

Cell Meter™ JC-10 Mitochondrial Membrane Potential Assay Kit

Optimized for Flow Cytometry

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 22801 (100 assays)	Keep in freezer and protect from light	Flow cytometer

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 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 exists in monomeric form and stains cells green. The kit is optimized to screen both apoptosis activators and inhibitors using flow cytometry while Kit 22800 is offered for microplate-based high throughput screening applications.

Kit Components

Components	Amount
Component A: 200X JC-10 in DMSO	1 vial (250 μL)
Component B: Assay Buffer	1 bottle (50 mL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells with test compounds at the density of 5×10^5 to 1×10^6 cells/mL → Resuspend the cells in 500 μL of 1X JC-10 dye-loading solution ($2-5 \times 10^5$ cells/tube) → Incubate at 37°C/ room temperature for 15-30 minutes → Analyze with flow cytometer in FL1 channel (green fluorescent monomeric signal) and FL2 channel (orange fluorescent aggregated signal)

1. Prepare cells:

Prepare cells at the density from 5×10^5 to 1×10^6 cells/mL.

Note: Each cell line should be evaluated on the 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 25 μL of 200X JC-10 (Component A) into 5 mL of Assay Buffer (Component B), and mix well.

Note: Aliquot and store the unused 200X JC-10 (Component A) at -20°C . Avoid repeated freeze/thaw cycles.

3. Run JC-10 assay:

3.1 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 $^\circ\text{C}$, 5% CO_2 incubator for 15 to 30 minutes.

Note: CCCP or FCCP can be added simultaneously with JC-10 dye-loading solution (from Step 2.2). Titration of the CCCP or FCCP may be required for optimal results with an individual cell lines.

3.2 Centrifuge the cells to get $2-5 \times 10^5$ 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 dye-loading solution (see Step 3.3).

3.3 Resuspend cells in 500 μL of JC-10 dye-loading solution (from Step 2.2).

3.4 Incubate the dye-loading cells at room temperature or in a 37 $^\circ\text{C}$, 5% CO_2 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.

3.5 Monitor the fluorescence intensity by using a flow cytometry in FL1 channel for the green fluorescent monomeric signal (in apoptotic cells), and the FL2 channel for the orange fluorescent 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 (See Appendix).

Data Analysis

1. In live non-apoptotic cells, JC-10 exists as a monomer in the cytosol stained green, and also accumulates as aggregates in the mitochondria stained red. In apoptotic and dead cells, JC-10 exists in the monomeric form only, staining the cytosol green.
2. Mitochondria containing red JC-10 aggregates in healthy cells are detectable by using the FL2 channel. Green JC-10 monomers in apoptotic cells are detectable by using the FL1 channel (FITC).
3. The intensity ratio of FL1 to FL2 is used to monitor the mitochondria membrane potential change induced by the compounds treatment

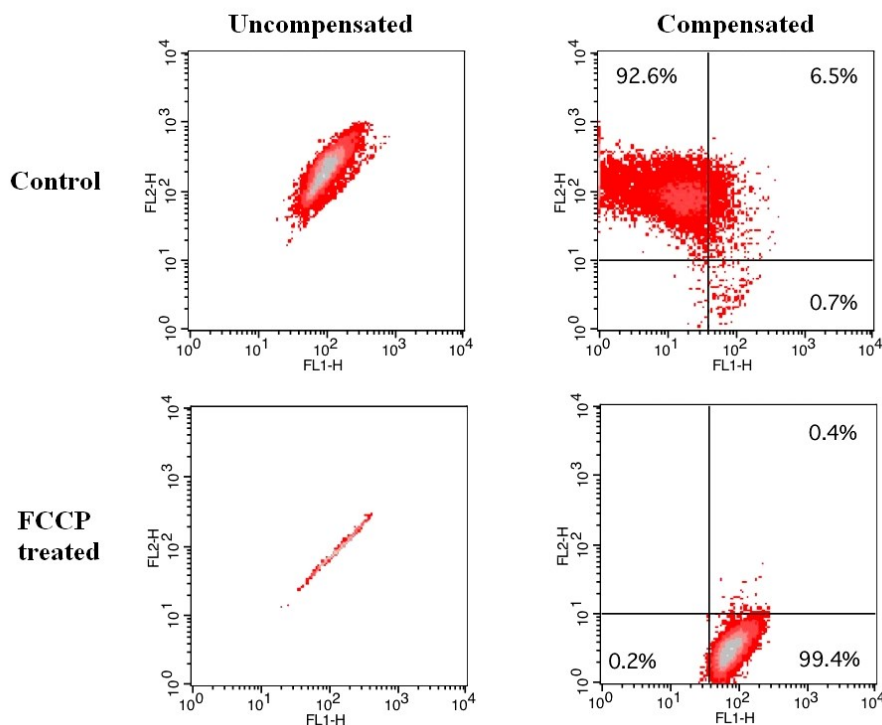


Figure 1. Effect of FCCP induced mitochondria membrane potential change in JurKat cells. JurKat cells were dye loaded with JC-10 dye-loading solution along with DMSO (Top) or 5 μ M FCCP (Low) for 10 minutes. The fluorescent intensities for both J-aggregates and monomeric forms of JC-10 were measured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

References

1. Jianxue Li, Lili Yu, Xuesong Gu, Yinghua Ma, Renata Pasqualini, Wadih Arap, Evan Y. Snyder, and Richard L. Sidman. Tissue plasminogen activator regulates Purkinje neuron development and survival. *PNAS*, Jun 2013; 110: E2410 - E2419.
2. Prantner D, Perkins DJ, Lai W, Williams MS, Sharma S, Fitzgerald KA, Vogel SN. 5,6-Dimethylxanthenone-4-acetic Acid (DMXAA) Activates Stimulator of Interferon Gene (STING)-dependent Innate Immune Pathways and Is Regulated by Mitochondrial Membrane Potential. *J Biol Chem*. 2012 Nov 16;287(47):39776-88. doi: 10.1074/jbc.M112.382986. Epub 2012 Oct 1.
3. Sakamuru S, Li X, Attene-Ramos MS, Huang R, Lu J, Shou L, Shen M, Tice RR, Austin CP, Xia M. Application of a homogenous membrane potential assay to assess mitochondrial function. *Physiol Genomics*. 2012 May 1;44(9):495-503. Epub 2012 Mar 20.
4. iCell® Cardiomyocytes: Assaying Mitochondrial Membrane Potential. http://www.cellulardynamics.com/products/lit/CDI_appnote_Cardiomyocytes_mitochondrial.pdf
5. Human iPS-derived Cardiomyocytes for Cardiotoxicity Screening (2011 SBS Molecular Devices). http://www.moleculardevices.com/Documents/general-documents/scientific-posters/imaging-posters/2011Poster_SBS%20Cardiotoxicity%20Imaging.pdf
6. Jinfang Liao, Qin Zhao, Xing Han, Zhenjun Diwu. Analyzing Cellular Apoptosis Through Monitoring Mitochondrial Membrane Potential Changes with JC-10. *Biophysical Journal*, Volume 100, Issue 3, Supplement 1, 2
7. Yu Shitara, Yuichi Tonohora, Takahiro Goto, Yasuhiro Yamada, Takashi Miki, Hirokazu Makino, Masanao Miwa, and Tohru Komiya. Mitochondrial P5, a member of protein disulphide isomerase family, suppresses oxidative stress-induced cell death. *J. Biochem.*, Jul 2012; 152: 73 - 85.

Appendix: Typical Flow Cytometer Settings and Two-Parameter Analysis

A 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

B Two-Parameter Analysis

B1. 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.

B2. The gate should be adjusted according to the conditions of the cells.

B3. Adjust compensation values.

Note 1: 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 (double positive top right quadrant Figure 1-A) and placed in a single positive quadrant Figure 1-B.

Note 2: Do not overcompensate. The FL2 background intensity of the negative control cells should be the same as that of a negative population (Figure 1-C).

Note 3: Compensate for any bleed from the orange channel into the green channel (FL1 - % FL2).

B4. 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 1: Do not change voltage after the compensation, any further voltage changes may unbalance the compensations and the process will have to be repeated.

Note 2: 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.

Note 3: If the induced cells exhibits only a minimal decrease in red emission as compared to the negative control cells, increase the FL2 - % FL1 compensation.