy]-2-oxoethyl]-N-[(7-hydroxy-4-methyl-2-oxo-2H-1-benzopyran-8-yl)methyl]-glycine, (acetyloxy)methyl ester		
SMILES	OC1=CC=C(C(C)=CC(O2)=O)C2=C1CN(CC(OCOC(C)=O)=O)CC(OCOC(C)=O)=O		
Formula	$C_{21}H_{23}NO_{11}$	M.Wt	465.4
Solubility	DMSO : 6 mg/mL (18.68 mM)	Storage	Store at -20°C,protect from light
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		

Structure



Protocol

Evaluation of cell viability using Calcein Blue AM

This protocol only provides a guideline, and should be modified according to your specific needs

Product Data Sheet

1. Prepare dyeing solution

(1) Prepare stock solution: Prepare Calcein Blue AM stock solution with a concentration of 1-10mM in high-quality anhydrous DMSO. Notice: Aliquot the unused stock solution and store it in the dark at -20°C or -80°C to avoid repeated freezing and thawing.

(2) Prepare working solution: Dilute the stock solution with a suitable buffer (such as serum- and phenol red-free culture medium or PBS) to prepare a working solution with a concentration of 1-10μM. Please adjust the concentration of the working fluid according to the actual situation, and prepare it now to avoid repeated freezing and thawing. Notice: The working solution must be prepared fresh on the day of the experiment. Before use, Calcein Blue AM solution needs to be rewarmed and centrifuged.

2. Cell suspension staining

(1) Suspension cells: centrifuge at 4°C and 1000g for 3-5 minutes, discard the supernatant, and wash twice with PBS or other buffer, 5 minutes each time.

(2) Add the Calcein Blue AM working solution, incubate at room temperature in the dark for a short period of time (30 minutes), and then wash twice with PBS.

(3) Select the appropriate instrument and conduct fluorescence measurement.

3. Cell adhesion staining

(1) Culture adherent cells on sterile coverslips;

(2) Add the Calcein Blue AM working solution, incubate at room temperature in the dark for a short period of time (30 minutes), and then wash twice with PBS.

(3) Remove the coverslip from the culture medium, suck out the excess culture medium, and place the coverslip in a humid environment;

(4) Select the appropriate instrument and conduct fluorescence measurement.

4. Precautions:

(1) Fluorescent dyes all have quenching problems. Please avoid light as much as possible to slow

down fluorescence quenching.

(2) For your safety and health, please wear a lab coat and disposable gloves when operating

(3) Calcein Blue AM should be stored in the dark throughout the experiment.

(4) This protocol and all reagents are for scientific research use only.

References:
[1] Grieshaber P, Lagrèze W A, Noack C, et al. Staining of fluorogold-prelabeled retinal ganglion cells with calcein-AM: A new method for assessing cell vitality[J]. Journal of neuroscience methods, 2010, 192(2): 233-239.

Background

Calcein Blue AM is a cell-permeant dye (E_x=360nm, E_m=455nm)[1]. Calcein Blue AM serves as a substrate for intracellular esterases, which hydrolyze the acetoxymethyl (AM) ester group to convert the non-fluorescent reagent into fluorescent Calcein Blue[2]. Calcein Blue AM has been widely used to detect cell viability, and the heterogeneous patterns of calcium deposition, growth and cell division within cell populations[3].

References:
[1] Huitink G M, Poe D P, Diehl H. On the properties of Calcein Blue[J]. Talanta, 1974, 21(12): 1221-1229.
[2] Tenopoulou M, Kurz T, Doulias P T, et al. Does the calcein-AM method assay the total cellular ‘labile iron pool’or only a fraction of it?[J]. Biochemical Journal, 2007, 403(2): 261-266.
[3] Fox E, Meyer E, Panasiak N, et al. Calcein staining as a tool to investigate coccolithophore calcification[J]. Frontiers in Marine Science, 2018, 5: 326.