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
Materials Supplied

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

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item</th>
<th>Quantity/Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>10009908</td>
<td>JC-1 Reagent</td>
<td>1 vial/500 μl</td>
<td>-20°C</td>
</tr>
<tr>
<td>10009322</td>
<td>Cell-Based Assay Buffer Tablet</td>
<td>3 tablets</td>
<td>RT</td>
</tr>
</tbody>
</table>

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.
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$_6$. More recently, a 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 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.

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 CO2 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 CO2 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.
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 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.
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 10⁶ 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 10⁴ - 5 x 10⁵ cells/well in 100 μl culture medium in a CO₂ 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 CO₂ 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.
Representative Staining Results

Figure 1. H9C2 cells were plated at a density of $2 \times 10^4$ 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 $2 \times 10^5$ 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

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

References


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.

This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from Cayman Chemical Company.

©01/10/2016, Cayman Chemical Company, Ann Arbor, MI, All rights reserved. Printed in U.S.A.