ab187966 –

CytoPainter Live Cell Labeling Kit - Blue Fluorescence

Instructions for Use

For uniformly labeling live cells in blue fluorescence with a dye whose fluorescence is strongly enhanced upon entering into live cells.

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

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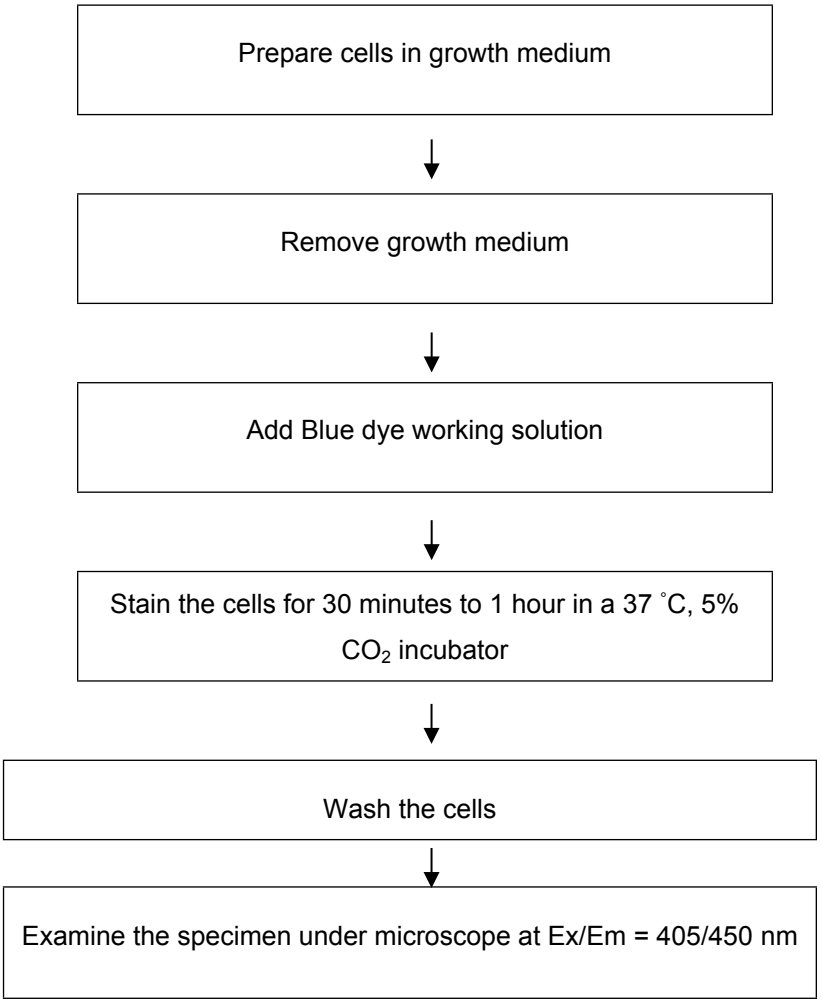
1. Introduction

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

The ab187966 CytoPainter Live Cell Labeling 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 labeling process is robust, requiring minimal hands-on time. ab187966 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-labeling 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: Labeling dye Blue	2 vials
Component B: HHBS (Hanks' buffer with 20 mM Hepes)	1 bottle (100 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/100 µl for 96-well plates or 2,500 to 10,000 cells/well/25 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/100 µl for 96-well poly-D lysine plates or 10,000-25,000 cells/well/25 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 Labeling dye Blue

1. Prepare Labeling dye Blue stock solution: Add 20 µl of DMSO into one of the Labeling dye Blue vials (Component A) to make stock solution.

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

2. Prepare Labeling dye Blue working solution: Dilute 20 µl of reconstituted Labeling Dye Blue stock solution from step 1 into 10 mL HHBS Buffer (Component B) to make a working solution. Mix well.

Note: The final concentration of the Labeling 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. Remove growth medium

2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) Labeling dye Blue working solution (from Step B 2) into the cell plate.

3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 min to 1 hour.

4. Remove the Labeling dye Blue working solution from the cells, and wash the cells with HHBS (Component B) for 2 to 3 times, and replace with HHBS.

5. Analyze the cells using a fluorescence microscope or flow cytometer with filter sets (Ex/Em = 405/4450 nm).

6. Data Analysis

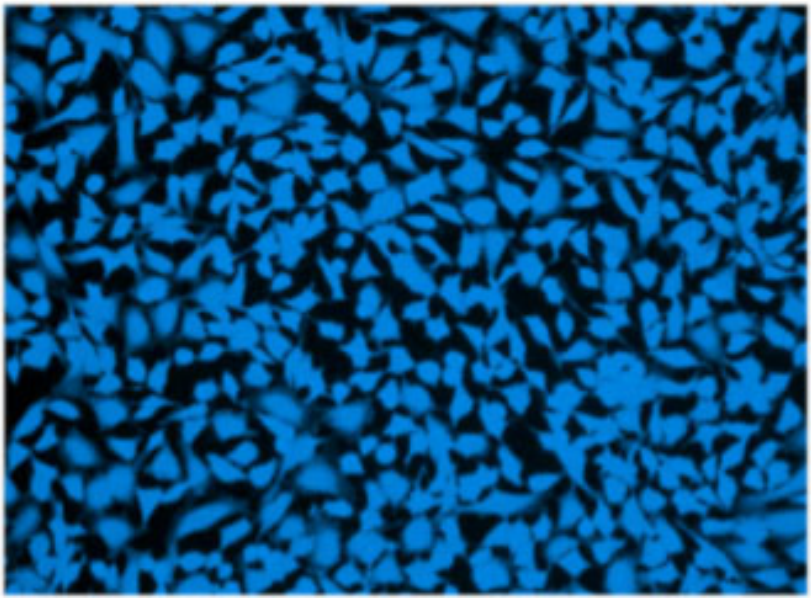


Figure 1. Image of HeLa cells stained with CytoPainter Live Cell Labeling Kit - Blue Fluorescence in a black wall/clear bottom 96-well plate.

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).

UK, EU and ROW
Email: technical@abcam.com
Tel: +44 (0)1223 696000
www.abcam.com

US, Canada and Latin America
Email: us.technical@abcam.com
Tel: 888-77-ABCAM (22226)
www.abcam.com

China and Asia Pacific
Email: hk.technical@abcam.com
Tel: Tel: 400 921 0189 / +86 21 2070 0500
www.abcam.cn

Japan
Email: technical@abcam.co.jp
Tel: +81-(0)3-6231-0940
www.abcam.co.jp