JC-10 Mitochondrial Membrane Potential Assay

(Cat. # 786-1541, 786-1549)

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INTRODUCTION

Mitochondrial membrane potential ($\Delta\psi_M$) is one of the key parameters of mitochondrial function and serves as an indicator of cell health. JC-10 Mitochondrial Membrane Potential Assay is based upon JC-10 dye, which is a superior alternative to JC-1 dye. Even though JC-1 dye is widely used in various labs for measuring mitochondrial membrane potential, it shows poor solubility in aqueous buffers and tends to precipitate at higher concentrations. On the contrary, JC-10 shows greater solubility even at higher concentrations. JC-10 dye, like JC-1, is a cationic, lipophilic dye that gets concentrated in healthy mitochondria to form reversible red-fluorescent JC-10 aggregates (Ex/Em=540/590nm). In apoptotic cells, the mitochondrial membrane potential collapses, which results in failure to retain JC-10 dye and the dye returns to its monomeric green form (EX/Em= 490/525 nm) in the cytosol.

The kit, not only measures mitochondrial membrane potential, but can be used for monitoring apoptosis and for screening of apoptotic activators and inhibitors.

ITEM(S) SUPPLIED

Description	Cat. # 786-1541 100 assays	Cat. # 786-1549 500 assays
JC-10 Dye Solution [3mM]	50 µl	5 x 50 µl
FCCP Control [20 mM]	5 µl	25 µl
MMP-Assay Buffer [5 X]	2 x 5ml	2 x 20 ml

STORAGE CONDITIONS

The kit is supplied on blue ice. Store all the reagents as indicated on the labels. If stored and used as directed this kit is stable for 12 months.

WARNING

Reagents JC-10 dye and FCCP should be considered as possible mutagens and should be handled carefully and disposed off as per local regulations. Wear protective clothing, gloves and eye wear when handling these reagents.

IMPORTANT INFORMATION

- JC-10 staining works on live cells only. It does not work on fixed cells.
- Recommended concentration of JC-10 for flow cytometry, microplate and microscopy assay is 15 µM.
  NOTE: *The concentration may vary depending upon culture conditions and cell types and should be determined by the user*
- Recommended FCCP working concentration is 20 µM.
- JC-10 is light sensitive. Do not expose to direct light when handling and staining cells.

- Avoid repeated thawing of JC-10 Dye Solution [3mM]. Store them at -20°C in preferably one time use aliquots

ADDITIONAL ITEMS REQUIRED

- Flow cytometer, fluorescence microscope or fluorometer microplate reader that can measure fluorescence of JC-10 dye aggregates using excitation of 535 ± 20 nm and an emission at 590 ±20 nm and monomers of JC-10 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.

PREPARATION BEFORE USE

- Bring the kit components to room temperature.
- Dilute the MMP-Assay Buffer [5X] to 1X with molecular grade water in ratio 1:4 to get required volume of MMP-Assay Buffer [1X] for the assay.
- Centrifuge the JC-10 Dye vial briefly. Prepare the required volume of working JC-10 Dye Solution by adding dye to prewarmed cell culture medium in ratio 1:200. For example add 5 µl JC-10 dye to 1 ml of prewarmed cell culture medium.
- FCCP Control use in assay is optional. Dilute the FCCP Control [20 mM] [1000X] to 20 µM [1X] in cell culture medium in ratio 1:1000.
  NOTE: *Avoid repeated thawing of FCCP. Preferably store it undiluted in small aliquots at -20°C.*

PROTOCOL

Flow cytometry assay

1. Culture cells in appropriate 6-, 12- or 24-well cell culture plates a density around 5 x 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. Use FCCP as positive control for apoptosis (optional).*
3. Replace the cell culture medium with same volume of working JC-10 Dye Solution under cell culture hood.
4. Incubate the plates in incubator (5% CO_2, 37°C) for 15-60 minutes.
   NOTE: *The optimum incubation time may vary depending upon cell type and cell concentration.*
5. Harvest cells by trypsinization or by scraping (determined by user).
   NOTE: *Cells can be directly analysed at this step under Flow Cytometer.*
   Optional steps:

6. Pellet cells at 1000 g for 5 minutes.
7. Add equal volume of prewarmed 1 X MMP-Assay Buffer and resuspend cells gently.
8. Pellet cells at 1000 g for 5 minutes and resuspend in fresh prewarmed 1 X MMP-Assay Buffer.
9. 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 x 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. Use FCCP as positive control for apoptosis (optional).*
3. Replace the cell culture medium with same volume of working JC-10 Dye Solution under cell culture hood.
4. Incubate the plates in incubator (5% CO_2, 37°C) for 15-60 minutes.
   NOTE: *The optimum incubation time may vary depending upon cell type and cell concentration.*
5. Observe the cells under fluorescence microscope using standard filters for FITC and Rhodamine.
   Optional steps:
6. 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.

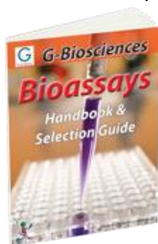
Microplate assay

1. Culture cells in 96-well black microtiter plate at a density of 5 x 10^4- 5 x 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. Replace the cell culture medium with 100 µl of working JC-10 Dye Solution under cell culture hood.
4. Incubate the plates in incubator (5% CO_2, 37°C) for 15-60 minutes.
   NOTE: *The optimum incubation time may vary depending upon cell type and cell concentration.*
5. Aspirate the supernatant and add 100 µl of prewarmed 1 X MMP-Assay Buffer per well.
6. Remove the buffer from wells and after that add fresh 100 µl of prewarmed 1 X MMP-Assay Buffer per well

7. Read the fluorescence at Ex/Em: 535nm/595 nm for JC-10 aggregates and at Ex/Em: 485 nm/535 nm for JC-10 monomers.
8. Plot the graph against the test compound concentration and ratio of red to green fluourescence to determine effects of compound on cell health.

RELATED PRODUCTS

Download our Bioassays Handbook.



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

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