# ab176738 - CytoPainter Fixable Cell Viability Assay Kit (Fluorometric - Blue Ex 405 nm)

Instructions for Use

For evaluation of the viability of mammalian cells by fluorescent labeling and flow cytometry.

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

Version: 1 Last Updated: 8 February 2019

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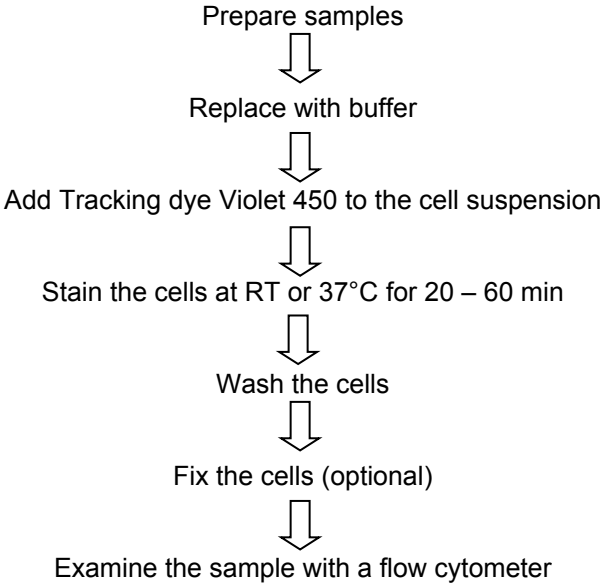
# 1. Introduction

Abcam's CytoPainter Fixable Cell Viability Assay Kit (Fluorometric – Blue Ex 405 nm) (ab176738) 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 fold and can be completely preserved after fixation. The approximate fluorescence excitation is 410 nm and emission maximum is 450 nm. The Excitation source is 405 nm.

## 2. Protocol Summary




## 3. Materials Supplied

| Item | 200 tests |
|---|---|
| Tracking dye Violet 450 | 1 vial |
| DMSO | 1 x 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-free 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 Violet 450.

*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-free and serum/protein-free buffer of your choice.

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

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

**d)** Add 1 µL of Tracking dye Violet 450 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

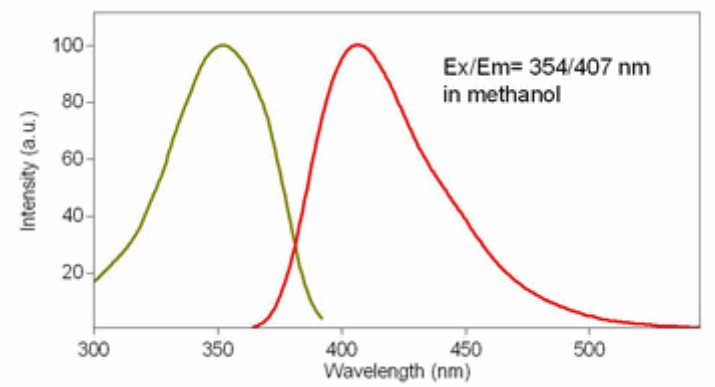



**Figure 1.** Spectrum for ab176738.

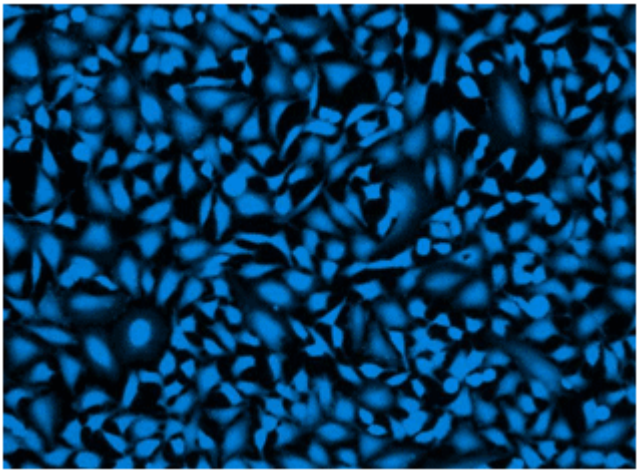


**Figure 2.** Fluorescent imaging of HeLa cells fixed with formaldehyde and labeled with ab176738 in a black wall/ clear bottom 96 well plate.

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