JC-1 Mitochondrial Membrane Potential Flow Cytometry Assay Kit

Item No. 701560

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. After opening kit, store individual components as stated below.

Item Number	Item	500 Tests Quantity/Size	Storage
10009908	JC-1 Reagent	1 vial/500 µl	-20°C
701311	FCCP Control (20 mM)	1 vial/25 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. Adherent or suspension cells
2. Flow cytometer equipped with a 488 nm laser line and filters capable of detecting 525 nm and 585 nm
3. FACS tubes or v-bottom staining plates
4. Distilled water

INTRODUCTION

Background

Mitochondrial membrane potential ($\Delta\psi_M$) is an important parameter of mitochondrial function and has been used as an indicator of cell health. Variations in $\Delta\psi_M$ 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-dazolylcarbocyanineiodide (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 Flow Cytometry Assay Kit can be used to study mitochondrial behavior in a variety of conditions, including apoptosis. Changes in $\Delta\psi_M$, reflected by aggregation level of JC-1, can be determined as a ratio of red to green mean fluorescence intensities using flow cytometry. Flow cytometry is an ideal way to assess the JC-1 aggregation at the single-cell level, and provides the added benefit of potential to multiplex other readouts from the same cells.[3] This kit additionally contains FCCP for treatment of cells as a compensation control. The reagents in this kit are sufficient for staining up to 500 samples for JC-1.

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 each Cell-Based Assay Buffer tablet (Item No. 10009322) in 100 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

2. **JC-1 Staining Solution (2X) Preparation**

   Thaw JC-1 Reagent (Item No. 10009908) at room temperature. Mix well to ensure there are no particles or flakes in the solution. Make a 2X staining solution by diluting the stock vial 1:100 into complete culture medium (containing FBS), and keep in the dark at room temperature until use.

   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.

2. **FCCP Control Preparation**

   Make an FCCP control solution of 10 µM by diluting 5 µl of the 20 mM stock into 10 ml assay buffer.

   NOTE: The FCCP control solution/samples are designed to provide a control for use in analyzing the flow cytometric data and are the best way to compensate for spectral overlap of green monomers into the red aggregates channel. 10 µM FCCP will typically uncouple mitochondria in most cell types, however, a titration may be necessary to find the optimal dose in your cells

ASSAY PROTOCOL

Flow Cytometry

1. Culture cells and treat as required by your experimental design in a CO_2 incubator at 37°C, running each sample (at least 5 x 10^4 cells) in duplicate or triplicate. If using adherent cells, they should be <80% confluent at the time of staining. Extra wells should be set aside without treatment for the FCCP Control.

2a. For adherent cells, gently remove from the cell culture vessel and transfer to FACS tubes or a staining plate. Trypsinization is compatible with this procedure.

2b. Suspension cells can be removed directly to the staining tubes or plate.

3. Centrifuge cells at 250 x g for 5 minutes at room temperature

4. Carefully remove the supernatant and resuspend cells in 100 µl assay buffer, except for the FCCP control samples, which are resuspended in 100 µl of the 10 µM FCCP control solution prepared on page 6.

5. Incubate the samples for 5 minutes at room temperature.

6. To each sample, add 100 µl of JC-1 Staining Solution (2X) prepared on page 6.

7. Immediately collect data on your flow cytometer, maintaining the samples at room temperature, and as dark as possible. Detect JC-1 aggregates (high $\Delta\psi_M$) in the PE channel and monomers (low $\Delta\psi_M$) in the FITC channel.

PERFORMANCE CHARACTERISTICS

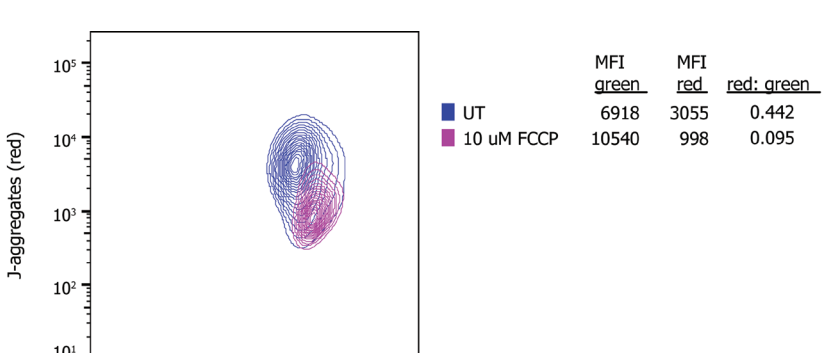

Figure 1. THP-1 cells respond to FCCP with a shift in JC-1 spectrum. THP-1 cells were incubated in PBS with or without 10 µM FCCP and stained as described in the kit booklet. Events were collected by a MACSQuant flow cytometer (Miltenyi) and digitally compensated and analyzed using FlowJo (Treestar). Live cells (gated by FSC/SSC) are shown overlaid from representative samples along with corresponding geometric mean fluorescence intensities (MFI). Addition of FCCP to cells induces a shift from red to green fluorescence, which is most evident in the ratio of red to green MFI.

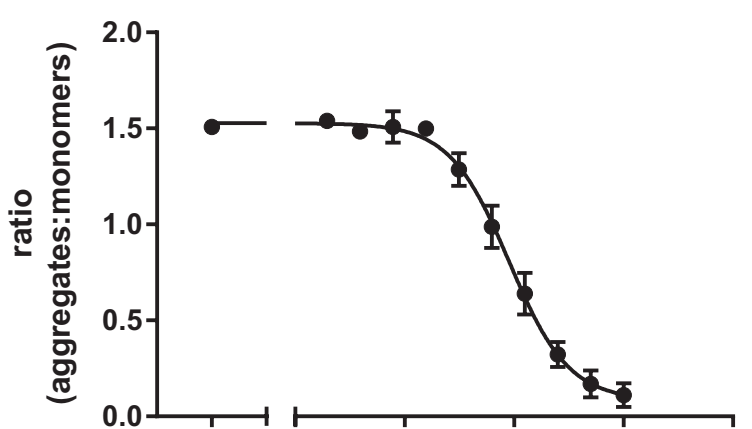

Figure 2. FCCP-mediated drop in mitochondrial membrane potential is detectable by ratiometric analysis of JC-1 aggregates and monomers. Huh-7 hepatocellular carcinoma cells were trypsinized, treated with varying concentrations of FCCP for 5 minutes and then stained with JC-1 according to the kit booklet protocol. Data were collected by a Miltenyi MACSQuant flow cytometer and compensated and analyzed in FlowJo by Treestar. The geometric mean fluorescence intensities (MFIs) of the red and green channels were obtained for the entire cell population and red was divided by green to generate the ratio plotted here against FCCP concentration.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
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
Cells appear to be double-stained or too bright	A. Uncompensated samples B. High voltage during sample collection	A. Use FCCP-treated control to compensate for spectral overlap between green and red channels B. Ensure that cells are visible in a red vs. green dot plot

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).
3. Troiano, L., Ferraresi, R., Lugli, E., *et al.* Multiparametric analysis of cells with different mitochondrial membrane potential during apoptosis by polychromatic flow cytometry. *Nature Protocols* **2(11)**, 2719-2727 (2007).

NOTES

Warranty and Limitation of Remedy

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