

ab138892

CytoPainter Cell Tracking Staining Kit – Orange Fluorescence

Instructions for Use

For labeling live cells in orange fluorescence for studies that require the fluorescent tag molecules retained inside cells for a relatively longer time.

This product is for research use only and is not intended for diagnostic use.

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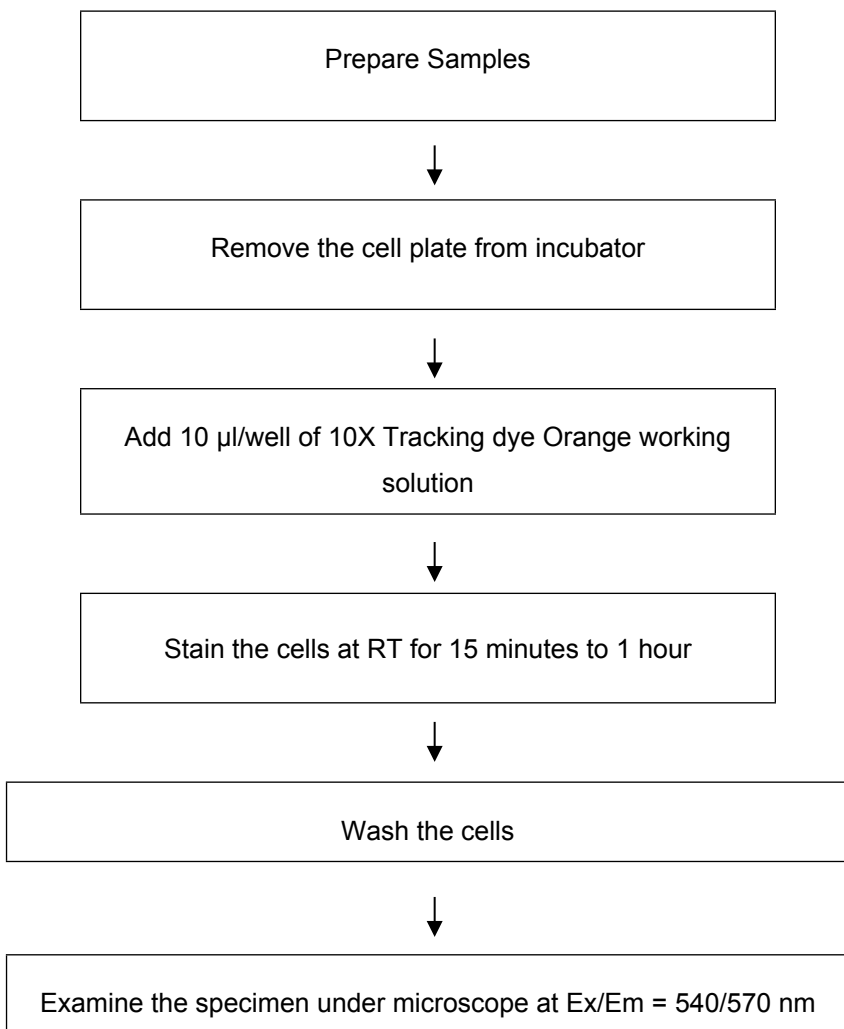
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1. Introduction

Abcam's CytoPainter Cell Tracking Staining 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.

ab138892 is designed to label live cells in orange 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 flow cytometry and fluorescence microscope (Ex/Em = 540/560). 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.

2. Protocol Summary



3. Kit Contents

Components	Amount
Component A: Tracking dye Orange (500X DMSO stock solution)	50 µl
Component B: Assay Buffer	20 ml

4. Storage and Handling

Keep at -20°C. Protect from moisture and light.

5. Assay Protocol

A. Prepare Cells

1. For adherent cells: Plate cells overnight in growth medium at 10,000 to 40,000 cells/well/90 μ l for 96-well plates or 2,500 to 10,000 cells/well/20 μ l for 384-well plates.
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 μ l for 96-well poly-D lysine plates or 10,000-25,000 cells/well/20 μ l 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.

B. Prepare 10X Tracking dye Orange stain solution

1. Dilute 500X Tracking dye Orange DMSO stock solution (Component A) into Assay Buffer (Component B) to make a 10 to 25X Tracking dye Orange working solution. The working solution should be prepared enough for all the wells at 10 μ l/well with the appropriate concentration. For example, to get a 1X final concentration of Tracking dye Orange for one 96-well microplate, dilute 20 μ l of the Tracking dye Orange DMSO stock solution into 1 ml of Assay Buffer (Component B) to make 1 ml of 10X Tracking dye Orange working solution.

Note 1: The unused portion of the Tracking dye Orange stock solution should be stored at -20 °C. Avoid repeated freeze/thaw cycles.

Note 2: The final concentration of the Tracking dye 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.

C. Stain the Cells

1. To the cell wells add 10X Tracking dye orange working solution which should be equal to 1/10 of the volume of cell culture medium. For example, for a 96-well plate, add 10 μ l/well of 10X Tracking dye Orange working solution into the cells.
2. Incubate the cells in a 37°C, 5% CO₂ 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 or flow cytometer with TRITC filter sets (Ex/Em = 540/570 nm).

6. Data Analysis



Figure 1. Image of HeLa cells stained with 1X CytoPainter Cell Tracking Staining Kit - Orange Fluorescence in a black wall/clear bottom 96-well plate.

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “*contact us*” on www.abcam.com for the phone number for your region).

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