ab176830

CytoPainter MitoGreen Indicator Reagent

Instructions for Use

For staining mitochondria in live cells with our proprietary Green probe.

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

Version: 3a Last Updated: 20 October 2020

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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 of a cellular DNA is contained in the cell nucleus, the mitochondrion has its own independent genome.

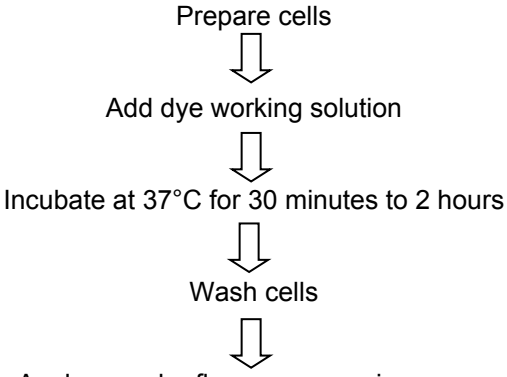
Abcam’s CytoPainter MitoGreen Indicator Reagent (ab176830) is part of a series of new fluorogenic probes to label mitochondria of live cells. The proprietary mitochondria dye selectively accumulates in mitochondria probably via the mitochondrial membrane potential gradient. The mitochondrial indicator is a hydrophobic compound that easily permeates intact live cells and becomes trapped in mitochondria after it gets into cells. The 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; make it useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity.

It can be readily adapted for a wide variety of fluorescence platforms such as microplate assays, immunocytochemistry and flow cytometry. It is suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells.

CytoPainter MitoGreen Indicator Reagent can be detected at Ex/Em = 498/520 nm.

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

2. Protocol Summary



3. Materials Supplied

Item	Quantity
MitoGreen Indicator (500X DMSO solution)	500 tests

4. Storage and Stability

Upon receipt, store kit at -20°C. Avoid exposure to light. Reagent is stable for at least 6 months if stored properly. Avoid repeated freeze/ thaw cycles.

Store reagent in 20 µL aliquots. Each aliquot is enough to stain 1x96-well plate.

5. Materials Required, Not Supplied

- HHBS Buffer (Hanks and 20 mM HEPES buffer) pH=7
- Pipettes and pipette tips
- Coverslips, petri dishes or well plates to grow cells

6. Assay Protocol

1. Reagent Preparation:

a) Warm MitoGreen Indicator to room temperature.

b) For a 1 x 96-well plate assay, prepare dye working solution by diluting 20 µL MitoGreen Reagent in 10 mL of HBSS buffer.

NOTE: The optional concentration of the fluorescent mitochondria indicator may vary 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.

2. Sample Staining and Analysis:

2.1 Adherent cells:

a) Grow cells either in a 96-well back wall/clear bottom plate (100 µL/well/96-well plate) or on cover-slips inside a petri dish filled with the appropriate culture media.

b) When cells reach the desired confluence, add equal volume of the dye-working solution (Step 1b).

c) Incubate the cells in a 37°C, 5% CO2 incubator for 30 min – 2 hours.

d) Replace the dye-loading solution or wash the cells with pre-warmed (37°C) Hanks and 20 mM Hepes buffer (HBSS)

or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration).

e) Fill the cell wells with HBSS or growth medium.

f) Observe cells using a fluorescence microscope fitted with the desired filter set.

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.

2.2 Suspension cells:

a) Centrifuge the cells at 1000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant.

b) Resuspend the cell pellets gently in pre-warmed (37 °C) growth medium, and add equal volume of the dye-working solution (from Step 1b).

c) Incubate the cells in a 37 °C, 5% CO2 incubator for 30 minutes to 2 hours.

d) Replace the dye-loading solution or wash the cells with pre-warmed (37°C) Hanks and 20 mM Hepes buffer (HBSS) or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration).

e) Fill the cell wells with HBSS or growth medium.

f) Observe cells using a fluorescence microscope fitted with the desired filter set. CytoPainter MitoGreen Indicator Reagent can be detected at Ex/Em = 498/520 nm.

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 that have been treated and stained as adherent cells (see Step 2.1).

7. Data Analysis

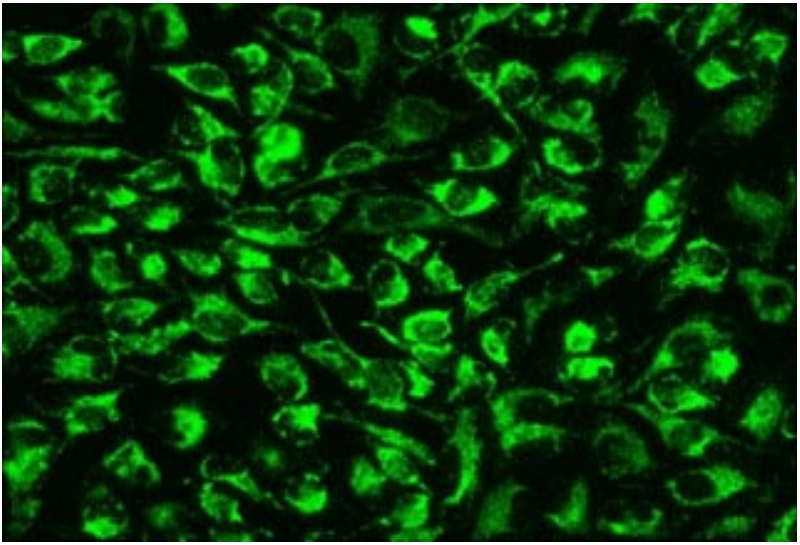


Figure 1. Image of U2OS cells stained with Abcam's CytoPainter MitoGreen Indicator Reagent (ab176830) in a Costar black wall/clear bottom 96-well plate.

8. Troubleshooting

Problem	Reason	Solution
Lysosomes not sufficiently stained.	Too low dye concentration or incubation time insufficient	Increase concentration or incubation time
	Cells observed at incorrect wavelength	Ensure you are using appropriate filter settings
Cells do not appear healthy	Cells require serum to remain healthy	Add serum to stain and wash solutions. Try range 2 – 10% serum.
Nuclear counterstain is too bright	Different microscopes, cameras and filters may make some signals appear very bright	Reduce concentration of nuclear counterstain or shorten exposure time.

Technical Support

For all technical or commercial enquiries please go to:

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