

## Cell Meter™ JC-10 Mitochondrial 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  $\mu$ M 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.

This Cell Meter™ JC-10 Mitochondrial 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 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 $\mu$ L)
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 540/590nm.
- 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  $\mu$ L/well/96-well plate or 12.5  $\mu$ L/well/384-well plate) → Incubate at 37 °C, 5% CO<sub>2</sub> incubator for 30 to 60 minutes → Add Assay Buffer B (50  $\mu$ L/well/96-well plate or 12.5  $\mu$ L/well/384-well plate) → Monitor fluorescence intensity at Ex/Em = 490/525 and 540/590 nm**

#### 1. Prepare cells:

- 1.1 For adherent cells: Plate cells overnight in growth medium at 20,000 to 80,000 cells/well/90 $\mu$ L for a 96-well plate or 5,000 to 20,000 cells/well/20 $\mu$ L 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  $\mu$ L for a 96-well poly-D lysine plate or 25,000-50,000 cells/well/20  $\mu$ L 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 kit components at room temperature before use.
- 2.2 Add 50 $\mu$ L of 100X JC-10 (Component A) into 5 mL of Assay Buffer A (Component B), and mix well.

*Note: Aliquot and store the unused 100X JC-10 (Component A) at -20°C. Avoid repeated freeze/thaw cycles.*

### 3. Run JC-10 assay:

- 3.1 Treat cells by adding 10  $\mu$ L of 10X test compounds (96-well plate) or 5  $\mu$ L 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  $\mu$ L for a 96-well plate or 20  $\mu$ L 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<sub>2</sub> 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.

- 3.3 Add 50  $\mu$ L/well (96-well plate) or 12.5  $\mu$ L/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<sub>2</sub> 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.*

- 3.5 Add 50  $\mu$ L/well (96-well plate) or 12.5  $\mu$ L/well (384-well plate) of Assay Buffer B (Component C) into the dye-loading plate (from Step 3.4) before reading the fluorescence intensity.

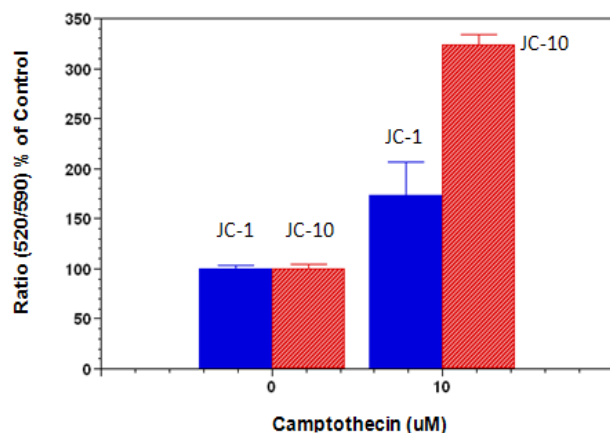
*Note 1: DO NOT wash the cells after loading.*

*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 (cut off at 515 nm) and 540/590 nm (cut off at 570 nm) with bottom read mode for ratio analysis.

### Data Analysis

The ratio of fluorescence intensities on Em at 525/590 was used for data analysis.



**Figure 1.** Camptothecin-induced mitochondria membrane potential changes were measured with JC-10 and JC-1 in Jurkat cells. After Jurkat cells were treated with camptothecin (10 mM) 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).

### References

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