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ab176741

Fixable Cell Viability Assay Kit (Blue Fluorescence, for 335 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: 3 Last Updated: 12 February 2026

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1. Introduction

Abcam's Fixable Cell Viability Assay Kit (Blue Fluorescence, for 335 nm excitation) (ab176741) 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 353 nm and emission maximum is 442 nm. The Excitation source is 335 nm.

2. Protocol Summary

Prepare samples

Replace with HH buffer

Add Tracking dye Blue to the cell suspension

Stain the cells at RT or 37°C for 20 – 60 min

Wash the cells

Fix the cells (optional)

Examine the sample with a flow cytometer

3. Materials Supplied

Item	200 tests
Tracking dye Blue	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 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 into the vial of Tracking dye Blue, mix it well by vortexing to have a 500X DMSO stock solution.

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

2. **Sample Analysis:**

   a) Prepare cells for flow cytometry staining 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 Blue 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

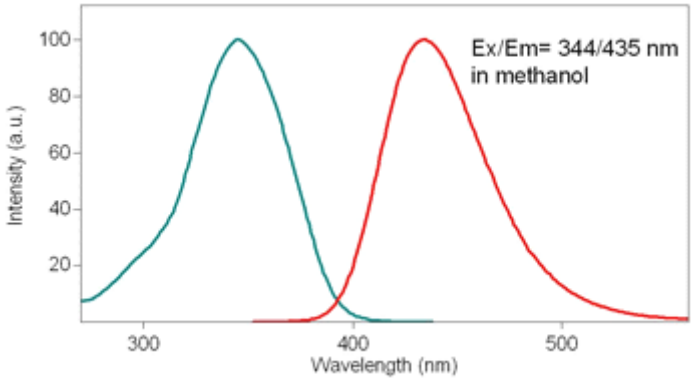

Figure 1. Spectrum for ab176741.

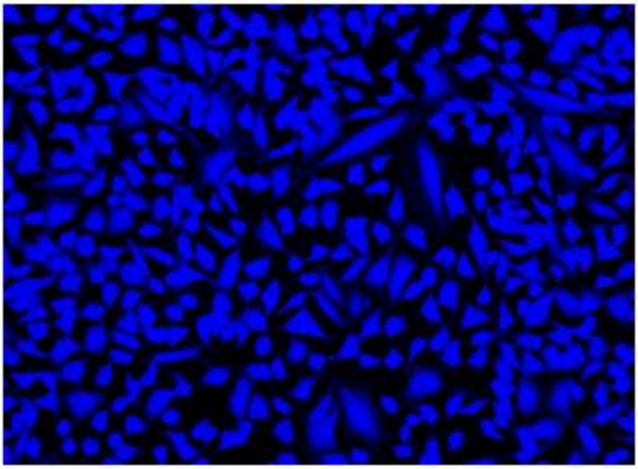

Figure 2. Fluorescent imaging of HeLa cells fixed with formaldehyde and labeled with Abcam's CytoPainter Fixable Cell Viability Assay Kit (Fluorometric - Blue) in a black wall/ clear bottom 96 well plate.

Technical Support

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