

NutriCulture JC-1 Mitochondrial Membrane Potential Assay Kit

100 tests

Cat No: JMM100

Shipping : Ship with blue/dry ice.
Storage : Store NutriCulture JC-1 (100x) and NutriCulture CCCP at -20°C protecting from light. NutriCulture JC-1 Assay Buffer (10x) should be stored at 2-8°C. NutriCulture JC-1 Mitochondrial Membrane Potential Assay Kit is stable for up to 1 year. Avoid repetitive freeze-thaw cycles while using. For immediate use, components may be stored at 2-8°C.

General Information

NutriCulture 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.

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

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

Carbonyl cyanide m-chlorophenylhydrazone (CCCP) is provided to induce a decrease in mitochondrial membrane potential as a positive control reagent.

Protocol

General Considerations before use

1. Warm the NutriCulture JC-1 Assay Buffer (10x) until any salt crystals are completely dissolved. Dilute JC-1 Assay Buffer (10x) 1:10 with molecular grade nuclease free ddH₂O (e.g. 1 mL JC-1 Assay Buffer (10x) + 9 mL dH₂O).

Note: Diluted NutriCulture JC-1 Assay Buffer should be sealed, stored at 2-8°C and used within one week.

2. Due to low solubility of NutriCulture JC-1 (100x) in aqueous solutions and tendency to form particulates that are difficult to remove, it is of importance to make sure that NutriCulture JC-1 (100x) is completely thawed and warmed to room temperature before diluting it into culture medium. Do not centrifuge the reagent.

3. JC-1 is light sensitive, we recommend that you make small aliquots and store them at -20°C with protection from light.

4. For all assay protocols, it is essential that samples be analyzed immediately upon completion of the staining.

5. Since JC-1 dye is very sensitive to alterations of temperature and pH, all reagents must be kept at 25°C and carefully checked for pH (7.4) during the experiment.

Assay Procedure for analysis with flow cytometry

1. Culture cells in 6-, 12-, 24-, or 96-well plates at a density of $5-10 \times 10^5$ cells/mL. Optimal conditions will be dependent on the cell type, it is recommended that cells be no more than 80% confluent by the time of visualization. The culture medium is recommended to be changed every other day until the cells reach the target confluency.
2. Culture and treat cells in duplicates or triplicates.
3. Collect cells using regular trypsinization technique.
4. Resuspend each sample in 1 ml warm *NutriCulture* JC-1 Assay Buffer (1x) not to exceed 1×10^6 cells/ml.
5. Centrifuge samples for 5 min at room temperature at 400 x g and remove the supernatant.
6. Resuspend each sample in 1 ml warm *NutriCulture* JC-1 Assay Buffer (1x).
7. Add 10 μ l of *NutriCulture* JC-1 (100x) and incubate the cells at 37°C, 5% CO₂ for 15-30 min.

Note: The amount of JC-1 dye might be optimized if needed.

8. To serve as a positive control, add 1 μ l of *NutriCulture* CCCP just to one sample and incubate at 37°C for 5 min.
9. Centrifuge samples for 5 min at room temperature at 400 x g and remove the supernatant.
10. Wash all samples by adding 2 ml of warm PBS, centrifuge for 5 min at room temperature at 400 x g, remove the supernatant.
11. Re-suspend the cell pellet again in 300 μ l *NutriCulture* JC-1 Assay Buffer (1x).
9. Begin observation and measuring immediately after completing the last step with flow cytometer.

Note: Healthy cells with mainly JC-1 J-aggregates can be detected with filter sets usually designed to detect rhodamine (excitation/emission = 540/570 nm). Apoptotic or unhealthy cells with mainly JC-1 monomers can be detected with filter sets designed to detect FITC (excitation/emission = 485/535 nm).

Assay Procedure for analysis with fluorescence microscopy or a fluorescence plate reader

1. Culture cells in 6-, 12-, 24-, or 96-well plates at a density of $5-10 \times 10^5$ cells/mL (cells can be cultured on pre-coated glass coverslips, 96 wells plate supports or chamber slides). Optimal conditions will be dependent on the cell type, it is recommended that cells be no more than 80% confluent by the time of visualization. The culture medium is recommended to be changed every other day until the cells reach the target confluency.

Note: A 96-well black culture plate should be used for analysis with a fluorescence plate reader.

2. Culture and treat cells in duplicates or triplicates.
 3. Remove the cell culture medium and then wash cells with a warm 1x PBS.
 4. Add *NutriCulture* JC-1 Assay Buffer (1x) to each well.
 5. Add 1 μ l of *NutriCulture* JC-1 (100x) for every 100 μ l *NutriCulture* JC-1 Assay Buffer (1x) and incubate the cells at 37°C, 5% CO₂ for 15-30 min.
- Note: The amount of JC-1 dye might be optimized if needed.*
8. To serve as a positive control, add 1 μ l of *NutriCulture* CCCP for every 1 ml *NutriCulture* JC-1 Assay Buffer (1x) just to one sample and incubate at 37°C for 5 min.
 10. Remove the culture medium of all samples and wash the cells once by adding warm 1x PBS.
 11. Add *NutriCulture* JC-1 Assay Buffer (1x) to samples (about 100 μ l into each of the 96 wells plate or corresponding amount in a petri dish or chamber slides depending on the culture platform and detection methods that was selected). Cover with a sheet of foil to keep the culture platform and its content out of light.
 9. Analyze the fluorescence of the test cultures (including controls) immediately.

Note: Healthy cells with mainly JC-1 J-aggregates can be detected with filter sets usually designed to detect rhodamine (excitation/emission = 540/570 nm). Apoptotic or unhealthy cells with mainly JC-1 monomers can be detected with filter sets designed to detect FITC (excitation/emission = 485/535 nm).