

**CytoCalcein™ Violet 450 \*Excited at 405 nm\***

 Catalog number: 22012  
 Unit size: 1 mg

Component	Storage	Amount (Cat No. 22012)
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**

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

**Fluorescence microscope**

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

**Fluorescence microplate reader**

Cutoff	435
Emission	450
Excitation	405
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**

1. 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.

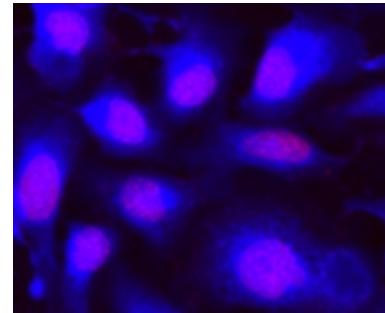
**PREPARATION OF WORKING SOLUTION**
**CytoCalcein™ Violet 450 Working Solution**

1. 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 sulfipyrazone (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).

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