Store at -20°C

#13296

Mitochondrial Membrane Potential Assay Kit (II)

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For Research Use Only. Not For Use In Diagnostic Procedures.

Description: The Mitochondrial Membrane Potential Assay Kit (II) is a fluorescent assay that detects the mitochondrial membrane potential in living cells. The kit includes the cationic dye TMRE (tetramethylrhodamine ethyl ester perchlorate) and a mitochondrial membrane potential disruptor CCCP (carbonyl cyanide 3-chlorophenylhydrazone). TMRE is a cell membrane permeable, fluorescent dye that accumulates in intact mitochondria. Depolarized or inactive mitochondria exhibit decreased membrane potential, resulting in reduced TMRE accumulation.

Background: Mitochondria are the main power house in cells and play important roles in processes such as steroid metabolism, calcium homeostasis, apoptosis and cellular proliferation. Mitochondrial membrane potential is a key indicator of its function and cell health (1,2). The dissipation of mitochondrial membrane potential is established as an early indicator for apoptosis (3).

TMRE (tetramethylrhodamine, ethyl ester) is a cell membrane permeable cationic dye. In normal cells, TMRE accumulates in the mitochondria in response to their high membrane potential and negative charge. When excited at 550 nm, TMRE emits an orange-red fluorescence with a maximum at 575 nm (orange-red). Cells that have lost membrane potential or mitochondria activity cannot accumulate TMRE. Therefore, the fluorescence intensity of the orange-red emission can be used to measure mitochondria membrane potential and is an indicator for cell health (4).

Specificity/Sensitivity: The Mitochondrial Membrane Potential Assay Kit (II) is expected to detect the mitochondrial membrane potential in living cells cross all species. For the best result, a cell number titration is recommended when using a plate-reader and a 96-well plate.

Background References:

(1) Perry, S.W. et al. (2011) *Biotechniques* 50, 98-115.

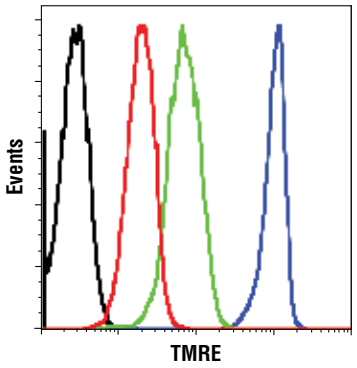
(2) Nesti, C. et al. (2007) *Biosci Rep* 27, 165-71.

(3) Petit, P.X. et al. (1995) *J Cell Biol* 130, 157-67.

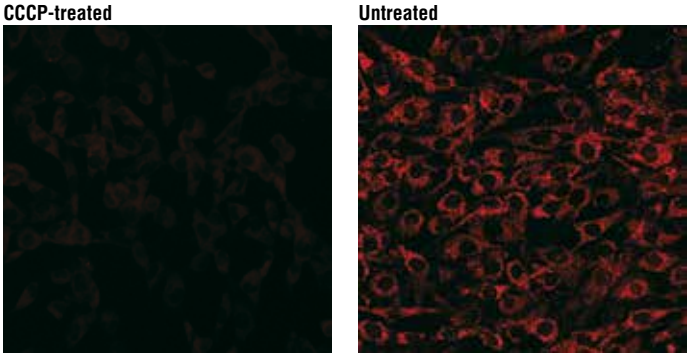
(4) O'Reilly, C.M. et al. (2003) *Biophys J* 85, 3350-7.

Products Included	Item	Quantity	Storage Temp
TMRE	13472	29 µg	–20°C
CCCP	13550	100 µl	–20°C
Phosphate Buffered Saline (PBS-20X)	9808	25 ml	Room Temp

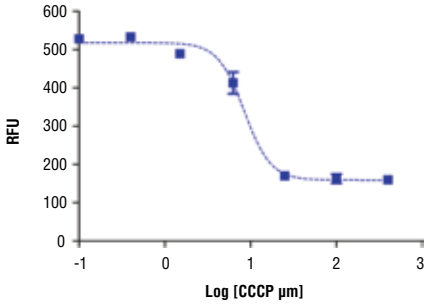
Note: All components in this kit are stable for at least 12 months when stored at the recommended temperature and left unused. Upon receipt, #9808 should be removed from kit box and stored at room temperature. Remaining components should be stored at -20°C.



◀ *Figure 1. Flow cytometric analysis of Jurkat cells, unlabeled (black) or labeled with 200 nM TMRE and treated with 0 µM CCCP (blue), 3.2 µM CCCP (green), or 80 µM CCCP (red).*



◀ *Figure 2. Confocal immunofluorescent analysis of NIH/3T3 cells (2x10^5 cell/ml) seeded in a 96-well black plate with a clear bottom and incubated overnight. Cells were untreated, or treated with CCCP (400 µM, 20 min) followed by labeling with TMRE (200 nM, 30 min).*



◀ *Figure 3. HeLa cells (3x10^5 cell/ml) were treated with various concentrations of CCCP for 15 minutes prior to labeling with 200 nM TMRE.*

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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Mitochondrial Membrane Potential Assay Kit (II) Protocol

Instrumentation Required

1. Flow cytometer with excitation approximately 550 nm and emission 580 nm. Very good results can also be achieved with a common argon blue line laser (488 nm).
2. Plate reader that can read 96-well plates with excitation approximately 550 nm and emission approximately 580 nm
3. 96-well plate (black with clear bottom)

Reagent Preparation

1. Prepare 1X PBS by diluting 20X PBS (included in each kit) in reverse osmosis deionized (RODI) or equivalently purified water.
   Note: For flow cytometry, adding 0.5% BSA to wash buffer may help to prevent cell loss during the process.
2. Add 55 µl DMSO to each vial of TMRE to make a 1000 µM stock solution. Each vial includes enough TMRE for five 96-well plates (0.1 µl/well) or 50 flow cytometry assays (10 µl/assay). Aliquot if desired and store at -20°C.
3. Dilute TMRE to 1:500 with full cell culture medium to make 10X TMRE Labeling Solution. 2 µM is used in this protocol; 0.1 to 10 µM is recommended based on different cell lines.
4. If CCCP is used as positive control, allow CCCP solution to equilibrate to room temperature before use.

A. Protocol for suspension cells

1. Suspend cells in warm medium or PBS at 1 x 10^6 cell/ml. Prepare 1 ml aliquots; each 1 ml cell aliquot is one assay point. Make sure there are enough cells for your experiment. For example, if one compound is going to be assayed at three different concentrations, a total of 4 x 1 ml samples will be needed (this includes a positive control).
2. Add test compound(s) to sample tubes at desired concentration and incubate cells for desired time. For best results, a compound titration and incubation time course can help to determine the best assay conditions. To prepare the positive control (mitochondrial membrane potential loss), add 1 µl of 50 mM CCCP (supplied with this kit) to the control tube for a 50 µM final concentration; incubate cells at 37°C for 15 min.
3. Add 100 µl of the 2 µM TMRE Labeling Solution to each sample (200 nM final concentration) and incubate cells in the incubator (37°C and 5% CO2) for 15 to 30 min.
   Note: 200 nM TMRE is recommended in this protocol. For best results, a titration of TMRE is recommended.
4. Centrifuge sample at 300 g for 5 min, then remove the supernatant.
5. Wash cells once with 1 ml warm 1X PBS wash buffer, repeat step 4.
6. Resuspend cells into 1000 µl warm 1X PBS.
7. Analyze sample on a flow cytometer. If samples are analyzed on plate reader, transfer 100 µl/ cell suspension/well to a black 96-well plate with a clear bottom and read plate on the plate reader. The settings are: excitation about 550 nm and emission about 580 nm.

B. Protocol for adherent cells

1. Plate cells to a 96-well plate in warm culture medium and culture cells in incubator overnight to allow cells to attach to the plate. A typical cell number is between 1-5 x to 10^4 cells/ well. A cell number titration may be necessary for optimal results.
2. Aspirate the medium from the plate and add test compounds in growth medium or 1X PBS to plate at 100 µl/well at desired concentration and incubate cells for desired time. Compound titration and incubation time course can help to determine the best assay conditions. For positive control (mitochondrial membrane potential loss), add CCCP (supplied with this kit) to the control wells for a 50 µM final concentration and incubate cells at 37°C for 15 min. For example, add 1 µl of 50 mM stock CCCP to 100 µl medium to make 500 µM CCCP; then add 10 µl of this 500 µM CCCP to each well containing 100 µl medium to get final concentration of 50 µM.
3. Add 10 µl of 2 µM TMRE Labeling Solution to each well to get a final concentration of 200 nM and place plate in an incubator (37°C and 5% CO_2) for 20 min.
   Note: 200 nM TMRE is recommended in this protocol. For best results, a titration of TMRE is recommended.
4. Aspirate the solution from the plate.
5. Wash plate 3 times with warm 1X PBS and then add 100 µl/well 1X PBS to the plate.
6. Analyze samples on the plate reader. The settings are: excitation about 550 nm and emission about 580 nm.