Cell Explorer™ Live Cell Labeling Kit *Red Fluorescence*

Catalog number: 22609
Unit size: 200 Tests

Component	Storage	Amount
Component A: Calcein Red™	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 in red fluorescence. The kit uses a proprietary non-fluorescent dye that becomes strongly fluorescence upon entering into live cells. The dye is a hydrophobic compound that easily permeates intact live cells. The hydrolysis of the non-fluorescent substrate by intracellular esterases generates a strongly red 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. The assay is more robust than the tetrazolium salt or Alarmar Blue™-based assays. 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 in 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 Red™ working solution (100 µL/well for 96-well plates or 25 µL/well for 384-well plates)
4. Incubate cells at 37°C for 30 minutes to 2 hours
5. Wash the cells
6. Examine the specimen under under fluorescence microscope with Cy5 filter (Ex/Em = 646/660 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	Cy5 filter set
Emission:	Cy5 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 Red™ stock solution:
Add 20 µL of DMSO into the vial of Calcein Red™ (Component A) and mix well to make Calcein Red™ stock solution.

Note 20 µL of Calcein Red™ stock solution is enough for 1 plate.

Note Unused Calcein Red™ stock solution can be aliquoted and stored at< -20 °C for 2 weeks if the tubes are sealed tightly. Avoid repeated freeze-thaw cycles and protect from light.

PREPARATION OF WORKING SOLUTION

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

   Note It is important to remove the growth medium in order to minimize the background fluorescence and increase the signal to background ratio.

2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) Calcein Red™ 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. Remove the Calcein Red™ working solution from the cells.

5. Wash the cells with HHBS (Component B) for 2 to 3 times, and replace with HHBS.

6. Image the cells using a fluorescence microscope with Cy5 filter (Ex/Em = 646/660 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

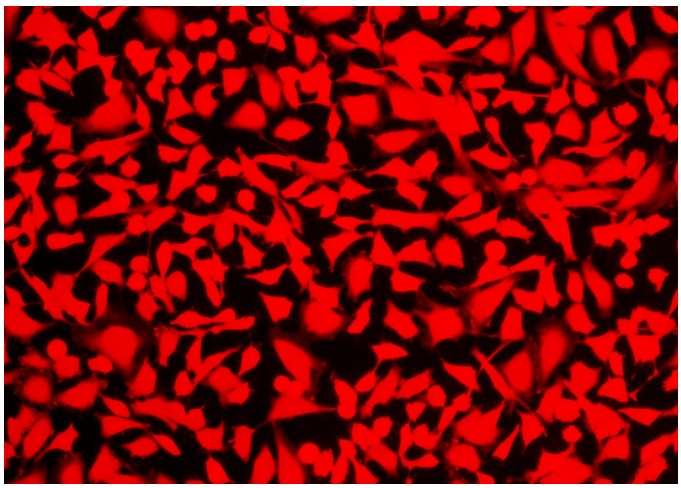

Figure 1. Image of HeLa cells stained with Cell Explorer™ Live Cell Labeling Kit *Red Fluorescence* (Cat#22609)in a Costar black wall/clear bottom 96-well plate. Cells were stained with Calcein Red™ for 30 minutes and image was aquired with fluorescence microscope using Cy5 filter.

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