

ab113852- TMRE Mitochondrial Membrane Potential Assay Kit

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

View kit datasheet: www.abcam.com/ab113852

(use www.abcam.cn/ab113852 for China, or www.abcam.co.jp/ab113852 for Japan) This product is for research use only and is not intended for diagnostic use.

Storage and Stability

Store kit at -20°C in dark upon receipt. Kit has a shelf life of 1 year from receipt, providing components have not been diluted. If stored for an extended period, aliquot stocks to working volumes, store at -20°C and Minimize freeze/thaw cycles.

Materials Supplied

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
1 mM TMRE (1,000X) in DMSO	40 µL	-20°C	-20°C
50 mM FCCP (2,500X) in DMSO	10 µL	-20°C	-20°C

Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components - performance cannot be guaranteed if utilized separately or substituted.

Materials Required, Not Supplied

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

- Microplate reader with 450 nm wavelength filter
- Deionized or distilled water
- Fluorometric microplate reader, fluorescence microscope or flow cytometer capable of measuring fluorescence at Ex/Em = 549/575 nm
- MilliQ water or other type of double distilled water (ddH₂O)
- Sterile PBS
- Bovine Serum Albumin (BSA)
- General tissue culture supplies
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- Sterile, tissue culture treated, 96 well plate with clear flat bottom, preferably black – if performing microplate assay

Technical hints

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure you have the right type of plate for your detection method of choice.

- Make sure all necessary equipment is switched on and set at the appropriate temperature.

1. Reagent preparation

- Briefly centrifuge small vials at low speed prior to opening.

1.1 Working TMRE Solution

Aliquot stock TMRE solution so that you have enough volume to perform the desired number of assays. Avoid freeze/thaw. Store stock solution at -20°C protected from light. Prepare a working TMRE solution by adding the appropriate volume of 1 mM TMRE in appropriate cell culture media: for example, to treat cells with a final volume of 10 mL cell culture media with 1 µM TMRE, prepare 1 mL of 10 µM TMRE (10 µL 1 mM TMRE + 1 mL media) and add this to 9 mL of cell culture media containing cells.

The exact concentration of TMRE required will depend on the cell lines being used. Exact concentrations should be determined on an individual basis by the end user.

Typical working concentrations for the different experiment types using Jurkat cell lines as follows:

Typical working concentrations	
Experiment type	nM
Microplate	200 – 1000
Flow Cytometry	50 – 400
Microscopy	50 - 200

1.2 20 µM FCCP (optional control compound)

Aliquot stock FCCP solution so that you have enough volume to perform the desired number of assays. Avoid freeze/thaw. Store stock solution at -20°C **protected from light**. Dilute 50 mM FCCP in appropriate cell culture media to reach 20 µM final concentration.

2. Assay procedure

- Equilibrate all materials and prepared reagents to correct temperature prior to use.
- We recommend to assay all controls and samples in duplicate.
- TMRE is a live cell stain and it is not compatible with fixation.
- TMRE is light sensitive and susceptible to photobleaching. Maintain TMRE solutions and TMRE-labeled cells in the dark.
- Use appropriate controls – FCCP serves a depolarization control (low mitochondrial membrane potential = low TMRE signal). Include non-TMRE stained controls for both untreated and treated cells.

2.1 Grow and treat cells of interest with compounds, culture conditions or other manipulation of interest.

- 2.1.1 Treatment times can vary depending on the experimental settings. 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 protein activation may take longer to manifest.
- 2.1.2 Control compound FCCP: add 20 µM FCCP to cells in media 10 minutes prior to staining with TMRE (next step). FCCP is an uncoupler that will eliminate the mitochondrial membrane potential and prevent staining by TMRE.

2.2 Microplate assay – Suspension cells

- 2.2.1 Prepare 10^5 – 2×10^5 cells/well in 100 – 200 µL: this should provide sufficient signal.
△ **Note:** User will need to determine optimal cell densities for the given cell lines.

2.2.2 Add TMRE to cells in media and incubate for 15 – 30 minutes. Guidelines for TMRE concentration: dependent on the cell lines used in the experiment. Recommended initial concentrations = 200 – 1000 nM TMRE.
Δ **Note:** 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 so that the final concentration is 1X. Return cells to incubator and culture for an additional 15 – 30 minutes.

2.2.3 Gently pellet the cells by centrifugation and remove the culture media by aspiration, being careful to not disturb the cell pellet

2.2.4 Resuspend in a like volume of PBS/0.2% BSA and pellet again.

2.2.5 Resuspend in PBS/0.2% BSA and transfer to a microplate.

2.2.6 Read the plate on a fluorescence plate reader with setting suitable for TMRE: Ex/Em = 549/575 nm.

2.3 Microplate assay – Adherent cells

2.3.1 Grow cells so that they are not overly confluent at the time of data collection.
Δ **Note:** User will need to determine optimal cell densities for the given cell lines.

2.3.2 Seed cells in microplate and allow to adhere prior to the TMRE staining.

2.3.3 Add TMRE to cells in media and incubate for 15 – 30 minutes. Guidelines for TMRE concentration: dependent on the cell line used in the experiment. Recommended initial concentrations = 200 – 1000 nM TMRE.
Δ **Note:** 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 so that the final concentration is 1X. Return cells to incubator and culture for an additional 15–30 minutes.

2.3.4 Gently aspirate the media and replace with 100 µL of PBS/0.2% BSA. Repeat. Read the plate on a fluorescence plate reader with setting suitable for TMRE: Ex/Em = 549/575 nm bottom read.

2.4 Flow cytometry assay

2.4.1 Ideally 10^5 cells should be analyzed and cells should not be overly dense during the experiment. Use $< 10^6$ cells/mL for suspension cells and not overly-confluent adherent cells.

2.4.2 Add TMRE to cells in media and incubate for 15 – 30 minutes. Guidelines for TMRE concentration: dependent on the cell line used in the experiment. Recommended initial concentrations = 50 – 400 nM TMRE.
Δ **Note:** Removing media is not required for flow cytometry assays' however, we do find an enhancement (up to 40% in some cases) of TMRE fluorescence relative to background if the media has been exchanged for PBS/0.2% BSA. The user should determine if washing away the media benefits their analysis.

Δ **Note:** 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 so that the final concentration is 1X. Return cells to incubator and culture for an additional 15 – 30 minutes.

2.4.3 While preparing cells for flow cytometry assessment, ensure that samples are non-aggregated and in a single cell solution. For adherent cells, this will require trypsinization or other means to remove cells from the culture vessel.

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

2.5 Microscopy assay

2.5.1 Plate cells 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.

2.5.2 Add TMRE to cells in media and incubate for 15 – 30 minutes. Guidelines for TMRE concentration: dependent on the cell line used in the experiment. Recommended initial concentrations = 50 – 200 nM TMRE. We recommend to use the least amount of TMRE that gives a reasonably detectable signal.
Δ **Note:** 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 so that the final concentration is 1X. Return cells to incubator and culture for an additional 15 – 30 minutes.

2.5.3 Gently aspirate the media and replace with 100 µL of PBS. Repeat. A PBS rinse step before imaging cells will avoid background caused by cell culture media.

2.5.4 Detect TMRE in a fluorescence microscope by using the appropriate filter set.

FAQs

Can I use TMRE to stain isolated mitochondria?

TMRE staining requires fully intact mitochondria that retain membrane potential. It is not recommended for staining isolated organelles.

Can I use TMRE to stain tissue?

TMRE is best suited for imaging cells grown in a monolayer (e.g. tissue culture cells). Advanced equipment and preparation would be required to image mitochondria in a tissue preparation.

What number of cells should be used per experiment?

The exact number of cells required per experiment will have to be optimized by individual users, as exact conditions will vary. Incubation times may also require optimization based on the cell line being used.

Could you provide a formula to calculate membrane potential? There is no formula to calculate membrane potential. It is a relative measurement within an experiment. Control samples need to be run to interpret experimental results – e.g. untreated cells vs FCCP treatment to knock membrane potential down. You can then see where other samples fall between FCCP and untreated cells. There will be slight experiment-to-experiment differences depending on cell density during the assay, amount of TMRE added, etc. An absolute quantification is not possible.

Technical Support

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