ab138894

CytoPainter Cell Tracking Staining Kit – Deep Red Fluorescence

Instructions for Use

For labeling live cells with deep red fluorescence.

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

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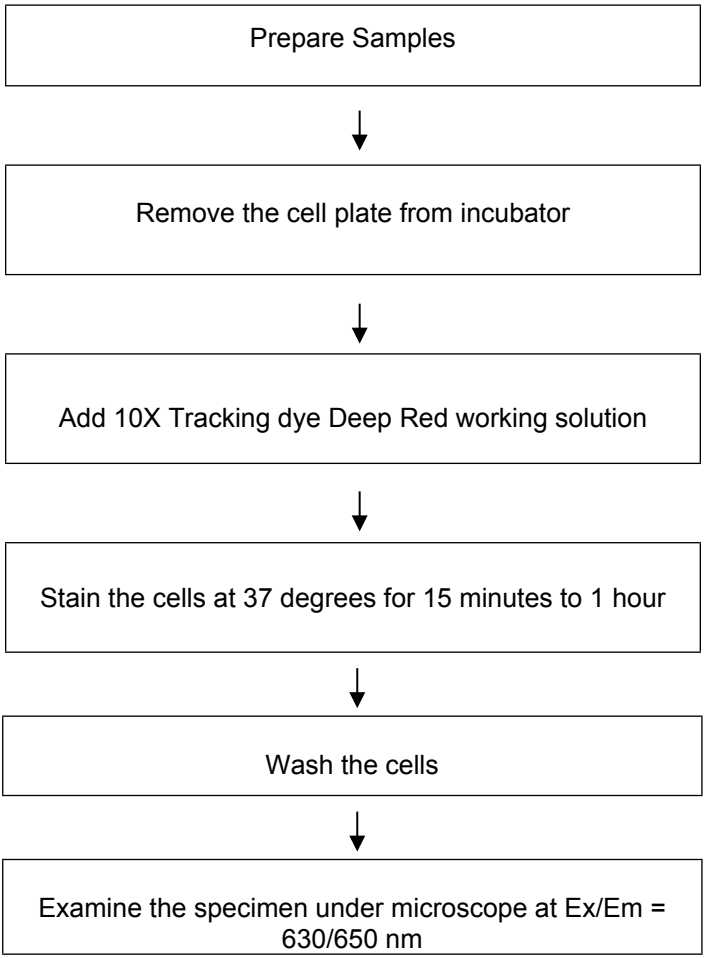
1. Introduction

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.

ab138894 is designed to label live cells in deep 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 flow cytometry and fluorescence microscope (Ex/Em = 637/650 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.

2. Protocol Summary



3. Kit Contents

Components	Amount
Component A: Tracking dye Deep Red (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 µL for 96-well plates or 2,500 to 10,000 cells/well/20 µL 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 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.

B. Prepare 10X Tracking dye Deep Red stain solution:

Note: Thaw all the components to room temperature, centrifuge the component A briefly before opening.

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

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

Note 2: The final concentration of the Tracking dye Deep 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 tenfold range.

C. Stain the cells:

1. To the cell wells add 10X Tracking dye Deep Red 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 µL/well of 10X Tracking dye Deep Red working solution into the cells.

2. Incubate the cells in a 37 ^{o}C, 5% CO_2 incubator for 15 minutes to 1 hour.

3. Wash cells with Hanks and 20 mM Hepes buffer (HHBS) or an appropriate buffer.

4. Fill the cell wells with growth medium.

5. Analyze the cells using a fluorescence microscope or flow cytometer with Cy5 filter sets (Ex/Em = 630/650 nm).

6. Data Analysis

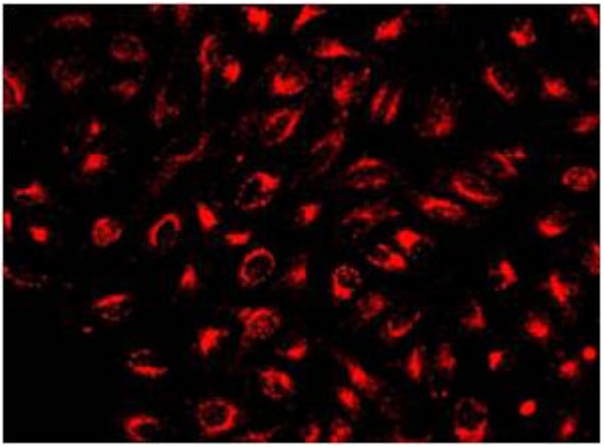


Figure 1. Hela cells stained with 1X CytoPainter Cell Tracking Staining Kit - Deep Red Fluorescence in a Costar black wall/clear bottom 96-well plate.

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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