Cell Explorer™ Live Cell Labeling Kit *Blue Fluorescence*

Catalog number: 22606
Unit size: 200 Tests

Component	Storage	Amount
Component A: Calcein UltraBlue™	Freeze (<-15 °C), Minimize light exposure	1 vial
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 in blue fluorescence. 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. Cells grown in black-walled plates can be stained and quantified in less than two hours. It can be readily adapted for high-throughput assays in a wide variety of fluorescence platforms such as microplate assays, immunocytochemistry and flow cytometry. 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.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Remove the medium
3. Add Calcein UltraBlue™ working solution (100 µL/well for 96-well plates or 25 µL/well for 384-well plates)
4. Incubate the cells at 37 °C for 30 minutes to 2 hours
5. Wash the cells
6. Examine the specimen under under fluorescence microscope with DAPI filter (Ex/Em = 360/445 nm)

Important Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	DAPI filter set
Emission:	DAPI filter set
Recommended plate:	Black wall/clear bottom

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. Calcein UltraBlue™ stock solution:
Add 20 µL of DMSO into the vial of Calcein UltraBlue™ (Component A) and mix well to make Calcein UltraBlue™ stock solution. Protect from light.

Note 10 µL of Calcein UltraBlue™ stock solution is enough for 1 plate. For storage, seal tubes tightly.

PREPARATION OF WORKING SOLUTION

Add 10 µL of Calcein UltraBlue™ stock solution into 10 mL of HHBS (Component B) and mix well to make Calcein UltraBlue™ working solution.

Note Protect from light.

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 from the cell plates.
2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Calcein UltraBlue™ working solution into the cell plate.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes to 2 hours.
4. Wash the cells with HHBS (Component B), and add growth medium or HHBS back to the cells.
5. Image the cells using a fluorescence microscope with DAPI filter (Ex/Em = 360/445 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

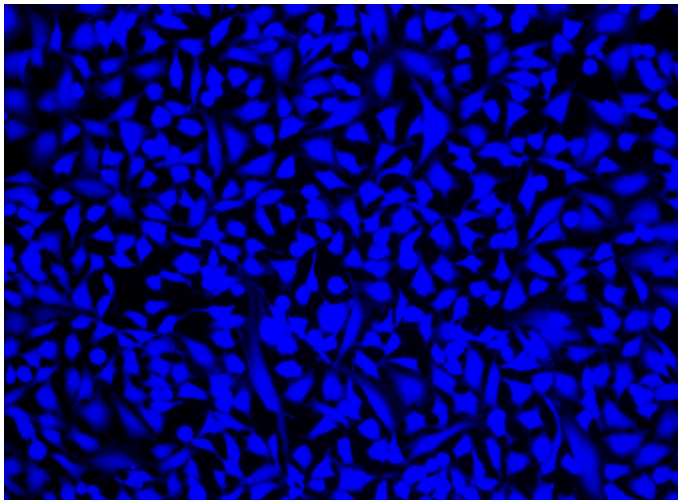

Figure 1. Image of HeLa cells stained with Cell Explorer™ Live Cell Labeling Kit *Blue Fluorescence* (Cat#22606) in a Costar black wall/clear bottom 96-well plate. Cells were stained with Calcein UltraBlue™ for 30 minutes at 37 °C. Images were aquired using a fluorescence microscope using DAPI filter.

DISCLAIMER

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com