Cell Explorer™ Fixable Live Cell Tracking Kit *Green Fluorescence*

Catalog number: 22621
Unit size: 200 Tests

Component	Storage	Amount
Component A: Track It™ Green	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 mL)
Component C: DMSO	Freeze (< -15 °C)	1 vial (100 µL)

OVERVIEW

Our Cell Explorer™ fluorescence imaging kits are a set of tools for labeling 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 relatively longer time. The cells can be fixed to retain the imaging pattern. 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 live cells to give a stable fluorescence signal for relatively long time. The dye is a hydrophobic compound that easily permeates intact live cells. The labeling process is robust, requiring minimal hands-on time. It can be readily adapted for a wide variety 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 kit provides all the essential components with an optimized cell-labeling protocol.

AT A GLANCE

Protocol Summary

1. Prepare samples
2. Add Track It™ Green working solution
3. Stain the cells at 37°C for 15 to 30 minutes
4. Wash the cells
5. Examine the specimen under fluorescence microscope with FITC filter (Ex/Em = 490/520 nm) or flow cytometer with 530/30 nm filter (FITC channel)

Important Thaw all the components at room temperature before opening.

KEY PARAMETERS

Flow cytometer

Excitation	488 nm laser
Emission	530/30 nm filter
Instrument specification(s)	FITC channel

Fluorescence microscope

Excitation	FITC filter
Emission	FITC filter
Recommended plate	Black wall/clear bottom

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Track It™ Green stock solution (1000X)
Add 25 µL of DMSO (Component C) into the vial of Track It™ Green (Component A) and mix well to make 1000X Track It™ Green stock solution.

Note The unused portion of 1000X Track It™ stock solution should be stored at -20 ° C. Avoid repeated freeze/thaw cycles.

PREPARATION OF WORKING SOLUTION

Track It™ Green working solution
Dilute 1000X Track It™ Green stock solution 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.

SAMPLE EXPERIMENTAL PROTOCOL

1. Remove Growth medium, wash cells with PBS once.
2. Add 100 µL Track It™ Green working solution to each well.
3. Incubate the cells in a 37 °C, 5% CO2 incubator for 15 to 60 minutes.
4. Wash cells with Hanks and 20 mM Hepes buffer (HHBS) or an appropriate buffer.
5. Fill the cell wells with Assay Buffer or an appropriate buffer.
6. 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 530/30 nm emission filter (FITC channel). Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

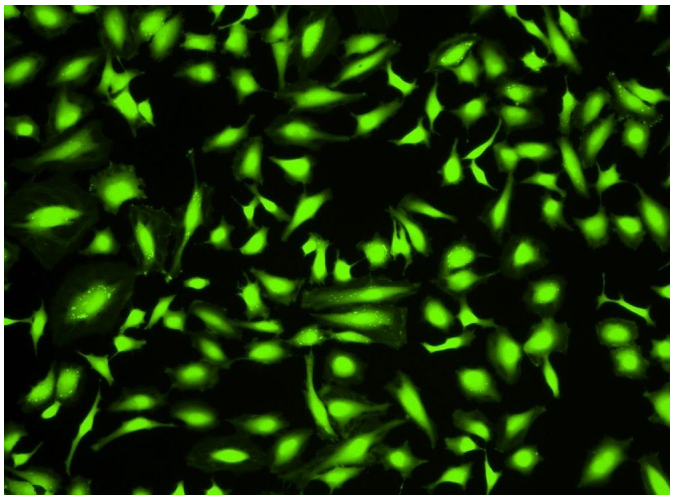

Figure 1. Image of HeLa cells stained with Cell Explorer™ Live Cell Tracking Kit in a Costar black wall/clear bottom 96-well plate. Cells were stained with Track It™ Green (Cat#22621) and incubated for 15 minutes. Images were aquired using fluorescence microscope with FITC filter set.

DISCLAIMER

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