TMRE Mitochondrial Membrane Potential Assay

(Cat. # 786-1313, 786-1314)

INTRODUCTION

TMRE Mitochondrial Membrane Potential Assay quantifies changes in mitochondrial membrane potential ($\Delta\psi_M$) of live cells, via microplate fluorometry, fluorescence microscopy and flow cytometry.

Tetramethylrhodamine, ethyl ester (TMRE) is a cell permeable, positively charged red-orange dye that accumulates in active mitochondria due to their relative negative charge. Inactive or depolarized mitochondria have decreased membrane potential and thus fail to retain the TMRE dye and as a result show low fluorescence signal.

TMRE is suitable for staining mitochondria in live cells only. It does not work with fixed cells.

Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) induced depolarization in mitochondria and eliminates mitochondrial membrane potential. FCCP is a potent mitochondrial oxidative phosphorylation uncoupler and serves as positive control for depolarization of mitochondrial membrane or for apoptosis.

Mitochondrial membrane potential is important for many mitochondrial processes and is linked to cell health. The $\Delta\psi_M$ regulates ATP synthesis, ROS production, calcium sequestration into mitochondria, import of mitochondrial proteins and mitochondrial membrane dynamics. On the contrary, $\Delta\psi_M$ itself is controlled by ATP utilization, mitochondrial proton conductance, calcium levels in mitochondria and capacity of respiratory chains. Hence, mitochondrial and cell health are interrelated and mitochondrial membrane potential is one of the feature too look for when studying mechanisms related to cell health and when testing drugs.

ITEM(S) SUPPLIED

Description	**Cat. # 786-1313 100 tests**	**Cat. # 786-1314 500 tests**
TMRE [0.5 mM]	20 µl	100 µl
FCCP Control [20 mM]	5 µl	25 µl
MMP-Assay Buffer [5 X]	5 ml	20 ml

STORAGE CONDITIONS

The kit is supplied at ambient temperature. Make small aliquots of TMRE and FCCP and store them in dark at -20°C. Store MMP-Assay Buffer at 4°C. Avoid repeated thawing of TMRE and FCCP. When stored as instructed, the kit is stable for 1 year.

WARNING

Reagents TMRE and FCCP should be considered as possible mutagens and should be handled carefully and disposed off as per local regulations. Wear protective clothing and gloves when handling these reagents.

IMPORTANT INFORMATION

- The kit components are sufficient for 100 (Cat. #786-1313) or 500 (Cat. # 786-1314) microwell assays. FCCP is an optional positive control for mitochondrial membrane depolarization.
- The exact concentration of TMRE required depends upon the cell lines being used. Typical recommended working concentrations for TMRE to start with cell lines is 200-1000 nM for microplate assay, 50-400 nM for flow cytometry and 50-200 nM for microscopy.
- TMRE is a live cell stain and is not compatible with fixed cells.
- FCCP working concentration is 20 µM.
- Optimization assay should be carried for appropriate TMRE concentration and appropriate cell density for a particular cell line. The fluorescence signal will increase proportionally with cell density until the cells reach confluence. The fluorescence signal will also increase proportionally with TMRE dye until self-quenching concentration is reached. The optimal experiment setup should have nearly confluent cell density with TMRE concentrations below self-quenching.

ADDITIONAL ITEMS REQUIRED

- Fluorometer microplate reader, fluorescence microscope or flow cytometer that can measure florescence at Ex/Em at 549/575 nm.
- Cell cultures on which drugs or the test chemical need to be tested.
- Cell culture medium and general cell supplies.
- Hemocytometer.
- Black 96 well microtiter plate for microplate assay.
- Drugs or compounds to be tested on cells.

PREPARATION BEFORE USE

- Bring the kit components to room temperature.
- Dilute the MMP-Assay Buffer [5X] to 1X with molecular grade water (Cat. #786-292) in ratio 1:4 to get required volume of MMP-Assay Buffer [1X] for the assay.
- Briefly centrifuge TMRE vial to bring the solution to the bottom of tube. Prepare appropriate working concentration of TMRE in pre-warmed cell culture medium. For example, for 500 nM working concentration, add 1 µl to 1 ml cell culture medium. Resuspend TMRE in cell culture medium using a pipette. Use 100 µl of this solution per microwell for the assay on adhered cells.
  NOTE: *Avoid repeated thawing of TMRE. Preferably store it undiluted in small one time use aliquots in dark at -20°C (TMRE is light sensitive).*

NOTE: *For suspension cells prepare 10-20X working concentration of TMRE in cell culture medium and add to well the TMRE such that final concentration is 1X.*

- FCCP Control use in assay is optional. Dilute the FCCP Control [20 mM] [1000X] to 20 µM [1X] in cell culture medium in ratio 1:1000.
  NOTE: *Avoid repeated thawing of FCCP. Preferably store it undiluted in small aliquots at -20°C.*

PROTOCOL

Microplate assay for suspension cells

1. Plate 1 x 10^5- 2 x 10^5 cell/well in 100 µl.
   NOTE: *Optimal densities for a given cell line needs to be determined by the user.*
2. Perform the assay in duplicate set. Keep two blanks with no cells as blank control and two baseline vehicle control cells with no drug/chemical treatment.
3. Treat the cell with targeted chemical compound or drug by adding the compound to the cell culture medium and incubating cells in the incubator (5% CO_2, 37°C). The incubation time for chemical compound varies depending upon compound.
   NOTE: *For chemical uncouplers like FCCP, the effect occurs in minutes. Drugs that affect protein synthesis need longer incubation time with cells.*
4. Treat the control cells (control for mitochondrial depolarization) with FCCP at final concentration of 20 µM for 10 minutes. This control is optional.
5. Add TMRE to the cells and incubate for 30 minutes in the incubator (5% CO_2, 37°C).
   NOTE: *For suspension cells prepare 10-20X working concentration of TMRE and add to well the TMRE such that final concentration is 1X.*
6. Pellet the cells by centrifuging at 1000 g for 5 minutes at room temperature.
7. Resuspend the cells in same volume MMP-Assay Buffer (1X) and pellet again.
8. Resuspend in same volume MMP-Assay Buffer (1X) and transfer to the black microplate.
9. Read the fluorescence at Ex/Em= 549/575 nm.

Microplate assay for adherent cells

1. Seed 1 x 10^5- 2 x 10^5 cell/well in 100 µl in a black 96-well microplate and incubate over night in incubator (5% CO_2, 37°C).
   NOTE: *Cell should be near confluency (monolayer) and not over-confluent. Optimal densities for a given cell line needs to be determined by the user.*
2. Perform the assay in duplicate set. Keep two blanks with no cells as blank control and two baseline vehicle control cells with no drug/chemical treatment.
3. Treat the cell with targeted chemical compound or drug by adding the compound to the cell culture medium and incubating cells in the incubator (5% CO_2, 37°C). The incubation time for chemical compound varies depending upon compound.

NOTE: *For chemical uncouplers like FCCP, the effect occurs in minutes. Drugs that affect protein synthesis need longer incubation time with cells.*

4. Treat the control cells (control for mitochondrial depolarization) with FCCP at final concentration of 20 µM for 10 minutes. This control is optional.
5. Remove the cell culture medium and add 100 µl of 1X TMRE per microwell. Incubate the cells after TMRE treatment in incubator (5% CO_2, 37°C) for 30 minutes.
   NOTE: *Make appropriate working stock of TMRE in pre warmed cell culture medium.*
6. Aspirate the media gently and add 100 µl of 1X MMP-Assay Buffer per microwell.
7. Aspirate MMP-Assay Buffer gently and add 100 µl of 1X MMP-Assay Buffer per microwell.
8. Read the fluorescence at Ex/Em= 549/575 nm.

Analysis

1. The percentage of TMRE fluorescence signal relative to baseline vehicle control is calculated as below

$$\text{Relative TMRE Fluorescence (\%)} = \frac{\text{Sample RFU - Blank RFU x 100}}{\text{Baseline vector control RFU - Blank RFU}}$$

   Sample RFU= Fluorescence of cells treated with drug or chemical compound
   Blank RFU = Fluorescence of microwells with no cells
   Baseline vector control RFU = Fluorescence of cells not treated with drug/chemical compound

2. IC_{50} value of each compound can be determined by plotting % TMRE fluorescence as a function of test compound concentration.

Flow cytometry assay

1. Seed 1 x 10^5- 2 x 10^5 cell/well in 100 µl in a black 96-well microplate and incubate over night in incubator (5% CO_2, 37°C).
   NOTE: *Cell should be near confluency (monolayer) and not over-confluent. Optimal densities for a given cell line needs to be determined by the user.*
   NOTE: *For suspension cells Plate 1 x 10^5- 2 x 10^5 cell/well in 100 µl.*
2. Perform the assay in duplicate set. Keep two blanks with no cells as blank control and two baseline vehicle control cells with no drug/chemical treatment.
3. Treat the cell with targeted chemical compound or drug by adding the compound to the cell culture medium and incubating cells in the incubator (5% CO_2, 37°C).

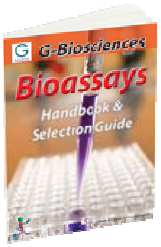
4. Add TMRE to the cells and incubate for 30 minutes in the incubator (5% CO_2, 37°C).
   NOTE: *For suspension cells prepare 10-20X working concentration of TMRE and add to well the TMRE such that final concentration is 1X.*
   NOTE: *For adherent cells prepare 1 X TMRE in cell culture medium. Replace medium with 100µl 1 X TMRE solution per well*
5. After treatment with TMRE, the adherent cells should be washed with PBS, trypsinized and brought to single cell suspendion.
6. Pellet cells and resuspend in 1 X MMP Assay Buffer.
   NOTE: *1 x 10^5 cells are usually optimal for Flow cytometry Assay. The concentration of adherent cell suspension and suspension cells should be < 1x 10^6 cells/ml*
7. Detect TMRE by excitation by 488 nm laser and detection in appropriate filter (peak emission in 575 nm). Usually this channel is FL2 in Flow Cytometer.

Microscopy assay

1. Plate the cells in accordance to the available microscope setup.
2. Add TMRE to cells in medium and incubate for 30 minutes in incubator (5%CO_2, 37°C).
   NOTE: *Prepare 10-20X working concentration of TMRE and add to well the TMRE such that final concentration is 1X.*
3. Aspirate medium and replace with 100 µl 1 X MMP Assay Buffer per microwell or same volume as of culture medium.
4. Detect fluorescence under fluorescence microscope by using appropriate filter.
   NOTE: *Cells should be imaged as quickly as possible along with appropriate controls as mitochondrial membrane potential is dependent on cell health and temperature.*

RELATED PRODUCTS

Download our Bioassays Handbook.



http://info2.gbiosciences.com/complete-bioassay-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

www.GBiosciences.com