Cell Explorer™ Live Cell Labeling Kit

Blue Fluorescence with 405 nm Excitation

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22614 (2 plates)	Keep in freezer Protect from moisture and light	Fluorescence microscope

Introduction

Our Cell Explorer™ Live Cell Labeling Kits are a set of tools which can be used to label cells for fluorescence microscopic and flow cytometric 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 in blue fluorescence. The kit uses our proprietary non-fluorescent CytoCalcein™ Violet 450 dye that becomes strongly fluorescent upon entering into live cells. CytoCalcein™ Violet 450 is a hydrophobic compound that easily permeates intact live cells. The hydrolysis of the non-fluorescent CytoCalcein™ Violet 450 by intracellular esterases generates a strongly fluorescent hydrophilic product that is well-retained in the cell cytoplasm. The blue fluorescence dye has the spectral properties similar to those of Pacific Blue® (trade mark of Invitrogen) at Ex/Em = ~400/450 nm. When excited with the Violet Laser at 405 nm, the dye emits intense blue fluorescence at ~450 nm. The kit is optimized to be used with a flow cytometer equipped with a Violet Laser and particularly suitable for multicolor flow cytometric analysis of cells. This kit can also be used with a fluorescence microscope with a Pacific Blue® filter set.

This Cell Explorer™ Live Cell labeling kit can be readily adapted for a wide variety of fluorescence platforms such as microplate assays, flow cytometer and fluorescence microscope. It is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. The kit provides all the essential components with an optimized cell-labeling protocol (Ex/Em = 405/450 nm), and can be used for both proliferating and non-proliferating cells (either suspension or adherent cells).

Kit Components

Components	Amount
Component A: CytoCalcein™ Violet 450	2 vials
Component B: 10X Assay Buffer	2 bottles (1 mL/bottle)
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	1 bottle (20 mL)

Protocol for one 96-well plate

Brief Summary

Prepare cells in growth medium → Add CytoCalcein™ Violet 450 working solution 100 µL/well for a 96-well plate or 25 µL/well for a 384-well plate → Stain the cells at RT for 30 minutes to 2 hours → Examine the specimen under microscope at Ex/Em = 405/450 nm

Note: Thaw all the components at room temperature before opening.

1. Prepare cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 10,000 to 40,000 cells/well/100 µL for a 96-well plate or 2,500 to 10,000 cells/well/25 µL for a 384-well plate.

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellets in culture medium at 50,000-100,000 cells/well/100 µL for a 96-well poly-D lysine plate or 10,000-25,000 cells/well/25 µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiment.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

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2. Prepare CytoCalcein™ Violet 450 stain solution:

2.1 Prepare CytoCalcein™ Violet 450 stock solution: Add 20 µL of DMSO into CytoCalcein™ Violet 450 vial (Component A), and mix them well.
Note: 20 µL of CytoCalcein™ Violet 450 stock solution is enough for 1 plate. Unused CytoCalcein™ Violet 450 stock solution can be aliquoted and stored at < -20 °C for more than one month if the tubes are sealed tightly. Avoid repeated freeze-thaw cycles and protect from light.

2.2 Make 1X assay buffer: Add **9 mL** of HHBS (Component C) into 10X Assay Buffer (Component B), and mix them well.
Note: 10 mL of 1X assay buffer is enough for one plate. Aliquot and store unused 1X assay buffer at < -20 °C. Avoid repeated freeze-thaw cycles and protect from light.

2.3 Prepare CytoCalcein™ Violet 450 working solution: Add 20 µL of DMSO reconstituted CytoCalcein™ Violet 450 stock solution (from Step 2.1) into 10 mL of 1X assay buffer (from Step 2.2), and mix them well. The working solution is stable for at least 2 hours at room temperature.

3. Stain the cells:

3.1 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) CytoCalcein™ Violet 450 working solution (from Step 2.3) into the cell plate.
Note: You may replace the culture medium with100 µL of HHBS buffer or an appropriate buffer.

3.2 Incubate the cells in a 37 °C, 5% CO_2 incubator for 30 minutes to 2 hours.

3.3 Image the cells using a fluorescence microscope with a Pacific Blue® filter set (Ex/Em = 405/450 nm).
Note 1: DO not wash the cells.
Note 2: Alternatively, fix the cells at this point. Store the fixed cells at 4 °C and image the cells later.

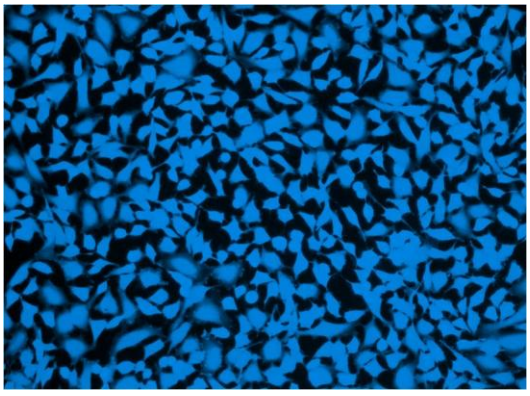

Figure 1. Image of CPA cells with Cell Explorer™ Live Cell Labeling Kit in a Costar black wall/clear bottom 96-well plate.

References

1. Wolff M, Wiedenmann J, Nienhaus GU, Valler M, Heilker R. (2006) Novel fluorescent proteins for high-content screening. Drug Discov Today, 11, 1054.
2. Lee S, Howell BJ. (2006) High-content screening: emerging hardware and software technologies. Methods Enzymol, 414, 468.
3. Haasen D, Schnapp A, Valler MJ, Heilker R. (2006) G protein-coupled receptor internalization assays in the high-content screening format. Methods Enzymol, 414, 121.