#12664 Store at RT and -20°C

Mitochondrial Membrane Potential Assay Kit (I)

1 Kit (500 assays (96 well format))

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Description: Mitochondrial Membrane Potential Assay Kit (I) is a fluorescent assay that detects the mitochondrial membrane potential in living cells. The kit includes the cationic dye JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide) and a mitochondrial membrane potential disruptor CCCP (carbonyl cyanide 3-chlorophenylhydrazone). JC-1 is a cell membrane permeable, fluorescent dye with green emission (~520 nm). When JC-1 accumulates in intact mitochondria, the dye forms aggregates that lead to orange-red fluorescence (~590 nm). The relative fluorescent units (RFU) of the orange-red emission, and the ratio of red to green emission, can be used as an indicator for mitochondria membrane potential.

Background: Mitochondria function as the main cellular powerhouse and play important roles in other processes, such as steroid metabolism, calcium homeostasis, apoptosis, and cellular proliferation. Mitochondrial membrane potential is a key indicator of mitochondrial function and cell health (1,2). The dissipation of mitochondrial membrane potential is considered an early indicator of apoptosis (3).

JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide) is a cell membrane permeable, cationic dye. In normal cells, JC-1 concentrates in mitochondria to form aggregates in response to relatively high membrane potential. Decreased mitochondrial membrane potential results in dispersal of mostly monomeric JC-1 throughout the cell. When excited at 490 nm, JC-1 monomers emit a green fluorescence with a maximum at ~520 nm. Aggregates of JC-1 emit an orange-red fluorescence with a maximum at ~590 nm. Therefore, the fluorescence intensity of the orange-red emission and the ratio of orange-red fluorescence to green fluorescence can be used to measure mitochondrial membrane potential and serve as an indicator of overall cell health (4).

Specificity/Sensitivity: Mitochondrial Membrane Potential Assay Kit (I) is expected to detect the mitochondrial membrane potential in living cells across all species. For the best result, a cell number titration is recommended when using a plate-reader and a 96-well plate.

Background References:

(1) Perry, S.W. et al. (2011) *Biotechniques* 50, 98-115.

(2) Nesti, C. et al. (2007) *Biosci Rep* 27, 165-71.

(3) Petit, P.X. et al. (1995) *J Cell Biol* 130, 157-67.

(4) Perelman, A. et al. (2012) *Cell Death Dis* 3, e430.

Products Included	Item	Quantity	Storage Temp
JC-1	14121	5 x 15 µg	–20°C
CCCP	13550	100 µl	–20°C
Phosphate Buffered Saline (PBS, 20X)	9808	25 ml	Room Temp

Note: All components in this kit are stable for at least 12 months when stored at the recommended temperature and left unused. Upon receipt, #9808 should be removed from kit box and stored at room temperature. Remaining components should be stored at -20°C.

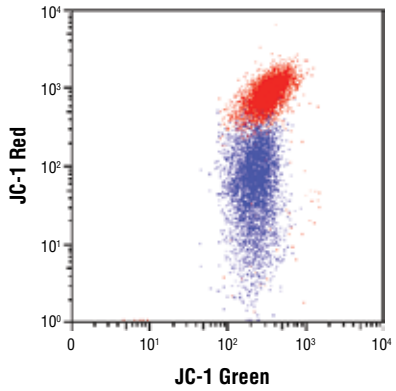
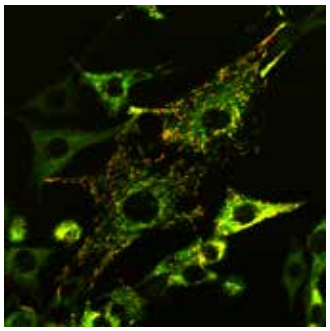


Figure 1. Flow cytometric analysis of Jurkat cells labeled with JC-1 (2 µM), untreated (red) or CCCP-treated (50 µM, 37°C, 30 min; blue).



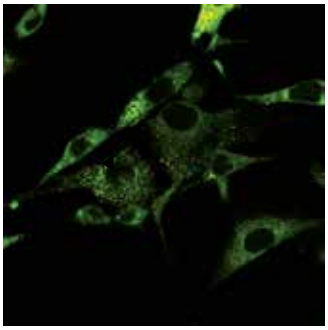


Figure 2. Confocal immunofluorescent analysis of NIH/3T3 cells seeded at 2x10^5 cell/ml in 96-well black plate with clear bottom and incubated overnight. Cells were then treated with JC-1 (2 µM, 30 min; upper) or with JC-1 (2 µM, 30 min) and CCCP (400 µM, 30 min; lower).

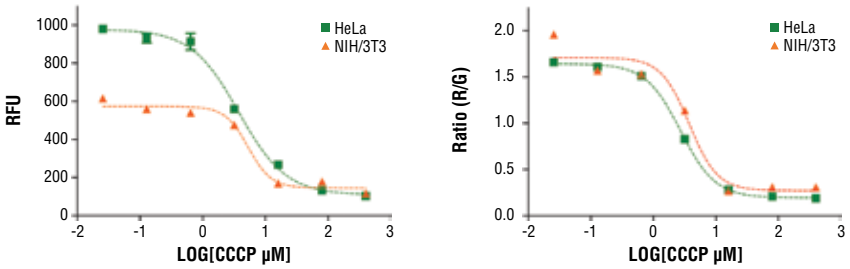


Figure 3. HeLa (3x10^5 cell/ml, green) and NIH/3T3 (2x10^5 cell/ml, orange) cells were incubated with JC-1 (2 µM, 30 min) and various concentrations of CCCP, as indicated. Relative fluorescent units (RFU) are shown on the left, while the RFU Ratio (RFU(red)/RFU(green)) is shown on the right.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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Mitochondrial Membrane Potential Assay Protocol

Instrumentation required

1. Flow cytometer with excitation between 480 nm and 490 nm, and emission at 520 nm and 590 nm.
2. Plate reader that can read 96-well plates with excitation between 480 and 490 nm, and emission at 520 nm and 590 nm.
3. 96-well plate (black with clear bottom).

Reagent Preparation

1. Prepare 1X PBS by diluting 20X PBS (included in each kit) in reverse osmosis deionized (RODI) or equivalently purified water.
   Note: For flow cytometry, adding 0.5% BSA to wash buffer may help to prevent cell loss during the process.
2. Add 110 µl DMSO to each vial of JC-1, this will make 200 µM stock solution and each vial has enough for one 96-well plate (1 µl/well), or 10 flow cytometry tests (10 µl/test).
3. If CCCP is used as a positive control, allow CCCP solution to equilibrate to room temperature before use.

A. Protocol for suspension cells

1. Suspend cells in warm medium or 1X PBS at $1x10^6$ cells/ml. Prepare 1 ml aliquots—each 1 ml cell aliquot is 1 assay point—making sure there are enough cells for your experiment. For example, if 1 compound is going to be assayed at 3 different concentrations, prepare 4 x 1 ml samples are needed (this includes a positive control).
2. Add test compound(s) to sample tubes at desired concentration and incubate cells for desired time. For best results, a compound titration and incubation time course can help to determine the assay conditions. To prepare the positive control (mitochondrial membrane potential loss), add 1 µl of 50 mM CCCP (supplied with this kit) to the control tube for a 50 µM final concentration, incubate cells at 37°C for 5 minutes.
3. Add 10 µl of the 200 µM JC-1 solution to each sample (2 µM final concentration) and incubate cells in an incubator (37°C, 5% CO_2) for 15 to 30 minutes.
   Note: Quick acting compounds, such as CCCP, can be added to sample at the same time as JC-1.
4. Centrifuge sample at 300 g for 5 min, then remove the supernatant.
5. Wash cells once with 1 ml warm 1X PBS wash buffer, repeat step 4.
6. Resuspend cells into 1000 µl warm 1X PBS.
7. Analyze sample on a flow cytometer. If samples are to be analyzed on plate reader, transfer 100 µl cell suspension/well to a black 96-well plate with a clear bottom. The settings are: excitation between 480 and 490 nm, and emission at 520 nm and 590 nm.

B. Protocol for adherent cells

1. Plate cells to a 96-well plate in warm culture medium and culture cells in an incubator overnight to allow cells to attach to the plate. A typical cell number is 1-5 x 10^4 cells/well, but a cell number titration may be necessary for optimal results.
2. Aspirate the medium from the plate and add test compounds in growth medium or 1X PBS to plate at 100 µl/well at desired concentration and incubate cells for the desired time. Compound titration and incubation time course can help to determine best assay conditions. For the positive control (mitochondria membrane potential loss), add CCCP (supplied with this kit) to the control wells for a 50 µM final concentration and incubate cells at 37°C for 5 minutes. For example, add 1 µl of 50 mM stock CCCP to 100 µl medium to make 500 µM CCCP; then add 10 µl of this 500 µM CCCP to each well containing 100 µl medium to get final concentration of 50 µM.
3. Add 1 µl of JC-1 stock (200 µM) to each well to get a final concentration of 2 µM and place the plate in an incubator (37°C, 5% CO_2) for 15 to 30 minutes.
   Note: JC-1 can be diluted 1:10 in medium to make 20 µM solution, add 10 µl of 20 µM JC-1 to each well containing 100 µl medium for a final concentration of 2 µM.
4. Aspirate the solution from the plate.
5. Wash plate once with warm 1X PBS, and then add 100 µl 1X PBS/well to the plate.
6. Analyze samples on the plate reader. The settings are: excitation between 480 and 490 nm, and emission at 520 nm and 590 nm.