abcam

Product datasheet

TMRE-Mitochondrial Membrane Potential Assay Kit ab113852

* ★ ★ ★ ★ 4 Abreviews 166 References 6 Images

Overview

Product name TMRE-Mitochondrial Membrane Potential Assay Kit

Detection method Fluorescent

Sample type Adherent cells, Suspension cells

Assay type Cell-based (qualitative)

Assay time 0h 35m

Product overview Store kit at 4°C in the dark immediately upon receipt.

TMRE-Mitochondrial Membrane Potential Assay Kit ab113852 is used for quantifying changes in mitochondrial membrane potential in live cells by flow cytometry, microplate spectrophotometry and fluorescent microscopy.

TMRE (tetramethylrhodamine, ethyl ester) is used 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. NB: TMRE is also available as free molecule as ab274305 (Tetramethylrhodamine ethyl ester).

The TMRE protocol also uses FCCP (carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone), which is a 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.

TMRE protocol summary:

- add FCCP to appropriate control cell samples and incubate for 10 min
- incubate with TMRE for 15-30 min, pellet (suspension cells) / remove media (adherent cells) and wash with PBS / 0.2% BSA
- analyze with micro-plate reader at Ex/Em 549/575 nm, flow cytometer using 488nm laser for excitation and at emission 575 nm, or fluorescent microscope.

TMRE is only suitable for use with live (not fixed) cells.

Related assays

Notes

Review the <u>cell health assay guide</u> to learn about kits to perform a <u>cell viability assay</u>, <u>cytotoxicity assay</u> and <u>cell proliferation assay</u>.

Review the <u>metabolism assay guide</u> to learn about assays for metabolites, metabolic enzymes, mitochondrial function, and oxidative stress, and also about how to assay metabolic function in live cells using your plate reader.

How other researchers have used TMRE assay kit ab113852

Maiti AK et al. of the University of Gothenburg, Sweden, used TMRE assay kit ab113852 to identify that C. rodentium infection in mice* reduced mitochondrial transmembrane potential. In a subsequent paper, they identified that this effect was reduced by treatment with vasoactive intestinal peptide (VIP). *a model for enteropathogenic E. coli

Microplate reader, Fluor. microscope, Flow cyt.

Platform

Properties

Storage instructions

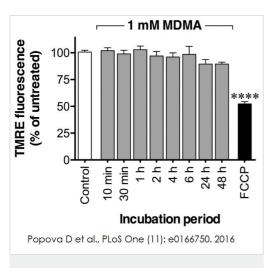
Store at +4°C. Please refer to protocols.

Components	200 tests
1mM TMRE (in DMSO)	1 x 40µl
50mM FCCP (in DMSO)	1 x 10µl

Relevance

Mitochondrial Membrane Potential is an important parameter of mitochondrial function used as an indicator of cell death. The collapse of the mitochondrial Membrane potential coincides with the opening of the mitochondrial permeability transition pores, leading to the release of cytochrome c into the cytosol, which in turn triggers other downstream events in the apoptotic cascade.

Images

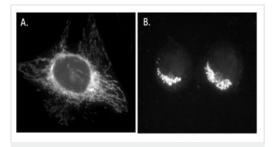


Mitochondrial membrane potential was measured using ab113852

Popova D et al., PloS One, 11(11). Fig 5. doi: 10.1371/journal.pone.0166750 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

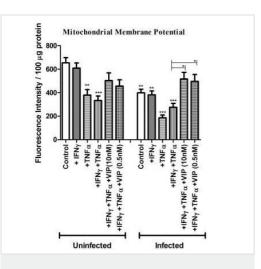
P19 neurons (750 cells/mm²) were exposed to MDMA on days 7–9 in serum-free medium for 10 min up to 48 hours. The positive control FCCP (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone), an uncoupler of mitochondrial oxidative phosphorylation, was applied at the concentration of 5 μ M

for 10 min. The cells were incubated with 500 μ M TMRE for 30–45 min at 37°C, 5% CO₂, followed by washing once with 100 μ l of HBSS containing 0.2% bovine serum albumin. A volume of 200 μ L of HBSS containing 0.2% bovine serum albumin was added to each well, and the fluorescence was measured with excitation/emission: 544/590 nm.



Immunocytochemistry/ Immunofluorescence - TMRE
—Mitochondrial Membrane Potential Assay Kit
(ab113852)

A: HeLa cells (adherent) were cultured on coverslips and stained with ab113852 (200nM 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.



Mitochondrial membrane potential assayed using

TMRE in the study of VIP

Image courtesy of Maiti AK et al. PLoS One. 2018; 13(9): e0204567. doi: 10.1371/journal.pone.0204567

Maiti AK et al (2018) used TMRE assay kit ab113852 to measure mitochondrial membrane potential in an in vitro mouse intestinal model treated with cytokines in the presence and absence of VIP (vasoactive intestinal peptide). VIP was induced by C. rodentium infection and cytokines.

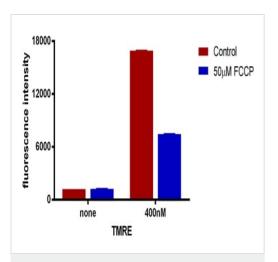
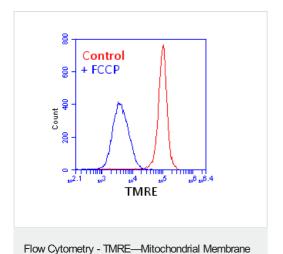


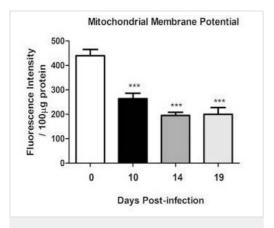
Chart showing mean fluorescent intensity +/- standard deviation from quadruplicate measurements of 400 nM TMRE stained Jurkat cells in a 96-well microplate +/- treatment with FCCP.

Analysis of TMRE staining using a fluorescent plate reader and a microplate.



Potential Assay Kit (ab113852)

Flow cytometry histogram of Jurkat cells stained with ab113852 (100nM TMRE) with (blue) or without (red) treatment with 100 μ M FCCP.



Maiti et al (2015) used TMRE assay kit ab113852 to assess mitochondrial membrane potential in murine distal colon after C. rodentium infection.

TMRE assay to assess mitochondrial function after

C. rodentium infection

Image courtesy of Maiti A K et al. Sci Rep. 2015; 5: 1543. doi: 10.1038/srep15434. Reproduced under the Creative Commons License http://creativecommons.org/licenses/by/4.0/

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