ab112145

CytoPainter Mitochondrial Staining Kit - Red Fluorescence

Instructions for Use

For staining mitochondria in live cells using our proprietary red fluorescence probe

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

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1. Introduction

Mitochondria are membrane-enclosed organelles found in most eukaryotic cells. Mitochondria are sometimes described as “cellular power plants” because they generate most of the cellular supply of ATP. In addition to supplying cellular energy, mitochondria are involved in a range of other processes, such as signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth. Mitochondria have been implicated in several human diseases, including mitochondrial disorders and cardiac dysfunction, and may play a role in the aging process. Although most cellular DNA is contained in the cell nucleus, the mitochondrion has its own independent genome.

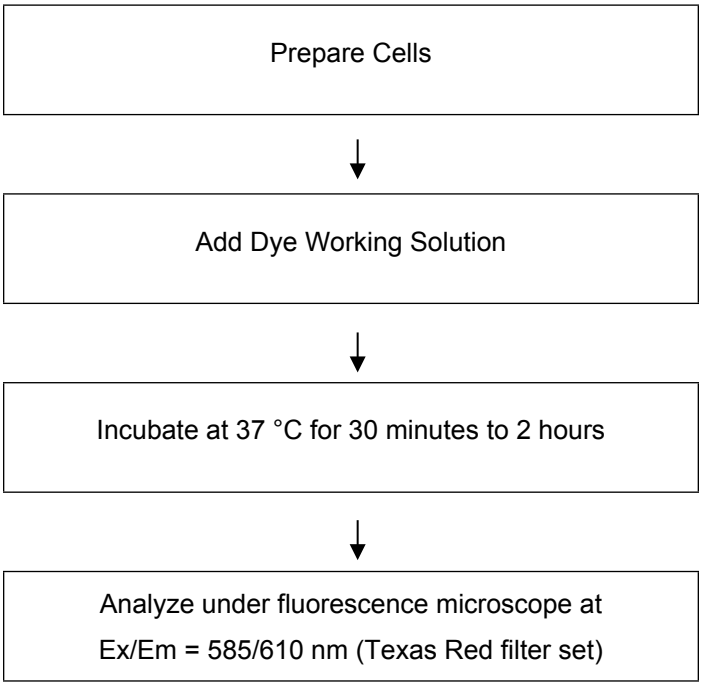
Abcam fluorescence imaging kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as membranes, lysosomes, mitochondria, nuclei, etc. The selective labeling of live cell compartments provides a powerful method for studying cellular events in a spatial and temporal context.

ab112145 is designed to label mitochondria in live cells with red fluorescence. The kit uses our proprietary dye that selectively accumulates in mitochondria probably via the mitochondrial membrane potential gradient. The red fluorescent mitochondrial stain used in the kit has Ex/Em = 580/600 nm. The mitochondrial indicator, a hydrophobic compound, easily permeates intact live cells

and becomes trapped in mitochondria after it gets into cells. This fluorescent mitochondrial indicator is retained in mitochondria for a long time since it carries a cell-retaining group. This key feature significantly increases the staining efficiency.

ab112145 can be readily adapted for many different types of fluorescence platforms, such as microplate assays, immunocytochemistry and flow cytometry. 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 proliferating and non-proliferating cells.

2. Protocol Summary



3. Kit Contents

Components	Amount
Component A: MitoRed Indicator (500X DMSO Stock)	100 µL
Component B: Live Cell Staining Buffer	50 mL

4. Storage and Handling

Keep at -20°C. Protect from light.

5. Assay Protocol

A. Prepare Mitochondrial Staining Solution

1. Warm all the components to room temperature.

2. Prepare dye working solution by diluting 20 µL of MitoRed Indicator (Component A) into 10 mL of Live Cell Staining Buffer (Component B).

Note 1: 20 µL of 500X MitoRed Indicator (Component A) is enough for one 96-well plate. Aliquot and store unused 500X MitoRed Indicator at < -20 °C. Protect from light and avoid repeated freeze-thaw cycles.

Note 2: The optimal concentration of the fluorescent mitochondrial indicator varies depending on the specific application. The staining conditions may be modified according to the particular cell type and the permeability of the cells or tissues to the probe.

B. Prepare serial dilutions of Aldehyde Standard

1. For adherent cells: Grow cells either in a 96-well black wall/clear bottom plate or on cover-slips inside a petri dish filled with the appropriate culture medium. When cells reach the desired confluence, add equal volume

(e.g. 100 µL for a 96-well plate and 25 µL for a 384-well plate) of the dye-working solution (from Step A2). Incubate the cells in a 37 °C, 5% CO_2 incubator for 30 minutes to 2 hours. Replace the dye-loading solution with Hanks and 20 mM Hepes buffer (HH buffer) or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration). Observe the cells by using a fluorescence microscope Ex/Em = 585/610 nm.

Note: It is recommended to increase either the labeling concentration or the incubation time to allow the dye to accumulate if the cells do not appear to be sufficiently stained.

2. For suspension cells: Centrifuge the cells at 1000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant. Resuspend the cell pellets gently in pre-warmed (37 °C) growth medium, and add equal volume of the dye-working solution (from Step A.2). Incubate the cells in a 37 °C, 5% CO_2 incubator for 30 minutes to 2 hours. Replace the dye-loading solution with Hanks and 20 mM Hepes buffer (HH buffer) or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration). Observe the cells using a fluorescence microscope fitted with a Texas Red filter set.

Note 1: It is recommended to increase either the labeling concentration or the incubation time to allow the dye to accumulate if the cells do not appear to be sufficiently stained.

Note 2: Suspension cells may be attached to cover-slips and stained as adherent cells (see Step B.1).

6. Data Analysis

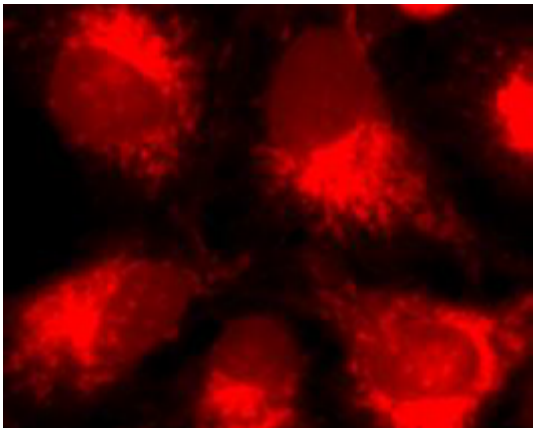


Figure 1. Image of U2OS cells stained with ab112145 in a black 96-well plate.

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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