INTRODUCTION

Tribioscience's Mitochondrial Membrane Potential Assay is based upon JC-10 dye, which is a superior alternative to JC-1 dye. JC-10 dye 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.

KIT CONTENT AND STORAGE CONDITIONS for 100 Assays

PART	PART#	Volume
JC-10 Dye Solution [200x]	TBS2049A	100 µl
MMP Assay Buffer [5 X]	TBS2049C	10 mL

Store the unopened kit at -20 ℃ stable for 12 months. Do not use past kit expiration date.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

- **MMP Assay Buffer:** Dilute the MMP-Assay Buffer [5X] to 1X with molecular grade water in ratio 1:4 to get a desired volume of MMP-Assay Buffer [1X] for the assay.
- **JC-10 Dye Working Solution:** Centrifuge the JC-10 Dye vial briefly. Prepare the desired volume of JC-10 Dye Working Solution by adding dye to prewarmed cell culture medium in ratio 1:100. For example, add 10 µl of JC-10 dye (100x) to 1 ml of prewarmed cell culture medium.

ASSAY PROCEDURE

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% CO2, 37°C).
2. Treat the cells with or without the test compound in duplicate manner.
3. Incubate the plates in incubator (5% CO2, 37°C) for a desired period time, like 4-6 hours. NOTE: The optimum incubation time may vary depending upon cell type and compound property.
4. Replace the cell culture medium with 100 µl of JC-10 Dye Working Solution under cell culture hood.
5. Incubate the plates in incubator (5% CO2, 37°C) for 15-60 minutes. NOTE: The optimum incubation time may vary depending upon cell type and cell concentration.
6. Aspirate the supernatant and add 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 (red fluorescence) and at Ex/Em: 485 nm/535 nm for JC-10 monomers (green Fluorescence).
8. Plot the graph against the test compound concentration and ratio of red to green fluorescence to determine the effects of compound on cell health.

Research Use only.