

JC-1 Mitochondrial Membrane Potential Assay Kit

1 Contents

Components	JC-1(200 μ M in DMSO)	Phosphate-buffered saline (10 \times)	CCCP (50 mM in DMSO)
HY-K0601-100T	230 μ L \times 5	25 mL	125 μ L

2 General Information

MCE JC-1 Mitochondrial Membrane Potential Assay Kit uses JC-1, a lipophilic cationic dye, to detect the mitochondrial membrane potential in variety of cell types, as well as intact tissues and isolated mitochondria.

$\Delta\Psi_m$, mitochondrial membrane potential, is an important parameter of mitochondrial function and has been used as an indicator of cell health. Variation of $\Delta\Psi_m$ would be studied using JC-1. In healthy cells with high $\Delta\Psi_m$, JC-1 forms complexes known as J-aggregates. While in cells with low $\Delta\Psi_m$, JC-1 remains in the monomeric form. When excited at 490 nm, JC-1 monomers emit a green fluorescence with a maximum at \sim 520 nm. Aggregates of JC-1 emit an orange-red fluorescence with a maximum at \sim 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.

MCE JC-1 Mitochondrial Membrane Potential Assay Kit consists of JC-1, Phosphate-buffered saline (10 \times) and CCCP (50 mM in DMSO). CCCP controls should be used to confirm that the JC-1 response is sensitive to changes in membrane potential.

MCE JC-1 Mitochondrial Membrane Potential Assay Kit can be analyzed by fluorescence microscopy, flow cytometer, or a fluorescence plate reader with appropriate filter sets.

3 General Protocol

1. Preparation of Phosphate-buffered saline (PBS) (1 \times)

- Warm the PBS (10 \times) until any salt crystals are completely dissolved.
- Dilute PBS (10 \times) 1:10 with dH₂O (e.g. 1 mL PBS + 9 mL dH₂O).

2. Labeling of Cells

- Culture cells in 6-, 12-, 24-, or 96-well plates at a density of 5-10 \times 10⁵ cells/mL. Incubate the cells according to your normal protocol.
- For the control tube, allow the vial of CCCP has come to room temperature, add 1 μ L of CCCP (50 mM). Incubate cells at 37 $^{\circ}$ C for 5 minutes.
- Add 10 μ L JC-1 (200 μ M) per well to make the final concentration at 2 μ M. Incubate cells at 37 $^{\circ}$ C, 5% CO₂, for 15-20 minutes.

Note: If additional labeling followed, for example with an annexin V, begin with step 3.a.

- After incubation, centrifuge cells for 3-4 minutes at 400 \times g at 4 $^{\circ}$ C, carefully aspirate the supernant.
- Wash cells twice with PBS (1 \times): add 2 mL PBS (1 \times) to suspend cells and vortex to mix thoroughly. Centrifuge cells for 3-4 minutes at 400 \times g at 4 $^{\circ}$ C, carefully aspirate the supernant.
- Add 500 μ L PBS (1 \times) to suspend cells. Analyze sample on a flow cytometer, fluorescence microscopy, or fluorescence microplate reader.

3. Additional Labeling with Annexin V

- After step 2.c, wash cells twice with PBS (1 \times): add 2 mL PBS (1 \times) to suspend cells and vortex to mix thoroughly. Centrifuge cells for 3-4 minutes at 400 \times g at 4 $^{\circ}$ C, carefully aspirate the supernant.
- Add 100 μ L Annexin V binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM CaCl₂, pH 7.4) to suspend the JC-1-stained cells.
- Add appropriate volume of Annexin V and incubate cells at 37 $^{\circ}$ C, 5% CO₂, for 15 minutes.

d. Add 400 μ L Annexin V binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM CaCl₂, pH 7.4) to suspend cells. Analyze sample on a flow cytometer, fluorescence microscopy, or fluorescence microplate reader.

4 Storage

Stored at -20 °C protecting from light, and is stable for up to 12 months.

For immediate use, components may be stored at 2-8 °C.

5 Precautions

1. A 'Test' simply refers to a single assay well. 100 tests based on labeling volumes of 1.0 mL.
2. JC-1 is light sensitive, we recommend that you make small aliquots and store them at -20 °C with light protected.
3. Phosphate-buffered saline (10 \times) could be stored at 4 °C.
4. This product is for R&D use only, not for drug, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.