CytoCalcein™ Violet 660, AM *Excited at 405 nm*

Catalog number: 21904
Unit size: 10x50 ug

Component	Storage	Amount (Cat No. 21904)
CytoCalcein™ Violet 660, AM *Excited at 405 nm*	Freeze (< -15 °C), Minimize light exposure	10x50 ug

OVERVIEW

CytoCalcein™ Violet 660 AM ester is designed for labeling live cells in the same way as Calcein, AM does. It is well excited with the Violet laser at 405 nm, which is now equipped in most flow cytometers. It is also well-excited by the excitation sources of fluorescence microscopes. Upon getting into live cells the CytoCalcein™ Violet 660 is hydrolyzed into a fluorescent dye of multiple negative charges, which makes the dye well retained in live cells. It has the largest Stokes Shift among the known live cell stains. This exceptional spectral separation from the typical existing FACS fluorophores provides additional options for multiplexing experiments with a flow cytometer or a fluorescence microscope. CytoCalcein™ Violet 660 has been developed for multiplexing flow cytometric and fluorescence imaging applications. It provides a new unique color for flow cytometric analysis of live cells.

KEY PARAMETERS

Flow cytometer

Emission	660/20 nm filter
Excitation	405 nm laser

Fluorescence microscope

Emission	675/30 nm
Excitation	405 nm laser
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Cutoff	630
Emission	660
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 660 Stock Solution

1. Prepare a 2 to 5 mM stock solution of CytoCalcein™ Violet 660 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 660 Working Solution

1. Prepare a CytoCalcein™ Violet 660 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 660 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 660 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 violet laser and 675/30 nm filter set, a flow cytometer equipped with a violet laser and a 660/20 nm filter, or a fluorescence plate reader at Ex/Em = 405/660 nm cutoff 630 nm.

DISCLAIMER

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