ab138898

Orange Mitochondrial Membrane Potential Assay Kit (Flow Cytometry)

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.

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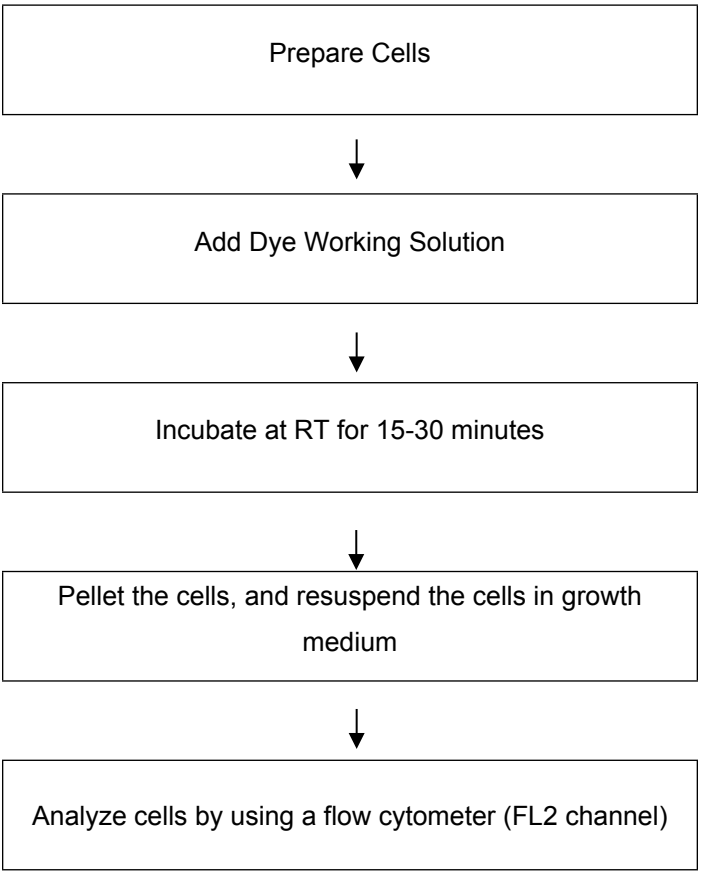
1. Introduction

Abcam assay kits are a set of tools for monitoring cell viability. 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.

ab138898 provides all the essential components with an optimized assay method. This fluorimetric assay uses our proprietary cationic MitoOrange Dye for the detection of apoptosis in cells with the loss of mitochondrial membrane potential. In normal cells, the red 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 visualized with a flow cytometer at 488 nm excitation with red emission (FL2 channel). The kit can be used together with other reagents for multi-parametric study of cell vitality and apoptosis. The kit is optimized for screening apoptosis activators and inhibitors with a flow cytometer.

2. Protocol Summary



3. Kit Contents

Components	Amount
Component A: MitoOrange Dye (500X DMSO Stock)	200 µL
Component B: Assay Buffer	100 mL

4. Storage and Handling

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

5. Assay Protocol

Prepare and Stain Cells

1. Warm all the components to room temperature.

2. For each sample, prepare cells in 1 mL of warm medium or buffer of your choice at the density of $5x10^5$ to $1x10^6$ cells/mL.

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

3. 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 37 °C, 5% CO2 incubator for 15 to 30 minutes.
   Note: CCCP or FCCP can be added simultaneously with MitoOrange Dye (See Step 4). To get the best result, titration of the CCCP or FCCP may be required for each individual cell line.

4. Add 2 µL of 200X MitoOrange Dye (Component A) into the treated cells (from Step 3), and incubate the cells in a 37 °C, 5% CO_2 incubator for 15 to 30 minutes.

   Note: For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact and wash the cells once with serum-containing media prior to the incubation with MitoOrange dye-loading solution

5. Centrifuge the cells at 1000 rpm for 4 minutes, and then re-suspend cells in 1 mL of Assay Buffer (Component B) or buffer of your choice.

6. Monitor the fluorescence intensity by using a flow cytometer in the FL 2 channel (Ex/Em = 540/590 nm). Gate on the cells of interest, excluding debris.

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.

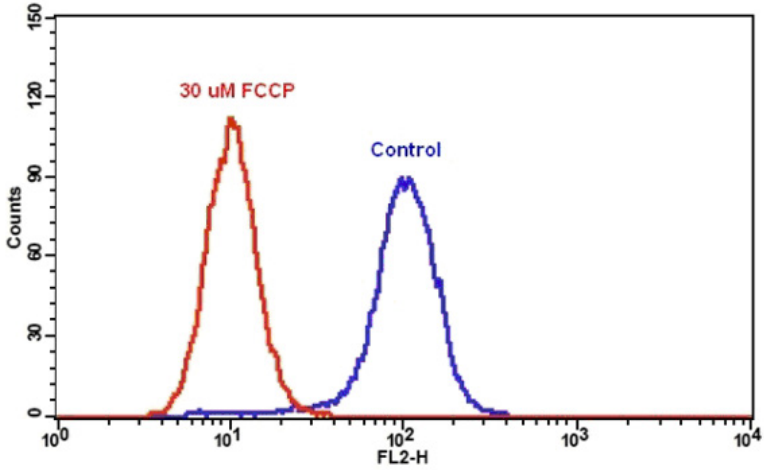


Figure 1. Flow Cytometric analysis demonstrating the decrease in fluorescence intensity of MitoOrange Dye with the addition of FCCP in Jurkat cells. Jurkat cells were loaded with MitoOrange Dye alone (Blue) or in the presence of 30 µM FCCP (Red) for 15 minutes. The fluorescence intensity of MitoOrange Dye was measured with a flow cytometer using the FL2 channel.

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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