Cell Explorer™ Live Cell Labeling Kit *Blue Fluorescence with 405 nm Excitation*

Catalog number: 22614
Unit size: 200 Tests

Component	Storage	Amount
Component A: CytoCalcein™ Violet 450	Freeze (<-15 °C), Minimize light exposure	2 vials
Component B: HHBS (Hanks' buffer with 20 mM Hepes)	Refrigerate (2-8 °C), Minimize light exposure	1 bottle (100 mL)

OVERVIEW

Our Cell Explorer™ fluorescence imaging kits are a set of tools for labeling cells for fluorescence microscopic investigations of cellular functions. The effective labeling of cells provides a powerful method for studying cellular events in a spatial and temporal context. This particular kit is designed to uniformly label live cells for the flow cytometric analysis of live cells with the violet laser (405 nm excitation). The kit uses a proprietary dye that gets enhanced fluorescence upon entering into live cells. The dye is a hydrophobic compound that easily permeates intact live cells. The hydrolysis of the weakly fluorescent substrate by intracellular esterases generates a strongly fluorescent hydrophilic product that is well-retained in the cell cytoplasm. It can be readily adapted for flow cytometry applications. The fluorescent dye used in the kit is well excited with the violet laser (405 nm excitation) to fluorescence at 460 nm. The kit provides all the essential components with an optimized cell-labeling protocol. It is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Remove growth medium
3. Add CytoCalcein™ Violet 450 working solution (100 µL/well for a 96-well plate or 25 µL/wellfor a 384-well plate)
4. Incubate the cells at 37°C for 30 minutes to 1 hour
5. Wash the cells
6. Examine the specimen under fluorescence microscope with DAPI filter or flow cytometer with 450/40 nm filter (Pacific Blue channel)

Important Thaw all the components at room temperature before opening.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	DAPI filter set
Emission:	DAPI filter set
Recommended plate:	Black wall/clear bottom
Instrument:	Flow cytometer
Excitation:	405 nm laser
Emission:	450/40 nm filter
Instrument specification(s):	Pacific Blue channel

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.

1. CytoCalcein™ Violet 450 stock solution:
Add 20 µL of DMSO into the vial of CytoCalcein™ Violet 450 (Component A) and mix well to make CytoCalcein™ Violet 450 stock solution.

Note 20 µL of CytoCalcein™ Violet 450 stock solution is enough for 1 plate.

Note Unused CytoCalcein™ Violet 450 stock solution can be aliquoted and stored at < -20 °C for one month if the tubes are sealed tightly. Avoid repeated freeze-thaw cycles and protect it from light.

PREPARATION OF WORKING SOLUTION

Add 20 µL of CytoCalcein™ Violet 450 stock solution into 10 mL of HHBS (Component B) and mix well to make CytoCalcein™ Violet 450 working solution.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Remove the growth medium.
2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) CytoCalcein™ Violet 450 working solution into the cell plate.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes to 1 hour.
4. Remove the CytoCalcein™ Violet 450 working solution from the cells, wash the cells with HHBS (Component B) for 2 to 3 times, and replace with HHBS.
5. Analyze the cells using a fluorescence microscope with DAPI filter set or flow cytometer with 450/40 nm filter (Pacific Blue channel).

EXAMPLE DATA ANALYSIS AND FIGURES

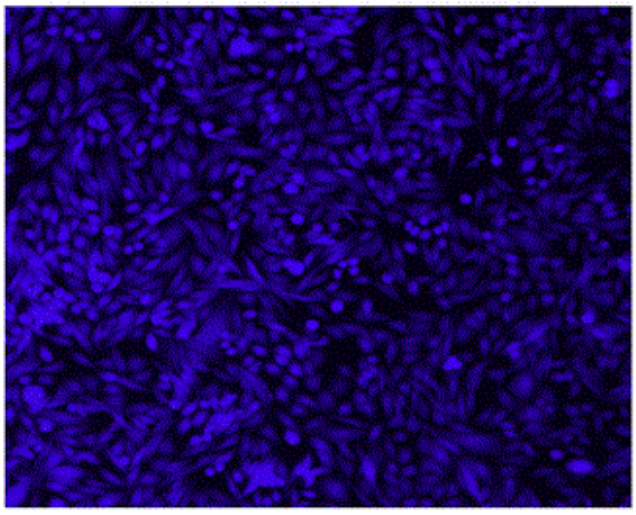

Figure 1. Image of CPA cells in 96-well Costar black wall/clear bottom plate stained with Cell Explorer™ Live Cell Labeling Kit *Blue Fluorescence with 405 nm Excitation*(Cat#22614). Cells were stained with CytoCalcein™ Violet 450 for 30 minutes. Images were aquired using fluorescence microscope using DAPI filter.

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