JC-10 *Superior Alternative to JC-1*

Ordering Information:	*Storage Conditions:*
Product Number: 22204 (1 mg, 2 mg/mL in DMSO) 5x100 uL(5 Vials)	Keep at -20 °C and desiccated Expiration date is 6 months from the date of receipt

Introduction

Although JC-1 is widely used in many labs, its poor water solubility makes it hard to use for some applications. Even at 1 µM concentration, JC-1 tends to precipitate in aqueous buffer. JC-10 has been developed to be an alternative to JC-1when high dye concentration is desired. Compared to JC-1, our JC-10 has much better water solubility. JC-10 is capable of entering selectively into mitochondria, and reversibly changes its color from green to orange as membrane potentials increase. This property is due to the reversible formation of JC-10 aggregates upon membrane polarization that causes shifts in emitted light from 520 nm (i.e., emission of JC-10 monomeric form) to 570 nm (i.e., emission of J-aggregate form). When excited at 490 nm, the color of JC-10 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 greenish orange emission in channel 2 (FL2). Besides its use in flow cytometry, JC-10 can also be used in fluorescence imaging. We have developed a protocol to use JC-10 in a fluorescence microplate platform. In some cell lines JC-10 has even superior performance to JC-1.

Chemical and Physical Properties

Molecular Weight: ~ 600
Solvent: Dimethylsulfoxide (DMSO)
Spectral Properties: Ex/Em = 515/529 and 590 nm

Assay Protocol with JC-10

Brief Summary

Prepare cells with test compounds → Add JC-10 working solution (100 µL/well for 96-well plates or 25 µL/well for 384-well plate) → Incubate at room temperature or 37 °C for 1 hr → Remove the JC-10 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-10 working solution:

1.1 Each vial of DMSO stock solution (100 µL, 2 mg/mL, 3 mM) should be used only once. Any unused vials should be stored at ≤ -20 °C.
Note: Avoid repeated freeze-thaw cycles, and protect from light.

1.2 Prepare a 1X JC-10 working solution: On the day of the experiment, thaw an aliquot of the JC-10 stock solution to room temperature. Prepare a 10 to 30 µM 1X working solution in Hanks and 20 mM Hepes buffer (HHBS) or buffer of your choice, pH 7-8 with 0.02% Pluronic® F-127. Mix them well by votexing.
Note: For some cell lines, working solution at pH 8 might prevent JC-10 leakage.

2. Run JC-10 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-10 working solution (from Step 1.2) into the cell plate.

2.3 Incubate the JC-10 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-10 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 Emission 525 and 590 nm (excitation at 490 nm) for ratio analysis.

3. Run JC-10 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-10 working solution (from Step 1.2).

3.4 Incubate at room temperature or in a 37 °C, 5%CO_2 incubator for 10 to 30 min, protected from light.
Note: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

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.
Note: It is not necessary to wash the cells after dye-loading if running the experiment with a flow cytometer.

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).

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