Cell Explorer™ Live Cell Labeling Kit *Red Fluorescence*

Catalog number: 22609
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 22609)
Component A: Calcein Deep Red™	Freeze (< -15 °C), Minimize light exposure	2 vials
Component B: HHBS (Hanks' buffer with 20 mM Hepes)	Refrigerated (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 Deep 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 Note

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

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

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

Calcein Deep Red™ stock solution

Add 20 µL of DMSO into the vial of Calcein Deep Red™ (Component A) and mix well to make Calcein Deep Red™ stock solution. **Note:** 20 µL of Calcein Deep Red™ stock solution is enough for 1 plate. **Note:** Unused Calcein Deep 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.

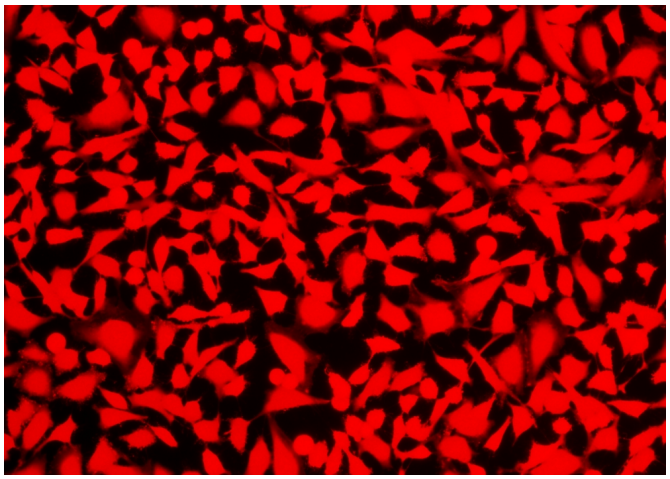
PREPARATION OF WORKING SOLUTION

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

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 Deep 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 Deep 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



DISCLAIMER

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