Cell Explorer™ Fixable Live Cell Tracking Kit

Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22621 (2 plates)	Keep in freezer Avoid moisture & light	Fluorescence microscope Flow cytometer

Introduction

Our Cell Explorer™ Fixable Live Cell Tracking Kits are a set of tools that provide well-retained cell-tracing reagents to label and track cells for investigations of cellular functions in a wide variety of colors. 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 green fluorescence for the studies that require the fluorescent tag molecules retained inside cells for a relatively longer time. The Track It™ Green used in the kit is 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 adduct that formed in labeled cells is retained by the cells throughout development and meiosis, and is inherited by daughter cells after cell division.

The advantage of Track It™ Green is compatible with cell culture medium in the staining cells. 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 = 490/520 nm). 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™ Green	1 vial
Component B: Assay Buffer	1 bottle (20 mL)
Component C: DMSO	1 vial (100 µL)

Assay Protocol

Brief Summary

Prepare samples → Add Track It™ Green working solution → Stain the cells at RT for 15 to 30 minutes → Wash the cells → Examine the specimen 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 1: For flow cytometry experiment, prepare cells in 0.5 mL warm medium or buffer of your choice at a density of 5×10^5 to 1×10^6 cells/mL.
Note 2: 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 1000X Track It™ Green stock solution: Add 20 µL of DMSO (Component C) into the vial of Track It™ Green (Component A) and mix well.
Note: The unused portion of 1000X Track It™ stock solution should be stored at -20°C. Avoid repeated freeze/thaw cycles.

2.2 Prepare Track It™ Green working solution: Dilute 1000X Track It™ Green stock solution (from Step 2.1) into Assay Buffer (Component B) at 1:1000 ratio to make 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. In general, long-term staining (more than about 3 days) or the use of rapidly dividing cells will require 1: 500 dilution to double the dye concentration. Dye at a lower concentration up to 1:2000 dilution may be 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.

3. Stain the cells:

3.1 Add equal volume of Track It™ Green working solution (from Step 2.2) into the cell wells. For example, for 96-well plate, add 100 µL/well of Track It™ Green working solution into the cells.
3.2 Incubate the cells in a 37°C, 5% CO_2 incubator for 15 to 30 minutes.
3.3 Wash cells with HHBS or an appropriate buffer for 3 times.
Note: Alternatively, fix the cells at this point. Store the fixed cells at 4°C, and image the cells later.
3.4 Image the cells using a fluorescence microscope with FITC filters (Ex/Em = 490/520 nm). Or monitor the fluorescence intensity with a flow cytometer using the FL1 channel (Ex/Em = 490/525 nm), gate on the cells of interest, excluding debris.

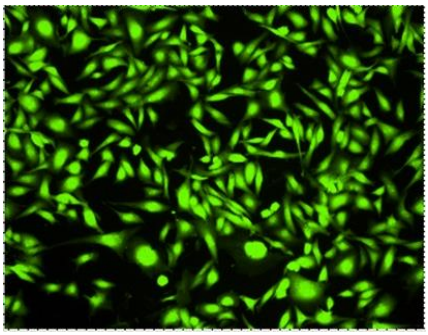

Figure 1. Image of U2OS cells stained with Cell Explorer™ Live Cell Tracking Kit in a Costar black 96-well plate.

References

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