MitoProbe™ JC-1 Assay Kit for Flow Cytometry (M34152)

Quick Facts

Storage upon receipt:

- 2–8ºC
- Protect from light

Ex/Em: 514/529 and 590 nm

Number of Assays: 100, based on labeling volumes of 1.0 mL

Introduction

The MitoProbe™ JC-1 Assay Kit supplies the cationic dye, JC-1 (5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide), and a mitochondrial membrane potential disrupter, CCCP (carbonyl cyanide 3-chlorophenylhydrazone), for the study of mitochondrial membrane potential. JC-1 (structure in Figure 1) exhibits potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from green (~529 nm) to red (~590 nm). Consequently, mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio. The potential-sensitive color shift is due to concentration-dependent formation of red fluorescent J-aggregates.[1-3] JC-1 can be used as an indicator of mitochondrial potential in a variety of cell types, including myocytes[3] and neurons,[4] as well as in intact tissues[5] and isolated mitochondria.[6] JC-1 is more specific for mitochondrial versus plasma membrane potential and more consistent in its response to depolarization than some other cationic dyes such as $DiOC_6(3)$ and rhodamine 123.[7]

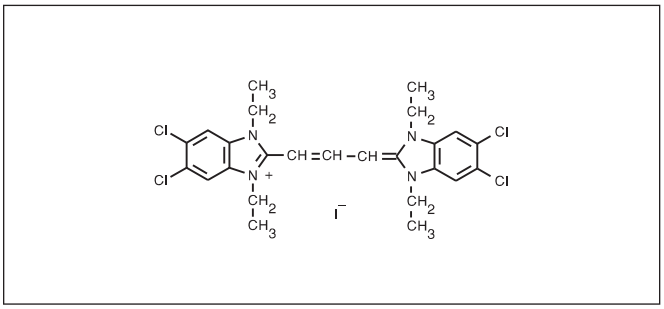

Figure 1. Structure of JC-1, molecular weight: 652.23.

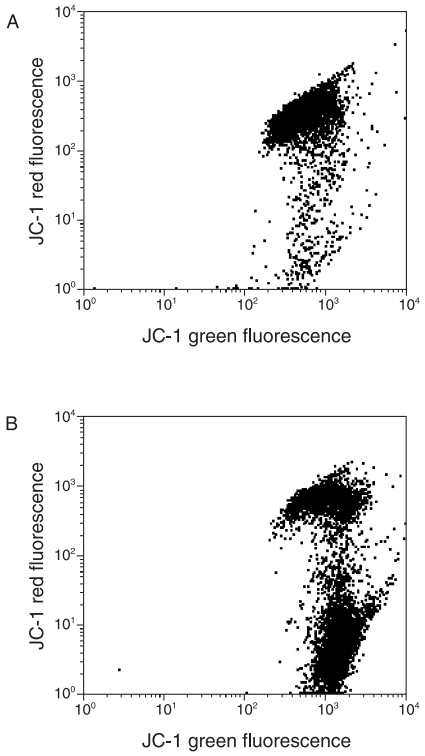

Figure 2. Jurkat cells stained with 2 µM JC-1. Cells were stained for 15 minutes at 37ºC, 5% CO_2, washed with PBS, and analyzed on a flow cytometer using 488 nm excitation with 530/30 nm and 585/42 nm bandpass emission filters. Untreated cultured cells are shown in panel A. Panel B shows cells induced to apoptosis with 10 µM camptothecin for 4 hours at 37ºC.

The ratio of green to red fluorescence is dependent only on the membrane potential and not on other factors such as mitochondrial size, shape, and density, which may influence single-component fluorescence signals. Use of fluorescence ratio detection therefore allows researchers to make comparative measurements of membrane potential and determine the percentage of mitochondria within a population that respond to an applied stimulus. Subtle heterogeneity in cellular responses can be discerned in this way.[1,6] For example, four distinct patterns of mitochondrial membrane potential change in response to glutamate receptor activation in neurons have been identified using confocal ratio imaging of JC-1 fluorescence.[4] The

most widely implemented application of JC-1 is for detection of mitochondrial depolarization occurring in apoptosis (Figure 2).[7-10]

Materials

Kit Contents

- JC-1 (5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide), 5 vials each containing 30 µg powdered dye
- DMSO, 1.5 mL dimethlysufoxide
- CCCP, 125 µL of 50 mM CCCP in DMSO
- 10X phosphate-buffered saline, 25 mL

Storage and Handling

Upon receipt, components should be stored at 2–8°C. Before opening, each vial must be at room temperature. When stored properly, the reagents should be stable for at least twelve months.

Spectral Characteristics

The approximate excitation peak of JC-1 is 488 nm. The approximate emission peaks of monomeric and J-aggregate forms are 529 nm and 590 nm, respectively. Cells labeled with JC-1 can be analyzed by flow cytometry using 488 nm excitation and green or orange-red emission, and by fluorescence microscopy using standard filters for Alexa Fluor® 488 dye and R-phycoerythrin.

Experimental Protocol

The following protocol describes introducing JC-1 reagent into the cultured cells and analyzing the stained cells by flow cytometry. Suggested initial conditions may require modifications because of differences in cell types and culture conditions. The concentration of probe for optimal staining will vary depending upon the application. A concentration range should be tested starting around 2 µM JC-1. CCCP controls should be used to confirm that the JC-1 response is sensitive to changes in membrane potential.

Reagent Preparation

Allow the JC-1 powder and DMSO solutions to come to room temperature before use. Prepare a 200 µM JC-1 stock solution immediately prior to use by dissolving the contents of one vial in 230 µL of the DMSO provided.

Labeling of Cells

Before beginning the experiment, ensure that the vial of CCCP has equilibrated to room temperature.

1.1 For each sample, suspend cells in 1 mL warm medium, phosphate-buffered saline, or other buffer at approximately 1×10^6 cells/mL.

1.2 For the control tube, add 1 µL of 50 mM CCCP (supplied with the kit, 50 µM final concentration) and incubate the cells at 37°C for 5 minutes.

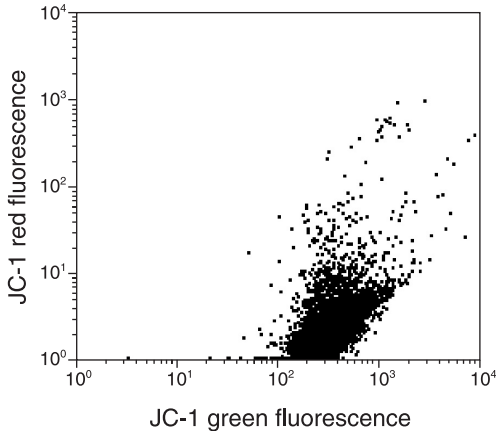

Figure 3. Jurkat cell response to CCCP. Cells were pretreated with 50 M CCCP for 5 minutes, stained with 2 M JC-1. Cells analyzed on a flow cytometer using 488 nm excitation with 530/30 nm and 585/42 nm band-pass emission filters.

Note: CCCP can be added simultaneously with JC-1. Titration of the CCCP may be required for optimal results with an individual cell system.

1.3 Add 10 µL of 200 µM JC-1 (2 µM final concentration) and incubate the cells at 37°C, 5% CO_2, for 15 to 30 minutes. If performing additional labeling, for example with an annexin V conjugate, follow the protocol below, beginning with step 2.1. If no additional staining is to be performed, proceed with step 1.4.

1.4 OPTIONAL: Wash cells once by adding 2 mL of warm phosphate-buffered saline (PBS) or other buffer to each tube of cells.

1.5 Pellet the cells by centrifugation.

1.6 Resuspend by gently flicking the tubes. Add 500 µL PBS (or other suitable buffer) to each tube.

1.7 Analyze on a flow cytometer with 488 nm excitation using emission filters appropriate for Alexa Fluor 488 dye and R-phycoerythrin. Gate on the cells, excluding debris. Using the CCCP-treated sample, perform standard compensation (Figure 3).

Additional Labeling with an Annexin V Conjugate

It is possible to label the JC-1–stained cells with other markers for apoptosis or viability, as long as the fluorescence emission of the additional label is spectrally resolved from JC-1. The example below is a protocol for labeling with annexin V–allophycocyanin.

2.1 After step 1.3 (above), wash cells once by adding 2 mL of warm phosphate-buffered saline or other buffer to each tube of cells.

2.2 Pellet the JC-1–stained cells and resuspend in 100 µL of

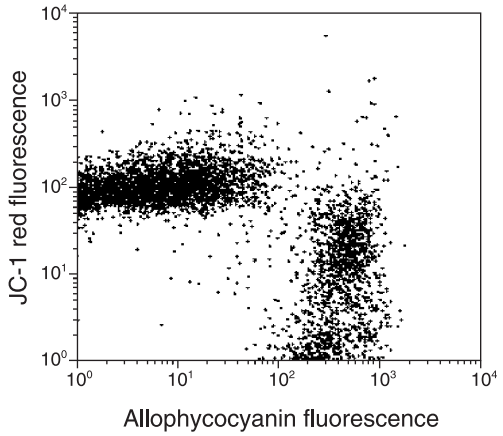

Figure 4. Camptothecin-treated Jurkat cells stained with JC-1 and annexin V–allophycocyanin. Cells were incubated for 4 hours with 10 µM camptothecin at 37°C, 5% CO_2, then stained with 2 µM JC-1 and annexin V–allophycocyanin. Cells were analyzed on a flow cytometer using 488 nm and 633 nm excitations with 530/30 nm, 585/42 nm, and 660/20 nm bandpass emission filters.

1X annexin binding buffer (10 mM HEPES, 140 mM NaCl and 2.5 mM $CaCl_2$, pH 7.4).

2.3 Add 5 µL annexin V conjugate (e.g. annexin V-allophycocyanin, A35110).

Note: 5 µL is appropriate for annexin V conjugates from Molecular Probes. Conjugates purchased from other suppliers may require a different volume to be effective.

2.4 Incubate the samples at 37°C for 15 minutes. (37°C is important to maintain membrane potential.)

2.5 Add 400 µL annexin binding buffer.

2.6 Analyze on a flow cytometer with 488 nm and 633 nm excitation using emission filters appropriate for fluorescein, R-phycoerythrin, and allophycocyanin (Figure 4).

References

1. Proc Natl Acad Sci U S A 88, 3671 (1991); 2. Biochemistry 30, 4480 (1991); 3. J Physiol 486, 1 (1995); 4. J Neurosci 16, 5688 (1996); 5. Methods 18, 104 (1999); 6. Exp Cell Res 222, 84 (1996); 7. FEBS Lett 411, 77 (1997); 8. J Neurosci 18, 932 (1998); 9. J Cell Biol 138, 449 (1997); 10. Exp Cell Res 245, 170 (1998).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
M34152	MitoProbe™ JC-1 Assay Kit *for flow cytometry* *100 assays*	1 kit

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