Cell Explorer™ Live Cell Tracking Kit

Orange Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22622 (2 plates)	Keep in freezer Protect from moisture and light	Fluorescence microscope Flow cytometry

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 label live cells in orange 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 is a hydrophobic compound that easily permeates intact live cells. It becomes strongly fluorescent upon entering into live cells, and trapped inside to give a stable fluorescence signal. The labeling process is robust and convenient, requiring minimal hands-on time. The kit can be readily adapted for many different types of fluorescence platforms such as flow cytometry and fluorescence microscope (Ex/Em = 540/560). It is useful for a variety of studies, including cell adhesion, chemotaxis, cell viability, apoptosis and cytotoxicity. The kit provides all the essential components with an optimized cell-labeling protocol, and can be used for both proliferating and non-proliferating cells.

Kit Components

Components	**Amount**
Component A: Track It™ Orange	50 µL (500X DMSO stock solution)
Component B: Assay Buffer	20 mL

Assay Protocol

Brief Summary

Prepare samples → Remove the cell plate from incubator → Add 10 µL/well of 10X Track It™ Orange working solution → Stain the cells at RT for 15 minutes to 1 hour → Wash the cells → Examine the specimen under microscope at Ex/Em = 540/570 nm

Note: Thaw all the components to room temperature, centrifuge the component A briefly 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 experiment.

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

2. Prepare 10X Track It™ Orange stain solution:

Dilute 500X Track It™ Orange DMSO stock solution (Component A) into Assay Buffer (Component B) to make a 10 to 25X Track It™ Orange 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 a 1X final concentration of Track It™ Orange for one 96-well microplate, dilute 20 µL of the Track It™ Orange DMSO stock solution into 1 mL of Assay Buffer (Component B) to make 1 mL of 10X Track It™ Orange working solution.

Note1: The unused portion of the Track It™ Orange stock solution should be stored at -20°C. Avoid repeated freeze/thaw cycles.
Note2: The final concentration of the Track It™ Orange working solution 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 ten fold range.

3. Stain the cells:

3.1 To the cell wells add 10X Track It™ orange working solution (from Step 2) which should be equal to 1/10 of the volume of cell culture medium. For example, for a 96-well plate, add 10 µL/well of 10X Track It™ Orange 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 Wash cells with Hanks and 20 mM Hepes buffer (HBSS) or an appropriate buffer.
3.4 Fill the cell wells with growth medium.
3.5 Analyze the cells using a fluorescence microscope or flow cytometer with TRITC filter sets (Ex/Em = 540/570 nm).

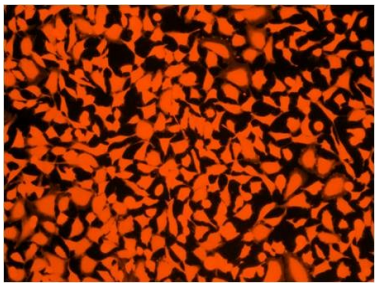

Figure 1. Image of Hela cells stained with 1X Cell Explorer™ Live Cell Tracking Kit *Orange Fluorescence* in a Costar black wall/clear bottom 96-well plate.

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.