

Cell Explorer™ Fixable Live Cell Tracking Kit

Red Fluorescence

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 22625 (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 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, and it can be fixed with 4% formaldehyde. 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 = 575/600 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™ Red	1 vial
Component B: Assay Buffer	20 mL
Component C: DMSO	100 µL

Assay Protocol

Brief Summary

Prepare samples → Remove the cell plate from incubator → Add 10 µL/well of 10X Track It™ Red working solution → Stain the cells at RT for 15 minutes to 1 hour → Wash the cells → Examine the specimen under microscope at Ex/Em = 570/600 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™ Red stain solution:

- 2.1 Make 500 X Track It™ Red DMSO stock solution by adding 50 µL DMSO (Component C) into the vial of Component A.
- 2.2 Dilute 500X Track It™ Red DMSO stock solution (From Step 2.1) into Assay Buffer (Component B) to make a 1X Track It™ Red working solution. The working solution should be prepared and used promptly.

For example, to get a 1 X final concentration of Track It™ Red for one 96-well microplate, dilute 20 µL of the Track It™ Red DMSO stock solution into 10 mL of Assay Buffer (Component B).

Note1: The unused portion of the Track It™ Red stock solution should be aliquoted as a single used vials stored at -20°C. Avoid light and 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

3. Stain the cells:

- 3.1 Remove the cell medium, and add 100 µL/well (for 96 well plate) of 1X Track It™ Red working solution (from Step 2.2).
- 3.2 Incubate the cells in a 37 °C, 5% CO₂ incubator for 15 minutes to 1 hour.
- 3.3 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 Texas Red filter sets (Ex/Em = 570/600 nm).

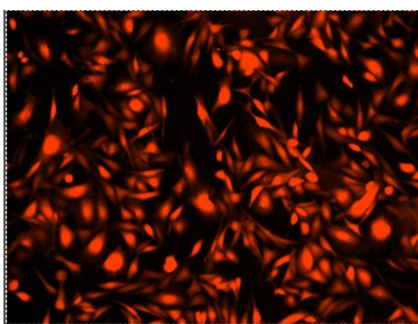


Figure 1. Image of HeLa cells stained with 1X Cell Explorer™ Live Cell Tracking Kit *Red Fluorescence* in a Costar black wall/clear bottom 96-well plate

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.

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.