JC-10 *Superior alternative to JC-1*

Catalog number: 22204
Unit size: 5x100 uL

Component	Storage	Amount (Cat No. 22204)
JC-10 *Superior alternative to JC-1*	Freeze (< -15 °C), Minimize light exposure	5x100 uL

OVERVIEW

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-1 where 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 changes reversibly 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). 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, so that green emission can be analyzed in fluorescence channel 1 (FL1) and greenish orange emission in channel 2 (FL2). Besides its potential use in flow cytometry, it can also be used in fluorescence imaging. We have developed a protocol to use JC-10 in fluorescence microplate platform. In some cell lines JC-10 has even superior performance to JC-1. Interestingly the performance of JC-10 is quite cell line-dependent. Our JC-10 is conveniently provided in DMSO solution at ~3 mM concentration (2 mg/mL).

AT A GLANCE

Protocol Summary

1. Prepare cells with test compounds
2. Add JC-10 working solution (100 μL/well for 96-well plates or 25 μL/well for 384-well plate)
3. Incubate at room temperature or 37°C for 1 hr
4. Read fluorescence intensity at Ex/Em = 490/525 nm and 540/590 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

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.

PREPARATION OF WORKING SOLUTION

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.

SAMPLE EXPERIMENTAL PROTOCOL

Run JC-10 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-10 working solution (from Step 1) into the cell plate.
3. Incubate the JC-10 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. Monitor the fluorescence change at Ex/Em = 490/525 nm (FITC channel) and 540/590 nm (TRITC channel) for ratio analysis.

   Optional: Remove the JC-10 working solution from the plate; add 100 μL/well/96-well plate or 25 μL/well/384-well plate of HHBS back to the cell plate before analysis.

Run JC-10 assay with a florescence microscope or a 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-10 working solution (from Step 2)
4. Incubate at room temperature or in a 37 °C, 5%CO2 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.
5. Monitor the fluorescence change at Ex/Em = 490/525 nm and 540/590 nm with a fluorescence microscope (using FITC and TRITC filters) or a flow cytometer (using FL1 and FL2 channels).

   Optional: Remove the JC-10 working solution from the plate; add 100 μL/well/96-well plate or 25 μL/well/384-well plate of HHBS back to the cell plate before analysis on fluorescence microscope.

EXAMPLE DATA ANALYSIS AND FIGURES

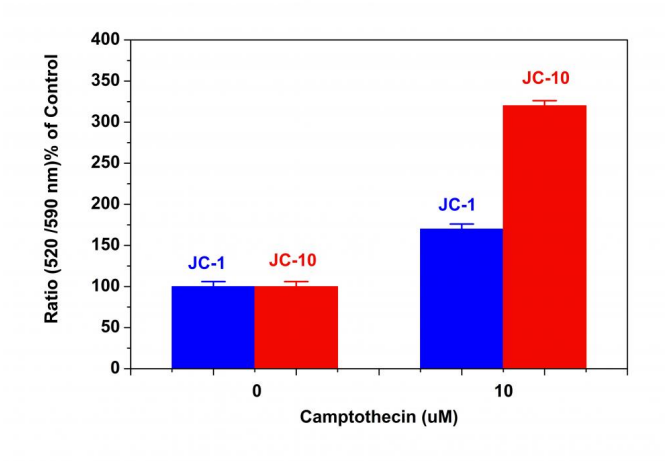

Figure 1. Campotothecin-induced mitochondria membrane potential changes were measured with JC-10™ and JC-1 in Jurkat cells. After Jurkat cells were treated with camptothecin (10 µM) for 4 hours, JC-1 and JC-10™ dye loading solutions were added to the wells and incubated for 30 minutes. The fluorescent intensities for both J-aggregates and monomeric forms of JC-1 and JC-10™ were measured at Ex/Em = 490/525 nm and 490/590 nm with NOVOstar microplate reader (BMG Labtech).

DISCLAIMER

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