# Cell Meter™ JC-10 Mitochondrion Membrane Potential Assay Kit *Optimized for Flow Cytometry Assays*

Catalog number: 22801
Unit size: 100 Tests

| Component | Storage | Amount |
|---|---|---|
| Component A: 200X JC-10 in DMSO | Freeze (<-15 °C), Minimize light exposure | 1 vial (250 µL) |
| Component B: Assay Buffer | Freeze (<-15 °C) | 1 bottle (50 mL) |

## OVERVIEW

Although JC-1 is widely used in many labs, its poor water solubility causes extraordinary 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 where high dye concentration is desired. Compared to JC-1, 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 and fluorescence microplate platform. This 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 exists in monomeric form and stains cells in green fluorescence. The kit is optimized for screening of apoptosis activators and inhibitors by flow cytometry. We also offer a convenient 96-well and 384-well fluorescence microtiter-plate format kit (cat#22800) for high through put screening.

## AT A GLANCE

**Protocol summary**

1. Prepare cells with test compounds at the density of $5 \times 10^5$ to $1 \times 10^6$ cells/mL
2. Resuspend the cells in 500 µL of JC-10 working solution ($2\text{-}5 \times 10^5$ cells/tube)
3. Incubate at 37°C or room temperature for 15-60 minutes
4. Analyze with flow cytometer using FL1 channel (green fluorescence monomeric signal) and FL2 channel (orange fluorescence aggregated signal)

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

## KEY PARAMETERS

| | |
|---|---|
| Instrument: | Flow cytometer |
| Excitation: | 488 nm laser |
| Emission: | 530/30 nm, 575/26 nm filter |
| Instrument specification(s): | FITC, PE channel |

## PREPARATION OF WORKING SOLUTION

Add 25 µL of 200X JC-10 (Component A) into 5 mL of Assay Buffer A (Component B) and mix well to make JC-10 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 with test compounds for a desired period of time to induce apoptosis. Set up parallel control experiments.

   **For Negative Control:** Treat cells with vehicle only.

   **For Positive Control:** Treat cells with FCCP or CCCP at 2-10 µM in a 37 oC, 5% CO2 incubator for 15 to 30 minutes.

   ***Note*** CCCP or FCCP can be added simultaneously with JC-10 working solution. Titration of the CCCP or FCCP may be required for optimal results with an individual cell lines.

2. Centrifuge the cells to get $2\text{-}5 \times 10^5$ cells per tube.

   ***Note*** For adherent cells, gently lift the cells by 0.5 mM EDTA to remain the cells intake, and wash the cells once with serum-containing media prior to incubation with JC-10 working solution.

3. Resuspend cells in 500 µL of JC-10 working solution.

4. Incubate the cells at room temperature or in a 37 oC, 5% CO2 incubator for 15 - 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. Monitor the fluorescence intensity using flow cytometer with FL1 channel for the green fluorescence monomeric signal (in apoptotic cells), and FL2 channel for the orange fluorescence aggregated signal (in healthy cells). Gate on the cells, excluding debris. It is recommended that compensation corrections be performed using the FCCP or CCCP-treated cells.

   - **Typical Flow Cytometer settings** for the analysis of JC-10 on a BD FACS Calibur System flow cytometer are as follows:
     Suggested initial conditions may require modifications because of differences in cell types and culture conditions, and also the individual instrumentation.
     FL1 PMT voltage 366
     FL2 PMT voltage 430
     Compensation: FL1 – 47.2% FL2; FL2 – 47.0% FL1

## EXAMPLE DATA ANALYSIS AND FIGURES

**Two-Parameter Analysis:**

1. Run the negative control cells (cells with vehicle treated only) first. Generate a log FL1 (X-axis) versus log FL2 (Y-axis) dot plot. Set PMT for FL1 and FL2 channels so both green and orange signals will fall between the 2nd and the 3rd log decade scale of FL1 and FL2. Set up quadrant gate to have a dual positive cell population in the upper right quadrant.

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2. The gate should be adjusted according to the conditions of the cells.

3. Adjust compensation values.

   ***Note*** The green JC-10 dye signal fluoresces mostly in the FL1 channel, but bleeds over into FL2 channel. This needs to be compensated (see Figure 1). To compensate, subtracting the FL1 bleed from FL2 (FL2 - % FL1). As the % compensation is increased, the green population was subtracted from the FL2 channel and placed in a single positive quadrant. Do not overcompensate. The FL2 background intensity of the negative control cells should be the same as that of a negative population. Compensate for any bleed from the orange channel into the green channel (FL1 - % FL2).

4. Run the induced cells (positive control), using the PMT settings established above for the negative control cells. A population of cells should appear in lower right quadrant. This reflects a loss of red emission on the FL2 axis, which corresponds to the loss of mitochondrial membrane potential in induced cells.

   ***Note*** Do not change voltage after the compensation, any further voltage changes may unbalance the compensations and the process will have to be repeated. The PMT settings for this assay may be low due to the strong fluorescence signals. Verify compensation values for each new experiment, or at least for each new cellular system tested. If the induced cells exhibits only a minimal decrease in red emission as compared to the negative control cells, increase the FL2 - % FL1 compensation.

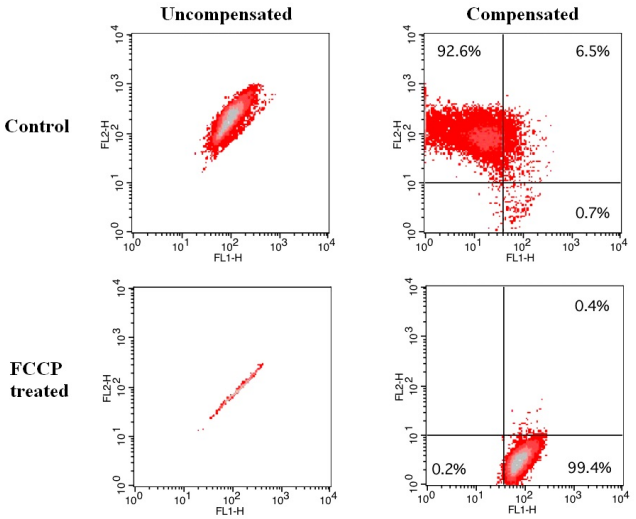


**Figure 1.** Effect of FCCP induced mitochondria membrane potential change in Jurkat cells. Jurkat cells were dye loaded with JC-10 dye working solution along with DMSO (Top) or 5 µM FCCP (Low) for 10 minutes. The fluorescence intensities for both J-aggregates and monomeric forms of JC-10 were measured with a FACSCalibur (Becton Dickinson) flow cytometer using FL1 and FL2 channels. Uncompensated data (left column) were compared with compensated data (right column).

## DISCLAIMER