# ab112143

# CytoPainter Mitochondrial Staining Kit - Green Fluorescence

## Instructions for Use

For staining Mitochondria in live cells using our proprietary green fluorescence probe

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

# Table of Contents

# 1. Introduction

ab112143 is designed to label mitochondria of live cells in green fluorescence at Ex/Em = 490/520 nm. The kit uses a proprietary dye that selectively accumulates in mitochondria probably via the mitochondrial membrane potential gradient. The mitochondrial indicator, a hydrophobic compound, easily permeates intact live cells and becomes trapped in mitochondria after it gets into cells. The 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.

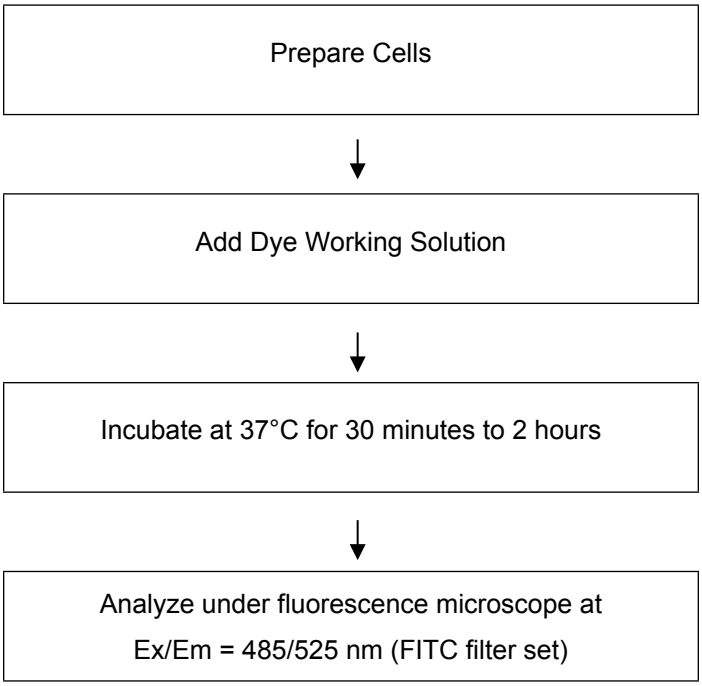
Abcam fluorescence imaging kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as membranes, lysosomes, mitochondria, nuclei, etc. The selective labeling of live cell compartments provides a powerful method for studying cellular events in a spatial and temporal context.

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.

**Note:** The dye will stain live cells but is not well-retained after fixation

## 2. Protocol Summary



## 3. Kit Contents

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

## 4. Storage and Handling

Keep at -20°C. Avoid exposure to 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 MitoGreen Indicator (Component A) into 10 mL of Live Cell Staining Buffer (Component B).

   *Note 1: 20 µL of 500X MitoGreen Indicator (Component A) is enough for one 96-well plate. Aliquot and store unused 500X MitoGreen 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 and Stain Cells

1. For adherent cells: Grow cells either in a black wall/clear bottom 96-well 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 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 (HHBS) or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration). Observe the cells by using a fluorescence microscope fitted with a FITC filter set.

*Note: It's 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 (HHBS) or buffer of your choice (e.g. the buffer with growth medium at 1:1

concentration). Observe the cells by using a fluorescence microscope fitted with a FITC 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

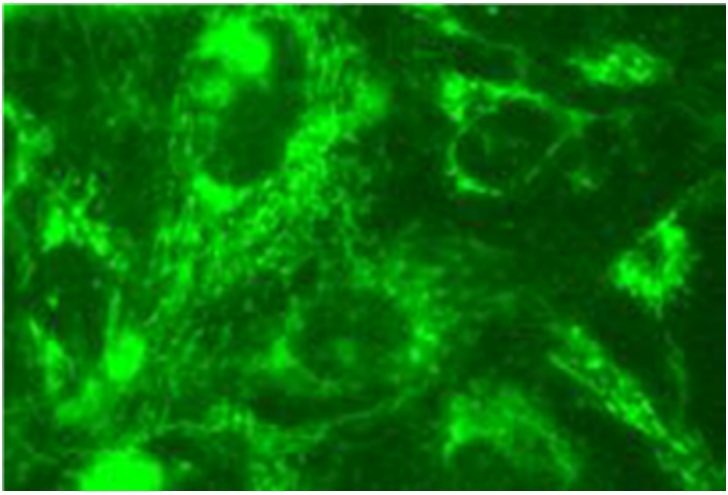


**Figure 1.** Image of U2OS cells stained with the ab112143 in a black 96-well plate

**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).**

**UK, EU and ROW**
Email:
technical@abcam.com
Tel: +44 (0)1223 696000
www.abcam.com

**US, Canada and Latin America**
Email: us.technical@abcam.com
Tel: 888-77-ABCAM (22226)
www.abcam.com

**China and Asia Pacific**
Email: hk.technical@abcam.com
Tel: 400 921 0189 / +86 21 2070 0500
www.abcam.cn

**Japan**
Email: technical@abcam.co.jp
Tel: +81-(0)3-6231-0940
www.abcam.co.jp