Calcein AM Solution

C1456514

Storage Temperature -20℃. Store in the dark. Avoid repeated freezing and thawing.
Shipping Conditions Shipped with ice packs.

Introduction

Calcein-AM is a cell-permeant green fluorescent probe commonly used for determining eukaryotic cell viability or the Mitochondrial Permeability Transition Pore (MPTP). In recent years, Calcein-AM has been widely applied in cell viability analysis experiments.

Calcein-AM is derived from Calcein by the addition of acetoxymethyl ester (AM) groups, which enhance its hydrophobicity, thereby allowing it to easily penetrate live cell membranes. Calcein-AM itself is non-fluorescent. Once inside the cell, it is hydrolyzed by endogenous esterases to generate calcein, a strongly negatively charged, polar molecule that cannot permeate the cell membrane and is therefore retained within the cell. Calcein emits intense green fluorescence (Ex/Em=494/517 nm). Compared to other similar probes, Calcein-AM is one of the most ideal fluorescent probes for staining live cells due to its very low cytotoxicity—it hardly affects cellular functions such as cell proliferation or lymphocyte chemotaxis—and its low sensitivity to pH changes.

Since dead cells lack esterase activity, Calcein-AM is used specifically for viability testing and short-term labeling of live cells. The red fluorescent nucleic acid dye Propidium Iodide (PI) can only stain dead cells with compromised membrane integrity, as it cannot penetrate the membranes of live cells. Therefore, Calcein-AM is often used in combination with Propidium Iodide (PI) for simultaneous dual-fluorescence staining of live and dead cells.

Product Information

Product Name	Specifications	Concentration	Storage Condition
Calcein AM solution	100 μL	2 mM in DMSO	-20℃. Store in the dark. Avoid repeated freezing and thawing

Procedure

1. **Preparation of Working Solution**

Dilute this product at a ratio of 1:1000 using an appropriate buffer, such as serum-free

medium, HBSS, or PBS. For example, add 1 μL of the staining solution to 1 mL of serum-free medium to obtain 1 mL of the working staining solution.

Note: The commonly used final concentration of Calcein AM is 0.1-5 μM. The final concentration of Calcein AM can be appropriately adjusted according to the cell type and actual experimental conditions.

2. **Staining Procedure**

a. For adherent cells:

(a) Seed cells in a culture dish, multi-well plate, or on glass coverslips. Treat the cells as required by the experimental design. Gently aspirate the culture medium from the wells and wash with PBS for approximately 10 seconds. Aspirate the PBS.

(b) Add an appropriate volume of Calcein-AM working solution. Gently swirl to ensure even coverage of all cells.

Note: For adherent cells cultured in a 6-well plate with a confluence exceeding 80%, it is recommended to add the staining working solution at a volume of 1 mL per well. This volume can be optimized based on the specific experimental system.

(c) Incubate cells at 37°C for 10-45 minutes. The optimal incubation time varies for different cell types. Consider starting with 10 minutes and optimize for ideal results based on specific experimental conditions.

(d) Aspirate the staining solution and wash the cells 2-3 times with PBS. Then, add serum-free culture medium before observing under a fluorescence microscope. Observe green fluorescence at Ex/Em=494/517nm. Alternatively, proceed with analysis by flow cytometry or measure fluorescence intensity using a fluorescence microplate reader after staining is complete.

b. For suspension cells:

(a) Treat cells as required by the experimental design and perform a cell count. Take an appropriate number of cells and centrifuge at 500 x g for 5 minutes at room temperature. Gently aspirate the medium, resuspend the pellet in an adequate volume of PBS, and centrifuge again at 500 x g for 5 minutes at room temperature to remove the PBS.

(b) Add an appropriate volume of Calcein-AM working solution to resuspend the cells at a density of approximately 1x10^6 cells/mL.

(c) Incubate cells at 37°C for 10-45 minutes. The optimal incubation time varies for different cell types. Consider starting with 10 minutes and optimize for ideal results based on specific experimental conditions.

(d) Place a drop of the cell suspension directly onto a glass slide, cover with a coverslip, and observe under a microscope. Observe green fluorescence at Ex/Em=494/517 nm. Alternatively,

proceed with analysis by flow cytometry or measure fluorescence intensity using a fluorescence microplate reader after staining is complete.

Note: If background interference is significant, centrifuge to remove the staining solution and wash 1-2 times with PBS before microscopic observation.

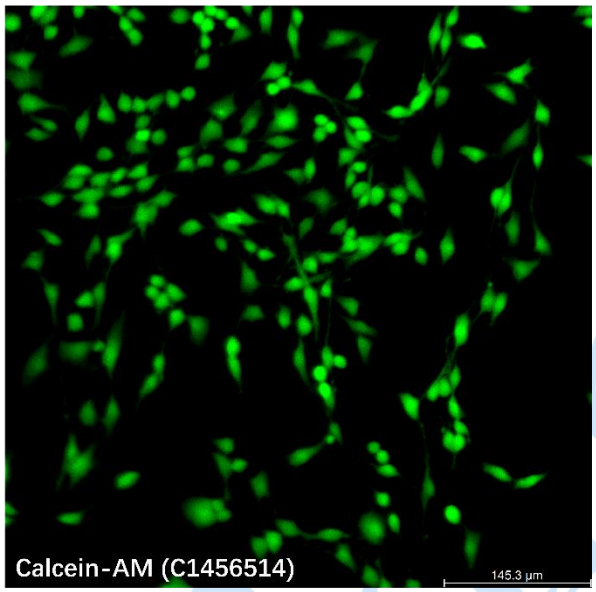

Figure 1. Staining results of HeLa cells treated with Calcein AM Solution.

Precautions

1. For your safety and health, wear a lab coat and disposable gloves.
2. Fluorescent dyes are susceptible to quenching. It is recommended to complete detection on the same day after staining.