

MitoPT JC-1 assay kit

MitoPT JC-1 Assay Kit (100 tests)

CATALOG #
AAA52718

PRODUCT SYNONYMS

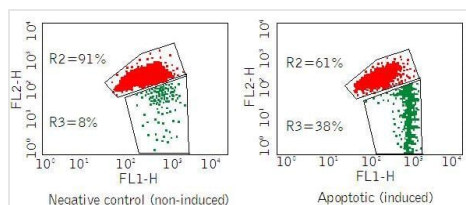
MitoPT JC-1; MitoPT JC-1 Assay Kit (100 tests); MitoPT JC-1 assay kit

PRODUCT SPECIFICATIONS

Preparation and Storage

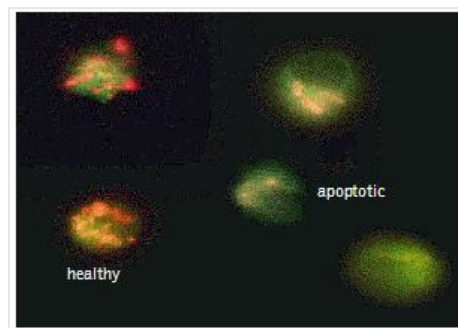
Store at -20 degree C

IMAGES



Application Data

Using a flow cytometer to analyze cells labeled with MitoPT JC-1, the instrument will measure apoptosis by monitoring the amount of red fluorescence in each region. Healthy cells with intact mitochondria fluoresce due to aggregated MitoPT JC-1 and appear in R2. As the mitochondrial membrane permeability collapses and cells enter apoptosis, MitoPT JC-1 reagent is dispersed throughout the cell, converting to a green fluorescent monomeric form. The amount of red fluorescence drops as these cells enter R3. In this example, Jurkat cells were either treated with DMSO (negative, non-induced cells) or with staurosporine (apoptotic, induced cells) for 4 hours and then labeled with MitoPT JC-1 for 15 minutes.



Application Data

Healthy cells fluoresce red, dying cells fluoresce green. Jurkat cells were stained with ICT's MitoPT JC-1 dye and analyzed with a fluorescence microscope containing a longband path filter (excitation at 490nm and emission >510 nm). In non-apoptotic healthy cells (2 cells at left), the reagent aggregates inside intact mitochondria and fluoresces red. As the mitochondrial membrane potential drops and cells enter apoptosis, the dye disperses throughout the cell. The reagent then assumes a monomeric form and fluoresces green (3 cells on right).

ADDITIONAL INFORMATION

Related Product Information for MitoPT JC-1 assay kit	Background/Introduction: 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 ($\Delta\psi_m$) 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 $\Delta\psi_m$ loss⁷. Moreover, a feedback mechanism that results in the generation of reactive oxygen species 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 $\Delta\psi_m$, 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; meaning 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. MitoPT JC-1 assay kits easily distinguish between healthy, non-apoptotic cell populations and those cell populations that are transitioning into an apoptotic state. Inside a healthy, non-apoptotic cell, the lipophilic JC-1 dye, bearing a delocalized positive charge, enters the negatively charged mitochondria where it accumulates¹⁰. When the mitochondrial $\Delta\psi_m$ collapses in apoptotic cells, MitoPT JC-1 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 and green, whereas cells with depolarized mitochondria fluoresce green and exhibit lower levels of orange fluorescence. MitoPT JC-1 kits can be used in conjunction with other existing research protocols. Grow cells following the usual cell cultivation protocol. If using an apoptosis induction model system, simply induce apoptosis according to the existing procedure, reserving a non-induced population of cells as a control. Once apoptosis has been induced in the cells, or the mitochondrial membrane has been depolarized by another method, such as using CCCP (included in the kit), spike MitoPT JC-1 dye solution into each sample and control. Incubate the cells for 15-30 minutes at 37 degree C to allow MitoPT JC-1 to equilibrate within the polarized mitochondria. If the cells are not undergoing some form of metabolic or apoptotic stress, the mitochondrial $\Delta\psi_m$ will remain intact, and MitoPT JC-1 will accumulate within the slightly negative/alkaline environment of the mitochondria and fluoresce brightly upon excitation. If the cells are apoptotic, the mitochondrial $\Delta\psi_m$ will break down, causing MitoPT JC-1 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.
Product Categories/Family for MitoPT JC-1 assay kit	Apoptosis Assays/Apoptosis Detection

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-jc-1/52718>