ab187967 –

CytoPainter Live Cell Labeling Kit - Green Fluorescence

Instructions for Use

For uniformly labeling live cells in green 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.

Version: 2 Last Updated: 3 June 2019

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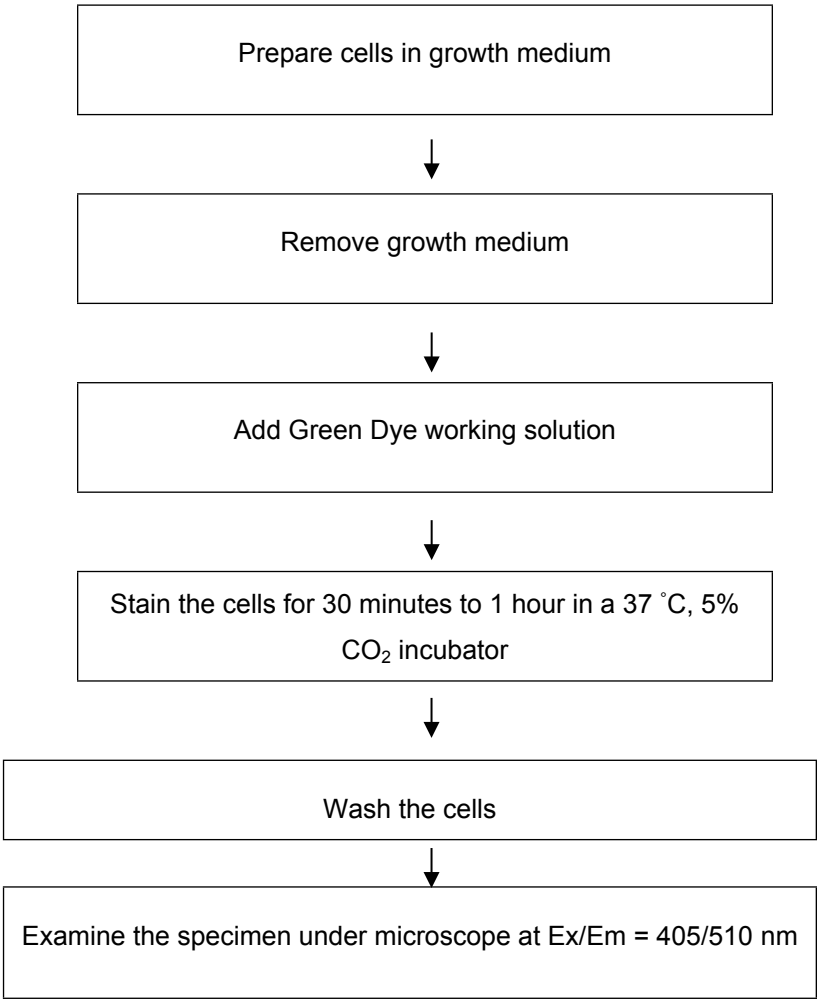
1. Introduction

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 labeling of cells provides a powerful method for studying cellular events in a spatial and temporal context. ab187967 CytoPainter Live Cell Labeling Kit – Green Fluorescence is designed to uniformly label live cells in Green 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. ab187967 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 green	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 Green

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

   Note: The unused portion of the Labeling Dye Green stock solution should be stored at -20˚C protected from light. Avoid repeated freeze/thaw cycles.

2. Prepare Labeling Dye Green working solution: Dilute 20 µl of reconstituted Labeling Dye Green 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 Green 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 Green 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 Green 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 = 405/510 nm).

   Note: Alternatively, cells might be fixed at this point for later image (fluorescent intensity might be decreased upon fixation).

6. Data Analysis

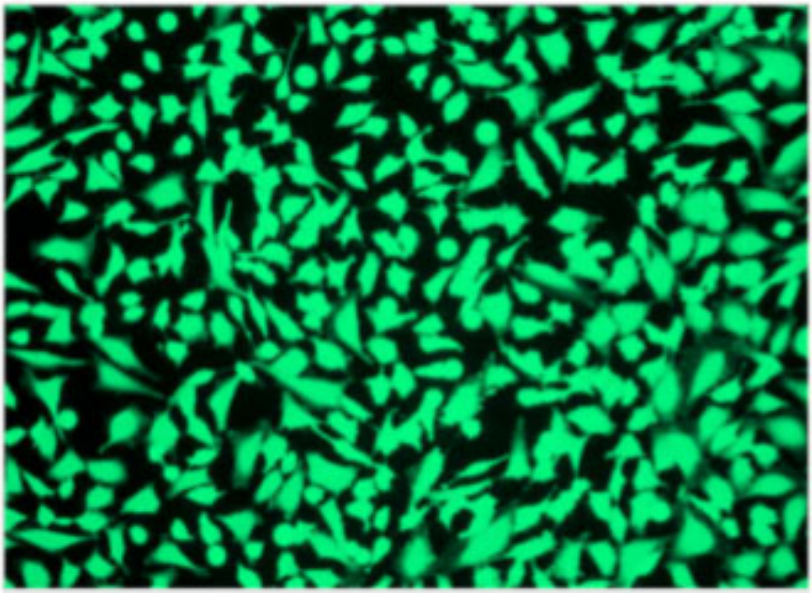


Figure 1. Image of HeLa cells stained with CytoPainter Cell Labeling Staining Kit - Green Fluorescence in a black wall/clear bottom 96-well plate.

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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