JC-1 [5,5,6,6-Tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide] *CAS#: 3520-43-2*

Catalog number: 22200, 22201
Unit size: 5 mg, 50 mg

Component	Storage	Amount (Cat No. 22200)	Amount (Cat No. 22201)
JC-1 [5,5,6,6-Tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide] *CAS#: 3520-43-2*	Freeze (< -15 °C), Minimize light exposure	1 vial (5 mg)	1 vial (50 mg)

OVERVIEW

JC-1 is widely used for determining mitochondrial membrane potential with flow cytometry. It is capable of entering selectively into mitochondria, and changes reversibly its color from green to orange as membrane potentials increase (over values of about 80-100 mV). This property is due to the reversible formation of JC-1 aggregates upon membrane polarization that causes shifts in emitted light from 530 nm (i.e., emission of JC-1 monomeric form) to 590 nm (i.e., emission of J-aggregate). When excited at 490 nm, the color of JC-1 changes reversibly from green to greenish orange as the mitochondrial membrane becomes more polarized. Both colors can be detected using the filters commonly mounted in all flow cytometers, so that green emission can be analyzed in fluorescence channel 1 (FL1) and greenish orange emission in channel 2 (FL2). The main advantage of the use of JC-1 is that it can be both qualitative, considering the shift from green to orange fluorescence emission, and quantitative, considering the pure fluorescence intensity, which can be detected in both FL1 and FL2 channels. Besides its wide use with flow cytometry, it is also used in fluorescence imaging. We have developed a protocol to use it in fluorescence microplate platform. Although JC-1 is widely used in many labs, its poor water solubility makes it hard to use for some applications. Our JC-10 has much better water solubility than JC-1, and in some cell lines JC-10 has even superior performance to JC-1. Interestingly the performance of JC-10 is quite cell line-dependent.

AT A GLANCE

Protocol Summary

1. Prepare cells with test compounds
2. Add JC-1 working solution (100 µL/well for 96-well plates or 25 µL/well for 384-well plates)
3. Incubate at room temperature or 37°C for 1 hr
4. Remove the JC-1 working solution
5. Read fluorescence intensity at Ex/Em = 490/525 nm and 490/590 nm

KEY PARAMETERS

Fluorescence microscope

Emission	FITC and TRITC filter set
Excitation	FITC and TRITC filter set
Recommended plate	Black wall/clear bottom

Flow cytometer

Emission	530/30 nm, 575/26 nm filter
Excitation	488 nm laser
Instrument specification(s)	FITC and PE channel

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Prepare a 2 to 10 mM stock solution of JC-1 in high-quality, anhydrous DMSO. The stock solution should be used promptly; any remaining solution should be aliquoted and frozen at < -20°C.

Note Avoid repeated freeze-thaw cycles, and protect from light.

PREPARATION OF WORKING SOLUTION

Prepare a 1X JC-1 working solution: On the day of the experiment, either dissolve JC-1 solid in DMSO or thaw an aliquot of the JC-1 stock solution to room temperature. Prepare a 10 to 30 µM 1X JC-1 working solution in Hanks and 20 mM Hepes buffer (HHBS) or buffer of your choice, pH 7 with 0.02% Pluronic® F-127. Mix them well by votexing.

Note JC-1is not water soluble, so it intends to aggregate in solution. It is recommended to filter the JC-1 working solution before loading it into the cells.

SAMPLE EXPERIMENTAL PROTOCOL

Run JC-1 assay with a fluorescence microplate reader

1. Treat cells with test compounds for a desired period of time (For example, Jurkat cells can be treated with camptothecin for 4-6 hours) to induce apoptosis. For blank wells (medium without the cells), add the corresponding amount of compound buffer.
2. Add 100 µL/well/96-well plate or 25 µL/well/384-well plate of JC-1 working solution (from Step 1) into the cell plate.
3. Incubate the JC-1 loading plate in a 37°C, 5% CO2 incubator for 15-60 min.

   Note The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.
4. Remove the JC-1 working solution from the plate, wash the cells with HHBS or buffer of your choice. Add 100 µL/well/96-well plate or 25 µL/well/384-well plate of HHBS back to the cell plate.
5. Monitor the fluorescence change at Ex/Em = 490/525 nm and 490/590 nm for ratio analysis.

Run JC-1 assay with a fluorescence microscope or flow cytometer

1. Treat cells with test compounds for a desired period of time (For example, Jurkat cells can be treated with camptothecin for 4-6 hours) to induce apoptosis.
2. Centrifuge the cells to get 1-5 × 105 cells per tube.
3. Resuspend cells in 500 µL of JC-1 working solution (from Step 1).
4. Incubate at room temperature or 37 °C for 10 to 30 min, protected from light.
5. Wash the cells with HHBS or buffer of your choice. Resuspend cells in 500 µL of HHBS to get 1-5 × 10^5 cells per tube.
6. Monitor the fluorescence change at Ex/Em = 490/525 nm and

490/590 nm with a fluorescence microscope (using FITC and TRITC filters) or a flow cytometer (using FL1 and FL2 channels).

EXAMPLE DATA ANALYSIS AND FIGURES

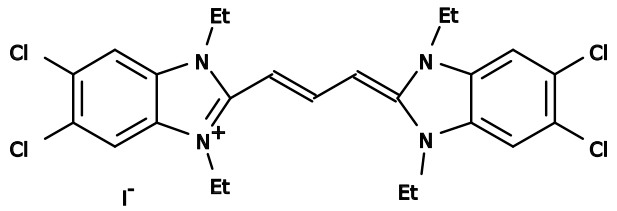

Figure 1. Chemical structure for JC-1 [5,5,6,6-Tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide] *CAS#: 3520-43-2*

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