ab138899

Orange Mitochondrial Membrane Potential Assay Kit (Microplate)

Instructions for Use

For staining Mitochondrial membrane in live cells using our proprietary orange fluorescence probe

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

Version 2 Last updated 6 February 2019

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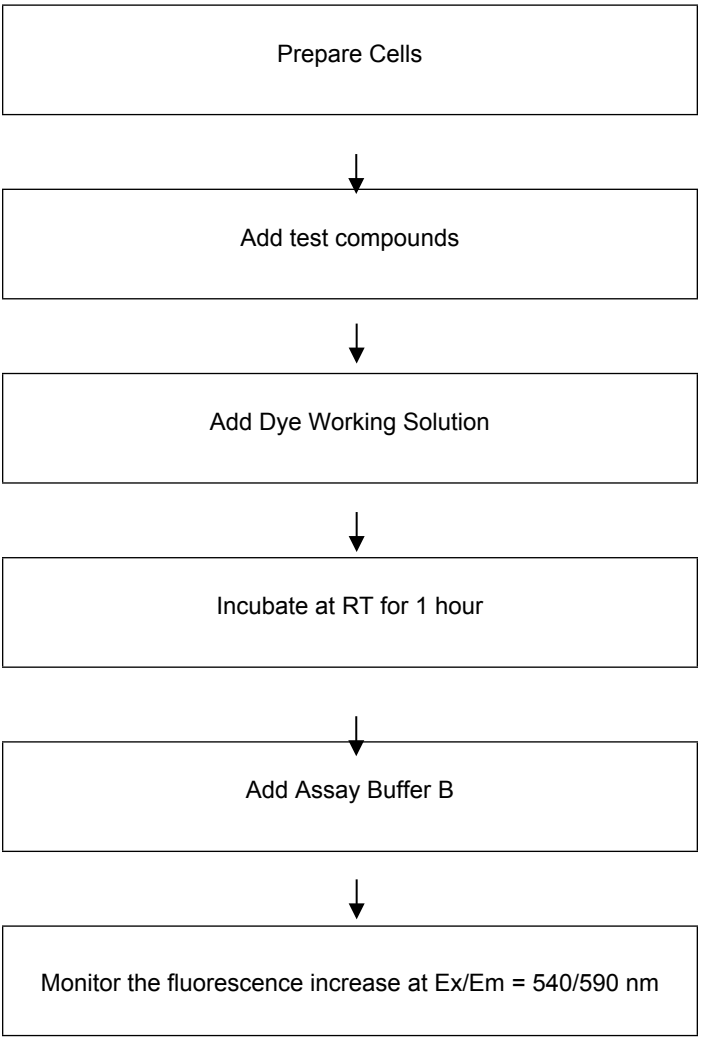
1. Introduction

Abcam assay kits are a set of tools for monitoring cellular functions. There are a variety of parameters that can be used. This particular kit is designed to detect cell apoptosis by measuring the loss of the mitochondrial membrane potential (MMP). The collapse of mitochondrial membrane potential coincides with the opening of the mitochondrial permeability transition pores, leading to the release of cytochrome C into the cytosol, which in turn triggers other downstream events in the apoptotic cascade.

ab138899 uses our proprietary cationic MitoOrange Dye for the detection of the mitochondrial membrane potential change in cells. In normal cells, the orange fluorescence intensity is increased when MitoOrange Dye is accumulated in the mitochondria. However, in apoptotic cells, the fluorescence intensity of MitoOrange Dye is decreased following the collapse of MMP. Cells stained with MitoOrange Dye can be fluorometrically monitored at Ex/Em = 540/590 nm. ab138899 provides all the essential components with an optimized assay method. The kit can be used for screening activators and inhibitors of apoptosis. And the assay can be performed in a convenient 96-well and 384-well fluorescence microtiter-plate format without a wash step.

2. Protocol Summary



3. Kit Contents

Components	Amount
Component A MitoOrange Dye (200X)	250 µL
Component B: Assay Buffer A	50 mL
Component C: Assay Buffer B	25 mL

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Assay Protocol

A. Prepare Cells

1. For adherent cells: Plate cells overnight in growth medium at 20,000 to 80,000 cells/well/100 µL for a 96-well plate or 5,000 to 20,000 cells/well/25 µL for a 384-well plate.

2. For suspension cells: Centrifuge the cells from the culture medium and then suspend the cell pellets in culture medium at 100,000-200,000 cells/well/90 µL for a 96-well poly-D lysine plate or 25,000-50,000 cells/well/20 µL for a 384-well poly-D lysine plate. Centrifuge the plates at 800 rpm for 2 minutes with brake off prior to the experiments.

   Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

B. Prepare MitoOrange Dye Solution

1. Warm all the components to room temperature.

2. Add 50 µL of 200X MitoOrange Dye (Component A) into 10 mL of Assay Buffer A (Component B), and mix them well.

   Note: Aliquot and store the unused 200X MitoOrange Dye (Component A) at -20 °C. Avoid repeated freeze/thaw cycles.

C. Run MitoOrange Assay

1. Treat cells with test compounds for a desired period of time to induce apoptosis, and set up parallel control experiments.
   For Negative Control: Treat cells with vehicle only.
   For Positive Control: Treat cells with FCCP or CCCP at 5-50 µM in a 5% CO2, 37 °C incubator for 15 to 30 minutes.
   Note: CCCP or FCCP can be added simultaneously with MitoOrange Dye. To get the best result, titration of the CCCP or FCCP may be required for each individual cell line.

2. Remove the cell medium.
   Note: It is important to remove the cell medium before adding MitoOrange dye-loading solution.

3. Add 100 µL/well/96-well plate or 25 µL/well/384-well plate of MitoOrange dye-loading solution (from Step B.2) into the cell plate (from Step C.2).

4. Incubate the dye-loading plate in a 5% CO_2, 37 °C incubator for 15-30 minutes, protected from light.

   Note: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

5. Add 50 µL/well/96-well plate or 12.5 µL/well/384-well plate of Assay Buffer B (Component C) into the dye-loaded cell plate (from Step C.4).

   Note 1: DO NOT wash the cells after loading.
   Note 2: For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with brake off after adding Assay Buffer B (Component C).

6. Monitor the fluorescence intensity at Ex/Em = 540/590 nm (bottom read) 10 to 30 minutes after Step C.5 either using the endpoint mode or using the kinetic mode.

6. Data Analysis

In live non-apoptotic cells, the orange fluorescence intensity is increased when MitoOrange Dye is accumulated in the mitochondria. In apoptotic and dead cells, MitoOrange Dye stain intensity is decreased following the collapse of MMP.

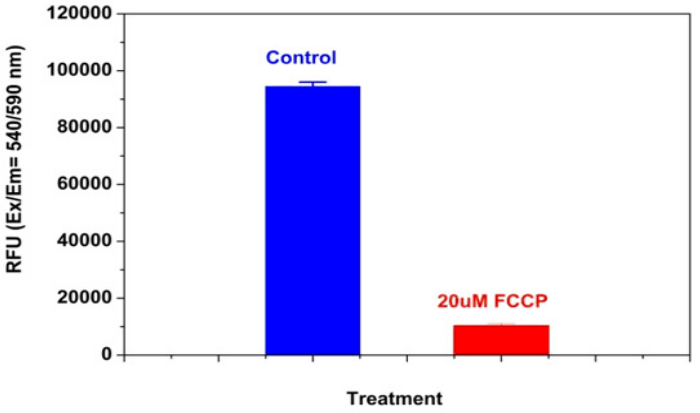


Figure 1. The decrease in MitoOrange Dye fluorescence with the addition of FCCP in HeLa cells. HeLa cells were dye loaded with MitoOrange Dye alone or in the presence of 20 µM FCCP for 15 minutes. The fluorescence intensity of MitoOrange Dye was measured 30 minutes after adding Assay Buffer B (Component C) with a microplate reader at Ex/Em = 540/590 nm (cut off 570 nm, bottom read).

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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