

**ab187965 –**

**Live Cell Staining Kit -  
Red Fluorescence  
(Ex/Em = 646/ 660 nm)**

**Instructions for Use**

For labelling live cells in Red 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

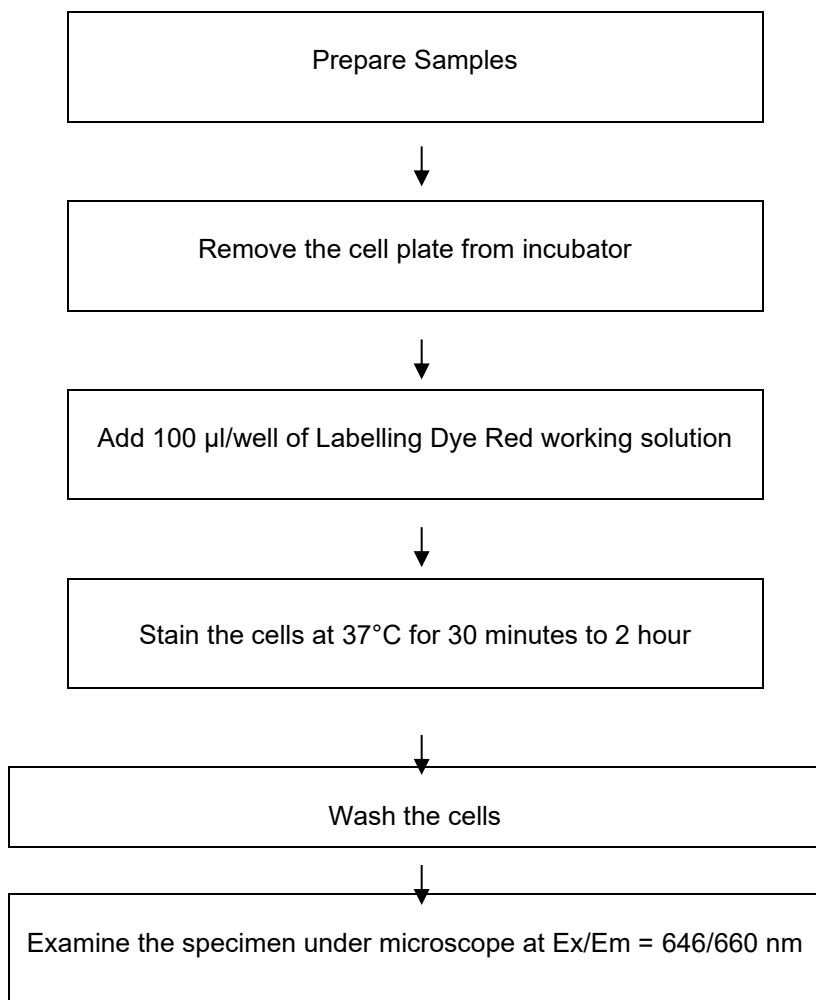
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Abcam's Live Cell Staining Kits are a set of tools used to label cells for fluorescence microscopic investigations and flow cytometric investigations of cellular functions. The effective labelling of cells provides a powerful method for studying cellular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

Live Cell Staining Kit - Red Fluorescence (Ex/Em = 646/ 660 nm) (ab187965) is designed to uniformly label live cells cells in red fluorescence with a proprietary non-fluorescent dye that becomes strongly fluorescence upon entering into live cells. The dye is a hydrophobic compound that easily permeates intact live cells. The hydrolysis of the non-fluorescent substrate by intracellular esterases generates a strongly red fluorescent hydrophilic product that is well-retained in the cell cytoplasm. Cells grown on slides or black wall/clear bottom plates can be stained and quantified in less than two hours. The assay is more robust than the tetrazolium salt or Alamar Blue™-based ones. It can be readily adapted for many different types of fluorescence platforms such as microplate assays, flow cytometry and fluorescence microscope with Ex/Em = 640/660 nm. And it is useful in a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. Live Cell Staining Kit - Red Fluorescence (Ex/Em = 646/ 660 nm) (ab187965) provides all the essential components and can be used for both proliferating and non-proliferating 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 Red	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 Red

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

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

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

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

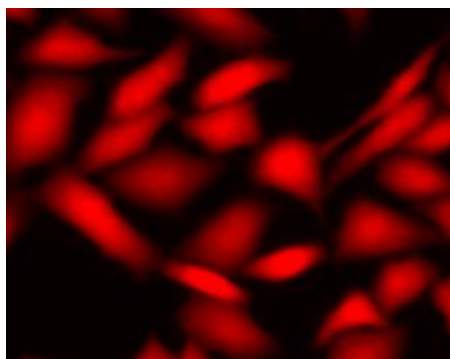
1. Remove the growth medium from the cell plates.

*Note: It is important to remove the growth medium in order to minimize the background fluorescence and increase the signal to background ratio.*

2. Add 100  $\mu\text{l}$ /well (96-well plate) or 25  $\mu\text{l}$ /well (384-well plate) Labelling Dye Red 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 HHBS (Component B) for 2 to 3 times.
5. Fill the cell wells with HHBS.
6. Analyze the cells using a fluorescence microscope or flow cytometer with FITC filter sets (Ex/Em = 646/660 nm).

## 6. Data Analysis

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**Figure 1.** Image of CPA cells stained with Live Cell Staining Kit - Red Fluorescence (Ex/Em = 646/ 660 nm) (ab187965) in a black wall/clear bottom 96-well plate.



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