Cell Explorer™ Live Cell Labeling Kit

Green Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 22607 (10 plates)	Keep in freezer Protected from moisture and light	Fluorescence microscope

Introduction

Our Cell Explorer™ fluorescence imaging kits are a set of tools which can be used to lable 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 in green fluorescence. The kit uses non-fluorescent Calcein Green™ that becomes strongly fluorescent upon entering into live cells. Calcein Green™ is a hydrophobic compound that easily permeates intact live cells. The hydrolysis of the non-fluorescent Calcein Green™ by intracellular esterases generates the strongly fluorescent hydrophilic calcein that is well-retained in the cell cytoplasm. Cells grown in black-walled plates can be stained and quantified in less than two hours. Our Cell Explorer™ fluorescence labeling kit can be readily adapted for a wide variety of fluorescence platforms such as microplate assays, immunocyto-chemistry and flow cytometry. And 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, and can be used for both proliferating and non-proliferating cells (either suspension or adherent cells).

Kit Key Features

Convenient: Formulated to have minimal hands-on time. It can be applied to a broad spectrum of samples.
Continuous: Easily adapted to automation without a separation step.

Kit Components

Components	Amount
Component A: Calcein Green™	1 vial
Component B: 10X Assay Buffer	10 bottles (1 mL/bottle)
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	1 bottle (100 mL)

Protocol

Brief Summary

Prepare cells in growth medium → Add Calcein Green™ working solution 100 µL/well for 96-well plates or 25 µL/well for a 384-well plates → Stain the cells at RT for 30 min to 2 hours → Examine the specimen under microscope at Ex/Em = 490/525 nm

Note: Thaw all the components to 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 96-well plates or 2,500 to 10,000 cells/well/25 µL for 384-well plates.

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 96-well poly-D lysine plates or 10,000-25,000 cells/well/25 µL for 384-well poly-D lysine plates. Centrifuge the plates 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.

2. Prepare Calcein Green™ stain solution:

2.1 Prepare Calcein Green™ stock solution: Add 200 µL of DMSO into Calcein Green™ vial (Component A) and mix them well.
Note: 20 µL of Calcein Green™ stock solution is enough for 1 plate. Unused Calcein Green™ 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 Component C (HHBS) into Component B (10X Assay Buffer), 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 Calcein Green™ working solution for one cell plate: Add 20 µL of DMSO reconstituted Calcein Green™ 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) Calcein Green™ 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 min to 2 hours.

3.3 Image the cells using a fluorescence microscope with FITC filters (Ex/Em = 490/525 nm).
Note1: DO not wash the cells.
Note2: Alternatively, fix the cells at this point. Store the fixed cells at 4 °C and image the cells later.

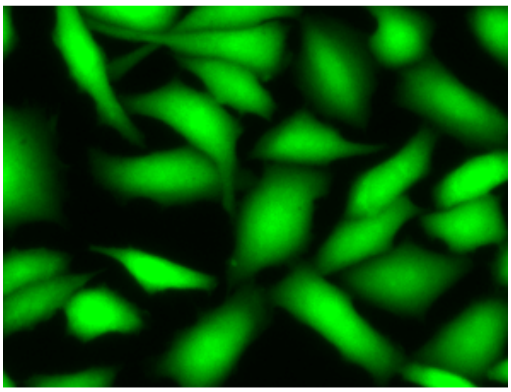

Figure 1. Image of CPA cells were stained with Cell Explorer™ Live Cell Labeling Kit *Green Fluorescence* in a 96-well Costar black plate

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.