Calcein violet, AM

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
Calcein violet AM	20 vials, 25 µg each	• ≤–20°C • Desiccate * • Protect from light	When stored as directed, product should remain stable for at least 1 year.
* Calcein violet AM may hydrolyze if exposed to moisture			
Number of labelings: At the recommended reagent concentrations and volumes, this kit contains sufficient material to perform approximately 1,000 assays using flow cytometry.			
Approximate fluorescence excitation/emission maxima: Calcein violet AM: 400/452 nm			

Introduction

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 violet AM to the intensely fluorescent calcein violet (ex/em 400/452 nm, Figure 1). Calcein violet AM is an optimal dye for this application; utilizing the violet laser allows other laser lines to be used with more conventional markers.

Principle of the Method

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 violet AM ester is colorless and non fluorescent until hydrolyzed. The polyanionic dye calcein violet is well retained within live cells, producing an intense uniform violet fluorescence in live cells (ex/em 400/452 nm, Figure 2).

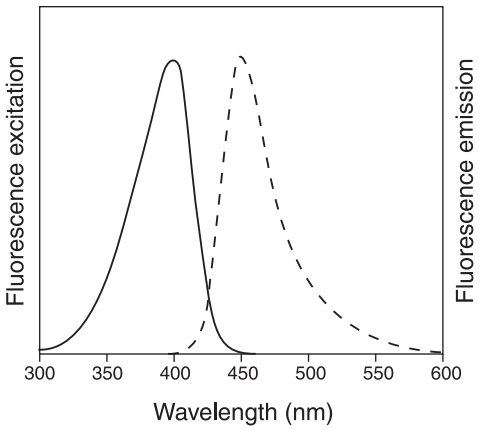

Figure 1. Fluorescence excitation and emission spectra for the Calcein violet stain in PBS, pH 7.2.

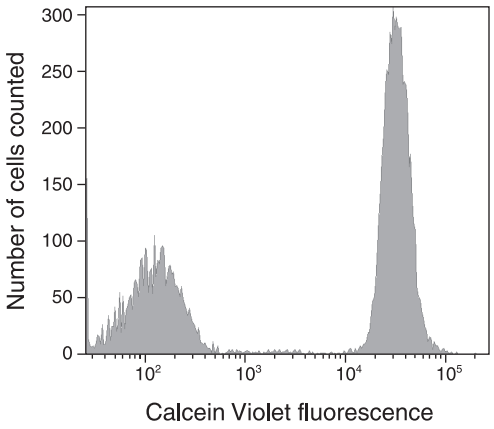

Figure 2. A mixture of heat-killed and untreated Jurkat cells (human leukemia T-cell) was stained according to the protocol in the Calcein violet, AM kit. Cells were analyzed using a flow cytometer equipped with a 405 nm laser and a 450/50 bandpass filter.

Before You Begin

Material Required but Not Provided

- Dimethylsulfoxide (DMSO): reconstitute calcein violet AM using high-quality, anhydrous DMSO.

Working with the Calcein Violet AM Stock Solution

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

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.

Experimental Protocols

For Flow Cytometry

This flow cytometry protocol has been optimized using Jurkat cells (human T-cell leukemia line) at a concentration of 1 x 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 (See Figure 3).

1.1 Allow one vial of calcein violet AM to come to room temperature.

1.2 Prepare a 1 mL suspension of cells with 0.1–5 × 10^6 cells/mL for each assay. Cells may be suspended in medium or buffer.

1.3 Add 42 µL high-quality, anhydrous DMSO to one vial calcein violet AM to prepare a stock solution. Add 40 µL of this stock solution to 1.25 mL buffer or media to make a working solution of calcein violet AM. Use this working solution within one day.

1.4 Add 5 µL of working solution to each mL cell suspension. Mix the sample.

1.5 Incubate the cells for 20–30 minutes on ice or at room temperature.

1.6 Analyze the stained cells by flow cytometry using violet (~405 nm) excitation and violet fluorescence emission (~450 nm).

For Fluorescence Microscopy

This microscopy protocol has been optimized using bovine pulmonary artery endothelial cells (BPAEC). 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.

2.1 Allow one vial of calcein violet AM to come to room temperature.

2.2 Add 42 µL high-quality, anhydrous DMSO to one vial calcein violet 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.

2.3 Prepare a working solution of dye in buffer which is free of serum. We recommend Hanks Balanced Salt Solution, HBSS (Invitrogen Cat. no. 14025-092) containing stain at a concentration range of 250 nM to 1 µM.

2.4 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.

2.5 Observe the samples in staining solution using a fluorescence microscope.

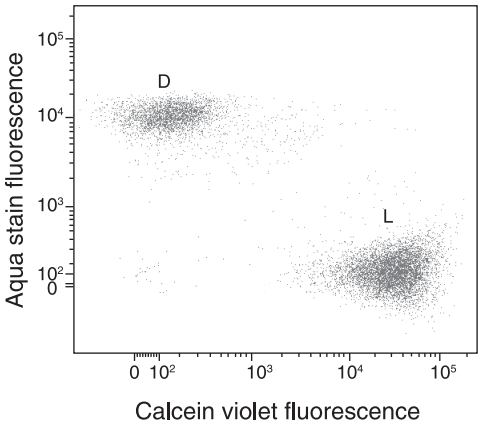

Figure 3. Staining pattern of a mixture of heat-killed and untreated Jurkat cells (human leukemia T-cell). Jurkat cells were stained according to the protocol in the LIVE/DEAD® Violet Viability/Vitality Kit, which combines the calcein violet fluorescence with the dead cell aqua-fluorescent reactive dye. Cells were analyzed using a flow cytometer equipped with a 405 nm laser and 450/50 bandpass for calcein violet labeling live cells (L) and 525/50 bandpass for the aqua-fluorescent reactive dye labeling dead cells (D).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
C34858	Calcein violet, AM *for flow cytometry* *for 405 nm excitation* *special packaging*	20 × 25 µg

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