ab112150

NIR Mitochondrial Membrane Potential Assay Kit (Microplate)

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

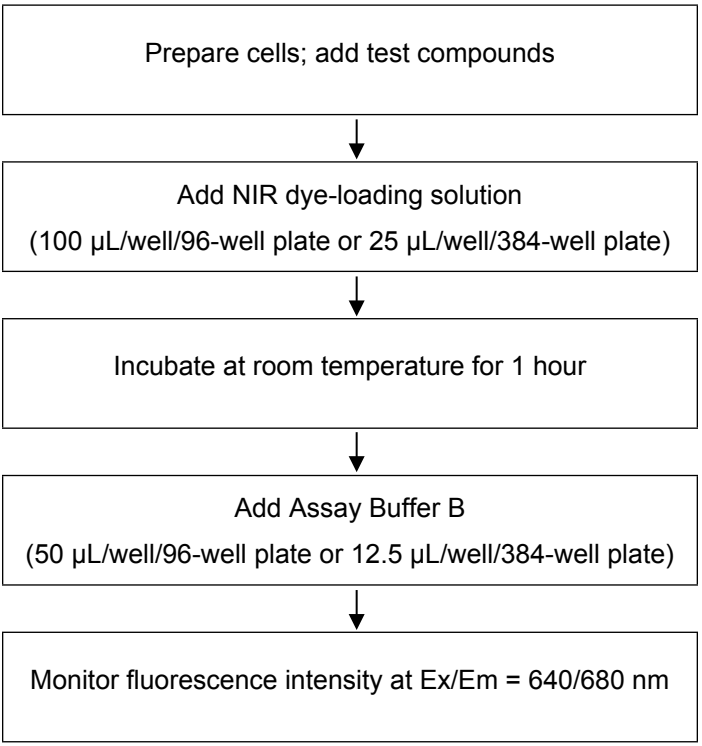
ab112150 is designed to detect cell apoptosis by measuring the loss of the mitochondrial membrane potential. The collapse of mitochondrial membrane potential coincides with the opening of the mitochondrial permeability transition pores, leading to the release of Cytochrome C into the cytosol, which in turn triggers other downstream events in the apoptotic cascade.

ab112150 NIR Mitochondria Membrane Potential Assay Kit provides all the essential components with an optimized assay method. This fluorometric assay uses our proprietary cationic a MitoNIR Dye for the detection of the mitochondrial membrane potential change in cells. In normal cells, the red fluorescence intensity is increased when MitoNIR Dye is accumulated in the mitochondria. However, in apoptotic cells, NIR stain intensity is decreased following the collapse of MMP. Cells stained with MitoNIR Dye can be monitored fluorometrically at 660-680 nm with excitation at 620-640 nm.

ab112150 can be used for screening apoptosis activators and inhibitors. The assay can be performed in a convenient 96-well and 384-well fluorescence microtiter-plate format

2. Protocol Summary

Summary for One 96-well Plate



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 250 µL
Component B: Assay Buffer A	1 x 50 mL
Component C: Assay Buffer B	1 x 25 mL

4. Storage and Handling

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

5. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Preparation of 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 non-adherent 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 plate 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. Preparation of NIR Dye-loading Solution

1. Thaw all the kit components at room temperature before use.

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

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

C. Run MitoNIR Dye 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 37°C, 5% CO_2 incubator for 15 to 30 minutes.

   Note: CCCP or FCCP can be added simultaneously with NIR 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 before adding NIR dye-loading solution (See Step C.3).

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

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

4. Incubate the dye-loading plate in a 37°C, 5% CO_2 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) before monitoring the fluorescence signal.

   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).

   Monitor the fluorescence intensity at Ex/Em = 640/680 nm (bottom read) either using the endpoint mode or using the kinetic mode 10 to 30 minutes after Step C.5.

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.

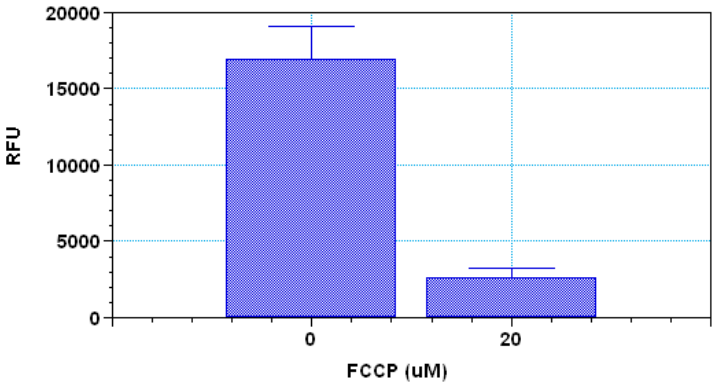


Figure 1. The decrease in NIR fluorescence with the addition of FCCP in HeLa cells. HeLa cells were dye loaded with MitoNIR Dye alone or in the presence of 20 μM FCCP for 15 minutes. The fluorescence intensity of MitoNIR Dye was measured 30 minutes after adding Assay buffer B (Component C) with a microplate reader at Ex/Em = 640/680 nm (cut off 665 nm, bottom read).

7. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

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