# Mitochondrial Membrane Potential Assay Kit(with JC-1)

**Cat. No: E-CK-A301** **Size: 20 Assays/50 Assays/100 Assays**

| Cat. | Products | 20 Assays | 50 Assays | 100 Assays | Storage |
|---|---|---|---|---|---|
| E-CK-A301A | JC-1 (500×) | 20 μL | 50 μL | 100 μL | -20℃, shading light |
| E-CK-A301B | JC-1 Assay Buffer (10×) | 4 mL | 10 mL | 10 mL×2 | 2~8℃ |
| E-CK-A301C | 10 mM CCCP | 40 μL | 40 μL | 40 μL | -20℃, shading light |
| | Manual | | | One Copy | |

## Storage

JC-1 Assay Buffer (10×) should be store at 2~8℃, other reagents should be stored at -20℃. The validity period of this kit is 12 months.

JC-1 (500×) and 10 mM CCCP should be stored in dark. Avoid repeated freezing and thawing.

## Introduction

Elabscience® Mitochondrial Membrane Potential Assay Kit (with JC-1) is developed to identify early apoptotic by detecting changes in mitochondrial membrane potential using JC-1 as a fluorescent probe. This kit provides carbonyl cyanide m-chlorophenylhydrazone (CCCP) [E-CK-A301C] to induce the decrease in mitochondrial membrane potential as a positive control reagent.

## Detection principle

The decrease of mitochondrial membrane potential is a marker event in the early stage of apoptosis. It occurs before the appearance of nuclear apoptotic features (chromatin condensation and DNA fragmentation). Once the mitochondrial membrane potential collapses, apoptosis is irreversible.

JC-1 is an ideal fluorescent probe widely used to detect mitochondrial membrane potential ΔΨm. In normal cells, the mitochondrial membrane potential is high, and JC-1 exists in the mitochondrial matrix in the form of multimers, producing red fluorescence; in the early stage of apoptosis, the mitochondrial membrane potential decreases, and JC-1 exists in the mitochondrial matrix in the form of monomers. , resulting in green fluorescence.

The decrease of cell membrane potential can be detected by the transition of JC-1 from red fluorescence to green fluorescence, and the transition of JC-1 fluorescence color can be used as an early detection indicator of cell apoptosis. The relative ratio of red and green fluorescence is commonly used to measure the ratio of mitochondrial depolarization.

The maximum excitation wavelength of JC-1 monomer is 514 nm and the maximum emission wavelength is 529 nm; the maximum excitation wavelength of JC-1 polymer is 585 nm and the maximum emission wavelength is 590 nm.


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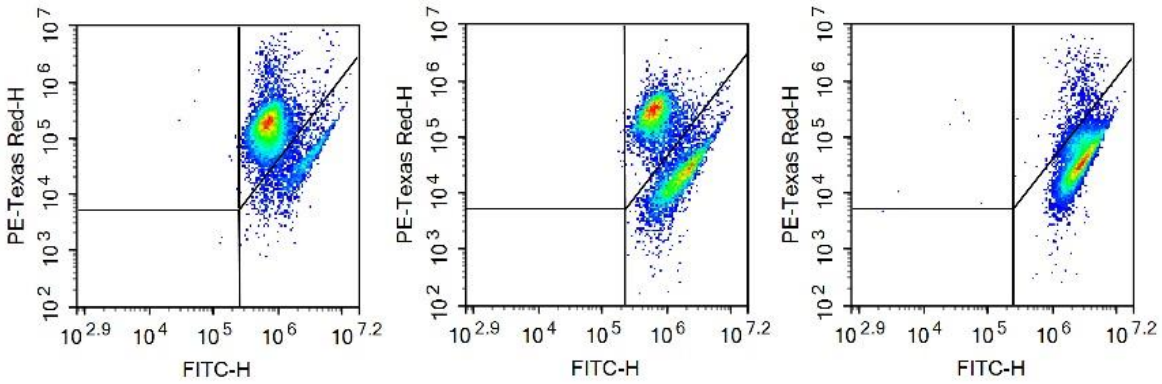
The effect of this kit on the apoptosis of Jurkat cells induced by camptothecin is shown in the figure below: normal cells (left) have a small amount of apoptosis, which is manifested as a small amount of mitochondrial membrane potential collapse cells; induced apoptotic cells (middle, 2.5 μM camptotheca Alkaline-treated Jurkat cells for 24 h) had a large number of mitochondrial membrane potential collapse cells; CCCP-treated cells (right, positive control) almost all cells had mitochondrial membrane potential collapse.




## Materials not supplied

Ultrapure water, vortex mixer, fluorescence microscope / laser scanning confocal microscope / flow cytometry.

## Reagent Preparation

**1. JC-1 working solution:**

Take an appropriate amount of JC-1 (500×), dilute JC-1 at the ratio of adding 9 mL of ultrapure water (self-prepared) for every 20 μL of JC-1 (500×), mix fully with a vortex mixer, and then add 1 mL of JC-1 Assay Buffer (10×) and mix fully with a vortex mixer. Prepare the fresh solution before use.

Note:

a) For 6-well plate, the volume of JC-1 working solution required for each well is 1 mL. And the volume of JC-1 working solution for other culture vessels can be deduced by analogy. For cell suspension, the volume of JC-1 working solution required for every $5\times10^5$~$1\times10^6$ cells is 0.5 mL.

b) JC-1 has a low solubility in water, it can be heated at 37 ℃ to promote dissolution.

**2. 1× JC-1 Assay Buffer**

Dilute the JC-1 Assay Buffer (10×) with ultrapure water for 10 times. The prepared the solution can be stored at 2~8 ℃.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## Experimental Procedure

➢ **Preparation procedure**

**Positive Control preparation (Only positive control samples require this step)**

Dilute 10 mM CCCP with cell culture medium for 1000 times, which the final concentration of CCCP is 10 μM, and incubate the cells with 10 μM CCCP for 20 min.

Note: For most cells, the mitochondrial membrane potential would be completely lost after 20 min of CCCP treatment at 10 μM and JC-1 stained cells showed green fluorescence, while normal cells showed red fluorescence after JC-1 staining. For specific cells, the concentration and the incubation time of CCCP may be different, please refer to the relevant literature to determine.

➢ **Operation for suspension cell**

1. Collect and count the cells, take $5\times10^5$~$1\times10^6$ cells, centrifuge at 300×g for 5 min, and discard the supernatant.
2. Resuspend the cells with 500 μL of JC-1 working solution, incubate the cells at 37 ℃ for 20 min.

Note: The incubation temperature varies with different cell types. Generally, the temperature for mammalian cells is 37 ℃. For other species, select the appropriate temperature according to the cell culture conditions.

3. After the incubation, centrifuge at 300×g for 5 min, discard the supernatant, wash the cells once with pre-cooled 1×JC- 1 Assay Buffer (300×g, 5 min), discard the supernatant.
4. Resuspend the cells with an appropriate amount of pre-cooled 1×JC-1 Assay Buffer, and analyze by flow cytometry, or observe by fluorescence microscope or laser confocal microscope.

Note:

a) In order to prevent fluorescence quenching, please perform observation as soon as possible (≤ 30 min), and store at 4 ℃ with shading light before detection.

b) When observing the results with a fluorescence microscope, in order to avoid the fluorescence quenching too fast, it is recommended to reduce the white light source and fluorescence power as much as possible, and then adjust the appropriate exposure time for observing /photographing.

c) If the fluorescence microscope filter is a long-pass filter, it is able to simultaneously detect normal cells and membrane potential-reduced cells in the green fluorescence channel.

➢ **Operation for adherent cells**

For the detection of adherent cells by flow cytometry, the cells can be collected first, and then perform the assay according to the operation for cell suspension above-mentioned. It should be noted that good cell growth is the key to the experiment. When detecting the apoptosis of adherent cells, mechanical operations such as trypsin digestion and pipetting may cause cell necrosis or apoptosis, which may affect the experimental results.

1. Discard the cell culture supernatant, wash the cells once with 1×JC-1 Assay Buffer.
2. Add 1 mL of JC-1 working solution, incubate the cells at 37 ℃ for 20 min.

Note:

a) For 6-well plate, the volume of JC-1 working solution required for each well is 1 mL. And the volume of JC-1 working solution for other culture vessels can be deduced by analogy.

b) The incubation temperature varies with different cell types. Generally, the temperature for mammalian cells is

37 ℃. For other species, select the appropriate temperature according to the cell culture conditions.

c) For cells with weak adhesion ability, it is recommended to do anti-detachment treatment before cell inoculation and staining, or directly dilute JC-1 (500×) into 1 ×with basal medium to prepare JC-1 working solution.

3. After the incubation, wash the cells once with 1×JC-1 Assay Buffer, then add 2 mL of cell culture medium or 1×JC-1 Assay Buffer.
4. Observe with a fluorescence microscope or laser scanning confocal microscope.

Note:

a) In order to prevent fluorescence quenching, please perform observation as soon as possible (≤ 30 min), and store at 4 ℃ with shading light before detection.

b) When observing the results with a fluorescence microscope, in order to avoid the fluorescence quenching too fast, it is recommended to reduce the white light source and fluorescence power as much as possible, and then adjust the appropriate exposure time for observing /photographing.

c) If the fluorescence microscope filter is a long-pass filter, it is able to simultaneously detect normal cells and membrane potential-reduced cells in the green fluorescence channel.

## Cautions

1. This kit is for research use only。
2. For your safety and health, please take safety precautions and follow the procedures of laboratory reagent operation. CCCP is an inhibitor of the mitochondrial electron transport chain, which is harmful to the human body. Please wear laboratory clothes and disposable gloves during operation, and avoid direct contact with the human body or inhalation of the body.
3. JC-1 may coagulate or precipitate at lower temperatures. Please heat at 20~25 ℃ water bath until it is completely dissolved.
4. Fluorescent substances are prone to quenching. When performing fluorescent observations, shorten the observation time as much as possible and pay attention to store the sample with shading light.


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