Cell Explorer™ Live Cell Tracking Kit

Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22621 (5 plates)	Keep in freezer Protect from moisture and light	Fluorescence microscope

Introduction

Our Cell Explorer™ Live Cell labeling kits are a set of tools used to label 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 green fluorescence for the studies that require the fluorescent tag molecules retained inside cells for a relatively longer time. The kit uses a non-fluorescent dye that carries a cell-retaining moiety. The dye becomes strongly fluorescent upon entering into live cells, and trapped inside cells to give a stable fluorescence signal. The labeling process is robust and convenient, requiring minimal hands-on time.

As kit 22620, this kit can be readily adapted for many different types 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 green fluorescent indicator used in the kit has Ex/Em = 490/520 nm, compatible with the FITC filter set that is installed with almost every major fluorescence instrument. The kit provides all the essential components with an optimized cell-labeling protocol, and can be used for both proliferating and non-proliferating cells (either suspension or adherent cells). Due to its minimal cytotoxicity of the dye, this kit can be used for monitoring the interaction of a drug compound with its cellular targets in live cells.

Kit Components

Components	**Amount**
Component A: Track It™ Green	5 vials
Component B: DMSO	1 vial (0.5 mL)
Component C: Assay Buffer	1 bottle (100 mL)

Assay Protocol

Brief Summary

Prepare samples (microplate wells) → Remove the cell plate from incubator → Add 10 µL/well of 10X Track It™ Green working solution → Stain the cells at RT for 30 minutes to 2 hours → Wash the cells → Examine the specimen under microscope at Ex/Em = 490/520 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/90 µL for 96-well plates or 2,500 to 10,000 cells/well/20 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/90 µL for 96-well poly-D lysine plates or 10,000-25,000 cells/well/20 µL for 384-well poly-D lysine plates. Centrifuge the plates at 800 rpm for 2 minutes with brake off prior to the experiments.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

2. Prepare Track It™ Green stain solution:

2.1 Prepare 2 mM Track It™ Green stock solution: Add 25 µL of DMSO (Component B) into one of Track It™ Green vial (Component A) to make 2 mM Track It™ Green stock solution.

Note: The unused portion of the 2 mM Track It™ stock solution should be stored at -20°C. Avoid repeated freeze/thaw cycles.

2.2 ~~Prepare 10X Track It™ Green working solution:~~ Dilute 2 mM Track It™ Green stock solution (from Step 2.1) into Assay Buffer (Component C) to make 1 to 50 µM Track It™ Green working solution. The working solution should be prepared enough for all the wells at 10 µL/well with the appropriate concentration. For example, to get Track It™ Green at the final concentration of 2 µM for one 96-well microplate: Dilute 10 µL of the Track It™ Green stock solution into 1 mL of Assay Buffer (Component C) to make 1 mL of 20 µM (10X) Track It™ Green working solution.

Note: The final concentration of the Track It™ Green should be empirically determined for different cell types and/or experimental conditions. It is recommended to test at the concentrations that are at least over a tenfold range. In general, long-term staining (more than about 3 days) or the use of rapidly dividing cells will require 5–25 µM dye. Dye at a lower concentration (0.5–5 µM) is needed for shorter experiments, such as viability assays. To maintain normal cellular physiology and reduce potential artifacts, the concentration of the dye should be kept as low as possible. The effects of overloading may not be immediately apparent. For example, peripheral blood lymphocytes respond normally to concanavalin A when treated with up to 1 µM dye, but not with more than 5 µM dye.

3. Stain the cells:

3.1 To the cell wells add 10X Track It™ Green working solution (from Step 2.2) which should be equal to 1/10 of the volume of cell culture medium. For example, for 96-well plate, add 10 µL/well of 10X Track It™ Green working solution into the cells.

3.2 Incubate the cells in a 37 °C, 5% CO_2 incubator for 15 minutes to 1 hour.

3.3 Replace the dye solution with fresh, pre-warmed culture medium and incubate the cells for another 30 minutes at 37 °C.

3.4 Wash cells with HHBS or an appropriate buffer.

Note1: Alternatively, fix the cells at this point. Store the fixed cells at 4 °C, and image the cells later.
Note2: When the cells are going to be subsequently labeled with an antibody, a permeabilization step is often required to enhance the antigen's accessibility. Cells can be permeabilized when incubated in ice-cold acetone for 10 minutes.

3.5 Image the cells using a fluorescence microscope with FITC filters (Ex/Em = 490/520 nm).

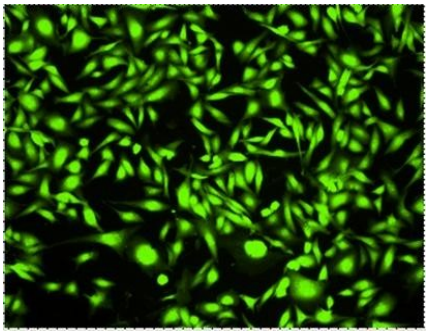

Figure 1. Image of U2OS cells stained with 2 µM Cell Explorer™ Live Cell Tracking Kit *Green Fluorescence* in a Costar black 96-well plate

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.