Cell Explorer™ Live Cell Tracking Kit

Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22620 (2 plates)	Keep in freezer Protect from moisture and light	Fluorescence microscope Flow Cytometer

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 blue 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 is trapped inside cells to give stable fluorescence signals. The dye is a hydrophobic compound that easily permeates intact live cells. The labeling process is robust, requiring minimal hands-on time. This Cell Explorer™ Live Cell labeling kit can be readily adapted for many different types of fluorescence platforms such as microplate assays, flow cytometry and fluorescence microscope. It is useful for 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, and can be used for both proliferating and non-proliferating cells (either suspension or adherent cells).

Kit Components

Components	Amount
Component A: Track It™ Blue	2 vials
Component B: DMSO	1 vial (0.5 mL)
Component C: Assay Buffer	1 bottle (20 mL)

Assay Protocol

Brief Summary

Prepare samples → Remove the cell plate from incubator → Add 10 µL/well of 10X Track It™ Blue working solution → Stain the cells at RT for 15 minutes to 1 hour → Wash the cells → Examine the specimen under microscope at Ex/Em = 360/445 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/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 Track It™ Blue stain solution:

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

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

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2.2 Prepare 10X Track It™ Blue working solution: Dilute 2 mM of Track It™ Blue stock solution (from Step 2.1) into Assay Buffer (Component C) to make 1 to 50 µM Track It™ Blue 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™ Blue at the final concentration of 2 µM for one 96-well microplate, dilute 10 µL of the Track It™ Blue stock solution into 1 mL of Assay Buffer (Component C) to make 1 mL of 20 µM (10X) Track It™ Blue working solution.
Note: The final concentration of the Track It™ Blue 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™ Blue working solution (from Step 2.2), 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™ Blue working solution into the cells.
3.2 Incubate the cells in a 37 ^{o}C, 5% CO_2 incubator for 15 min to 1 hour.
3.3 Wash Wash cells with Hanks and 20 mM Hepes buffer (HHBS) 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 DAPI filter sets (Ex/Em = 360/445 nm).

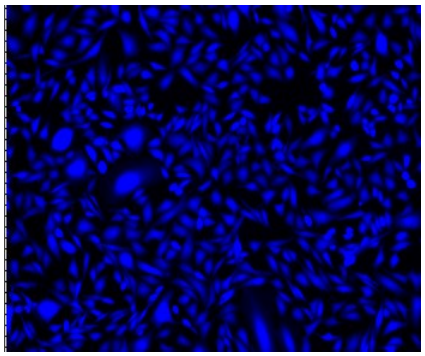

Figure 1. Image of U2OS cells stained with 2 µM Cell Explorer™ Live Cell Tracking Kit *Blue Fluorescence* in a Costar black wall/clear bottom 96-well plate.

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.