

## Cell Explorer™ Live Cell Tracking Kit \*Orange Fluorescence\*

Catalog number: 22622  
Unit size: 200 Tests

Component	Storage	Amount
Component A: Track It™ Orange	Freeze (<-15 °C), Minimize light exposure	1 vial (50 µL - 500X DMSO stock solution)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 mL)

### OVERVIEW

Our Cell Explorer™ Live Cell Tracking Kit uses a proprietary orange fluorescent dye that gets enhanced fluorescence upon entering into live cells. The dye is a hydrophobic compound that easily permeates intact live cells. The hydrolysis of the weakly fluorescent substrate by intracellular esterases generates a strongly fluorescent hydrophilic product that is well-retained in the cell cytoplasm. The tracking dye has good photostability with robust imaging performance. The kit is particularly suitable for multicolor flow cytometric analysis of cells. It can also be used with a fluorescence microscope equipped with a TRITC filter set. This kit provides an effective tool of labeling cells for flow cytometric and fluorescence microscopic investigations of cellular functions. The effective labeling of cells offers a powerful method for studying cellular events in a spatial and temporal context. This kit 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 and remove cells from incubator
2. Add 10 µL/well of 10X Track It™ Orange working solution in each well
3. Stain the cells at 37°C for 15 minutes to 1 hour
4. Wash the cells
5. Examine the specimen under fluorescence microscope with Cy3/TRITC filter or flow cytometer with 575/26 nm filter (PE channel)

**Important** Thaw all the components to room temperature, centrifuge the component A briefly before opening.

### KEY PARAMETERS

Instrument: Fluorescence microscope  
Excitation: 540 nm  
Emission: 570 nm  
Instrument specification(s): Cy3/TRITC filter  
Recommended plate: Black wall/clear bottom

Instrument: Flow cytometer  
Excitation: 488 nm or 532 nm laser  
Emission: 575/26 nm filter  
Instrument specification(s): PE channel

### PREPARATION OF WORKING SOLUTION

Dilute 500X Track It™ Orange DMSO stock solution (Component A) into Assay Buffer (Component B) to make a 10X to 25X Track It™ Orange 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 1X final concentration of Track It™ Orange for one 96-well microplate, dilute 20 µL of the Track It™ Orange DMSO stock solution into 1 mL of Assay Buffer (Component B) to make 1 mL of 10X Track It™ Orange working solution.

**Note** The final concentration of the Track It™ Orange 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.

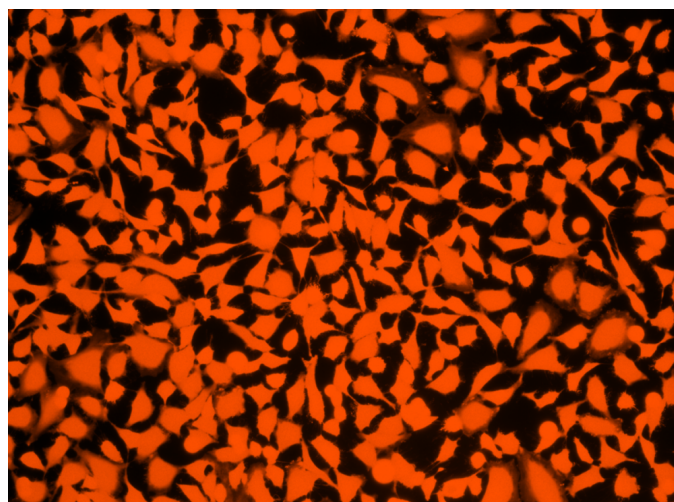
### PREPARATION OF CELL SAMPLES

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

### SAMPLE EXPERIMENTAL PROTOCOL

1. Add 10X Track It™ Orange working solution to the cells, the volume 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™ Orange working solution into the cells.
2. Incubate the cells in a 37°C, 5% CO<sub>2</sub> incubator for 15 minutes to 1 hour.
3. Wash cells with Hanks and 20 mM Hepes buffer (HBSS) or an appropriate buffer.
4. Fill the cell wells with growth medium.
5. Analyze the cells using a fluorescence microscope with Cy3/TRITC filter sets of flow cytometer with 575/26 nm filter (PE channel).

### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Image of HeLa cells stained with Cell Explorer™ Live Cell Labeling Kit \*Orange Fluorescence\* (Cat#22622) in 96-well Costar black wall/clear bottom. Cells were stained with Track It™ Orange for 15 minutes and image was acquired with fluorescence microscope using TRITC filter.

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