MQ Mitochondrial Membrane Potential Kit (JC-10 Assay)

Cat No. MMP-M-001-100
Size: 100 tests

MOLEQULE-ON®

Components	Quantity
JC-10 (200X)	100μL x 5 tubes
CCCP (10mM)	20μL
Dye buffer (5X)	80ml
Ultrapure water	90ml

Storage

Store at -20℃ for 1 year, protect from light, avoid refrozen as soon as possible, ultrapure water and Dye buffer (5X) can be storage at 4℃.

Description

JC-10 are cationic dyes that exhibit potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from green (~525 nm) to red (~590 nm). Consequently, mitochondrial depolarization is indicated by a decrease in the red/ green fluorescence intensity ratio. The potential-sensitive color shift is due to concentration dependent formation of red fluorescent J-aggregates. JC-10 can be used as an indicator of mitochondrial potential in a variety of cell types, including myocytes 3 and neurons, 4 as well as in intact tissues 5 and isolated mitochondria. Use of fluorescence ratio detection therefore allows researchers to make comparative measurements of membrane potential and determine the percentage of mitochondria within a population that respond to an applied stimulus. Subtle heterogeneity in cellular responses can be discerned in this way. The most widely implemented application of JC-10 is for detection of mitochondrial depolarization occurring in the early stages of apoptosis.

Reagent Preparation

1. JC-10 dye working solution:
Prepare dyeing working solution in 15ml tube. Transfers 50ul JC-10 (200X) in 15ml tube and add 8ml ultrapure water, shake vigorously and mix thoroughly then add 2ml dye buffer. For six well plate, the amount of dyeing working solution can be calculated in the following ratio. If 1~2 million cells are present in each well then add 1ml dyeing working solution or if 0.5~1 million cells are present in each well then add 0.5ml dyeing working solution.

2. Positive control preparation:
Prepare positive control by adding CCCP (10mM) in a cell suspension with a ratio of 1:1000, so it will be diluted to 10μM, incubate for 20 minutes and detect membrane potential. For most cells, membrane potential decreases after adding with 10μM CCCP for 20 minutes and gives green fluorescent. For different cells, CCCP may have different concentrations and duration of action, which should be determined by referring to relevant literature.

Protocol

1. For suspended cell:

1. Prepare (1X) working Dye buffer by adding 1ml Dye buffer (5X) to 4ml ultrapure water in ice bath.
2. Take 0.1~0.6 million cell suspension in cell culture medium, serum and phenol red are allowed to add.
3. Add 0.5 ml JC-10 dyeing working solution, mix thoroughly, incubate at 37℃ for 20 minutes.
4. Centrifuge at 600g for 3~4min at 4℃, collect cells and discard supernatant.
5. Wash twice with working Dye buffer (1X) : Add 1ml JC-10 dyeing buffer (1X) and centrifuge at 600g for 3~4 min at 4℃, collect cells and discard supernatant.
6. Repeat step 5.
7. Re-suspend with appropriate dyeing buffer (1X), then observe with fluorescence microscope or laser confocal microscope, detect with fluorescent spectrophotometer or flow analysis of cytometry.

2. For Adherent cells:

1. Prepare working Dye buffer (1X) by adding 1ml Dye buffer (5X) to 4ml ultrapure water in ice bath.
2. Remove cell culture medium in each well, wash with PBS or other solution, add 1ml cell culture medium, serum and phenol red are allowed to add.
3. Add 1ml JC-10 dyeing working solution, mix thoroughly, incubate at 37℃ for 20 minutes.
4. Centrifuge at 600g for 3~4min at 4℃, collect cells and discard supernatant.
5. Wash twice with working Dye buffer (1X) : Add 1ml Dye buffer (1X) and centrifuge at 600g for 3~4 min at 4℃, collect cells and discard supernatant.
6. Repeat step 5.
7. Add 2ml cell culture medium, serum and phenol red are allowed to add.
8. Observe with fluorescence microscope or laser confocal microscope.

3. For purify mitochondria:

1. Prepare working Dye buffer (1X) by adding 1ml Dye buffer (5X) to 4ml ultrapure water in ice bath.
2. Add 0.1ml purify mitochondria which include 10～100 μg protein to 0.9ml diluted Dye working solution.
3. Detect with fluorescent spectrophotometer or microplate reader: Time scan with fluorescent spectrophotometer after mix thoroughly, excitation wavelength is 485nm, emission wavelength is 590 nm. When fluorescent microplate reader is used, the excitation wavelength can be set within the range of 475 ~ 520nm if the excitation wavelength cannot be set to 485nm. Or you can reference step 6 to detect fluorescent.
4. Observe with fluorescence microscope or laser confocal microscope.

4. Fluorescence observation and result analysis:

Detect JC-10 monomer, set the excitation wavelength to 490 nm, emission wavelength to 530 nm; Detect JC-10 component, set the excitation wavelength to 525 nm, emission wavelength to 590 nm.

Note

1. JC-10 (200X) will be solidified and stick on the tube at low temperature like 4 ℃ ice bath. Use 20~25 ℃ water bath to dissolve thoroughly.
2. Keep the temperature 4 ℃ when wash with Dye buffer (1X).
3. Finish detection in 30 min after washing as soon as possible, storage in ice bath before detection.
4. Do not dissolve all Dye buffer (5X) to (1X).
5. If precipitation is found in Dye buffer (5X), it must be dissolved before use. Heat at 37℃ to facilitate dissolution when necessary.
6. CCCP is mitochondrial electron transport chain inhibitor, toxic, be careful.
7. Wear lab coat and disposable glove for your safety and health.
8. JC-10 produce green fluorescent after dyeing, but normal cells have red fluorescent.