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A Geno Technology, Inc. (USA) brand name

JC-1 Mitochondrial Membrane Potential Assay

(Cat. # 786-1321, 786-1322)



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INTRODUCTION

Mitochondrial membrane potential ($\Delta\psi_M$) is one of the key parameter of mitochondrial function and serves as an indicator of cell health. JC-1 Mitochondrial Membrane Potential Assay comprise of lipophilic cationic fluorescent dye; 5,5,6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimi-dazolycarbocyanine iodide (JC-1). In healthy cells, JC-1 enters the energized mitochondria and form aggregates which change the fluorescent property of JC-1 dye. JC-1 monomers exhibit green fluorescence where as the JC-1 dye aggregates also called J-aggregates show intense red fluorescence. Unhealthy or apoptotic cells have low $\Delta\psi_M$. JC-1 does not form aggregates in mitochondria's with low $\Delta\psi_M$ and remain in monomeric form and exhibit green fluorescence. Thus higher the ratio of red to green fluorescence, the higher is the polarization of mitochondrial membrane.

Mitochondrial membrane potential is important for many mitochondrial processes and is linked to cell health. The $\Delta\psi_M$ regulates ATP synthesis, ROS production, Calcium sequestration into mitochondria, import of mitochondrial proteins and mitochondrial membrane dynamics. On contrary, $\Delta\psi_M$ itself is controlled by ATP utilization, mitochondrial proton conductance, calcium levels in mitochondria and capacity of respiratory chains. Hence, mitochondrial and cell health are interrelated and mitochondrial membrane potential is one of the feature too look for when studying mechanisms related to cell health and when testing drugs.

ITEM(S) SUPPLIED

Description	Cat. # 786-1321 25 tests	Cat. # 786-1322 100 tests
JC-1 Dye	1 vial	4 vials
DMSO [Sterile Filtered]	250 μ l	1 ml
MMP-Assay Buffer [5 X]	2 x 5ml	2 x 20 ml

STORAGE CONDITIONS

The kit is supplied at ambient temperature. Reconstitute the JC-1 with DMSO (Sterile Filtered) provided in kit and aliquot in preferable one time use aliquots. Store the aliquots of JC-1 at -20°C in dark. Store MMP-Assay Buffer [5 X] at 4°C. When stored as instructed, the kit is stable for 1 year.

WARNING

JC-1 should be considered as possible mutagen and should be handled carefully and disposed off as per local regulations. Wear protective clothing and gloves when handling this reagent.

IMPORTANT INFORMATION

- The JC-1 Mitochondrial Membrane Potential Assay are available in 25 (Cat. #786-1321) or 100 (Cat. # 786-1322) flow cytometry assays format with 1ml as labeling volume.
- JC-1 staining works on live cells only. It does not work on fixed cells.
- Recommended concentration of JC-1 for flow cytometry is 2 μ M.
NOTE: *The concentration may vary depending upon culture conditions and cell types and should be determined by the user*
- Recommended concentration of JC-1 for microplate and microscopy assay is 1-10 μ M.
NOTE: *The concentration may vary depending upon culture conditions and cell types and should be determined by the user.*
- JC-1 is light sensitive. Do not expose to direct light when handling and staining cells.

ADDITIONAL ITEMS REQUIRED

- Flow cytometer, fluorescence microscope or fluorometer microplate reader that can measure fluorescence of J-aggregate using excitation of 535 ± 20 nm and an emission at 590 ± 20 nm and monomers of JC-1 at excitation/ emission wavelength 485/535 nm respectively.
- Cells on which drugs or the test chemical need to be tested.
- Cell culture medium and general cell supplies.
- Haemocytometer.
- Black 96 well microtiter plate for microplate assay.
- Drugs or compounds to be tested on cells.
- Optional control for membrane depolarization: FCCP (Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone) (Cat. #786-1316).

PREPARATION BEFORE USE

- Bring the kit components to room temperature.
- Dilute the MMP-Assay Buffer [5X] to 1 X with molecular grade water in ratio 1:4 to get required volume of MMP-Assay Buffer [1X] for the assay.
- Centrifuge the JC-1 Dye vial briefly. Add 250 μ l DMSO to 1 vial of JC-1 and mix well with a pipette. Aliquot in small, preferably one time use aliquots in brown vials. The prepared stock solution of JC-1 in 200 μ M. Store the aliquots at -20°C in dark.

NOTE: *Avoid repeated thawing of JC-1 stock solutions.*

PROTOCOL

Flow cytometry assay

1. Culture cells in appropriate 6-, 12- or 24-well cell culture plates a density around 5×10^5 cells/ml overnight in incubator (5% CO_2 , 37°C).

2. Treat the cells with or without the test compound. Perform each assay in duplicate set.
NOTE: *Treatment with or without test compound is determined by end user.*
3. Prepare 2 μM JC-1 working stock in cell culture medium. Add 10 μl of 200 μM JC-1 stock solution per 1 ml cell culture medium to get 2 μM JC-1 solutions.
4. Replace the cell culture medium with same volume of 2 μM JC-1 solution under cell culture hood.
5. Incubate the plates in incubator (5% CO_2 , 37°C) for 15-30 minutes.
6. Harvest cells by trypsinization or by scraping (determined by user).
NOTE: *Cells can be directly analysed at this step under Flow Cytometer.*
- Optional steps:**
7. Pellet cells at 1000 g for 5 minutes.
8. Add equal volume of prewarmed 1 X MMP-Assay Buffer and resuspend cells gently.
9. Pellet cells at 1000 g for 5 minutes and resuspend in fresh prewarmed 1 X MMP-Assay Buffer.
10. Analyze cells on flowcytometry using 488 nm excitation and green (FL1) or orange-red emission (FL2).

Microscopy assay

1. Culture cells on cover slips on appropriate 6-, 12- or 24-well cell culture plates a density around 5×10^5 cells/ml overnight in incubator (5% CO_2 , 37°C).
2. Treat the cells with or without the test compound. Perform each assay in duplicate set.
NOTE: *Treatment with or without test compound is determined by end user.*
3. Prepare 1-10 μM JC-1 working stock in cell culture medium. For example, for 1 μM working stock, add 5 μl of JC-1 (200 μM) per 1 ml cell culture medium.
NOTE: *Optimal concentration depends on cell types and density and should be determined by user. Recommended range 1-10 μM .*
4. Replace the cell culture medium with same volume of JC-1 working solution under cell culture hood
5. Incubate the plates in incubator (5% CO_2 , 37°C) for 15-30 minutes.
6. Observe the cells under fluorescence microscope using standard filters for FITC and Rhodamine.
Optional steps:
7. Rinse the cells with prewarmed 1 ml of 1 X MMP-Assay Buffer and observe cells in 1 X MMP-Assay Buffer under fluorescence microscope with appropriate filters.

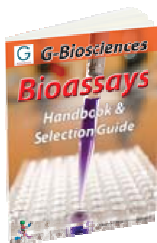
Microplate assay

1. Culture cells in 96-well black microtiter plate at a density of 5×10^4 - 5×10^5 cells per well in 100 μl cell culture medium overnight in incubator (5% CO_2 , 37°C).

2. Treat the cells with or without the test compound. Perform each assay in duplicate set.
NOTE: *Treatment with or without test compound is determined by end user.*
3. Prepare 1-10 μM JC-1 working stock in cell culture medium. For example, for 1 μM working stock, add 5 μl of JC-1 (200 μM) per 1 ml cell culture medium.
NOTE: *Optimal concentration depends on cell types and density and should be determined by user. Recommended range 1-10 μM .*
4. Replace the cell culture medium with 100 μl of JC-1 working solution per well under cell culture hood.
5. Incubate the plate in incubator (5% CO_2 , 37°C) for 15-30 minutes.
6. Aspirate the supernatant and add 100 μl of prewarmed 1 X MMP-Assay Buffer per well.
7. Remove the buffer from wells and after that add fresh 100 μl of prewarmed 1 X MMP-Assay Buffer per well
8. Read the fluorescence at Ex/Em: 535nm/595 nm for JC-1 aggregates and at Ex/Em: 485 nm/535 nm for JC-1 monomers.
9. Plot the graph against the test compound concentration and ratio of red to green fluorescence to determine effects of compound on cell health.

RELATED PRODUCTS

Download our Bioassays Handbook.



<http://info2.gbiosciences.com/complete-bioassay-handbook>

For other related products, visit our website at www.GBiosciences.com or contact us.



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