

**ab187966 –**

**Live Cell Staining Kit -  
Blue Fluorescence  
(Ex/Em= 405/450 nm)**

**Instructions for Use**

For uniformly labeling live cells in blue fluorescence with a dye whose fluorescence is strongly enhanced upon entering into live cells.

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



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# 1. Introduction

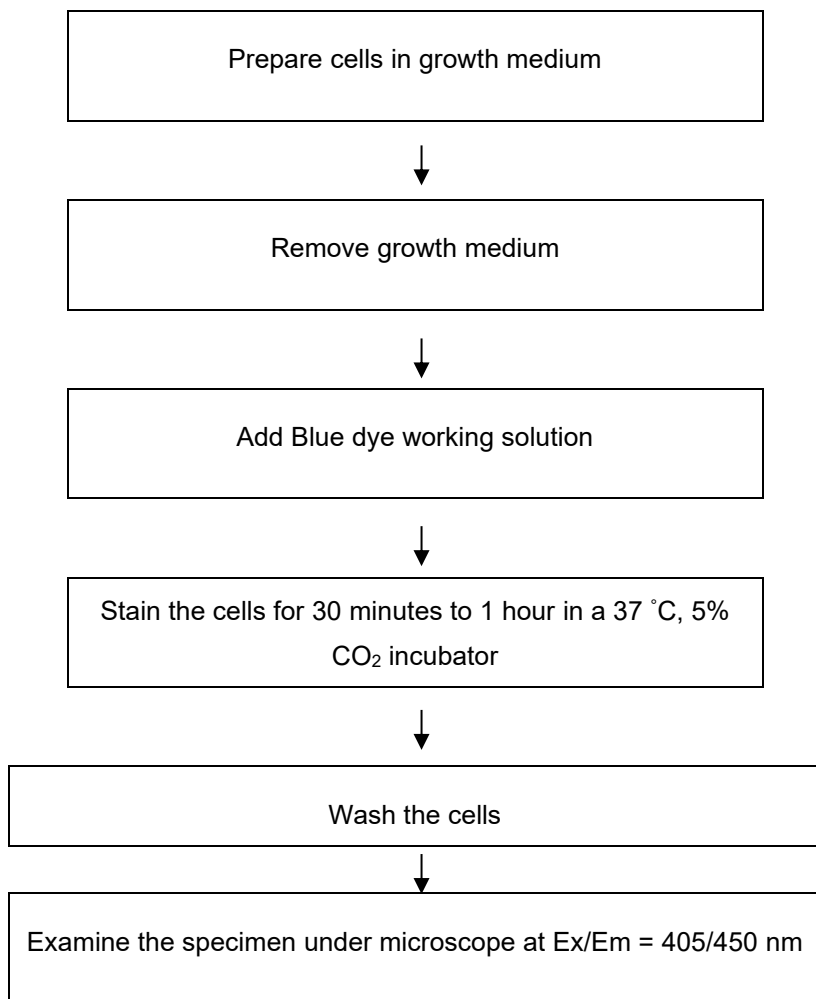
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Abcam's Live Cell Staining Kits are a set of tools used to label cells for fluorescence microscopic investigations of cellular functions. The effective labeling of cells provides a powerful method for studying cellular events in a spatial and temporal context.

The ab187966 Live Cell Staining Kit - Blue Fluorescence (Ex/Em= 405/450 nm) is designed to uniformly label live cells in blue fluorescence for the studies that require the fluorescent tag molecules retained inside cells for a relatively longer time. The kit uses a non-fluorescent dye that carries a cell-retaining moiety. The dye becomes strongly fluorescent upon entering into live cells, and is trapped inside cells to give stable fluorescence signals. The dye is a hydrophobic compound that easily permeates intact live cells. The labeling process is robust, requiring minimal hands-on time. ab187966 can be readily adapted for many different types of fluorescence platforms such as microplate assays, flow cytometry and fluorescence microscope. It is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. The kit provides all the essential components with an optimized cell-labeling protocol, and can be used for both proliferating and non-proliferating cells (either suspension or adherent cells).

## 2. Protocol Summary

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### 3. Kit Contents

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<b>Components</b>	<b>Amount</b>
Component A: Labeling dye Blue	2 vials
Component B: HHBS (Hanks' buffer with 20 mM Hepes)	1 bottle (100 ml)

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### 4. Storage and Handling

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Keep at -20°C. Protect from moisture and light.

## 5. Assay Protocol

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### A. Prepare Cells

1. For adherent cells: Plate cells overnight in growth medium at 10,000 to 40,000 cells/well/100  $\mu$ l for 96-well plates or 2,500 to 10,000 cells/well/25  $\mu$ l 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/100  $\mu$ l for 96-well poly-D lysine plates or 10,000-25,000 cells/well/25  $\mu$ l for 384-well poly-D lysine plates. Centrifuge the plates at 800 rpm for 2 minutes with brake off prior to the experiment.

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

### B. Prepare Labeling dye Blue

1. Prepare Labeling dye Blue stock solution: Add 20  $\mu$ l of DMSO into one of the Labeling dye Blue vials (Component A) to make stock solution.

*Note: The unused portion of the Labeling dye Blue stock solution should be stored at -20°C. Avoid repeated freeze/thaw cycles.*

2. Prepare Labeling dye Blue working solution: Dilute 20  $\mu$ l of reconstituted Labeling Dye Blue stock solution from step 1 into 10 mL HHBS Buffer (Component B) to make a working solution. Mix well.

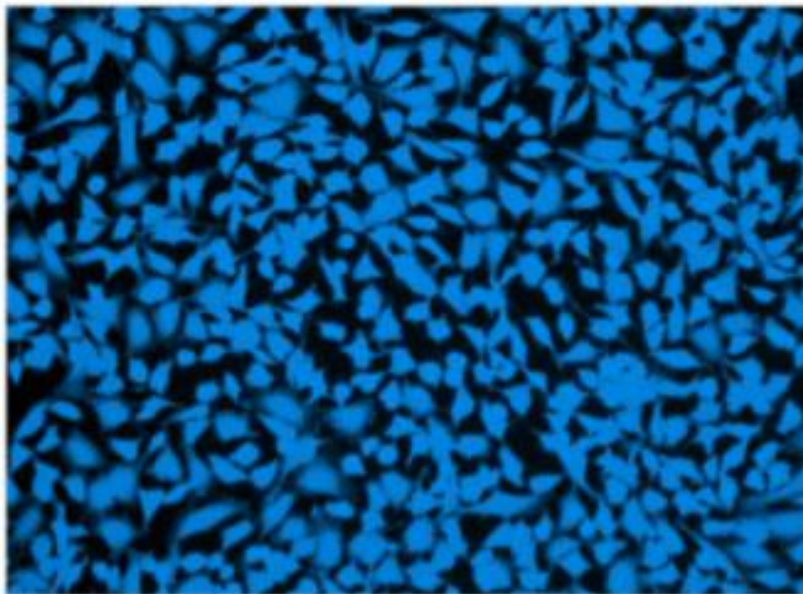
*Note: The final concentration of the Labeling dye Blue should be empirically determined for different cell types and/or experimental conditions. It is recommended to test at the concentrations that are at least over a ten-fold range.*

### **C. Stain the cells**

1. Remove growth medium
2. Add 100  $\mu\text{L}$ /well (96-well plate) or 25  $\mu\text{L}$ /well (384-well plate) Labeling dye Blue working solution (from Step B 2) into the cell plate.
3. Incubate the cells in a 37°C, 5% CO<sub>2</sub> incubator for 30 min to 1 hour.
4. Remove the Labeling dye Blue working solution from the cells, and wash the cells with HHBS (Component B) for 2 to 3 times, and replace with HHBS.
5. Analyze the cells using a fluorescence microscope or flow cytometer with filter sets (Ex/Em = 405/4450 nm).

## 6. Data Analysis

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**Figure 1.** Image of HeLa cells stained with Live Cell Staining Kit - Blue Fluorescence (Ex/Em= 405/450 nm) in a black wall/clear bottom 96-well plate.

**For further technical questions please do not hesitate to contact us by email ([technical@abcam.com](mailto:technical@abcam.com)) or phone (select “*contact us*” on [www.abcam.com](http://www.abcam.com) for the phone number for your region).**

## **Technical Support**

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