ab176742

CytoPainter Fixable Cell Viability Assay Kit (Fluorometric - Green)

Instructions for Use

For evaluation of the viability of mammalian cells by flow cytometry.

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

Version: 1 Last Updated: 6 February 2019

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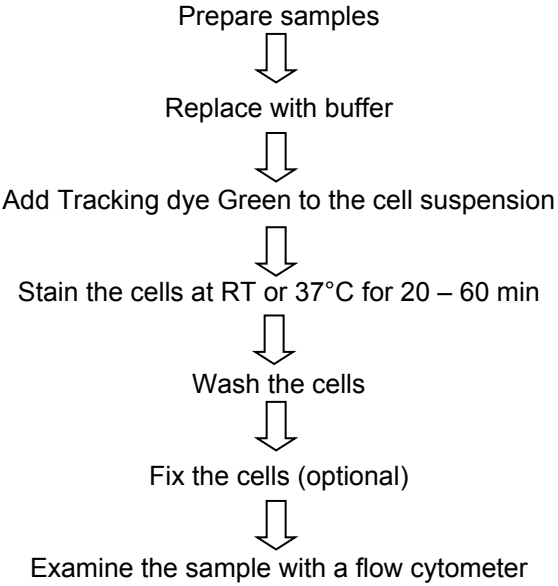
1. Introduction

Abcam's CytoPainter Fixable Cell Viability Assay Kit (Fluorometric - Green) (ab176742) is used to evaluate the viability of mammalian cells by flow cytometry. The fluorescent dye provided in the kit is retained in cells by reacting with cellular components. For viable cells, only the cell-surface amines are available to react with the dye while for the necrotic cells or the other cells with compromised membranes, the reactive dye reacts with cell surface amines and intracellular amines, resulting in more intense fluorescent staining. The difference in fluorescence intensity between the live and dead cell populations is ~100-500 folds and can be completely preserved after fixation. The approximate fluorescence excitation is 498 nm and emission maximum is 521 nm. The Excitation source is 488 nm.

2. Protocol Summary



3. Materials Supplied

Item	200 tests
Tracking dye Green	1 vial
DMSO	1 vial (200 µL)

4. Storage and Stability

Upon receipt, store kit at -20°C. Avoid exposure to light and moisture (store desiccated).

5. Materials Required, Not Supplied

- Sodium azide and serum/protein free buffer such as HHBS Buffer (1X Hanks and 20 mM HEPES buffer)
- CO_2 incubator
- Pipettes and pipette tips
- FACS tubes

6. Assay Protocol

1. Reagent Preparation:

a) 500X DMSO stock solution

Add 200 µL DMSO to the vial of Tracking dye Green, mix it well by vortexing to have a 500X DMSO stock solution.

NOTE: The unused stock solution should be aliquoted and stored at -20°C. Avoid repeated freeze/thaw cycles.

2. Sample Analysis:

a) Prepare cells for flow cytometry using 1X Hanks and 20 mM HEPES buffer (HHBS) or a sodium azide- and serum/protein free buffer of your choice.

b) Wash cells once with HHBS or sodium azide- and serum/protein free buffer of your choice.

c) Resuspend cells at 5-10 x 10^6 /mL in HHBS or sodium azide- and serum/protein free buffer of your choice.

d) Add 1 µL of Tracking dye Green stock solution (see Reagent Preparation) to 0.5 mL of cells /assay and mix it well.

e) Incubate for 20-60 min at room temperature or 37°C, 5% CO_2 incubator, protected from light.

NOTE: The optimal stain concentrations and incubation time should be experimentally determined for different cell lines.

f) Wash cells twice and resuspend cells in with HHBS or buffer of your choice.

g) Fix cells as desired (optional).

h) Analyze cells with a flow cytometer.

7. Data Analysis

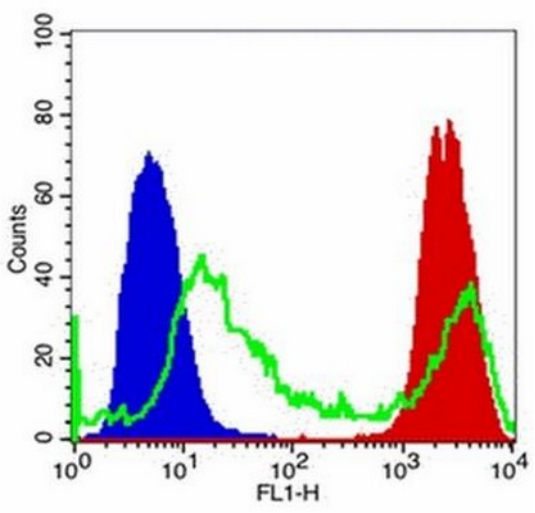


Figure 1. Detection of Jurkat cell viability using Abcam's CytoPainter Fixable Cell Viability Assay Kit (Fluorometric - Green) (ab176742). Jurkat cells were treated and stained with Tracking Dye Green. The cells were fixed in 3.7% formaldehyde and analyzed by flow cytometry. Live (Blue solid peak), staurosporine treated (green line) and heat-treated (red solid peak) cells were distinguished with Ex/Em = 488 nm /520 nm (FL1). The live cell population is easily distinguished from the dead cell population, and nearly identical results were obtained using unfixed cells.

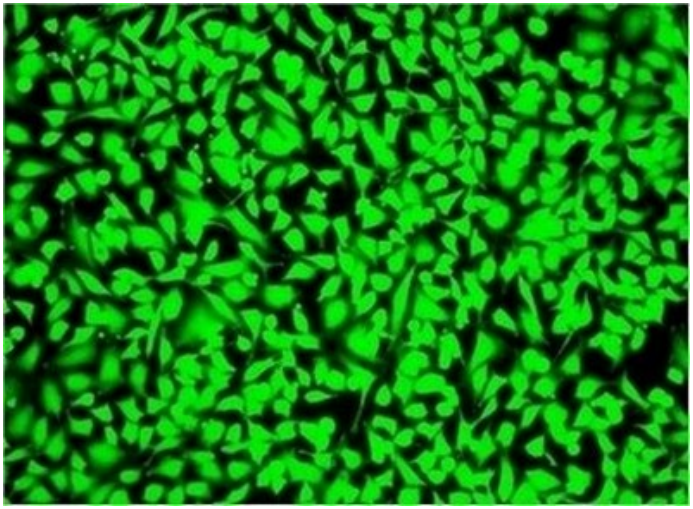


Figure 2. Fluorescent imaging of HeLa cells using ab176742. HeLa cells were treated and stained with Tracking Dye Green. The cells were fixed in 3.7% formaldehyde and analyzed by fluorescence microscopy.

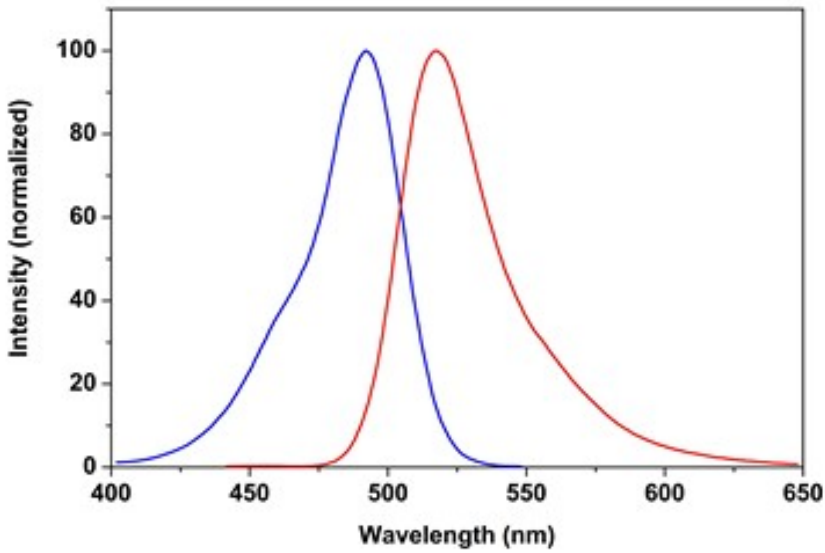


Figure 3. Excitation and Emission Spectra for CytoPainter Fixable Cell Viability Assay Kit (Fluorometric - Green) (ab176742)

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