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TMRE Mitochondrial Membrane Potential Assay Kit manual

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TMRE Mitochondrial Membrane Potential Assay Kit manual

The **TMRE Mitochondrial Membrane Potential Assay Kit** is designed for measuring changes in mitochondrial membrane potential in live cells using microplate fluorometry, fluorescence microscopy, or flow cytometry.

LumiTracker® Mito TMRE is a cell-permeant, positively charged red-orange dye that selectively accumulates in active mitochondria due to their negative membrane potential. When mitochondria become inactive or depolarized, their membrane potential decreases, preventing them from retaining TMRE and resulting in a reduced fluorescence signal.

The kit contains FCCP, an ATPase inhibitor that is commonly used as a positive control for mitochondrial depolarization or apoptosis.

Kit components

Kit component	Count 45372 200 assays
B9410, FCCP, ATPase inhibitor, 10 uL, 50 mM/DMSO	1
B3615, LumiTracker® Mito TMRE, 40 uL, 1 mM/DMSO	1

Transportation: at room temperature for 1 week. Store at -20 °C.

Shelf life 12 months.

Before you start

- One assay in the kit specification refers to a single well.
- Component volumes are provided in excess to allow for additional dilutions, evaporation, or instrument needs.
- TMRE requires intact mitochondria with preserved membrane potential and is not suitable for staining isolated organelles.
- TMRE stains only living mitochondria and is not suitable for use with fixed cells.
- Keep TMRE solutions and stained cells protected from light to prevent photobleaching.

Reagent Preparation

- Centrifuge all small vials briefly before opening.
- Avoid foaming when mixing or reconstituting reagents.

1. Working TMRE Solution

- Aliquot the stock TMRE solution to prevent repeated freeze-thaw cycles. Store aliquots at -20 °C, protected from light.
- Prepare the TMRE staining working solution in two steps. First, dilute the 1 mM stock solution in culture medium to a 10-20× intermediate solution, then dilute it to the required working concentration. For example, to achieve 1 µM TMRE in 10 mL of media, first prepare 1 mL of 10 µM TMRE (10 µL of 1 mM TMRE plus 990 µL of media), then add this to 9 mL of culture media.
- Optimal working concentration of TMRE depends on the cell line and should be determined empirically. Typical concentration ranges are:
    - Microplate Assay: 200-1000 nM
    - Flow Cytometry: 50-400 nM
    - Microscopy: 50-200 nM

2. Working FCCP Solution

- Aliquot the 50 mM FCCP stock solution and store at -20 °C, protected from light.
- Dilute the FCCP stock in cell culture media to a final concentration of 20 µM. For example, to prepare 10 mL of working solution, dilute 4 µL of the stock solution into the culture medium.

Assay Procedure

- Process all samples and controls in duplicate.
- Include an FCCP-treated depolarization control (low signal) and unstained cells for background subtraction.
- Seal plates securely during incubation.
- Change pipette tips between samples, standards, and reagents to prevent cross-contamination.

1. Cell Preparation

- *Treatment:* Culture and treat cells as required by your experimental design. Treatment times vary depending on the experimental settings. Uncouplers like FCCP act within minutes, while indirect treatments may require more prolonged incubation.
- *FCCP Control:* Treat cells with 20 µM FCCP for 10 minutes before TMRE staining to dissipate mitochondrial membrane potential.

2. Microplate Assay (Suspension Cells)

1. Prepare 1×10^5 to 2×10^5 cells in 100-200 µL per well. Optimize density for your cell line.
2. Add TMRE (200-1000 nM recommended starting concentration) using an intermediate 10-20× working solution in the appropriate culture medium.

Incubate for 15-30 minutes at 37 °C.

3. Centrifuge at 300 *g* for 5 min and aspirate the media without disturbing the cell pellet.
4. Resuspend in the same volume of PBS/0.2% BSA and centrifuge again.
5. Resuspend in PBS/0.2% BSA, transfer to a microplate, and measure fluorescence (Ex/Em ~552/575 nm).

3. Microplate Assay (Adherent Cells)

1. Seed cells at an optimal sub-confluent density in a microplate and allow them to adhere.
2. Add TMRE (200-1000 nM recommended starting concentration) using an intermediate 10-20× working solution in the appropriate culture medium. Incubate for 15-30 minutes at 37 °C.
3. Aspirate media and wash twice with PBS/0.2% BSA.
4. Measure fluorescence immediately (Ex/Em ~552/575 nm; bottom read recommended).

4. Flow Cytometry Assay

1. Use approximately 1×10^5 cells per sample. Keep suspension cells below 1×10^6/mL and adherent cells sub-confluent.
2. Add TMRE (50-400 nM recommended starting concentration) using an intermediate 10-20× working solution in the appropriate culture medium. Incubate for 15-30 minutes at 37 °C.

Optionally. Removing media is not required for flow cytometry assays; however, washing with PBS/0.2% BSA can reduce background.

3. Prepare a single-cell suspension. Trypsinize adherent cells if necessary.

4. Analyze using a 488 nm laser and detect emission at approximately 575 nm (for example, FL2 channel).

5. Microscopy Assay (Live-Cell Imaging)

Important! Image promptly after staining to maintain physiological conditions.

1. Grow cells as appropriate for your imaging system.
2. Add TMRE using an intermediate 10-20× working solution in appropriate culture medium (50-200 nM recommended starting concentration; use the minimal signal-saturating concentration). Incubate for 15-30 minutes at 37 °C.
3. Gently replace media with pre-warmed PBS. Repeat as needed to reduce background.
4. Image immediately using an appropriate fluorescence filter set (Ex/Em ~552/575 nm).

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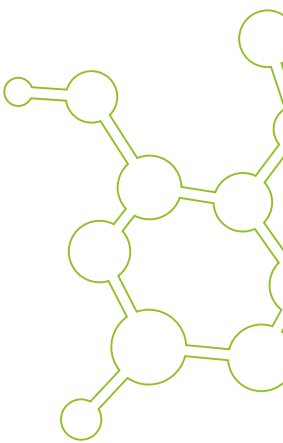

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