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ab228569 TMRM Assay Kit (Mitochondrial Membrane Potential)

For the measurement of TMRM in suspension or adherent cells.

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

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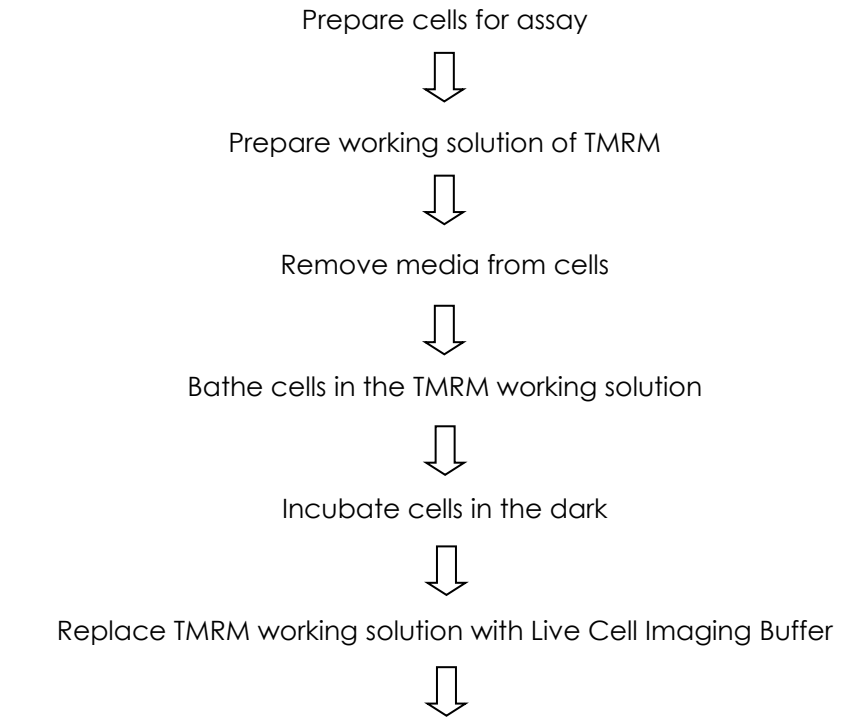
1. Overview

ab228569 TMRM Assay Kit (Mitochondrial Membrane Potential) is designed to quantify changes in mitochondrial membrane potential in living cells using flow cytometry, fluorescence microtiter plate reader analysis, or by fluorescence microscopy. It utilizes an established red-fluorescent probe tetramethylrhodamine, methyl ester (TMRM) that localizes in mitochondria and detects mitochondrial membrane depolarization.

The kit provides enough reagents to analyze 5 x 96 wells (500 samples) using the methods as described.



2. Materials Supplied and Storage

- Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components
- Prolonged exposure of labeled cells to fluorescence light sources may result in photobleaching of the dyes therein
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
TMRM Reagent	100 µl	Store below -20°C in the dark. Avoid repeated freeze-thaws.	Store below -20°C in the dark. Avoid repeated freeze-thaws.
FCCP	25 µl	-20°C	-20°C
5X Live Cell Imaging Buffer	30 ml	4°C	4°C

3. Materials Required, Not Supplied

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 548/573 nm
- Flow Cytometer with filter channel for peak emission at 575 nm
- Fluorescence microscope system equipped with bandpass filters capable of visualizing Ex/Em = 548/573 nm
- 96 well plate with clear flat bottom, preferably black (for fluorometric assay)

4. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet

- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines

- For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

All reagent preparation and cell staining procedures should be performed under sterile conditions, such as in a laminar flow hood.

Any prepared solutions should be discarded after use.

5.1 TMRM Reagent (1 mM in DMSO) 1 x 100 µl:

Prepare a working solution of the TMRM Reagent by diluting to 20 nM – 1000 nM in serum free media appropriate for the cell type being tested.

5.2 FCCP (50 mM in DMSO) 1 x 25 µl:

Dilute as necessary.

5.3 5X Live Cell Imaging Buffer 30 ml:

Dilute 1:5 in sterile DI water to prepare a 1X working solution.

6. Microscopy Procedure

All reagent preparation and cell staining procedures should be performed under sterile conditions, such as in a laminar flow hood.

Recommended TMRM reagent concentrations for microscopy assays are 50 – 200 nM.

Δ Note If a biological agent is being tested for its effects on mitochondrial membrane potential, treat cells in culture prior to use of this kit. Include non-TMRM stained controls for both untreated and treated cells.
Δ Note If you choose to use a positive control: Prepare a working solution of FCCP by diluting to 20 µM. Add 20 µM FCCP to cells in serum free media 10 minutes prior to staining with TMRM. FCCP is an un-coupler that will reduce the mitochondrial membrane potential and prevent staining by TMRM.

6.1 Prepare a working solution of the TMRM Reagent by diluting to 50 nM – 200 nM in serum free media appropriate for the cell type being tested.
6.2 Remove the growth medium from cells by aspiration.
6.3 Wash cells with 1X Phosphate Buffered Saline (PBS) (not provided). Remove by aspiration.
6.4 Bath cells in an appropriate volume of the TMRM working solution prepared in Step 6.1.
6.5 Incubate cells for in the incubator at 15-45 minutes at 37° in the dark.
6.6 Aspirate the staining medium.
6.7 Prepare enough 1X Live Cell Imaging Buffer for all wells based upon the same volumes as used in Step 6.1.
6.8 Bath cells with the prepared 1X Live Cell Imaging Buffer.
6.9 View cells using a fluorescence microscope system equipped with bandpass filters capable of visualizing Ex/Em = 548/573.

7. Microplate Assay Procedure for Suspension Cells

All reagent preparation and cell staining procedures should be performed under sterile conditions, such as in a laminar flow hood.

Recommended TMRM reagent concentrations for microplate assays are 200 – 1,000 nM.

7.1 Prepare 1 x 10^5 – 2 x 10^5 cells/ well in 100 – 200 µL of media: this should provide sufficient signal.
Δ Note User may need to determine optimal cell densities for the given cell lines.

7.2 Add TMRM to the cells in media by preparing a 10 – 20X working solution of TMRM in the appropriate media and overlay this to the experimental cultures so that the final concentration is 1X.

7.3 Return the cells to incubator and incubate for 15 – 45 minutes.

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

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

7.6 Resuspend the cells in a like volume of 1X Live Cell Imaging Buffer and transfer to an optical microplate.

7.7 Read the microplate on a fluorescence plate reader with settings suitable for TMRM dye wavelengths: Ex/Em = 548/573 nm.

8. Microplate Assay Procedure for Adherent Cells

All reagent preparation and cell staining procedures should be performed under sterile conditions, such as in a laminar flow hood.

Recommended TMRM reagent concentrations for microplate assays are 200 – 1,000 nM

8.1 Seed cells to give approximately 70% confluency at the time of data collection in microplate wells and allow to adhere prior to the TMRM staining.
Δ Note User will need to determine optimal cell densities for the given cell lines.

8.2 Remove the culture media from plate.

8.3 Add TMRM to the cells in media by preparing a 10 – 20X working solution of TMRM in the appropriate media and overlay this to the experimental cultures so that the final concentration is 1X.

8.4 Return the cells to incubator and incubate for 15 – 45 minutes.

8.5 Gently aspirate the media and wash with 100 µL of PBS/ 0.2% BSA.

8.6 Gently aspirate the PBS/0.2% BSA and then bathe the cells in 1X Live Cell Imaging Buffer. Prepare enough for all wells based upon the same volumes as used in Step 8.3.

8.7 Read the microplate on a fluorescence plate reader with settings suitable for TMRM dye wavelengths: Ex/Em = 548/575 nm.

9. Flow Cytometry Procedure

All reagent preparation and cell staining procedures should be performed under sterile conditions, such as in a laminar flow hood.

Recommended TMRM reagent concentrations for flow cytometry assays are 50 – 400 nM.

9.1 Optimally, 1 x 10^5 cells should be analyzed. Use < 1 x 10^6 cells/mL for suspension cells and <70% confluent adherent cells for all assays.

9.2 Add diluted TMRM Reagent to cells in appropriate media and incubate for 15 – 30 minutes.
Δ Note User will need to determine optimal dilution of TMRM Reagent for the cell lines used in the experiment.

9.3 Removing the media is not required for flow cytometry assays. However, we do find an enhancement of TMRM fluorescence relative to background if the media has been exchanged for Live Cell Imaging Buffer. Prepare enough for all wells based upon the same volumes as used in Step 9.2.

9.4 Prior to analysis, ensure that samples remain non-aggregated and in a single cell solution.

9.5 Detection: TMRM is excited by the 488 nm laser and should be detected in the appropriate filter channel for peak emission at 575 nm.

10. Notes

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)