TMRE Mitochondrial Membrane Potential Assay Kit (Fluorometric) (#BN00506)

(Catalog #BN00506, 200 assays; Store kit at -20°C)

I. Introduction:

Mitochondria are the powerhouse of the cells and generate energy in the form of ATP. Mitochondria utilize oxidizable substrates to produce a membrane potential in the form of a proton gradient across the mitochondrial inner membrane. Mitochondrial membrane potential is tightly interlinked to many mitochondrial processes, including ATP synthesis, ROS, calcium sequestration and import of proteins into the mitochondrion. Therefore, pharmacological changes in mitochondrial membrane potential are associated with several pathological parameters. Assay Genie's TMRE Mitochondrial Membrane Potential Assay Kit uses TMRE (tetramethylrhodamine, ethyl ester) to label active mitochondria. TMRE is a cell-permeable, positively-charged, red-orange dye that readily accumulates in active mitochondria. Protonophores like FCCP and CCCP induces dissipation of mitochondrial membrane thus results in compromised staining.

II. Applications:

- Labeling of active mitochondria in cells
- Screening of compounds which may compromise mitochondrial membrane potential

III. Sample Type:

- Adherent and suspension cells

IV. Kit Contents:

Components	BN00506	Cap Code	Part number
Assay Buffer	100 ml	NM	BN00506-1
TMRE Dye (1 mM, in DMSO)	10 µl	Blue	BN00506-2
Negative Control (FCCP, 40 mM)	10 µl	Red	BN00506-3

V. User Supplied Reagents and Equipment:

- 96-well white plate with clear bottom
- Plate reader

VI. Storage Conditions and Reagent Preparation:

Store kit at - 20°C, protected from light. Centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Assay Buffer:** Store at 4°C or -20°C. Warm to 37°C and mix well before use.
- **TMRE Dye (1 mM):** Thaw at room temperature (RT). Protect from light. Open under sterile conditions.
- **Negative Control (FCCP, 40 mM):** Aliquot and store at -20°C. Thaw at RT.

VII. TMRE Mitochondrial Membrane Potential Assay protocol:

1. **Sample Preparation:**
   a. Grow cells ($1x10^5$-$5x10^5$ cells per ml) of interest in a 96-well clear plate in 100 µl culture media according to the desired protocol. For flow cytometry or microscopy experiments grow the cells in 6-well plates ($1-5x10^6$ cells per ml) or as desired.
   b. Treat cells with compounds, varied culture conditions or other manipulation of interest. Treatment time can vary depending on the compound or manipulation method. Dilute Negative Control in provided Assay Buffer or appropriate serum free cell culture media 1:100, to reach a final concentration of 400 µM. Treat cells with 5 µl of diluted Negative Control (FCCP, 20 µM), for 10-20 min. at 37°C in serum free media.

   Notes:

   - Cells seeded at densities between 10,000-50,000 cells per well should reach optimal population densities within 48-72 hrs. We recommend using appropriate incubation time depending on the individual cell type and cell concentrations used. Therefore it is recommended to determine the optimal incubation time for each experiment.
   - Chemical uncouplers act instantaneously (e.g. FCCP can depolarize mitochondria within min.). In contrast, treatments that may have a less direct effect on the mitochondrial electron transport chain or require changes in protein synthesis or activation may take longer to manifest a change in mitochondrial membrane potential.

2. **Cell labeling:** Dilute the TMRE Dye in Assay Buffer 1:100, to get 10 µM solution. Dilute further to 200 nM by adding 2 µl of 10 µM TMRE dye to 98 µl of Assay Buffer. Make as much as needed. Add 100 µl of 200 nM TMRE dye solution to each well of a 96 well plate. Use appropriate amount of volume depending on the plate use. For background control, don't add TMRE Dye solution to the wells containing cells. Incubate the plate at 37°C for 15-30 min.

   Note: We recommend adding TMRE Dye in a range of 200-1000 nM (final conc.) per well.

3. **Data Analysis:** After incubation, for adherent cells carefully discard the media. For suspension cells, spin the samples at 1,000 x g for 5 min. in a microplate compatible centrifuge and carefully discard the media. Wash the 96-well plates 3-4 times using 100 µl of Assay Buffer. After washing, add 100 µl of Assay Buffer and read fluorescence at Ex/Em = 549/575 nm. For flow cytometry, we recommend analyzing the data in FL-3 (Red) channel. For fluorescent microscopy, we recommend using red filter (Ex/Em = 549/575 nm) set up and quickly acquiring the image.

Note:

- Culture conditions such as age of the culture, number of passages, growth media can affect the results and must be taken into consideration when analyzing the data.

A. **B.** **C.**

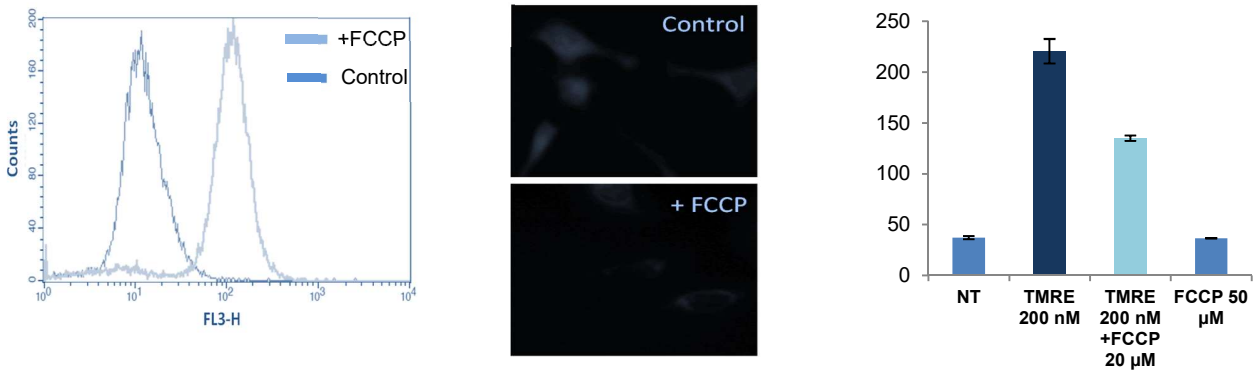

Figure: TMRE Mitochondrial Membrane Potential Assay: Hela cells (10^4 cell/well) were cultured overnight in a cell plate (White Plate Clear Bottom) with media containing 10% FCS and assayed for Mitochondrial Membrane Potential according to the kit protocol. NT: No treatment. **(B)** Flow cytometry histogram of Jurkat cells stained with 100 nM TMRE with (red) or without (green) treatment with 40 µM FCCP. **(C)** HeLa cells were treated with or without FFCP (20 µM) and stained with 100 nM TMRE for 20 min. in media, washed briefly with PBS and immediately imaged.

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