

ab138890 –

**CytoPainter Cell Tracking
Staining Kit –
Blue Fluorescence**

Instructions for Use

For labelling live cells in blue fluorescence for the 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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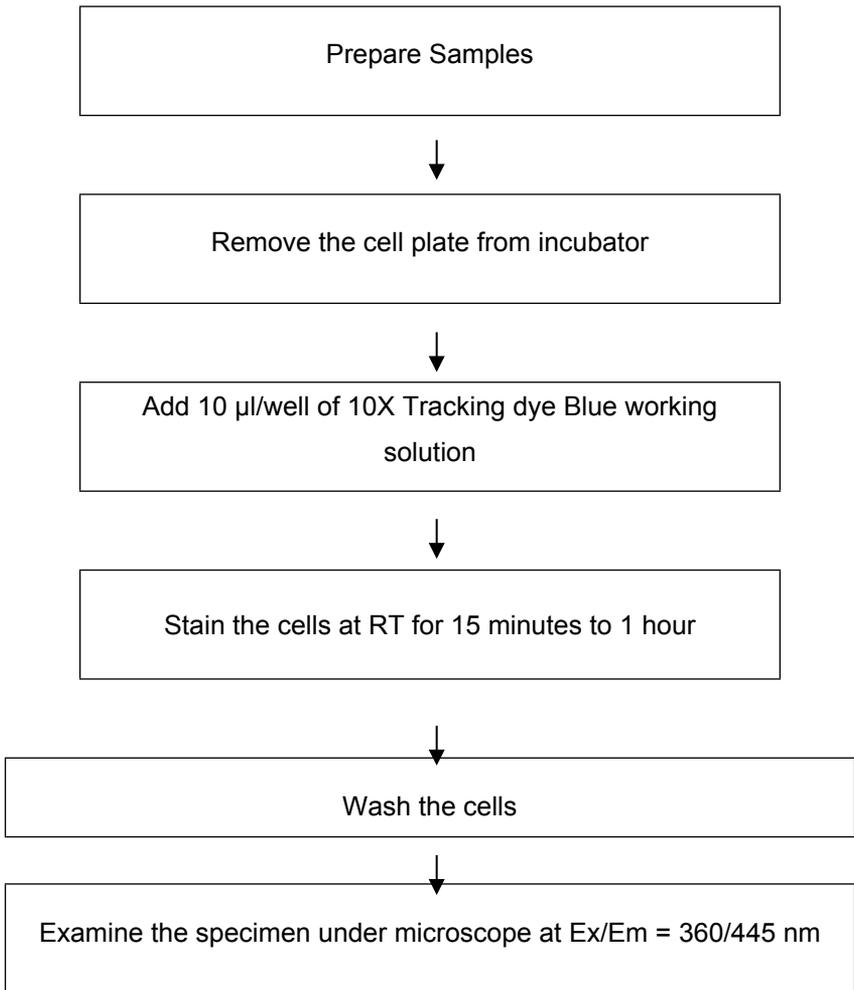
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1. Introduction

Abcam's CytoPainter Cell Tracking Staining Kits are a set of tools used to label cells for fluorescence microscopic investigations of cellular functions. The effective labelling of cells provides a powerful method for studying cellular events in a spatial and temporal context.

ab138890 CytoPainter Cell Tracking Staining Kit – Blue Fluorescence is designed to uniformly label live cells in blue 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 becomes strongly fluorescent upon entering into live cells, and is trapped inside cells to give stable fluorescence signals. The dye is a hydrophobic compound that easily permeates intact live cells. The labelling process is robust, requiring minimal hands-on time. ab138890 can be readily adapted for many different types of fluorescence platforms such as microplate assays, flow cytometry and fluorescence microscope. 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-labelling protocol, and can be used for both proliferating and non-proliferating cells (either suspension or adherent cells).

2. Protocol Summary



3. Kit Contents

Components	Amount
Component A: Tracking dye Blue	2 vials
Component B: DMSO	1 vial (0.2 ml)
Component C: Assay Buffer	1 bottle (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 Tracking dye Blue

1. Prepare 2 mM Tracking dye Blue stock solution: Add 25 μ l of DMSO (Component B) into one of the Tracking dye Blue vials (Component A) to make 2 mM stock solution.

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

2. Prepare 10X Tracking dye Blue working solution: Dilute 2 mM of Tracking dye Blue stock solution into Assay Buffer (Component C) to make 1 to 50 μM Tracking dye Blue 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 Tracking dye Blue at the final concentration of 2 μM for one 96-well microplate, dilute 10 μl of the Tracking dye Blue stock solution into 1 ml of Assay Buffer (Component C) to make 1 ml of 20 μM (10X) Tracking dye Blue working solution.

Note: The final concentration of the Tracking dye Blue 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 Blue 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 Blue working solution into the cells.
2. Incubate the cells in a 37°C, 5% CO₂ incubator for 15 min 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 DAPI filter sets (Ex/Em = 360/445 nm).

6. Data Analysis

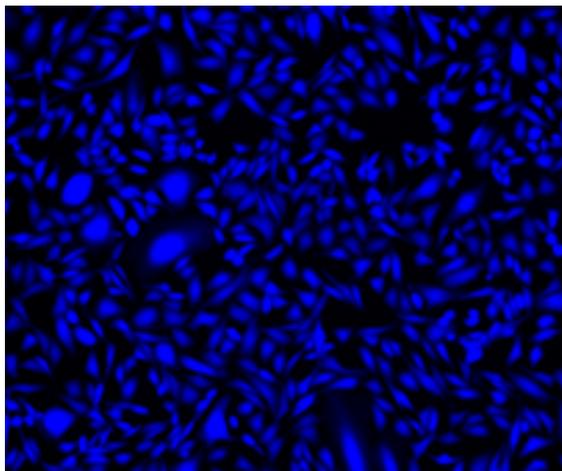


Figure 1. Image of U2OS cells stained with 2 μ M CytoPainter Cell Tracking Staining Kit - Blue Fluorescence in a black wall/clear bottom 96-well plate.

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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