

**JC-1 Mitochondrial Membrane  
Potential Assay Kit**

Item No. 10009172

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## GENERAL INFORMATION

### Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	100 Tests Quantity/Size	Storage
10009908	JC-1 Reagent	1 vial/500 µl	-20°C
10009322	Cell-Based Assay Buffer Tablet	3 tablets	Room Temperature

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.



**WARNING:** This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

## Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

## If You Have Problems

### Technical Service Contact Information

**Phone:** 888-526-5351 (USA and Canada only) or 734-975-3888

**Fax:** 734-971-3641

**Email:** techserv@caymanchem.com

**Hours:** M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. Adjustable pipettes and a repeat pipettor
2. A 6-, 12-, 24-, or 96-well plate for culturing cells
3. A flow cytometer, fluorescence microscope, or plate reader equipped with laser/fluorescent filters capable of detecting the J-aggregates form of JC-1 using an excitation of 520-570 nm and an emission at 570-610 nm as well as the monomeric form of JC-1 at excitation and emission wavelengths of 485 and 535 nm, respectively.
4. Distilled water

## INTRODUCTION

### Background

Apoptosis is a cellular process involving a genetically programmed series of events leading to the death of a cell. During this process, several key events occur in mitochondria, including the release of caspase activators such as cytochrome c, changes in electron transport, and loss of mitochondrial transmembrane potential ( $\Delta\Psi_m$ ).<sup>1</sup> For this reason,  $\Delta\Psi_m$  is an important parameter of mitochondrial function and has been used as an indicator of cell health.

Variation of  $\Delta\Psi_m$  has been previously studied by evaluating the changes in fluorescence intensity of cells stained with cationic dyes such as rhodamine-123 (Rh123) and DiOC<sub>6</sub>.<sup>2</sup> However, data obtained by using these probes may not be reliable. For example, Rh123 is relatively insensitive to  $\Delta\Psi_m$  changes and DiOC<sub>6</sub> dye cannot distinguish between depolarization of the plasma membrane and changes at  $\Delta\Psi_m$  in several physiological or pathological conditions when both events can take place.<sup>3</sup> More recently, a new cytofluorimetric, lipophilic cationic dye, 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1), has been developed. JC-1 has advantages over other cationic dyes in that it can selectively enter into mitochondria and reversibly change color from green to red as the membrane potential increases. In healthy cells with high mitochondrial  $\Delta\Psi_m$ , JC-1 spontaneously forms complexes known as J-aggregates with intense red fluorescence. On the other hand, in apoptotic or unhealthy cells with low  $\Delta\Psi_m$ , JC-1 remains in the monomeric form, which shows only green fluorescence.

### About This Assay

Cayman's JC-1 Mitochondrial Membrane Potential Assay Kit can be used to study the behavior of mitochondria in a variety of conditions, including apoptosis. The main advantage of this assay is that the changes in  $\Delta\Psi_m$  reflected by different forms of JC-1 as either green or red fluorescence can be both qualified and quantified by fluorescence microscopy, flow cytometry, or a fluorescence plate reader with appropriate filter sets.

## PRE-ASSAY PREPARATION

*NOTE: JC-1 is light sensitive. Do not expose to direct intense light.*

Thaw the JC-1 Reagent at room temperature. Mix well. To avoid repeated freeze/thawing of this solution, we recommend that you make small aliquots and store them at -20°C.

### Reagent Preparation

#### 1. Assay Buffer Preparation

Dissolve three Cell-Based Assay Buffer tablets (Item No. 10009322) in 300 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

#### 2. JC-1 Staining Solution Preparation

Thaw an aliquot of the JC-1 Reagent (Item No. 10009908) at room temperature. Prepare a staining solution by diluting the reagent 1:10 in the culture medium you are using for your cells. Mix well to make sure there are no particles or flakes in the solution.

*NOTE: JC-1 Staining Solution is difficult to prepare due to its low solubility in aqueous medium and tendency to form particulates that are difficult to remove. Make sure JC-1 Reagent is completely thawed and warmed to room temperature before diluting it into culture medium. Do not centrifuge the reagent.*

#### NOTES

- JC-1 is light sensitive. All staining procedures must be performed without direct exposure to intense light. Therefore, incubations need to be done in the dark.
- For all assay protocols, on pages 7-9, it is imperative that samples be analyzed immediately following completion of the staining.

## ASSAY PROTOCOL

### Flow Cytometry

1. Culture cells in 6-, 12-, or 24-well plates at a density of  $5 \times 10^5$  cells/ml in a CO<sub>2</sub> incubator overnight at 37°C. Treat the cells with or without experimental compounds (each sample should be run in duplicate or triplicate). Incubate the cells according to your normal protocol.
2. Add 100 µl of the JC-1 Staining Solution (prepared on page 6) per ml of culture medium to each well of the plate. For example, if you culture cells in 2 ml of culture medium in a 6-well plate, add 200 µl of the JC-1 Staining Solution into each well. Mix gently. Further dilution, such as adding only 50 µl of JC-1 Staining Solution to 1 ml of culture medium, may be used in cases when the staining is too intense.
3. Incubate samples in a CO<sub>2</sub> incubator at 37°C for 15-30 minutes. Sufficient staining is usually obtained after 15 minutes of incubation.
4. Harvest cells from each well into a plastic tube fitted for the flow cytometer. The samples can be directly analyzed in the culture medium.
5. Analyze the samples immediately. Healthy cells with functional mitochondria contain red JC-1 J-aggregates and are detectable in the FL2 channel. Apoptotic or unhealthy cells with collapsed mitochondria contain mainly green JC-1 monomers and are detectable in the FL1 channel.

The following steps are optional:

6. Alternatively, centrifuge the samples obtained in step 4 (above) for five minutes at 400 x g at room temperature. Carefully aspirate the supernatant. Add 1 ml of Assay Buffer to each tube and vortex to ensure that all cells are suspended.
7. Centrifuge the samples for five minutes at 400 x g at room temperature. Carefully aspirate the supernatant.
8. Repeat steps 6-7 one more time.
9. Add 500 µl of Assay Buffer to each tube and vortex to ensure that all cells are suspended in the assay solution.
10. Analyze the samples immediately. Healthy cells with functional mitochondria contain red JC-1 J-aggregates and are detectable in the FL2 channel. Apoptotic or unhealthy cells with collapsed mitochondria contain mainly green JC-1 monomers and are detectable in the FL1 channel.

## Fluorescence Microscopy

A 6-, 12-, 24-, or 96-well culture plate can be used for this method. We recommend that the cell density be  $\leq 1 \times 10^6$  cells/ml. Optimal conditions will be dependent on the cell type.

1. Culture cells in 6-, 12-, 24-, or 96-well plates at a density of  $5 \times 10^5$  cells/ml in a CO<sub>2</sub> incubator overnight at 37°C. Treat the cells with or without experimental compounds (each sample should be run in duplicate or triplicate). Incubate the cells according to your normal protocol.
2. Add 100  $\mu$ l of the JC-1 Staining Solution (prepared on page 6) per ml of culture medium to each well of the plate. For example, if you culture cells in 2 ml of culture medium in a 6-well plate, add 200  $\mu$ l of the JC-1 Staining Solution into each well. Mix gently. Further dilution, such as adding only 50  $\mu$ l of JC-1 Staining Solution to 1 ml of culture medium, may be used in cases when the staining is too intense.
3. Incubate samples in a CO<sub>2</sub> incubator at 37°C for 15-30 minutes. Sufficient staining is usually obtained after 15 minutes of incubation. The cells can be analyzed directly in the culture medium since phenol red does not interfere with fluorescent staining. Healthy cells with mainly JC-1 J-aggregates can be detected with fluorescence settings usually designed to detect rhodamine (excitation/emission = 540/570 nm) or Texas Red (excitation/emission = 590/610 nm). Apoptotic or unhealthy cells with mainly JC-1 monomers can be detected with settings designed to detect FITC (excitation/emission = 485/535 nm).

The following steps are optional:

4. Centrifuge the plate for five minutes at 400 x g at room temperature. Discard the supernatant by careful aspiration.
5. Add 2 ml, 1 ml, 500  $\mu$ l, or 200  $\mu$ l of Assay Buffer to each well of 6-, 12-, 24-, or 96-well plate respectively.
6. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate the supernatant.
7. Repeat steps 5-6 one more time.

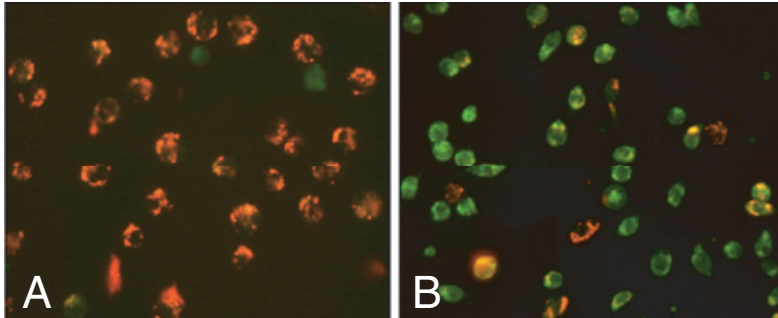
8. Add 1 ml, 500  $\mu$ l, 250  $\mu$ l, or 100  $\mu$ l of Assay Buffer to each well of 6-, 12-, 24-, or 96-well plate, respectively. The cells are now ready for analysis by fluorescent microscopy and must be analyzed immediately. Healthy cells with mainly JC-1 J-aggregates can be detected with fluorescence settings usually designed to detect rhodamine (excitation/emission = 540/570 nm) or Texas Red (excitation/emission = 590/610 nm). Apoptotic or unhealthy cells with mainly JC-1 monomers can be detected with settings designed to detect FITC (excitation/emission = 485/535 nm).

## Plate Reader

A 96-well **Black** culture plate should be used for this method. We recommend that cell density be  $\leq 1 \times 10^6$  cells/well. Optimal conditions will be dependent on the cell type.

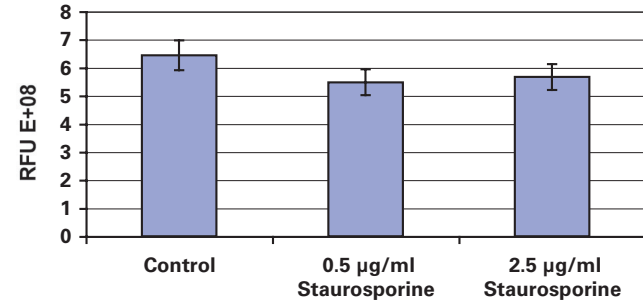
1. Culture cells in a 96-well black plate at a density of  $5 \times 10^4$  -  $5 \times 10^5$  cells/well in 100  $\mu$ l culture medium in a CO<sub>2</sub> incubator overnight at 37°C. Treat the cells with or without experimental compounds (each sample should be run in duplicate or triplicate). Incubate the cells according to your normal protocol.
2. Add 10  $\mu$ l of the JC-1 Staining Solution (prepared above) to each well and mix gently. Further dilution, such as adding 5  $\mu$ l of JC-1 Staining Solution to 100  $\mu$ l of culture medium, may be used in cases where the staining is too intense.
3. Incubate the cells in a CO<sub>2</sub> incubator at 37°C for 15-30 minutes. Sufficient staining is usually obtained after 15 minutes of incubation.
4. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate the supernatant.
5. Add 200  $\mu$ l of Assay Buffer to each well and centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate the supernatant.
6. Repeat step 5 one more time.
7. Add 100  $\mu$ l of Assay Buffer to each well. The cells are now ready for analysis by a fluorescent plate reader. In healthy cells, JC-1 forms J-aggregates which display strong fluorescent intensity with excitation and emission at 560 nm and 595 nm, respectively. In apoptotic or unhealthy cells, JC-1 exists as monomers which show strong fluorescence intensity with excitation and emission at 485 nm and 535 nm, respectively. The ratio of fluorescent intensity of J-aggregates to fluorescent intensity of monomers can be used as an indicator of cell health.

Representative Staining Results

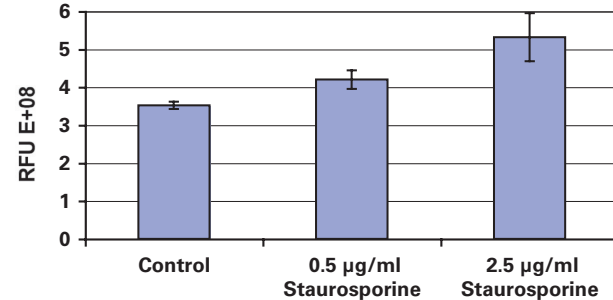


**Figure 1. Effect of staurosporine on mitochondrial potential in Jurkat cells.** Jurkat cells were plated at a density of  $5 \times 10^4$  cells/well. The next day, cells were treated with 2.5  $\mu\text{g/ml}$  of staurosporine or vehicle for two hours in a  $\text{CO}_2$  incubator at  $37^\circ\text{C}$ . JC-1 Staining Solution was then added to the wells and staining was visualized after a 15 minute incubation. *Panel A:* untreated cells showing most of cells had strong J-aggregation (red). *Panel B:* staurosporine treated cells showing a majority of cells stained green due to low  $\Delta\Psi\text{m}$ .

J-aggregates in control cells vs apoptotic cells (induced by staurosporine)



JC-1 Monomers in control cells vs apoptotic cells (induced by staurosporine)



**Figure 2. Measurement of JC-1 fluorescence in Jurkat cells in a 96-well plate format.** Jurkat cells were plated at a density of  $5 \times 10^4$  cells/well. The next day, cells were treated with staurosporine or vehicle for two hours in a  $\text{CO}_2$  incubator at  $37^\circ\text{C}$ . JC-1 Staining Solution was then added to the wells and incubated at  $37^\circ\text{C}$  for 15 minutes. The fluorescent intensity for both J-aggregates and monomeric forms of JC-1 was measured with a 96-well plate reader (J-aggregates: excitation/emission = 560/595 nm; JC-1 monomers: excitation/emission = 485/535 nm).

## Troubleshooting

Problem	Possible Causes	Recommended Solutions
No staining	Samples have been fixed with a fixative such as paraformaldehyde	Assays should be performed without any fixation
Poor staining	A. JC-1 staining solution has been centrifuged. B. Stained cells have been exposed to strong light	A. Do not centrifuge JC-1 staining solution as this will precipitate the reagent. B. Analyze the stained cells immediately after washing
Control cells without treatment show low ratio of red to green signal	Control cells are not healthy	Use only healthy cells
Staining is too strong	JC-1 staining solution is too concentrated for this cell type	Dilute JC-1 staining solution (see Assay Protocols for more details)

## References

- Green, D.R. and Reed, J.C. Mitochondria and apoptosis. *Science* **281**, 1309-1312 (1998).
- Petit, P.X., Lecoqeur, H., Zorn, E., *et al.* Alterations in mitochondrial structure and function are early events of dexamethasone-induced thymocyte apoptosis. *J. Cell Biol.* **130(1)**, 157-167 (1995).
- Salvioli, S., Ardizzoni, A., Franceschi, C., *et al.* JC-1, but not DiOC6(3) or rhodamine 123, is a reliable fluorescent probe to assess  $\Delta\Psi$  changes in intact cells: Implications for studies on mitochondrial functionality during apoptosis. *FEBS Lett.* **411**, 77-82 (1997).

## Related Products

Adipogenesis Assay Kit - Item No. 10006908

Apoptotic Blebs Assay Kit - Item No. 10010750

Glycolysis Cell-Based Assay Kit - Item No. 600450

LDH Cytotoxicity Assay Kit - Item No. 10008882

Multi-Drug Resistance Assay Kit (Calcein AM) - Item No. 600370

Multi-Parameter Apoptosis Assay Kit - Item No. 600330

WST-1 Cell Proliferation Assay Kit - Item No. 10008883

## Warranty and Limitation of Remedy

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Buyer's exclusive remedy and Cayman's sole liability hereunder shall be limited to a **refund** of the purchase price, or at Cayman's option, the **replacement**, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

## NOTES

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