JC-1 [5,5,6,6-Tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide]

Ordering Information	*Storage Conditions*
Product Number: 22200 (5 mg)	Keep at -20°C and desiccated Expiration date is 12 months from the date of receipt

Introduction

JC-1 is widely used for determining mitochondrial membrane potential with flow cytometry. It is capable of selectively entering mitochondria, and reversibly changes its color from green to orange as mitochondria membrane potentials increase (over values of about 80-100 mV). This property is due to the reversible formation of JC-1 aggregates upon mitochondria 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 form). 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. The green emission can be analyzed in fluorescence channel 1 (FL1) and the greenish orange emission in channel 2 (FL2). The main advantage of the use of JC-1 is that it can give both qualitative information, considering the shift from green to orange fluorescence emission, and quantitative information, considering the pure fluorescence intensity, which can be detected in both FL1 and FL2 channels. Besides its wide use in flow cytometry, JC-1 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 JC-10 has even superior performance to JC-1 in some cell lines.

Chemical and Physical Properties

Molecular Weight: 652.23
Solvent: Dimethylsulfoxide (DMSO)
Spectral Properties: Ex/Em = 515/529 and 590 nm

Assay Protocol with JC-1

Brief Summary

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

Note: Following is our recommended protocol for live cells. This protocol only provides a guideline, and should be modified according to your specific needs.

1. Prepare JC-1 working solution:

1.1 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.

1.2 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.

2. Run JC-1 assay with a fluorescence microplate reader:

2.1 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.

2.2 Add 100 µL/well/96-well plate or 25 µL/well/384-well plate of JC-1 working solution (from Step 1.2) into the cell plate.

2.3 Incubate the JC-1 loading plate in a 37 ^{o}C, 5% CO_2 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.

2.4 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.

2.5 Monitor the fluorescence change at Ex/Em = 490/525 nm and 490/590 nm for ratio analysis.

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

3.1 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.

3.2 Centrifuge the cells to get 1-5 × 10^5 cells per tube.

3.3 Resuspend cells in 500 µL of JC-1 working solution (from Step 1.2).

3.4 Incubate at room temperature or 37 °C for 10 to 30 min, protected from light.

3.5 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.

3.6 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).