ab113852 –
TMRE Mitochondrial Membrane Potential Assay Kit

Instructions for Use

For the measurement of mitochondrial membrane potential by flow cytometry, fluorescence plate reader and fluorescence microscopy

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

TMRE is suitable for interrogating mitochondrial membrane potential in live cells for analysis by flow cytometry, microplate spectrophotometry and fluorescent microscopy. Each assay kit contains sufficient materials for at least 200 measurements.

Mitochondrial membrane potential ($\Delta \psi_m$) is highly interlinked to many mitochondrial processes. The $\Delta \psi_m$ controls ATP synthesis, generation of ROS, mitochondrial calcium sequestration, import of proteins into the mitochondrion and mitochondrial membrane dynamics. Conversely, $\Delta \psi_m$ is controlled by ATP utilization, mitochondrial proton conductance, respiratory chain capacity and mitochondrial calcium. Hence pharmacological changes in $\Delta \psi_m$ can be associated with a multitude of other mitochondrial pathological parameters which may require further independent evaluation.

Depolarization can be found in the presence of ionophores that could induce nonselective cation channels or become selective mobile ionic carriers. Protonophores such as FCCP and CCCP induce reversal of the ATPase, as a compensatory mechanism that tries to maintain $\Delta \psi_m$, which will deplete ATP even in the presence of a normal glycolytic pathway. Hyperpolarization could be found in the presence of ATPase inhibition, inadequate supply of ADP, increased supply of NADH, apoptosis due to oxidative stress and potentially
proton slippage due to cytochrome c oxidase dephosphorylation. In either scenario, OXPHOS uncoupling ensues.

**Principle:** This mitochondrial membrane potential kit uses TMRE (tetramethylrhodamine, ethyl ester) to label active mitochondria. TMRE is a cell permeant, positively-charged, red-orange dye that readily accumulates in active mitochondria due to their relative negative charge. Depolarized or inactive mitochondria have decreased membrane potential and fail to sequester TMRE.

FCCP (carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone) is an ionophore uncoupler of oxidative phosphorylation. Treating cells with FCCP eliminates mitochondrial membrane potential and TMRE staining.

TMRE is suitable for the labeling of mitochondria in live cells and is not compatible with fixation.

**Limitations:**

- FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC PROCEDURES.
- Use this kit before expiration date.
- Do not mix or substitute reagents from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
2. Assay Summary

Seed cell line(s) and add treatment(s) for desired amount of time

\[\downarrow\]

Optional: 10min before adding TMRE (next step), add 20µM FCCP to one sample in media

\[\downarrow\]

Bring TMRE to room temperature

Add TMRE to cells in media to final concentration of 50-1000nM

Incubate 20min at 37 °C

\[\downarrow\]

Measure TMRE staining of mitochondria *in live cells.*

[peak exitation=549 nm, peak emission=575 nm]
For Flow cytometry: collect data from single cell suspension in media or 0.2% BSA in PBS.

For Fluorescence plate reader: wash cells once with 0.2% BSA in PBS, then read cells in a microplate.

For Fluorescence microscope: wash cells once with PBS, then image live cells.

3. Kit Contents

- 1mM TMRE (in DMSO) (~1000x) : 0.04 mL
- 50mM FCCP (in DMSO) (~2500x) : 0.01 mL

4. Storage and Handling

Store all components at 4°C in the dark. For longer term storage, keep at -20°C.
5. Additional Materials Required

- Flow cytometer and/or fluorescence microscope (required excitation/emission wavelengths 594 nm/575 nm)
- General tissue culture supplies
- PBS (sterile)
- Bovine serum albumin (BSA)
6. Assay Procedure

1. Treat cells of interest with compounds, culture conditions or other manipulation of interest.
   a. Treatment times can vary depending on the experiment at hand. For example, chemical uncouplers essentially act instantaneously (e.g. FCCP can depolarize mitochondria within minutes). In contrast, treatments that may have a less direct effect on the mitochondrial electron transport chain or that require changes in protein synthesis or activation may take longer to manifest a change in mitochondrial membrane potential.
   b. Control compound FCCP. Add 20 µM FCCP to cells in media 10 minutes prior to staining with TMRE (step 2). FCCP is an uncoupler that will eliminate the mitochondrial membrane potential and prevent staining by TMRE.

2. Add TMRE to cells in culture and incubate for 15-30 minutes.
   a. TMRE should be added to cells in media. An efficient means to do this is to prepare a 10-20X working solution of TMRE in the appropriate media
and overlay this to the experimental cultures such that the final concentration is 1X.

b. Return cells to incubator and culture for an additional 10-30 minutes.

3. Data collection and analysis

Microplate assay:

i. Removing the culture media is required to eliminate background fluorescence from the media:
   - **Suspension cells:** Gently pellet the cells by centrifugation and remove the culture media. Resuspend in a like volume of 0.2% BSA in PBS and pellet again. Resuspend in 0.2% BSA in PBS and transfer to microplate.
   - **Adherent cells:** Cells should be seeded in microplates and allowed to adhere prior to the TMRE staining. After TMRE staining, gently aspirate the media and replace with 0.2% BSA in PBS. Repeat.

ii. Read the plate on a fluorescence plate reader with settings suitable for TMRE (Ex: 549nm, Em: 575nm).

iii. **Guidelines for cell numbers:** For suspension cells, 100,000 - 200,000 cells per well in 100 - 200µL should provide sufficient signal. For adherent cells, culture
such that the cells are not overly confluent at the time of data collection. The user will need to determine optimal cell densities for the given cell lines.

iv. **Guidelines for TMRE concentration:** Dependent on the cell line at hand. Recommending starting concentrations to test are 200 - 1000 nM TMRE.

v. Black wall microtiter plates are recommended.

**Flow cytometry:**

i. Removing media as described above for the microplate reader is not required; however, we do find an enhancement (in some cases up to 40%) of TMRE fluorescence relative to background if the media has been exchanged for 0.2% BSA in PBS. The user should determine if washing away the media benefits their analysis.

ii. Before assessing cells by flow cytometry, be certain that the samples are non-aggregated and in a single cell solution. For adherent cells this will require trypsinization or other means to remove cells from their culture vessel.

iii. **Guidelines for cell numbers:** Ideally 10,000 cells should be analyzed and cells should not be overly dense during the experiment (<1x10^6 cells/mL for
suspension cells and not overly-confluent for adherent cells).

iv. **Guidelines for TMRE concentration:** Dependent on the cell line at hand. Starting concentrations to test are 50 - 400 nM TMRE.

v. TMRE is excited by the 488nM laser and should be detected in the appropriate filter channel (peak emission is 575nm). This is commonly FL2.

**Microscopy:**

i. Cells should be plated in a manner consistent with the available microscope setup for live cell imaging. Cells should be imaged as quickly as possible after being removed from the culture conditions as mitochondrial morphology and function is dependent on temperature and cell health. It is recommended to remove media by a PBS rinse before imaging cells to avoid background caused by the media.

ii. **Guidelines for TMRE concentration:** 50 - 200 nM, as determined by the user. It is recommended to use the least amount of TMRE that gives a reasonably detectable signal.

iii. Use appropriate filter set for TMRE.
4. Appropriate controls

a. FCCP serves as a control for depolarized mitochondria (low mitochondrial membrane potential).

b. At minimum, also include control cells that are not stained with TMRE. Ideally both treated and untreated cells should be included as non-TMRE stained controls.

7. Sample Data

i. **Microplate assay:**

![Bar chart showing TMRE staining decreased by FCCP in Jurkat cells](chart.jpg)

**Figure 1. Analysis of TMRE staining using a fluorescent plate reader and a microplate.** Chart showing mean fluorescent intensity +/- standard deviation from quadruplicate measurements of 400 nM
TMRE stained Jurkat cells in a 96-well microplate +/- treatment with FCCP.

ii. Flow cytometry:

![Flow cytometry histogram of Jurkat cells stained with 100 nM TMRE with (blue) or without (red) treatment with 100µM FCCP.]

**Figure 2. Analysis of TMRE staining by flow cytometry.** Flow cytometry histogram of Jurkat cells stained with 100 nM TMRE with (blue) or without (red) treatment with 100µM FCCP.
iii. **Microscopy:**

![Microscopy Image](image)

**Figure 3. Fluorescence microscopy of TMRE labeled mitochondria in live cells.** A. HeLa cells (adherent) were cultured on coverslips and stained with 200 nM TMRE for 20 minutes in media, washed briefly with PBS and immediately imaged. B. Jurkat cells (suspension) were stained and washed as above and then transferred to a slide and immobilized under a coverslip for imaging.
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