

**ab187963 –**

**Live Cell Staining Kit -  
Blue Fluorescence  
(Ex/Em = 360/445 nm)**

**Instructions for Use**

For labelling live cells in blue fluorescence for the studies that require the fluorescent tag molecules retained inside cells for a relatively longer time.

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



# Table of Contents

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1. Introduction	3
2. Protocol Summary	4
3. Kit Contents	5
4. Storage and Handling	5
5. Assay Protocol	6
6. Data Analysis	8

# 1. Introduction

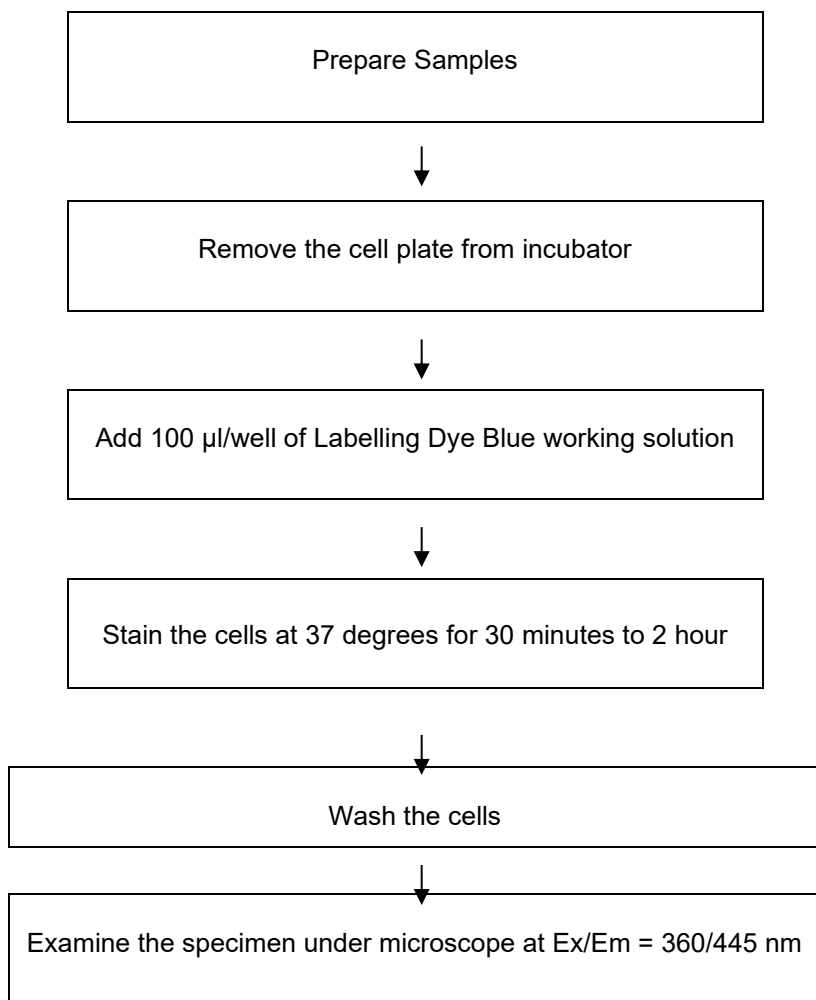
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Abcam's Live Cell Staining Kit are a set of tools used to label cells for fluorescence microscopic investigations and flow cytometric investigations of cellular functions. The effective labelling of cells provides a powerful method for studying cellular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

Live Cell Staining Kit - Blue Fluorescence (Ex/Em = 360/445 nm) (ab187963) is designed to uniformly label live cells in blue fluorescence with a proprietary dye whose fluorescence is strongly enhanced upon entering into live cells. The dye is a hydrophobic compound that easily permeates intact live cells. The hydrolysis of the weakly fluorescent substrate by intracellular esterases generates a strongly fluorescent hydrophilic product that is well-retained in the cell cytoplasm. Cells grown on black wall/clear bottom plates or slides can be stained and quantified in less than two hours. This Live Cell Staining Kit - Blue Fluorescence (Ex/Em = 360/445 nm) (ab187963) can be readily adapted for a wide variety 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 and can be used for both suspension and adherent cells.

## 2. Protocol Summary

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### 3. Kit Contents

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

### 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 Labelling Dye Blue

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

*Note: The unused portion of the Labelling Dye Blue stock solution should be stored at -20°C. Avoid repeated freeze/thaw cycles and protect from light.*

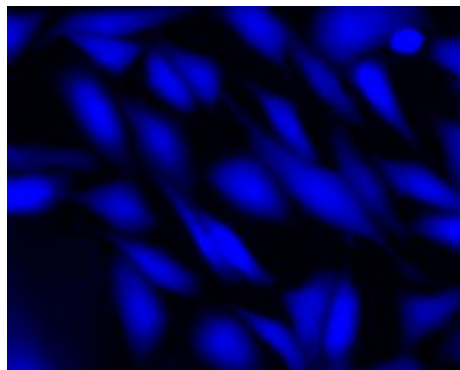
2. Prepare Labelling Dye Blue working solution for one cell plate:  
Add 10  $\mu$ L of DMSO reconstituted Labelling Dye Blue stock solution (from Step B.1) into 10 mL of HHBS (Component B), and mix them well.

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

1. Remove the growth medium from the cell plates.
2. Add 100  $\mu$ l/well (96-well plate) or 25  $\mu$ l/well (384-well plate) Labelling 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 2 hour.
4. Wash cells with Hanks and 20 mM Hepes buffer (HHBS) or an appropriate buffer.
5. Fill the cell wells with growth medium.
6. Analyze the cells using a fluorescence microscope or flow cytometer with DAPI filter sets (Ex/Em = 360/445 nm).

## 6. Data Analysis

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**Figure 1.** Image of CPA cells stained with Live Cell Staining Kit - Blue Fluorescence (Ex/Em = 360/445 nm) (ab187963) in a black wall/clear bottom 96-well plate.



## **Technical Support**

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