ab138891

CytoPainter Cell Tracking Staining Kit – Green Fluorescence

Instructions for Use

For labelling live cells in green 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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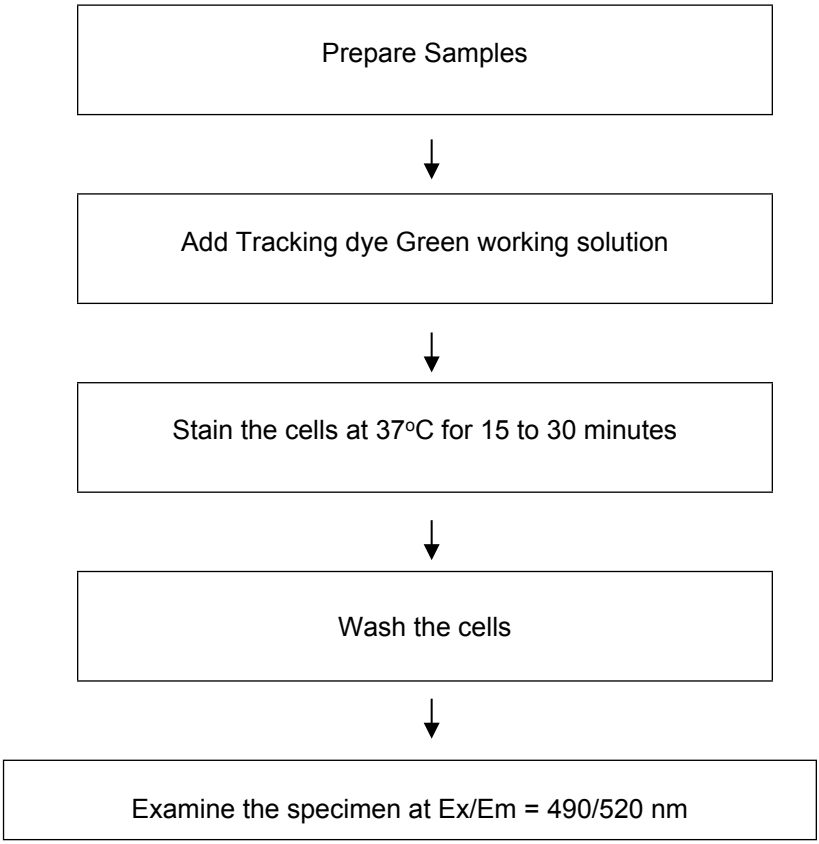
1. Introduction

Abcam's CytoPainter Cell Tracking Staining Kits are a set of tools that provide well-retained cell-tracing reagents to label and track cells for investigations of cellular functions in a wide variety of colors. The effective labelling of cells provides a powerful method for studying cellular events in a spatial and temporal context. This particular kit is designed to label live cells in green fluorescence for the studies that require the fluorescent tag molecules retained inside cells for a relatively longer time. The Tracking dye Green used in the kit is a non-fluorescent dye that carries a cell-retaining moiety. The dye becomes strongly fluorescent upon entering into live cells, and becomes trapped inside cells to give a stable fluorescence signal. The adduct that formed in labelled cells is retained by the cells throughout development and meiosis, and is inherited by daughter cells after cell division.

ab138891 can be readily adapted for many different types of fluorescence platforms such as microplate assays, and flow cytometry analysis. For the long term tracking of cell proliferation or cell division studies, Tracking dye Green has the similar features as traditional cell proliferation reagent CFSE, but eliminates the dye efflux drawback associated with CFSE. The CFSE-stained cells show continued efflux of the dye molecules after 24 hours and the fluorescence intensity of daughter generations reduces by >10 times from first generation. Another advantage of Tracking dye Green is

that it is compatible with cell culture medium in the staining cells prior to imaging or flow cytometric analysis, and those features make this kit more convenient and less hands-on operations than CFSE staining. The green fluorescent indicator used in the kit has Ex/Em = 490/520 nm, compatible with the FITC filter set that is installed with almost every major fluorescence instrument and flow cytometer.

2. Protocol Summary



3. Kit Contents

Components	Amount
Component A: Tracking dye Green	1 vial
Component B: Assay Buffer	1 bottle (20 ml)
Component C: DMSO	1 vial (100 µl)

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 experiments.

   Note 1: For flow cytometry experiment, prepare cells in 0.5 ml warm medium or buffer of your choice at a density of 5×10^5 to 1×10^6 cells/ml.

   Note 2: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

B. Prepare Tracking dye Green Stain Solution

1. Prepare 1000X Tracking dye Green stock solution: Add 100 µl of DMSO (Component C) into the vial of Tracking dye Green (Component A) and mix well.

   Note: The unused portion of 1000X Tracking dye Green stock solution should be stored at -20°C. Avoid repeated freeze/thaw cycles.

2. Prepare Tracking dye Green working solution: Dilute 1000X Tracking dye Green stock solution into Assay Buffer (Component B) at 1:1000 ratio to make Tracking dye Green working solution.

   Note: The final concentration of the Tracking dye Green should be empirically determined for different cell types and/or experimental conditions. In general, long-term staining (more than about 3 days) or the use of rapidly dividing cells will require 1: 500 dilution to double the dye concentration. Dye at a lower concentration up to 1:2000 dilution may be needed for shorter experiments, such as viability assays. To maintain normal cellular physiology and reduce potential artifacts, the concentration of the dye should be kept as low as possible.

C. Stain the cells

1. Add equal volume of Tracking dye Green working solution into the cell wells. For example, for 96-well plate, add 100 µl/well of Tracking dye Green working solution into the cells.

2. Incubate the cells in a 37°C, 5% CO_2 incubator for 15 to 30 minutes.

3. Wash cells with HHBS or an appropriate buffer for 3 times.

   Note: Alternatively, fix the cells at this point. Store the fixed cells at 4 °C, and image the cells later.

4. Image the cells using a fluorescence microscope with FITC filters (Ex/Em = 490/520 nm). Or monitor the fluorescence intensity with a flow cytometer using the FL1 channel (Ex/Em = 490/525 nm), gate on the cells of interest, excluding debris.

6. Data Analysis

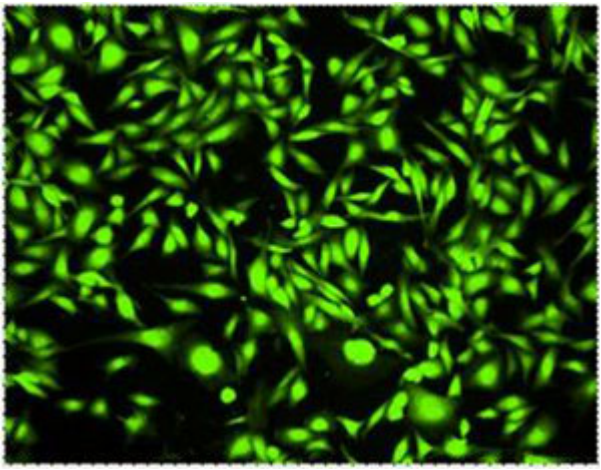


Figure 1. Image of U2OS cells stained with CytoPainter Cell Tracking Staining Kit - Green Fluorescence in a black 96-well plate.

Technical Support

For all technical or commercial enquiries please go to:

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