ab176736

CytoPainter Cell Proliferation Staining Reagent – Red Fluorescence

Instructions for Use

For fluorescent labeling and analysis on heterogeneous cell populations.

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

Version: 5 Last Updated: 31 January 2019

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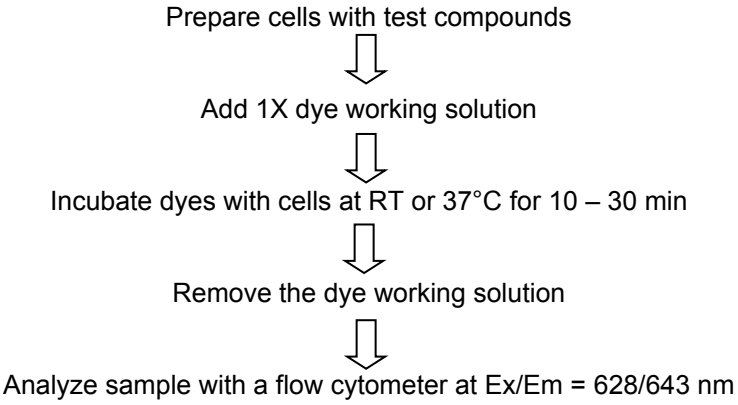
1. Introduction

It is widely recognized that fluorescent labeling of cells is an effective method for detecting the presence of viable cells in a sample. Flow cytometry combined with fluorescent staining is a powerful tool to analyze heterogeneous cell populations. Among all the existing fluorescent dyes, CFSE is frequently used. The non-fluorescent CFSE molecule diffuses into cells and is hydrolyzed by intracellular non-specific esterases to give the highly fluorescent fluorescein product. The fluorescent product is generated and accumulated only in the cells that have intact cell membranes and active esterase activities while dead cells are not stained. The precise kinetics of membrane transport and intracellular hydrolysis of CFSE are related to cellular functions. However, it is impossible to use CFSE and its fluorescein analogs for GFP-transfected cells or for the applications where a FITC-labeled antibody is used since CFSE and analogs have the excitation and emission spectra almost identical to GFP or FITC.

Abcam's CytoPainter Cell Proliferation Staining Reagents – Red Fluorescence (ab176736) is functionally similar to CFSE and can be used for the multicolor applications where either GFP or FITC-labeled antibody is used since the CytoPainter cell tracking staining reagents have either excitation and emission spectra distinct from CFSE and its fluorescein analogs. Moreover, our CytoPainter cell tracking staining reagents not only eliminate the dye efflux

drawback associated with CFSE, but also are compatible with the cell culture medium in the staining cells prior to imaging or flow cytometric analysis.

2. Protocol Summary



3. Materials Supplied

Item	500 tests	1000 tests
Cyto Labeling Red Reagent	1 vial	2 vials

4. Storage and Stability

Upon receipt, store kit at -20°C. Avoid exposure to light.

Lyophilized powder should be stable for at least 6 months if stored at -20°C. Avoid freeze/ thaw cycles.

5. Materials Required, Not Supplied

- DMSO
- HHBS Buffer (Hanks and 20 mM HEPES buffer) pH=7
- Microcentrifuge
- Pipettes and pipette tips
- FACS tubes
- PBS

6. Assay Protocol

1. Reagent Preparation:

a) 500X dye working solution

Add 500 µL DMSO into the dye powder vial of Cyto Labeling Red Reagent, mix it well by vortexing to have a 500X DMSO stock solution.

NOTE: The stock solution should be used promptly. Any remaining solution should be aliquoted and frozen at -20°C. Avoid repeated freeze/thaw cycles, and protect from light.

b) 1X dye working solution:

Prepare enough 1X dye working solution for your experiment by diluting the 500X DMSO stock solution at 1/500 in HHBS buffer, pH=7. Example: 1 µL 500X DMSO stock solution in 500 µL buffer.

2. Sample Analysis:

a) If needed for your experiment, treat cells with test compounds for a desired period of time.

b) Harvest cells and add 10^5 – 5 x10^5 cells/tube.

Adherent cells: scrap cells in cold PBS or trypsinize briefly to collect cells. Centrifuge cells at 1000 rpm for 5 minutes and aspirate supernatant.

Suspension cells: simply collect cells and centrifuge at 1000 rpm for 5 minutes and aspirate supernatant.

c) Resuspend cells in 500 µL of the dye solution (Step 1b).

NOTE: you can add the 500X DMSO stock solution into the cells directly without removing cell media. Example: add 1 µL 500X DMSO stock solution into 500 µL cells.

d) Incubate cells with the dye solution at room temperature or 37°C for 10 – 30 minutes, protected from light.

e) Remove the dye working solution from the cells, wash the cells with HHBS. Resuspend cells in 500 µL of pre-warmed HHBS in order to get 10^5 – 5 x 10^5 cells/tube.

f) Monitor the fluorescence change at Ex/Em = 628/643 nm with a flow cytometer or a fluorescence microscope fitted with a red laser (Ex=633 nm).

7. Data Analysis

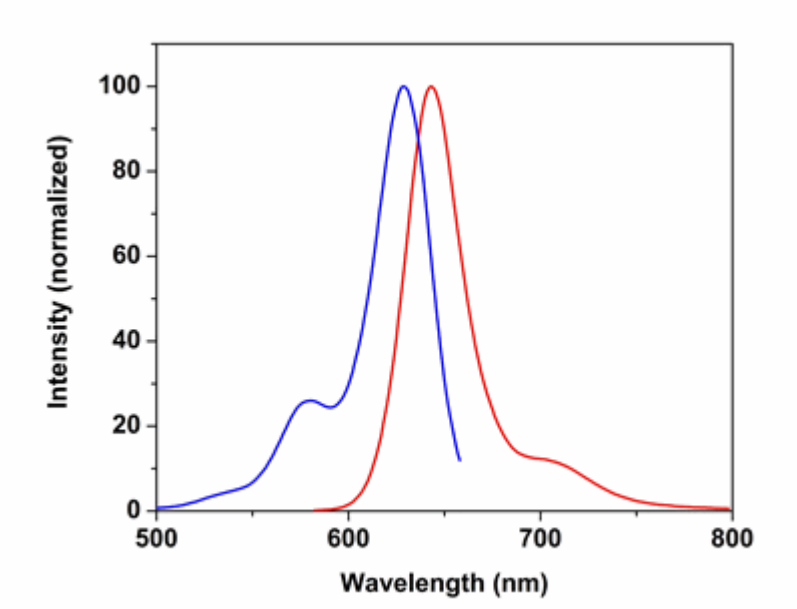


Figure 1. Emission and Excitation spectra for CytoPainter Cell Proliferation Staining Reagents – Red Fluorescence (ab176736).

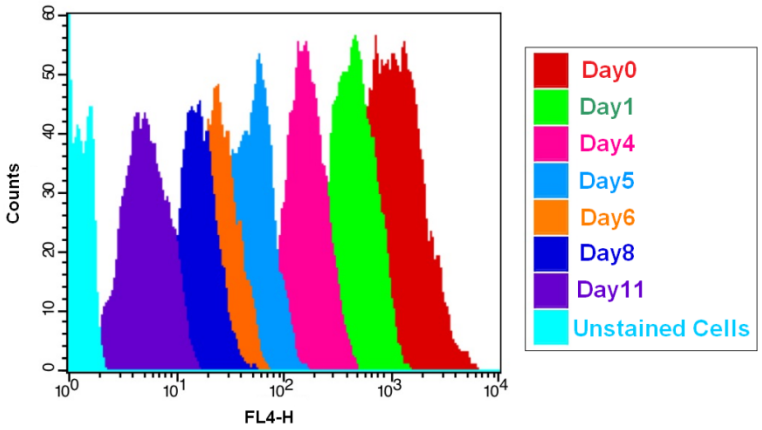


Figure 2. Cell tracking assay with CytoPainter Cell Proliferation Staining Reagents – Red Fluorescence (ab176736). Jurkat cells ($2x10^6$ cells/mL) were stained with ab176736 on Day0. The cells were passed serially at 1:1 ratio for 11 days. Fluorescence intensity was measured with a FACS flow cytometer in FL1 channel on the day after passage. Successive generations were represented by different colors.

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