Cell Explorer™ Live Cell Labeling Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22609 (10 plates)	Keep in freezer Protect from moisture and light	Fluorescence microscope

Introduction

Our Cell Explorer™ fluorescence labeling kits are a set of tools 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 red fluorescence with 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 on slides or black wall/clear bottom plates can be stained and quantified in less than two hours. The assay is more robust than the tetrazolium salt or Alarmar Blue™-based ones. It can be readily adapted for many different types of fluorescence platforms such as microplate assays, flow cytometry and fluorescence microscope with Ex/Em = 646/660 nm. And 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 and can be used for both proliferating and non-proliferating 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 Red™	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)

Assay Protocol

Brief Summary

Prepare cells in growth medium → Remove the medium → Add Calcein Red™ 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 with Cy5 filters (Ex/Em = 646/660 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.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

2. Prepare Calcein Red™ stain solution:

2.1 Prepare Calcein Red™ stock solution: Add 200 µL of DMSO into Calcein Red™ vial (Component A) and mix them well.
Note: 20 µL of Calcein Red™ stock solution is enough for 1 plate. Unused Calcein Red™ 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 Red™ working solution for one cell plate: Add 20 µL of DMSO reconstituted Calcein Red™ 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 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.

3.2 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) Calcein Red™ working solution (from Step 2.3) into the cell plate.

3.3 Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes to 2 hours.

3.4 Image the cells using a fluorescence microscope with Cy5 filters (Ex/Em = 646/660 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.

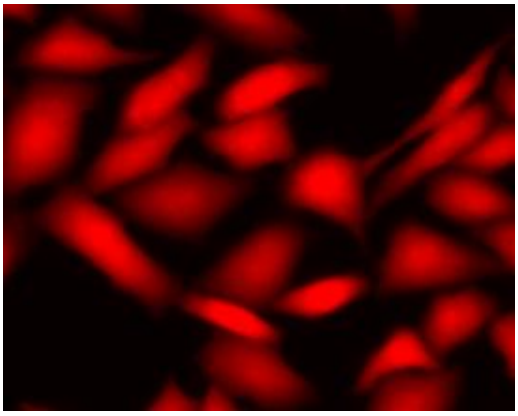

Figure 1. Image of CPA cells stained with Cell Explorer™ Live Cell Labeling kit *Red Fluorescence* in a Costar black 96-well plate

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.