Calcein, AM

Cat. No. CTG-CV0009 Store the kit at -15 to -25°C

Product Description

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 a green-fluorescent calcein, after acetoxymethyl ester hydrolysis by intracellular esterases. The acetomethoxy (AM) derivate of calcein (calcein AM) is widely used for labeling live cells as it can be transported through the cellular membrane into live cells. The AM ester groups mask the part of the molecule that chelates calcium. Upon transporting into live cells cellular esterases cut off the AM groups, the molecule binds to calcium within cell (resulting in acquiring strong green fluorescence), and gets trapped inside. As dead cells lack esterases, only live cells are marked. This feature makes it very useful for testing of cell viability and for short-term marking of cells.

Speciifications

CAS 148504-34-1

Excitation:	495
Emission:	515
Molecular Weight:	994.86
Solvent:	DMSO

Application

Compared with other live cell-labeling reagents (such as BCECF-AM and carboxy-fluorescein diacetate), calcein-AM is the most suitable fluorescent probe for staining viable cells because of its low cytotoxicity. Calcein does not significantly affect cellular functions such as proliferation or chemotaxis of lymophocyte. In addition, viability assays using calcein are reliable and correlate well with the standard 51Cr-release assay.

Kit Components

2X 50ug

Kit storage/stability

It is recommened to prepare and use Calcein, AM stock solution on the same day. However, if stock solutions need to be prepared in advanced we recommend storing the Calcein, AM stock solution as aliquots in tightly sealed vials at -20°C, dessicated and protected from light. Under these conditions, AM esters should be stable for 3 months. 10% Pluronic F-127 stock solution must be stored at room temperature (DO NOT FREEZE) for up to 6 months. 20 mM Probenecid may be stored at -20°C and protected from light for up to 6 months. Avoid repeated freeze-thaw cycles.

Usage

Celltechgen provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please request the Safety Data Sheet (SDS) provided for the product.

Experimental Protocol

Prepare these materials

IMPORTANT NOTE: This protocol includes the non-ionic detergent Pluronic® F-127 and the organic anion-transport inhibitor probenecid. Both reagents are not required, but highly recommended. To remove a reagent from the loading protocol, uncheck the appropriate box:

Required Reagents

Calcein, AM
Hanks and Hepes Buffer *(HHBS) or a buffer of your choice
100% DMSO

Optional Reagents

10% Pluronic® F-127
25 mM Probenecid

Procedure

1. Prepare an HHBS buffer, a 10% Pluronic® F-127 solution, and a 25 mM Probenecid solution.

2. Prepare a 2 mM to 5 mM Calcein, AM stock solution in high quality anhydrous DMSO.

3. Prepare a 2X working solution in HHBS with 20 µM Calcein, 0.08% Pluronic® F-127 and 2 mM Probenecid.

4. Add 100 µL of the dye working solution into the desired wells already containing 100 µL of culture medium.

5. Incubate the dye-loading plate5.

6. Prepare an HHBS buffer (or a buffer of your choice) with 1.0 mM Probenecid.

7. Replace the dye working solution with the HHBS buffer or a buffer of your choice with 1.0 mM Probenecid.

8. Run your assay.

Notes

- Volumes can be adjusted according to the need and volume of the experiment setups.
- Pluronic® F-127 (PF-127) is a nonionic surfactant and relatively non-toxic to cells. PF-127 is commonly used with dye AM esters to improve their aqueous solubility.
- If your cells contain organix anion-transporters, Probenecid (0.5-1.0 mM) may be added to the dye working solution to reduce the leakage of the de-esterified indicators.
- The exact concentration of the indicator required for cell loading must be determined empirically.