Cell Meter™ JC-10 Mitochondria Membrane Potential Assay Kit

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 22800 (5 plates)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

Although JC-1 is widely used in many labs, its poor water solubility causes great inconvenience. Even at 1 µM concentration, JC-1 tends to precipitate in aqueous buffer. JC-10 is developed to be a superior alternative to JC-1 when high dye concentration is desired. Compared to JC-1, JC-10 has much better water solubility. JC-10 is capable of selectively entering 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, it can also be used in fluorescence imaging and fluorescence microplate platform. This Cell Meter™ Mitochondria Membrane Potential Assay Kit enables researchers to run JC-10 assay in the format of microplate reader, and it provides the most robust assay method for monitoring mitochondria membrane potential changes.

The kit provides all the essential components with an optimized assay method for the detection of apoptosis in cells with the loss of mitochondrial membrane potential. This fluorometric assay is based on the detection of the mitochondrial membrane potential changes in cells by the cationic, lipophilic JC-10 dye. In normal cells, JC-10 concentrates in the mitochondrial matrix where it forms **red** fluorescent aggregates. However, in apoptotic and necrotic cells, JC-10 diffuses out of mitochondria. It changes to monomeric form and stains cells in **green** fluorescence. The kit can be used to screen both apoptosis activators and inhibitors. The assay can be performed in a convenient 96-well and 384-well fluorescence microtiter-plate format.

Kit Components

Components	**Amount**
Component A: 100X JC-10 in DMSO	1 vial (250 µL)
Component B: Assay Buffer A	1 bottle (25 mL)
Component C: Assay Buffer B	1 bottle (25 mL)

Materials Required (but not provided)

- 96 or 384-well microplates: Tissue culture microplates with black wall and clear bottom.
- Fluorescence microplate readers with a filter set of Ex/Em = 490/525 and 590nm.
- HHBS (1X Hank's with 20 mM Hepes Buffer, pH 7.0) or PBS.

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells → Add test compounds → Add JC-10 dye-loading solution (50 µL/well/96-well plate or 12.5 µL/well/384-well plate) → Incubate at room temperature for 1 hour → Add Assay Buffer B (50 µL/well/96-well plate or 12.5 µL/well/384-well plate) → Read fluorescence intensity at Ex/Em = 490/525 and 590 nm

1. Prepare cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 20,000 to 80,000 cells/well/90µL for a 96-well plate or 5,000 to 20,000 cells/well/20µL for a 384-well plate.

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellet in culture medium at 100,000-200,000 cells/well/90 µL for a 96-well poly-D lysine plate or 25,000-50,000 cells/well/20 µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

2. Prepare JC-10 dye-loading solution:

2.1 Thaw all the components at room temperature before use.

2.2 Add 50µL of 100X JC-10 (Component A) into 5 mL of Assay Buffer A (Component B), and mix well.
Note: Aliquot and store the unused Component A at -20 °C. Avoid repeated freeze/thaw cycles.

3. Run JC-10 assay:

3.1 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). For blank wells (medium without the cells), add the corresponding amount of compound buffer.
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.

3.2 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 (4-6 hours for Jurkat cells treated with camptothecin) to induce apoptosis.

3.3 Add 50 µL/well (96-well plate) or 12.5 µL/well (384-well plate) of JC-10 dye-loading solution (from step 2.2) into the cell plate (from Step 3.2).

3.4 Incubate the dye-loading plate in a 37 °C, 5% CO_2 incubator for 15-30 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 Add 50 µL/well (96-well plate) or 12.5 µL/well (384-well plate) of Assay Buffer B (Component C) into the dye-loading plate (from Step 3.4) before reading the fluorescence intensity.
Note 1: DO NOT wash the cells after loading.
Note 2: 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).

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

Data Analysis

The fluorescence intensities in the blank wells only filled with the growth medium were subtracted from the fluorescence intensity values for the wells filled with cells treated with the test compounds. The background fluorescence of the blank wells may vary depending on the sources of the microtiter plates or of the growth media.

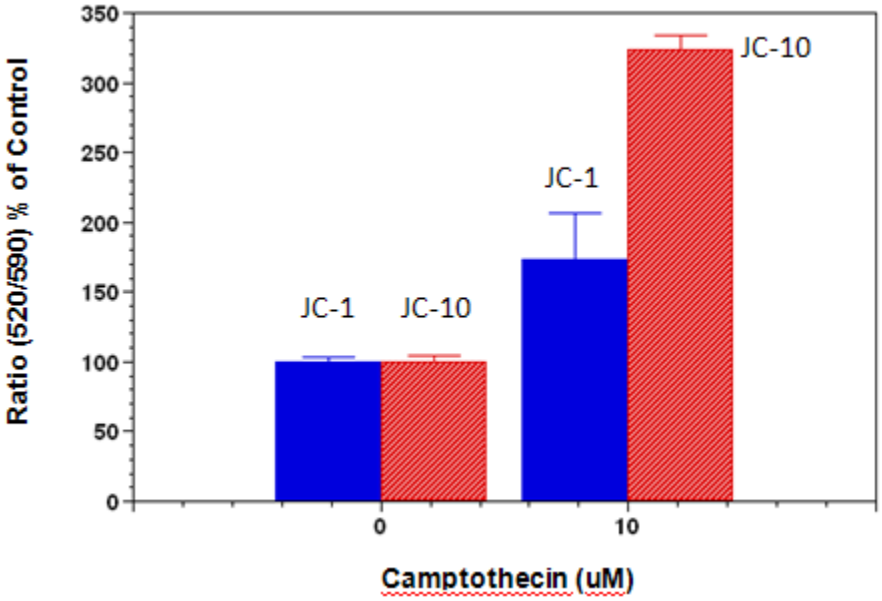

Figure 1. Campotothecin-induced mitochondria membrane potential changes were measured with JC-10 and JC-1 in Jurkat cells. After Jurkat cells were treated with camptothecin (10 µM) for 4 hours, JC-1 and JC-10 dye loading solutions were added to the wells and incubated for 30 minutes. The fluorescent 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).