Calcein AM

Catalog Numbers C1430, C3099, and C3100

Pub. No. MAN0019058 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

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 green-fluorescent calcein, after acetoxymethyl ester hydrolysis by intracellular esterases. This dye is also available in our special packaging (Cat. No. C3100) and resuspended in DMSO (Cat. No. C3099). For a longer wavelength version of this dye check out our new CellTrace™ calcein red-orange AM (Cat. No. C34851).

Cell vitality as measured by intracellular esterase activity is a recognized parameter of cell health. Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually non-fluorescent cell-permeant calcein AM to the intensely fluorescent calcein (ex/em 494/517 nm, Figure 1). Calcein AM is an optimal dye for this application; utilizing the blue laser.

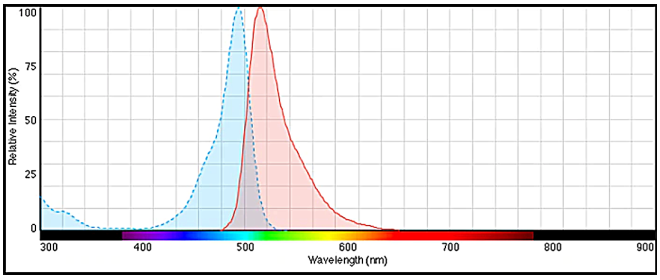

Fig. 1 Excitation and emission spectra as shown in the Fluorescence SpectraViewer.

The acetoxymethyl (AM) ester derivatives of fluorescent indicators make up one of the most useful groups of compounds for the study of live cells. Modification of carboxylic acids with AM ester groups results in an uncharged molecule that can permeate cell membranes. Once inside the cell, the lipophilic blocking groups are cleaved by nonspecific esterases, resulting in a charged form that is retained in cells to a much greater extent than its parent compound. The calcein AM ester is colorless and non- fluorescent until hydrolyzed. The polyanionic dye calcein is well retained within live cells, producing an intense uniform green fluorescence in live cells (ex/em 494/517 nm, Figure 2).

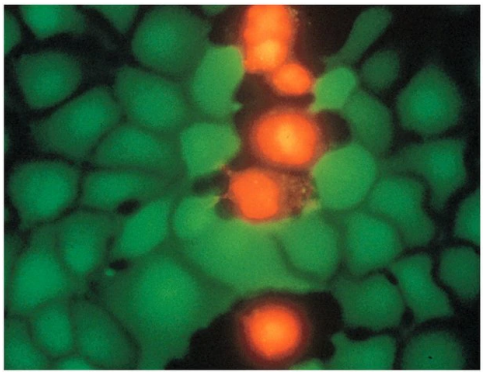

Fig. 2 Live and dead kangaroo rat (PtK2) cells stained with ethidium homodimer-1 and the esterase substrate calcein AM, both of which are provided in our LIVE/DEAD Viability/Cytotoxicity Kit (L3224). Live cells fluoresce a bright green, whereas dead cells with compromised membranes fluoresce red-orange.

Contents and storage

Contents	Catalog Number	Amount	Storage [1,2]
Calcein, AM	C1430	1 mg	Store at or below -20°C in desiccant. Protect from light. When stored as directed, product should remain stable for at least 1 year.
	C3099	1 mg/mL solution in dry dimethylsulfoxide (DMSO)	
	C3100	20 × 50 µg	

[1] Calcein AM may hydrolyze if exposed to moisture.
[2] Approximate fluorescence excitation/emission maxima for calcein AM are 494/517 nm.

Materials required but not provided

Dimethylsulfoxide (DMSO) - Reconstitute calcein AM using high-quality, anhydrous DMSO.

Important information

- Allow the vial to warm to room temperature before opening.
- Calcein AM is susceptible to hydrolysis when exposed to moisture. Aqueous working solutions containing calcein AM should be prepared immediately prior to use and used within one day. Once prepared, use DMSO stock solutions of calcein AM within a short time period for one set of experiments.

CAUTION! Hazards posed by these stains have not been fully investigated. DMSO is known to facilitate entry of organic molecules into tissue. These reagents should be handled using equipment and practices appropriate for the hazards posed by such material. Please dispose of reagents in compliance with all pertaining local regulations.

Basic loading protocol

1. Dissolve dye using high-quality, anhydrous dimethylsulfoxide (DMSO) at 1 mg/mL.
2. Dilute dye to a final working concentration of 1-10 µM in buffer or serum-free medium.
3. Add the dye to the cells and incubate for 15-60 minutes. Longer times (1-4 Hours) may be required.

   Note: Longer incubation times (1-4 hours) may be required for optimal results.
4. Remove the dye and put the cells in normal medium for 15-60 minutes to allow for AM ester removal.
5. Image the cells.

Fluorescence microscopy protocol

This example protocol makes 10 mL of an approximately 2 µM calcein AM. These dye concentrations are suitable for NIH 3T3, PtK2, and MDCK cells when incubated at room temperature for 20–40 minutes. Cultured mouse leukocytes (J774A.1), which have higher esterase activity, require 5–10 times less calcein AM than that required for the three other cell types. **This is an example protocol only; the optimal dye concentrations for any experiment will vary.**

1. Remove one 50 µg calcein AM from the freezer and allow to warm to room temperature.
2. Add 50 µL high-quality, anhydrous DMSO to one vial calcein AM, yielding a 1 mM stock solution. Once prepared, the DMSO stock solution should be used within a short time period for one series of experiments.
3. Transfer 20 µL of the 1 mM calcein AM stock solution to the 10 mL of buffer which is free of serum. We recommend Hanks Balanced Salt Solution, HBSS (Invitrogen Cat. no. 14025-092). Vortex the resulting solution to ensure thorough mixing.
4. The resulting approximately 2 µM calcein AM working solution is then added directly to cells. The final concentration of DMSO is ≤ 0.1%, a level generally innocuous to most cells.

   Note: Aqueous solutions of calcein AM are susceptible to hydrolysis and should be used within one day.
5. Adherent cells may be stained on coverslips after rinsing once with HBSS to remove residual serum present in the culture medium. Add a sufficient amount of stain solution to adequately cover the adherent cells. Cells in suspension should be pelleted by centrifugation and washed once in HBSS. Incubate for 30 minutes at 37°C.
6. Observe the samples in staining solution using a fluorescence microscope.

Flow cytometry protocol

This flow cytometry protocol has been optimized using Jurkat cells (human T-cell leukemia line) at a concentration of 1×10^6 cells/mL. Use of other cell types or other cell concentrations may require optimization of staining. If another staining reaction is to be performed on the sample, the user must determine the optimal staining sequence for the two procedures (Figure 3).

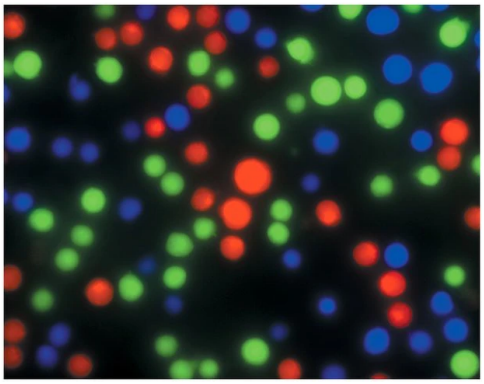

Fig. 3 Jurkat cells incubated with green-fluorescent calcein AM, CellTrace™ calcein red-orange AM (Cat. No. C34851) or calcein blue AM (Cat. No. C1429). The labeled cells were then combined and imaged with the appropriate filters.

1. Allow one 50 µg vial of calcein AM to come to room temperature.
2. Add 50 µL high-quality, anhydrous DMSO to one vial calcein AM to prepare a 1 mM stock solution. Add 10 µL of this stock solution to 190 µL DMSO to make a 50 µM working solution of calcein AM. Use this working solution within one day.
3. Prepare a 1 mL suspension of cells with $0.1–5 \times 10^6$ cells/mL for each assay. Cells may be suspended in medium or buffer.
4. Add 2 µL of working solution to each mL of cell suspension. Mix the sample.
5. Incubate the cells for 15–20 minutes on ice or at room temperature, protected from light.
6. Analyze the stained cells by flow cytometry using blue (~488 nm) excitation and fluorescence emission (~517 nm).

Limited product warranty

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