JC1 Mitochondrial Membrane Potential Assay Kit

Catalog No.: abx090685

Size: 100 tests

Storage: Store all reagents at -20 °C for up to one year.

Introduction: Abbexa's JC1 Mitochondrial Membrane Potential Assay Kit contains tetraethylbenzimidazolylcarbocyanine iodide (JC1), a cationic dye that accumulates in polarized mitochondria. At low concentrations (due to low mitochondrial membrane potential) JC1 is predominantly a monomer that emits green fluorescence with an emission wavelength of 530 ± 15 nm. At high concentrations (due to high mitochondrial membrane potential) the dye aggregates, emitting a red-orange color (emission wavelength of 590 ± 17.5 nm). Therefore, a decrease in the aggregate fluorescent count is indicative of depolarization whereas an increase is indicative of hyperpolarization. The ratio of red to green fluorescence of JC1 is dependent only on membrane potential, and not influenced by mitochondrial size, shape, or density.

Kit components

1. 200X JC1: 5 × 100 µl
2. Ultra-Pure Water: 90 ml
3. 5X JC1 Staining Buffer: 80 ml
4. CCCP (10 mM): 20 µl

Materials Required But Not Provided

1. Fluorescence microscope, laser confocal microscope or fluorescence spectrophotometer
2. Cell incubator
3. Phosphate-Buffered Saline (PBS)
4. Dulbecco's Modified Eagle Medium (DMEM)
5. Distilled water
6. High-precision pipette and sterile pipette tips
7. Centrifuge and centrifuge tubes
8. Timer
9. Ice
10. Vortexer

Procedure

A. Preparation of JC1 Staining Working Solution

- To each of the 5 vials of 200X JC1, add 8 ml of Ultra-Pure Water. Vortex to mix thoroughly, then add 2 ml of 5X JC1 Staining Buffer to each vial. Mix thoroughly.
- For one well in a 6-well plate, the volume of JC1 staining working solution required is 1 ml.
- For cell suspension samples, 0.5 ml of JC1 staining working solution is required for each 50-1000 × 10^3 cells.

B. Preparation of JC1 Staining Working Solution

- Prepare the 1X JC1 Staining Buffer working solution by diluting the 5X JC1 Staining Buffer with distilled water (e.g. add 1 ml 5X JC1 Staining Buffer to 4 ml distilled water). Store the solution in an ice bath.

C. Positive Control

- Add CCCP (10 mM) into DMEM at 1/1000 for a CCCP concentration of 10 µM. Treat cells for 20 minutes. Load JC1 according to one of the following methods and conduct testing of the mitochondrial membrane potential (MMP). For most cells, the MMP will be lost completely after conducting 10 µM CCCP for 20 minutes. Cells treated with CCCP should exhibit green fluorescence, normal cells should exhibit red fluorescence. **Note:** For specific cells, the time and CCCP concentration will vary, these should be determined by the end user.

D. Suspension Cells

1. Take 10-600 × 10^3 cells and resuspend in DMEM. The DMEM can contain serum and phenol red.
2. Add 0.5 ml of JC1 Staining Working Solution. Mix thoroughly and incubate for 20 minutes at 37 °C.
3. Centrifuge at 600 × g at 4 °C for 3-4 minutes to precipitate the cells. Discard the supernatant. Avoid disturbing the pellet.
4. Add 1 ml of 1X JC1 Staining Buffer to the suspension cells. Centrifuge at 600 × g at 4 °C for 3-4 minutes to precipitate the cells. Discard the supernatant. Avoid disturbing the pellet. Repeat this step one more time for a total of two times.
5. Resuspend in 1X JC1 Staining Buffer and observe using a fluorescence microscope or laser confocal microscope. The cells may also be analyzed using a fluorescence spectrophotometer or via flow cytometry. If using a fluorescence spectrophotometer or fluorescence microplate reader, the readings should be taken as soon as possible. To detect the JC-1 monomer, set the excitation wavelength to 490 nm and the emission wavelength to 530 nm. To detect the JC-1 polymer, set the excitation wavelength to 525 nm and the emission wavelength to 590 nm. The occurrence of red fluorescence indicates normal cell activity.

E. Adherent Cells

1. Wash cells with PBS (or other solution if required by specific experiments) and add 1 ml of DMEM. The DMEM can contain serum and phenol red.
2. Add 1 ml of JC1 Staining Working Solution. Mix thoroughly and incubate for 20 minutes at 37 °C.
3. Transfer the supernatant to a new tube and wash twice with 1X JC1 Staining Buffer.
4. Add 2 ml of DMEM. The DMEM can contain serum and phenol red.
5. Observe using a fluorescence microscope or laser confocal microscope. The cells may also be analyzed using a fluorescence spectrophotometer or via flow cytometry. If using a fluorescence spectrophotometer or fluorescence microplate reader, the readings should be taken as soon as possible. To detect the JC-1 monomer, set the excitation wavelength to 490 nm and the emission wavelength to 530 nm. To detect the JC-1 polymer, set the excitation wavelength to 525 nm and the emission wavelength to 590 nm. The occurrence of red fluorescence indicates normal cell activity.

F. Purified Mitochondria

1. Dilute samples 5-fold using 1X JC1 Staining Buffer.
2. Dilute the solution 10-fold using JC1 Staining Working Solution. The solution should contain 10-100 µg purified mitochondria.
3. Observe using a fluorescence microscope or laser confocal microscope. The cells may also be analyzed using a fluorescence spectrophotometer or via flow cytometry. If using a fluorescence spectrophotometer or fluorescence microplate reader, the readings should be taken as soon as possible. To detect the JC-1 monomer, set the excitation wavelength to 490 nm and the emission wavelength to 530 nm. To detect the JC-1 polymer, set the excitation wavelength to 525 nm and the emission wavelength to 590 nm. The occurrence of red fluorescence indicates normal cell activity.

Notes

- The 200X JC1 may appear as a solid at the bottom of the tube when stored at or below 4 °C. If this occurs, allow the reagent to stand at room temperature (20-25 °C) until there is no precipitate observed.
- The 200X JC1 reagent must first be diluted with Ultra-Pure Water before adding 5X JC1 Staining Buffer. The 5X JC1 Staining Buffer should **not** be diluted as the JC1 reagent will not dissolve easily in 1X JC1 Staining Buffer.
- Do not dilute all of the 5X JC1 Staining Buffer to 1X, as the 5X JC1 Staining Buffer reagent is used undiluted in preparing the JC1 Staining Working Solution.
- The 5X JC1 Staining Buffer may appear as a solid at the bottom of the tube when stored at or below 4 °C. If this occurs, heat the reagent using a water bath set to 20-25 °C until there is no precipitate observed.
- CCCP is a mitochondrial electron transport chain inhibitor. It is therefore toxic, use appropriate personal protective equipment (PPE) when handling this reagent.
- Samples should be analyzed as soon as possible and within 30 minutes after completing the assay procedure. Samples should be stored on ice before being tested.