JC-1 Mitochondrial Membrane Potential Assay Kit

Item No. 10009172

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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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	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

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
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-aggregate form of JC-1 using an excitation of 535 +/- 20 nm and an emission at 590 +20 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

$\Delta\psi_M$ is an important parameter of mitochondrial function and has been used as an indicator of cell health. Variations of $\Delta\psi_M$ have been previously studied using cationic dyes such as rhodamine-123 (Rh123) and $DiOC_6$.[1] More recently, a cytofluorimetric, lipophilic cationic dye, 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimi-dazolylcarbocyanine iodide (JC-1), has been developed. JC-1 has advantages over other cationic dyes in that it enters the mitochondria and changes its fluorescent properties based on the aggregation of the probe. In healthy cells with high $\Delta\psi_M$, JC-1 forms complexes known as J-aggregates with intense red fluorescence. However, in cells with low $\Delta\psi_M$, JC-1 remains in the monomeric form, which exhibits green fluorescence. The higher the ratio of red to green fluorescence, the higher the polarization of the mitochondrial membrane.[2]

About This Assay

Cayman's JC-1 Mitochondrial Membrane Potential Assay Kit can be used to study mitochondrial behavior in a variety of conditions, including apoptosis. Changes in $\Delta\psi_M$ reflected by different forms of JC-1 as either green or red fluorescence can be determined as a ratio of green:red using 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 7-10, 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 x 10^5 cells/ml in a CO_2 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_2 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.
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 ≤1 x 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 x 10^5 cells/ml in a CO_2 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_2 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 µl, or 200 µl 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.
8. Add 1 ml, 500 µl, 250 µl, or 100 µl 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 ≤1 x 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 x 10^4 - 5 x 10^5 cells/well in 100 µl culture medium in a CO_2 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 µl of the JC-1 Staining Solution (prepared above) to each well and mix gently. Further dilution, such as adding 5 µl of JC-1 Staining Solution to 100 µl of culture medium, may be used in cases where the staining is too intense.
3. Incubate the cells in a CO_2 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 µl 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 µl 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 535 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.

PERFORMANCE CHARACTERISTICS

Representative Staining Results

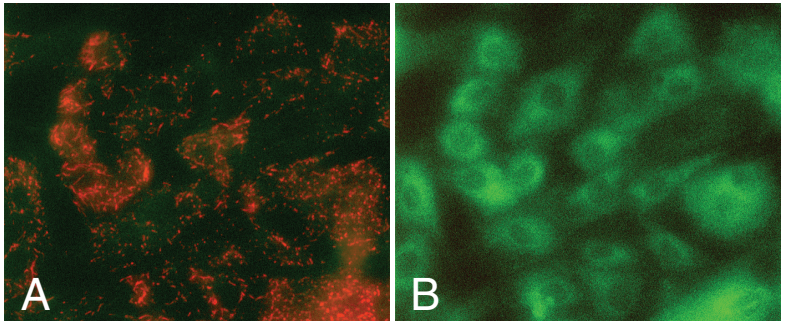

Figure 1. H9C2 cells were plated at a density of 2 x 10^4 and cultured overnight. The next day, cells were stained with JC-1 according to the protocol on page 9. *Panel A:* an image was taken of cells prior to treatment with FCCP, showing red J-aggregated accumulated in the mitochondria, with the more diffuse J-monomer in green. Immediately following this, 1 µM FCCP was added to the well followed by a 10 minute incubation to uncouple the mitochondria. *Panel B:* this image shows a more fluorescent diffuse, J-monomer (green) with no J-aggregate (red) staining present.

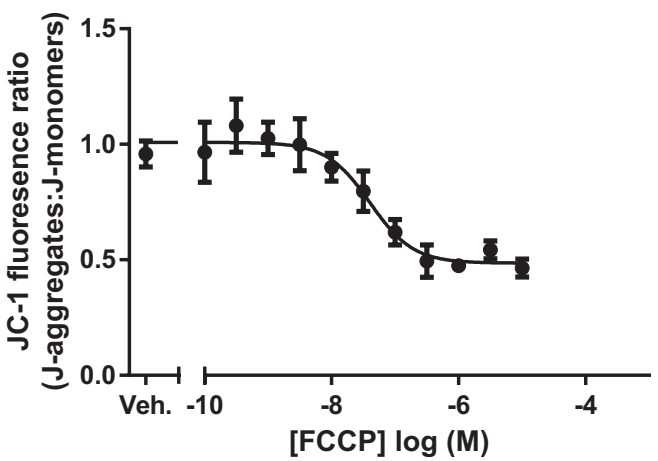

Figure 2. Ratio of J-aggregates to J-monomers in a 96-well plate format. H9C2 cells were plated at a density of 2 x 10^5 cells/well and cultured overnight. Cells were stained with JC-1 according to the protocol on page 11. Post staining, cells were treated with FCCP in and incubated in the dark for 10 minutes. Florescence of J-aggregates and J-monomers was measured using excitation/emission wavelengths of 535/595 nm and 485/535 nm, respectively. Data are shown as a ratio of J-aggregates to J-monomers.

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

1. Petit, P.X., Lecoeur, 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).
2. Reers, M., Smith, T.W., and Chen, L.B. J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential. *Biochemistry* **30(18)**, 4480-4486 (1991).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.