

MitoPT TMRM Mitochondrial Depolarization Assay Kit | MitoPT TMRM assay kit

MitoPT TMRM Mitochondrial Depolarization Assay Kit

CATALOG #
AAA52710

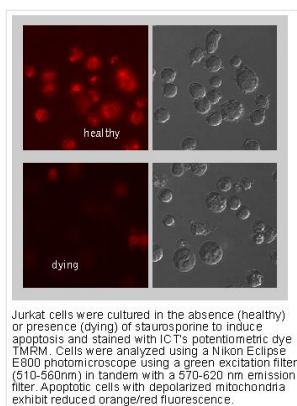
PRODUCT SYNONYMS

MitoPT TMRM Mitochondrial Depolarization; MitoPT TMRM Mitochondrial Depolarization Assay Kit; MitoPT TMRM assay kit

PRODUCT SPECIFICATIONS

Reagents	TMRM
Sample Protocol	Culture cells up to 1×10^6 cells/mL. Expose cells to apoptosis inducing agent or metabolic stress environment using your experimental protocol. Reconstitute the TMRM reagent with 100 μ L DMSO to form the stock concentrate (may be frozen for future use). Dilute the stock concentrate with 1X PBS, cell culture media, or wash buffer to achieve a 10 μ M intermediate stock for spiking cell aliquots. Spike cell cultures with 10 μ M TMRM to achieve a 20-200 nM TMRM concentration. Incubate cells in TMRM (20-200 nM) solution, 15-20 minutes at 37 degree C in dark. Wash cells once in wash buffer if using > 50 nM TMRM staining concentration. Analyze data using a fluorescence microscope, plate reader, or flow cytometer.
Target	Mitochondrial Depolarization
Excitation / Emission	548 nm / 573 nm
Method of Analysis	Flow Cytometer, Fluorescence Microscope, Fluorescence Plate Reader
Types of Samples	Cell Culture, Tissue
Preparation and Storage	Mixed Storage Conditions: CCCP at -20 degree C Other Kit Components at 2 - 8 degree C

IMAGES



Standard Curve (Sample)

ADDITIONAL INFORMATION

Related Product Information for MitoPT TMRM assay kit	Background: Mitochondria play a central role in the biochemical processes associated with the life and death stages of eukaryotic cells¹. Under normal physiological conditions, a membrane-based proton pump generates an electrochemical gradient, enabling the production of ATP to drive cellular energy dependent processes². The oxidation of glucose and fatty acids by enzymes associated with the mitochondrial respiratory chain establishes a proton and pH gradient across the mitochondrial inner membrane, resulting in a transmembrane electrical potential gradient ($\Delta\psi$) of -80 to -120 mV and a pH gradient of 0.5-1.0 pH units^{3, 4}. Depolarization of the inner mitochondrial membrane can lead to an opening of the mitochondrial permeability transition pore (PTP)⁵. This results in the leakage of intermembrane proteins, including cytochrome c, that facilitate the induction of apoptosis through apoptosome formation⁶. Caspase activation has been shown to accelerate the process of $\Delta\psi$ loss⁷. Moreover, a feedback mechanism that results in the generation of reactive oxygen species (ROS) further accelerates the rate of cell death⁷. Because mitochondrial dysfunction has been closely tied to such neurodegenerative diseases as Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis, mitochondria remain an important organelle of study⁸. Loss of mitochondrial $\Delta\psi$, indicative of apoptosis, can easily be detected using lipophilic, cationic fluorescent redistribution dyes such as MitoPT reagents: tetramethylrhodamine ethyl ester (TMRE), tetramethylrhodamine methyl ester (TMRM), and 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1)⁹. These dyes have a delocalized positive charge dispersed throughout their molecular structure, and yet their lipophilic solubility enables them to be readily membrane permeant and penetrate living cells⁹⁻¹¹. They redistribute across cell membranes according to the Nernst equation in a voltage-dependent manner⁹⁻¹¹. Accordingly, they possess a low membrane partition coefficient: a low tendency to non-specifically associate with intracellular organelles and macromolecules. These excellent potentiometric dyes also exhibit minimal self-quenching, low cytotoxicity, and are reasonably photostable¹¹. The MitoPT dyes exhibit very low toxicity and display rapid and reversible membrane equilibration properties¹¹. TMRE and TMRM assay kits easily distinguish between healthy, non-apoptotic cell populations and cell populations that are transitioning into an apoptotic state. Inside a healthy, non-apoptotic cell, the lipophilic TMRE or TMRM dye, bearing a delocalized positive charge, enters the negatively charged mitochondria where it accumulates and fluoresces orange upon excitation¹⁰. When the mitochondrial $\Delta\psi$ collapses in apoptotic cells, TMRE or TMRM no longer accumulates inside the mitochondria, instead becoming more evenly distributed throughout the cytosol. When dispersed in this manner, overall cellular fluorescence levels drop dramatically. Healthy cells fluoresce orange, whereas cells with depolarized mitochondria exhibit lower levels of orange fluorescence. TMRE and TMRM kits can be used in conjunction with existing research protocols. Grow cells following the usual cell cultivation protocol. If using an apoptosis induction model system, induce apoptosis according to the existing procedure, reserving a noninduced population of cells as a control. Once apoptosis has been induced or the mitochondrial membrane has been depolarized by a known method, such as using CCCP (included), spike MitoPT dye solution into each sample and control. Incubate the cells for 15-30 minutes at 37 degree C to allow MitoPT to equilibrate within the polarized mitochondria. If cells are not undergoing some form of metabolic or apoptotic stress, the mitochondrial $\Delta\psi$ will remain intact, and MitoPT will accumulate within the slightly negative/alkaline environment of the mitochondria and fluoresce brightly upon excitation. If the cells are apoptotic, the mitochondrial $\Delta\psi$ will break down, causing MitoPT to disperse throughout the cell cytosol. This results in a dramatic reduction in the fluorescence of the affected mitochondria, and as a result, overall cellular fluorescence is diminished significantly. MitoPT is for research use only. Not for use in test procedures.
Product Categories/Family for MitoPT TMRM assay kit	Apoptosis Assays/Apoptosis Detection
References	Plasek, J. and K. Sigler. 1996. Slow fluorescent indicators of membrane potential: a survey of different approaches to probe response analysis. J. Photoch. Photobio. B. 33: 101-124. Ehrenberg, B., V. Montana, M. Wei, J.P. Wuskell, and L.M. Loew. 1988. Membrane potential can be determined in individual cells from the Nernstian distribution of cationic dyes. Biophys. J. 53: 785-794. Waggoner, A.S. 1979. Dye indicators of membrane potential. Ann Rev. Biophys Bioeng. 8: 47-68. o, R.C. and L.W. Grotyohann. 1999. Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. Biophys. J. 76: 469-477. Wlodkowic, D., J. Skommer, and J. Pelkonen. 2006. Multiparametric analysis of HA14-1 induced apoptosis in follicular lymphoma cells. Leukemia Res. 30: 1187-1192.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

AAA Biotech | Tel: +1 (619) 604-9440 | Fax: +1 (619) 604-9441 | contact@aaabiotech.com
 Online datasheet: <https://stage.aaabiotech.com/assay-kits/mitopt-tmr-mitochondrial-depolarization/52710>