MitoProbe™ TMRM Kit for Flow Cytometry

Catalog No. M20036

Pub. No. MAN0017633 **Rev.** B.0

Product information

Presence of a mitochondrial membrane potential (ΔΨm), the electrochemical gradient between the interior and exterior of the mitochondria, is a sign of healthy cells. As a cationic and lipophilic fluorescent dye, TMRM can be used to detect changes in the membrane potential by tracking the redistribution of the dye. A healthy cell will display a robust fluorescent signal; however, the fluorescence diminishes when the mitochondrial membrane depolarizes due to changes such as induction of apoptosis.

Table 1. Contents and storage

Product	Amount	Concentration	Storage*
TMRM (Component A)	100 µL	20 µM solution in DMSO	• Store in freezer (–5°C to –30°C) • Desiccate • Protect from light
CCCP (Carbonyl cyanide 3-chlorophenylhydrazone) (Component B)	125 µL	50 mM solution in DMSO	
* When stored as directed, the product is stable for at least 6 months from the date receipt. **Approximate Ex/Em maxima:** 561 nm/585 nm			

Materials required, but not provided

- Cells of interest
- Phosphate-buffered saline (PBS) or similar protein-free buffer
- Culture media containing protein such as FBS or BSA
- Flow cytometer

Caution

No data are available addressing the mutagenicity or toxicity of the TMRM reagent. Handle the DMSO and DMSO containing reagents with caution because DMSO is known to facilitate the entry of organic molecules into tissues. Always wear suitable protective clothing, gloves and eye/face protection when handling this reagent. Dispose of the reagents in compliance with all pertaining local regulations.

Storage and handling

Upon receipt, store the kit components desiccated at ≤–20°C until required for use. When stored as directed, TMRM and CCCP reagents are stable for at least 6 months. Allow the products to warm to room temperature before opening the vials. Stock solution can be frozen after use, but should be aliquoted to avoid repeated freezing and thawing.

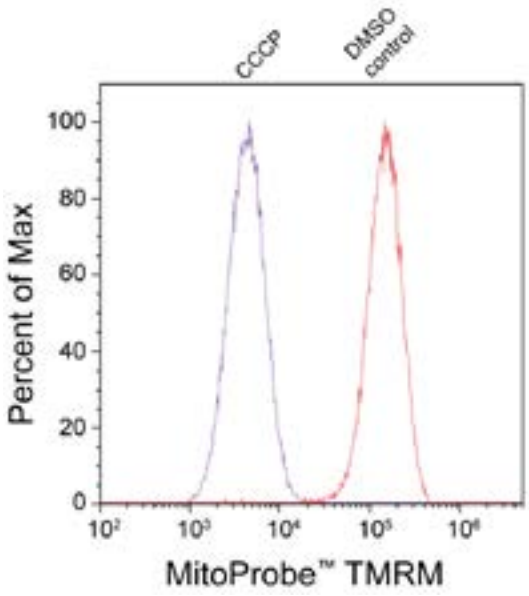

Figure 1. MitoProbe™ TMRM detects changes in mitochondrial membrane potential. Jurkat cells, a human T-lymphocyte cell line, were treated with DMSO (control) or 50 µM CCCP to induce mitochondrial membrane depolarization. Cells were subsequently stained with MitoProbe™ TMRM reagent and analyzed on an Attune™ NxT Flow Cytometer using a 561-nm laser for excitation and 585/16BP-nm filter for emission.

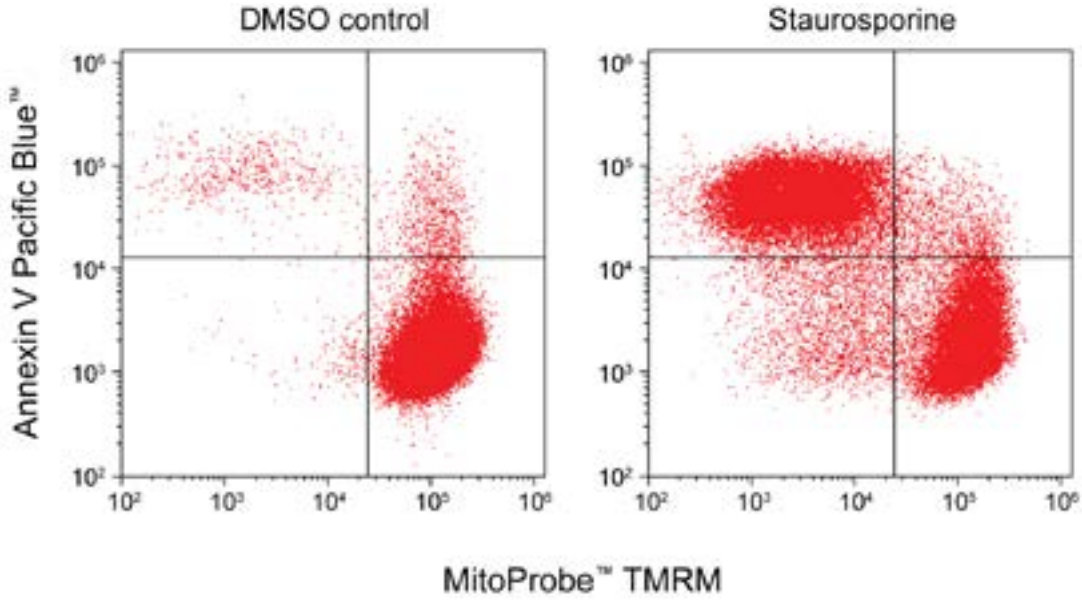

Figure 2. Detection of mitochondrial membrane potential changes in apoptotic cells. Jurkat cells, a human T-lymphocyte cell line, were treated with DMSO (control) or 500 nM staurosporine for 2 hours. Cells were subsequently stained with 20 nM MitoProbe™ TMRM reagent for 30 minutes at 37°C, followed by a wash and additional stain with Annexin V Pacific Blue™ reagent. Data were acquired on an Attune™ NxT Flow Cytometer using a 561-nm laser and a 585/16-nm emission filter for the MitoProbe™ TMRM reagent and a 405-nm laser and 440/50-nm emission filter for the Annexin V Pacific Blue™ reagent. Staurosporine induced apoptosis, resulting in a mixed population of cells containing a population of healthy MitoProbe™ TMRM-positive cells as well as a population of apoptotic, Annexin V Pacific Blue™-positive, MitoProbe™ TMRM-low cells.

Methods

Prepare reagent

MitoProbe™ TMRM reagent is provided as a 1000X stock solution at a concentration of 2 µM in DMSO. To use it, simply add 1 µL of 20 µM TMRM reagent to 1 mL of 1×10^6 cells.

Stain cultured cells with TMRM reagent

Before beginning your experiment, ensure that the vial of CCCP has equilibrated to room temperature.

1. For each sample, resuspend the cells in cell culture medium or PBS at approximately 1×10^6 cells/mL.
2. For the control sample, add 1 µL of 50 mM CCCP to the cells, then incubate for 5 minutes at 37°C, 5% CO_2.
3. For experimental samples, add 1 µL from the 20 µM stock TMRM reagent solution (20 nM final concentration) and incubate for 30 minutes at 37°C, 5% CO_2.
4. *Optional*: Wash the cells once in 1 mL of culture medium or PBS, then resuspend the cells in 500 µL of PBS (or other suitable buffer).
5. Analyze the cells on a flow cytometer with 561-nm excitation using emission filters appropriate for R-phycoerythrin.

   Note: TMRM is optimally excited at 561 nm; however, it can also be excited by the 532-nm or 488-nm laser lines. In each case, the PE equivalent bandpass filter should be used to detect TMRM.

Ordering information

Cat. No.	Product	Unit size
M20036	MitoProbe™ TMRM Kit for Flow Cytometry	100 tests

Documentation and support

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision	Date	Description
B.0	21 June 2018	Product name correction in the Methods section
A.0	16 February 2018	New user guide

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