Cell Meter™ JC-10 Mitochondrion Membrane Potential Assay Kit *Optimized for Microplate Assays*

Catalog number: 22800
Unit size: 500 Tests

Component	Storage	Amount
Component A: 100X JC-10 in DMSO	Freeze (<-15 °C), Minimize light exposure	1 vial (250 µL)
Component B: Assay Buffer A	Freeze (<-15 °C), Minimize light exposure	1 bottle (25 mL)
Component C: Assay Buffer B	Freeze (<-15 °C), Minimize light exposure	1 bottle (25 mL)

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 a superior 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. This Cell Meter™ JC-10 Mitochondrial Membrane Potential Assay Kit enable you to monitor mitochondrial membrane potential changes using a simple microplate reader while all the other commercial JC-1 assay kits require the use of a flow cytometer. Our kit provides the most robust method to monitor mitochondrial membrane potential changes, and can be readily used for screening a large compound library.

AT A GLANCE

Protocol summary

1. Prepare cells
2. Add test compounds
3. Add JC-10 dye-working solution (50 µL/well/96-well plate or 12.5 µL/well/384-well plate)
4. Incubate at 37°C, 5% CO_2 incubator for 30 to 60 minutes
5. Add Assay Buffer B (50 µL/well/96-well plate or 12.5 µL/well/384-well plate)
6. Monitor fluorescence intensities (bottom read mode) at Ex/Em = 490/525 nm (Cutoff = 515 nm) and 540/590 nm (Cutoff = 570 nm)

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490/540 nm
Emission:	525/590 nm
Cutoff:	515/570 nm
Instrument specification(s):	Bottom read mode
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

Add 50 µL of 100X JC-10 (Component A) into 5 mL of Assay Buffer A (Component B) and mix well to make JC-10 dye-working solution. Protect from light.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat cells by adding 10 µL of 10X test compounds (96-well plate) or 5 µL of 5X test compounds (384-plate) into the desired buffer (such as PBS or HHBS).

   Note It is not necessary to wash cells before adding compound. However, if tested compounds are serum sensitive, growth medium and serum factors can be aspirated away before adding compounds. Add the same volume of HHBS into the wells (such as 90 µL for a 96-well plate or 20 µL for a 384-well plate) after aspiration. Alternatively, cells can be grown in serum-free media.

2. Incubate the cell plate at room temperature or in a 37°C, 5% CO_2 incubator for at least 15 minutes or a desired period of time (for Jurkat cells, 4 - 6 hours with camptothecin or 3 - 5 hours with staurosporine treatment) to induce apoptosis.

3. Add 50 µL/well (96-well plate) or 12.5 µL/well (384-well plate) of JC-10 dye-working solution into the cell plate.

4. Incubate the plate in a 37°C, 5% CO_2 incubator for 30 - 60 minutes, 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. Add 50 µL/well (96-well plate) or 12.5 µL/well (384-well plate) of Assay Buffer B (Component C) into JC-10 dye-working solution plate before reading the fluorescence intensity.

   Note DO NOT wash the cells after loading. For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with brake off after adding Assay Buffer B (Component C).

6. Monitor the fluorescence intensities with a fluorescence microplate reader (bottom read mode) at Ex/Em = 490/525 nm (Cutoff = 515 nm) and 540/590 nm (Cutoff = 570 nm) for ratio analysis.

EXAMPLE DATA ANALYSIS AND FIGURES

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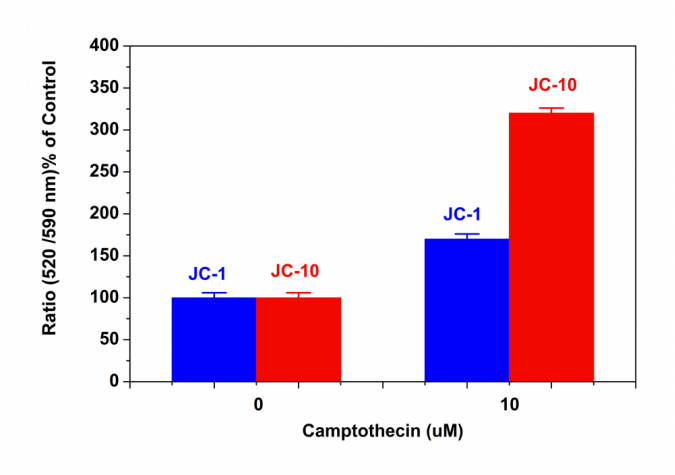

Figure 1. Campotothecin-induced mitochondria membrane potential changes were measured with JC-10™ and JC-1 in Jurkat cells. After Jurkat cells were treated with camptothecin (10 μM) for 4 hours, JC-1 and JC-10™ dye working solutions were added to the wells and incubated for 30 minutes. The fluorescence intensities for both J-aggregates and monomeric forms of JC-1 and JC-10™ were measured at Ex/Em = 490/525 nm (Cutoff = 515 nm) and 490/590 nm (Cutoff = 570 nm) with NOVOstar microplate reader (BMG Labtech).

DISCLAIMER