

JC-1 Mitochondrial Membrane Potential Detection Kit

Catalog Number: A048

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
JC-1 (Component A)	5×100 ~L	100X	<ul style="list-style-type: none"> -20 °C, Protect from light 	The product is stable for 1 year when stored as directed.
CCCP (Component B)	125 ~L	25 mM in DMSO		

Number of assays: 100 flow cytometry assays.

Approximate fluorescence excitation/emission maxima: JC-1: 514/529 and 590 nm.

Introduction

The loss of mitochondrial membrane potential is a hallmark for apoptosis. JC-1 Mitochondrial Membrane Potential Detection Kit measures the mitochondrial membrane potential in cells. The JC-1 Assay Kit uses a unique cationic dye (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) to signal the loss of mitochondrial membrane potential. JC-1 exhibits potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from green (529 nm) to red (590 nm). Consequently, mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio. In healthy cells, the JC-1 accumulates in the mitochondria to form J-aggregates, which become red fluorescence. In apoptotic cells, the JC-1 cannot accumulate within the mitochondria and remains in the cytoplasm in monomeric form to show green fluorescence.

The ratio of green to red fluorescence is dependent only on the membrane potential and not on other factors such as mitochondrial size, shape, and density, which may influence single-component fluorescence signals. Use of fluorescence ratio detection therefore allows researchers to make comparative measurements of membrane potential and determine the percentage of mitochondria within a population that respond to applied stimulus.

Both apoptotic and healthy cells can be visualized simultaneously by fluorescence microscopy using a wide band-pass filter suitable for detection of fluorescein and rhodamine emission spectra. JC-1 reagent is easy to use. Simply add the reagent into the cells. After 15 minute incubation, wash the cells and analyze by flow cytometry or fluorescence microscopy or fluorescence plate reader.

Experimental Protocols

Staining Protocol for Flow Cytometry

1.1 For each sample, suspend cells in 0.5 mL warm phosphate-buffered saline (PBS), or other buffer at approximately 1×10^6 cells/mL.

1.2 Induce apoptosis in cells according to your specific protocol. A negative control should be prepared by incubating cells in the absence of inducing agent. For positive control, add 1 µL of 25 mM CCCP (Component B) and incubate the cells at 37°C for 10 minutes.

1.3 Pellet the cells by centrifugation, and resuspend in 0.5 mL warm phosphate-buffered saline (PBS), or other buffer.

1.4 Add 5 µL of JC-1 stock solution (Component A) to each 0.5 mL of cell suspension, and incubate the cells at 37°C, 5% CO₂ for 15 to 30 minutes.

1.5 Pellet the cells by centrifugation, and resuspend in 0.5 mL warm phosphate-buffered saline (PBS), or other buffer.

1.6 Repeat step 1.5.

1.7 Analyze on a flow cytometer with 488 nm excitation using emission filters appropriate for fluorescein and rhodamine. Gate on the cells, excluding debris.

Staining Protocol for Fluorescence Microscopy

Staining of Cells in Suspension

2.1 For each sample, suspend cells in 0.5 mL warm phosphate-buffered saline (PBS), or other buffer at approximately 1×10^6 cells/mL.

2.2 Induce apoptosis in cells according to your specific protocol. A negative control should be prepared by incubating cells in the absence of inducing agent. For positive control, add 1 μ L of 25 mM CCCP (Component B) and incubate the cells at 37°C for 10 minutes.

2.3 Pellet the cells by centrifugation, and resuspend in 0.5 mL warm phosphate-buffered saline (PBS), or other buffer.

2.4 Add 5 μ L of JC-1 stock solution (Component A) to each 0.5 mL of cell suspension, and incubate the cells at 37°C, 5% CO₂ for 15 to 30 minutes.

2.5 Pellet the cells by centrifugation, and resuspend in 0.5 mL warm phosphate-buffered saline (PBS), or other buffer.

2.6 Repeat step 2.5.

2.7 Observe immediately with a fluorescence microscope using a dual-bandpass filter designed to simultaneously detect fluorescein and rhodamine. In live non-apoptotic cells, the mitochondria will appear red fluorescence with an emission at 590 nm. In apoptotic and dead cells, the dye will remain in its monomeric form and will appear green fluorescence with an emission at 530 nm.

Staining of Adherent Cells

3.1 Grow cells on a glass cover slip in a petri dish or in a chamber slide.

3.2 Induce apoptosis in cells according to your specific protocol. A negative control should be prepared by incubating cells in the absence of inducing agent. For positive control, mix 1 μ L of 25 mM CCCP (Component B) with 0.5 mL warm phosphate-buffered saline (PBS), and incubate the cells at 37°C for 10 minutes.

3.3 Remove the cell culture media.

3.4 Prepare 1X JC-1 solution by diluting 100X JC-1 stock solution (Component A) at 1:100 in warm phosphate-buffered saline (PBS), and add 0.5 mL 1X JC-1 solution to each dish, and incubate the cells at 37°C, 5% CO₂ for 15 to 30 minutes.

3.5 Remove media and wash once with warm phosphate-buffered saline (PBS), or other buffer.

3.6 Add one drop of PBS and cover with a coverslip.

3.7 Observe immediately with a fluorescence microscope using a dual-bandpass filter designed to simultaneously detect fluorescein and rhodamine. In live non-apoptotic cells, the mitochondria will appear red fluorescence with an emission at 590 nm. In apoptotic and dead cells, the dye will remain in its monomeric form and will appear green fluorescence with an emission at 530 nm.

Staining Protocol for Fluorescence Ratio Detection

Cells in Suspension

4.1 For each sample, suspend cells in 0.5 mL warm phosphate-buffered saline (PBS), or other buffer at approximately 1×10^6 cells/mL.

4.2 Induce apoptosis in cells according to your specific protocol. A negative control should be prepared by incubating cells in the absence of inducing agent. For positive control, add 1 μ L of 25 mM CCCP (Component B) and incubate the cells at 37°C for 10 minutes.

4.3 Pellet the cells by centrifugation, and resuspend in 0.5 mL warm phosphate-buffered saline (PBS), or other buffer.

4.4 Add 5 μ L of JC-1 stock solution to each 0.5 mL of cell suspension, and incubate the cells at 37°C, 5% CO₂ for 15 to 30 minutes.

4.5 Pellet the cells by centrifugation, and resuspend in 0.5 mL warm phosphate-buffered saline (PBS), or other buffer.

4.6 Pellet the cells by centrifugation, and resuspend in 600 μ L warm phosphate-buffered saline (PBS).

4.7 Transfer 200 μ L cell suspension into each of three wells of a black 96-well plate.

4.8 Measure red fluorescence (excitation 550 nm, emission 600 nm) and green fluorescence (excitation 485 nm, emission 535 nm) using a fluorescence plate reader.

4.9 Determine the ratio of red fluorescence divided by green fluorescence.

Adherent Cells

5.1 Plate cells into a 96-well plate and grow cells into your desired density.

5.2 Add your test chemicals and positive control into cell medium and incubate the cell culture plate at 37°C for required amount of time.

5.3 Remove the cell culture media, and replace with 200 μ L warm phosphate-buffered saline (PBS), or other buffer.

5.4 Add 2 μ L of JC-1 stock solution to each well, and incubate the plate at 37°C, 5% CO₂ for 15 to 30 minutes.

5.5 Wash cells with 200 μ L PBS twice.

5.6 Add 200 μ L PBS to each well.

5.7 Measure red fluorescence (excitation 550 nm, emission 600 nm) and green fluorescence (excitation 485 nm, emission 535 nm) using a fluorescence plate reader.

5.8 Determine the ratio of red fluorescence divided by green fluorescence.