CytoCalcein™ Violet 450 *Excited at 405 nm*

Catalog number: 22012
Unit size: 1 mg

Component	Storage	Amount
CytoCalcein™ Violet 450 *Excited at 405 nm*	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

CytoCalcein™ Violet 450 is designed for labeling live cells in the same way to calcein, AM. It has a maximum excitation at 405 nm, which perfectly matches the violet laser line equipped in most flow cytometers, and it is well-excited by the excitation sources of fluorescence microscopes. Upon getting into live cells the weakly fluorescent CytoCalcein™ Violet 450 is hydrolyzed into a strongly fluorescent dye that has an excitation/emission maxima of 405/450 nm. This exceptional spectral separation from the typical FACS fluorophores provides additional options for multiplexing experiments. Compared to calcein blue, CytoCalcein™ Violet 450 is brighter and is better excited by the 405 nm laser line. CytoCalcein™ Violet 450 and CytoCalcein™ Violet 500 have been developed for flow cytometric applications. CytoCalcein™ dyes exhibit similar biological properties to calcein, AM. They are optimized for the excitation wavelengths of a variety of flow cytometers, providing additional colors for flow cytometric analysis of live cells. CytoCalcein™ Violet 450 and CytoCalcein™ Violet 500 are well excited by 405 nm of violet laser and emit fluorescence at 450 nm and 500 nm respectively.

KEY PARAMETERS

Flow cytometer

Excitation	405 nm laser
Emission	450/40 nm filter
Instrument specification(s)	Pacific Blue channel

Fluorescence microscope

Excitation	DAPI filter set
Emission	DAPI filter set
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	405
Emission	450
Cutoff	435
Recommended plate	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

CytoCalcein™ Violet 450 Stock Solution
Prepare a 2 to 5 mM stock solution of CytoCalcein™ Violet 450 in high-quality, anhydrous DMSO.

Note The nonionic detergent Pluronic® F-127 can be used to increase the aqueous solubility of AM esters. In the staining buffer, the final Pluronic® F-127 concentration should be approximately 0.02%. A variety of Pluronic® F-127 products can be purchased from AAT Bioquest. Avoid long-term storage of AM esters in the presence of Pluronic® F-127.

PREPARATION OF WORKING SOLUTION

CytoCalcein™ Violet 450 Working Solution
Prepare a CytoCalcein™ Violet 450 working solution of 1 to 10 µM in the buffer of your choice (e.g., Hanks and Hepes buffer). For most cell lines, CytoCalcein™ Violet 450 at the final concentration of 4 to 5 µM is recommended. The exact concentration of indicators required for cell loading must be determined empirically.

Note If your cells contain organic anion-transporters, probenecid (1–2.5 mM) or sulfinpyrazone (0.1–0.25 mM) may be added to the working solution to reduce leakage of the de-esterified indicators.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare cells for imaging.
2. Remove the cell culture medium and wash cells once with serum-free buffer to remove any remaining media.

   Note Serum in cell culture media may contain esterase activity, which can increase background interference.
3. Add CytoCalcein™ Violet 450 working solution to the culture.
4. Incubate cells at 37 °C for 30 to 60 minutes.
5. Replace the dye working solution with HHBS or buffer of your choice (containing an anion transporter inhibitor, such as 1 mM probenecid, if applicable) to remove any excess probes.
6. Measure the fluorescence intensity using either a fluorescence microscope equipped with a DAPI filter set, a flow cytometer equipped with a violet laser and a 450/40 nm filter (Pacific Blue channel), or a fluorescence plate reader at Ex/Em = 405/450 nm cutoff 435 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

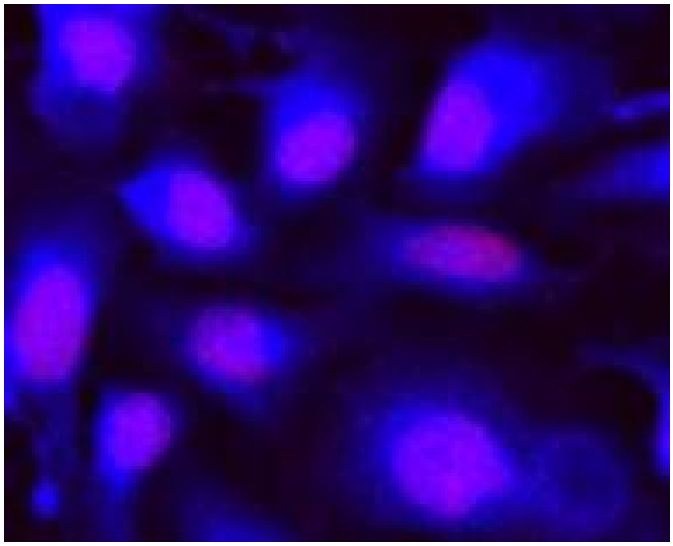

Figure 1. Image of Live HeLa cells stained with CytoCalcein™ Violet 450 *Excited at 405 nm*. Cell nuclei were stained with Nuclear Red LCS1 (Cat#17542).

DISCLAIMER

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