Cell Explorer™ Live Cell Labeling Kit

Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22606 (10 plates)	Keep in freezer Protect from moisture and light	Fluorescence microscope

Introduction

Our Cell Explorer™ fluorescence 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 with a proprietary dye whose fluorescence is strongly enhanced 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. Cells grown on black wall/clear bottom plates or slides can be stained and quantified in less than two hours. This Cell Explorer™ fluorescence labeling kit can be readily adapted for a wide variety of fluorescence platforms such as microplate assays, flow cytometry 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 and can be used for both suspension and 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 UltraBlue™	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 UltraBlue™ working solution 100 µL/well for 96-well plates or 25 µL/well for 384-well plates → Stain the cells at RT for 30 minutes to 2 hours → Examine the specimen under microscope at Ex/Em= 360/445 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 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.

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Technical Support: support@aatbio.com; Tel: 408-733-1055

2. Prepare Calcein UltraBlue™ stain solution:

2.1 Prepare Calcein UltraBlue™ stock solution: Add 200 µL of DMSO into Calcein UltraBlue™ vial (Component A) and mix them well.
Note: 20 µL of Calcein UltraBlue™ stock solution is enough for one plate. Unused Calcein UltraBlue™ 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 Calcein UltraBlue™ working solution for one cell plate: Add 20 µL of DMSO reconstituted Calcein UltraBlue™ stock solution (from Step 2.1) into 10 mL of 1X assay buffer (from Step 2.2), and mix them well. This 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 UltraBlue™ 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 Wash cells with HHBS or an appropriate buffer, and add growth medium or HHBS back to the cells.
Note 1: Many cells contain organic-anion transporters. It's highly recommended to add additional 2.5 mM probenecid, an inhibitor of organic-anion transporters in HHBS buffer, when wash and replace the Calcein UltraBlue™ solution in the cells.
Note 2: Alternatively, fix the cells at this point. Store the fixed cells at 4 °C, and image the cells later.

3.4 Image the cells using a fluorescence microscope with FITC filters (Ex/Em = 360/445 nm).

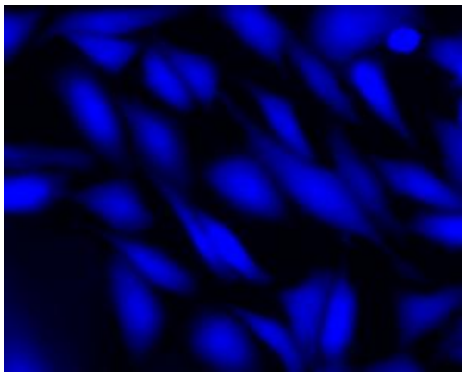

Figure 1. Image of CPA cells stained with Cell Explorer™ Live Cell Labeling Kit *Blue Fluorescence* in a Costar black 96-well plate

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.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.