

Cell Explorer™ Live Cell Tracking Kit

Red Fluorescence

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 22623 (5 plates)	Keep in freezer Protect from moisture and light	Fluorescence microscope

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 uniformly label live cells in red 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 microplate assays, flow cytometry and fluorescence microscope (Ex/Em = 575/600 nm). 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.

Kit Components

Components	Amount
Component A: Track It™ Red	100 µL (1 mM DMSO stock solution)
Component B: Assay Buffer	100 mL

Assay Protocol

Brief Summary

Prepare samples (microplate wells) → Remove the cell plate from incubator → Add 10 µL/well of 10X Track It™ Red working solution → Stain the cells at RT for 30 minutes to 2 hours → Wash the cells → Examine the specimen under microscope at Ex/Em = 570/600 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 10X Track It™ Red stain solution:

Dilute 1 mM Track It™ Red stock solution (from Step 2.1) into Assay Buffer (Component C) to make a 1 to 50 µM Track It™ Red 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 2 µM final concentration of Track It™ Red for one 96-well microplate: Dilute 20 µL of the Track It™ Red stock solution into 1 mL of Assay Buffer (Component B) to make 1 mL of 20 µM (10X) Track It™ Red working solution.

Note1: The unused portion of the Track It™ Red stock solution should be stored at -20 °C. Avoid repeated freeze/thaw cycles.

Note2: The final concentration of the Track It™ Red 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. 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™ Red 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™ Red working solution into the cells.
- 3.2 Incubate the cells in a 37 °C, 5% CO₂ 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 = 360/445 nm).

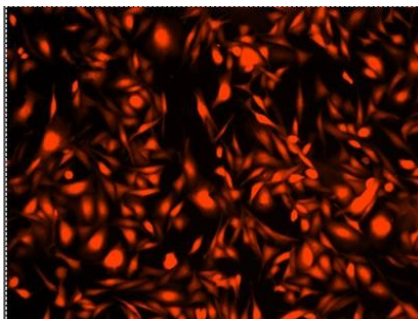


Figure 1. Image of U2OS cells stained with 2 μM Cell Explorer™ Live Cell Tracking Kit *Red Fluorescence* in a Costar black 96-well plate

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.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.