# ab287864 – TMRE Mitochondrial Membrane Potential Assay Kit (Fluorometric)

For the labeling of active mitochondria in cells, and screening of compounds which may compromise mitochondrial membrane potential.
For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit:**
http://www.abcam.com/ab287864

## Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 6 months.

## Materials Supplied

The materials supplied are sufficient for 200 assays.

| Item | Quantity | Storage Condition |
|---|---|---|
| Assay Buffer | 100 ml | -20°C |
| TMRE Dye (1 mM, in DMSO) | 10 µl | -20°C |
| Negative Control (FCCP, 40 mM) | 10 µl | -20°C |

## Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

## Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

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.

## Assay Protocol

**Sample Preparation:**

1. Grow cells ($1x10^5$-$5x10^5$ cells per ml) of interest in a 96-well clear plate in 100 µl of culture media, according to the desired protocol.

**Δ Note**: *For flow cytometry or microscopy experiments grow the cells in 6-well plates (1x106-5x106 cells per ml) or as desired.*

2. Treat test cells with compounds, varied culture conditions, or other manipulation of interest. Treatment time can vary depending on the compound or manipulation method.
3. 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 negative control cells with 5 µl of diluted Negative Control (FCCP, final conc. 20 µM in well), for 10-20 min at 37°C in serum-free media.



**Δ Note**: *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.*

**Δ Note**: *Chemical uncouplers 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 require changes in protein synthesis or activation may take longer to manifest a change in mitochondrial membrane potential.*

**Cell Labeling**:

- Make as much diluted labelling dye solution as is needed to provide 100 µl of solution for each used well on the plate. This does not include the background control wells, which do not include dye.

1. Dilute the TMRE Dye in Assay Buffer 1:100, to get a 10 µM solution.
2. Dilute further to 200 nM by adding 2 µl of 10 µM TMRE dye to 98 µl of Assay Buffer.
3. Add 100 µl of 200 nM TMRE dye solution to each well. Do not add the TMRE dye solution to the background control wells.
4. Incubate the plate at 37°C for 15-30 min.

**Δ Note**: *We recommend adding TMRE Dye to achieve a final concentration in the range of 200-1000 nM in each well.*

**Measurement**:

1. After incubation, for adherent cells carefully discard the media. For suspension cells, spin the samples at 1,000 x g for 5 mins in a microplate compatible centrifuge and then carefully discard the media.
2. Wash the 96-well plates 3-4 times using 100 µl of Assay Buffer.
3. After washing, add 100 µl of Assay Buffer to each well.
4. Read fluorescence at Ex/Em = 549/575 nm.

**Δ Note**: *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.*

**Δ 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.*

## Technical Support


For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)