

ab112149

**NIR Mitochondrial
Membrane Potential
Assay Kit**

Instructions for Use

For measuring Mitochondrial membrane potential in cells using our proprietary fluorescence probe

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

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1. Introduction

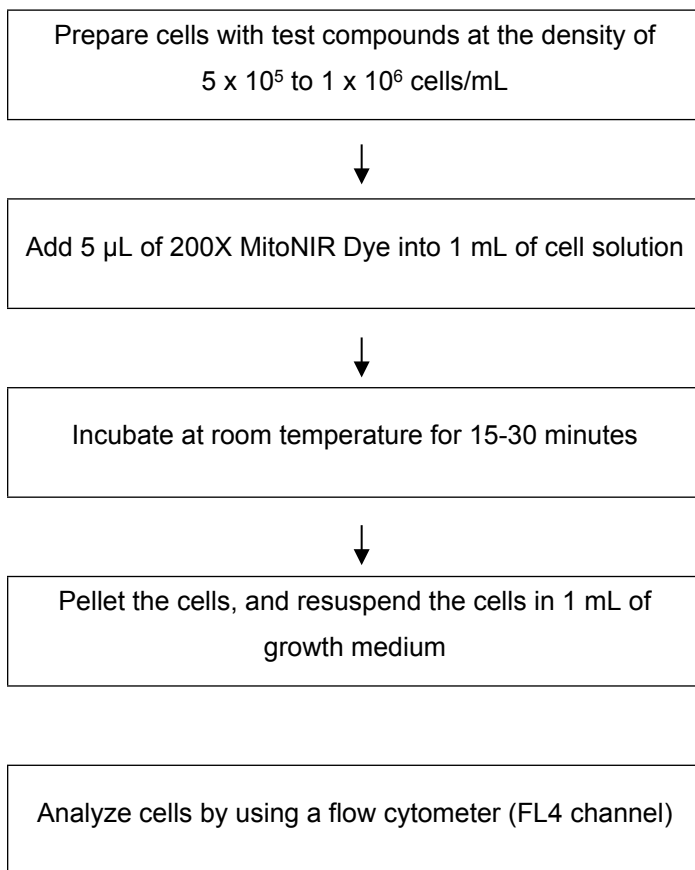
ab112149 is designed to detect cell apoptosis by measuring the loss of the mitochondrial membrane potential. The collapse of mitochondrial membrane potential coincides with the opening of the mitochondrial permeability transition pores, leading to the release of Cytochrome C into the cytosol, which in turn triggers other downstream events in the apoptotic cascade.

ab112149 uses our proprietary cationic NIR probe for the detection of apoptosis in cells with the loss of mitochondrial membrane potential. In normal cells, the red fluorescence intensity is increased when the MitoNIR Dye is accumulated in the mitochondria. However, in apoptotic cells, the NIR stain intensity is decreased following the collapse of MMP. Cells stained with MitoNIR Dye can be visualized with a flow cytometer at red excitation and far red emission (FL4 channel).

ab112149 provides all the essential components. ab112149 can be used together with other reagents, such as blue laser excited Propidium iodide and for studying cell vitality and apoptosis. ab112149 is optimized for screening apoptosis activators and inhibitors with a flow cytometer.

2. Protocol Summary

Summary for Flow Cytometer



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: 200X MitoNIR Dye in DMSO	1 x 500 μ L
Component B: Assay Buffer	1 x 100 mL

4. Storage and Handling

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

5. Assay Protocol

Note: This protocol is for Flow Cytometer

- A.** For each sample, prepare cells in 1 mL of warm medium or buffer of your choice at the density of 5×10^5 to 1×10^6 cells/mL.

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

- B.** 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 μ M in a 37°C, 5% CO₂ incubator for 15 to 30 minutes.

Note: CCCP or FCCP can be added simultaneously with MitoNIR Dye (See Step C). To get the best result, titration of the CCCP or FCCP may be required for each individual cell line.

- C.** Add 5 μ L of MitoNIR Dye (Component A) into the treated cells (from Step B), and incubate the cells in a 37°C, 5% CO₂ 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 NIR dye-loading solution.

- D.** 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.

- E.** Monitor the fluorescence intensity by using a flow cytometer in the FL 4 channel (Ex/Em = 635/660 nm). Gate on the cells of interest, excluding debris.

6. Data Analysis

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

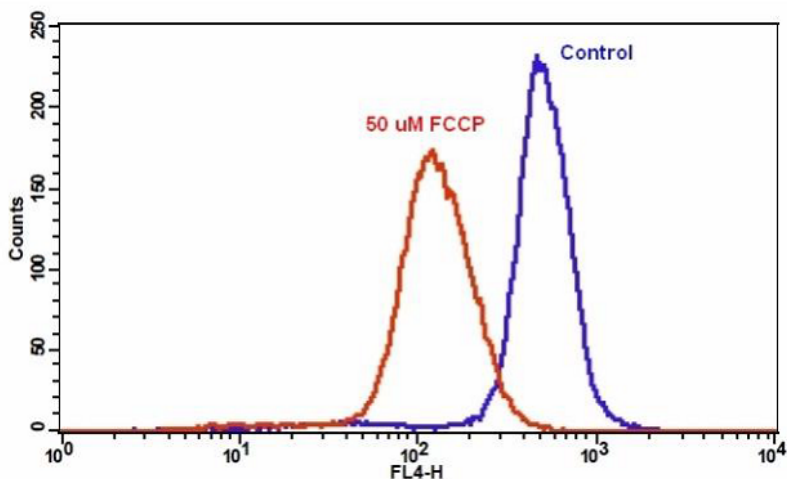


Figure 1. The decrease in fluorescence intensity of MitoNIR Dye with the addition of FCCP in Jurkat cells. Jurkat cells were loaded with MitoNIR Dye alone (blue line) or in the presence of 50 μ M FCCP (red line) for 10 minutes. The fluorescence intensity of MitoNIR Dye was measured with a flow cytometer using FL4 channel.

For 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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