

**ab187964 –**

**Live Cell Staining Kit -  
Green Fluorescence  
(Ex/Em = 490/ 525 nm)**

**Instructions for Use**

For labelling live cells in green 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.

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# 1. Introduction

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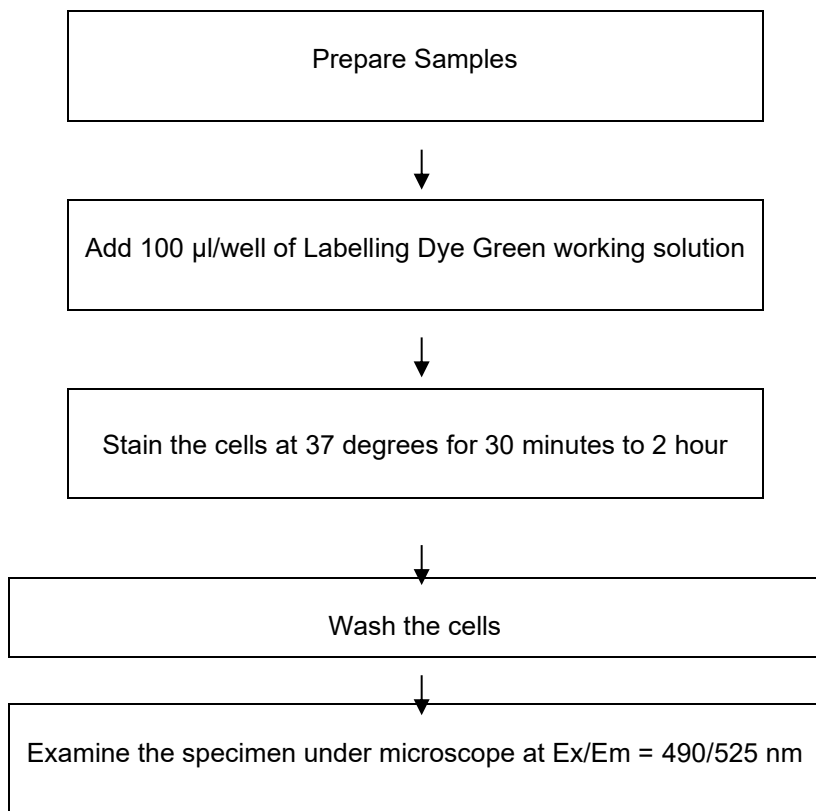
Abcam's Live Cell Staining Kits are a set of tools used to label cells for fluorescence microscopic investigations and flow cytometric investigations of cellular functions. The effective labelling of cells provides a powerful method for studying cellular events in a spatial and temporal context.

Live Cell Staining Kit - Green Fluorescence (Ex/Em = 490/ 525 nm) (ab187964) is designed to uniformly label live cells in green 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 - Green Fluorescence (Ex/Em = 490/ 525 nm) (ab187964) 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. Live Cell Staining Kit - Green Fluorescence (Ex/Em = 490/ 525 nm) (ab187964) provides all the essential components with an optimized cell-labeling protocol

(Ex/Em=490/525), 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: Labelling Dye Green	2 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 Green

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

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

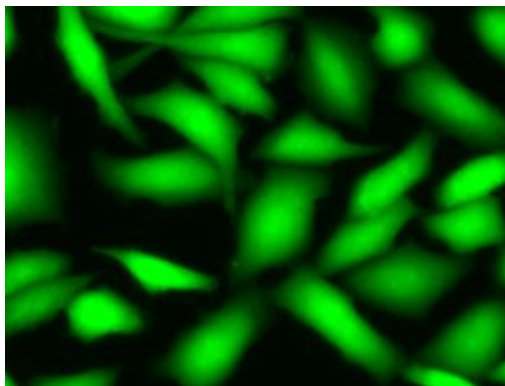
2. Prepare Labelling Dye Green working solution for one cell plate: Add 20  $\mu\text{L}$  of DMSO reconstituted Labelling Dye Green stock solution (from Step B.1) into 10 mL of HHBS (Component B), and mix them well.

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

1. Remove the growth medium from the cell plates.
2. Add 100  $\mu\text{l}$ /well (96-well plate) or 25  $\mu\text{l}$ /well (384-well plate) Labelling Dye Green 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 FITC filter sets (Ex/Em = 490/525 nm).

## 6. Data Analysis

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**Figure 1.** Image of CPA cells stained with Live Cell Staining Kit - Green Fluorescence (Ex/Em = 490/ 525 nm) (ab187964) 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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