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×)	25 μL	25 μL×2	100 μL	-20°C, shading light
E-CK-A301B	JC-1 Assay Buffer (10×)	10 mL	10 mL	10 mL×2	2-8°C
E-CK-A301C	10 mM CCCP	40 μL	40 μL	40 μL	-20°C, shading light
	Manual			One Copy	

Storage

JC-1 Assay Buffer (10×) should be store at 2-8°C, other reagents should be stored at -20°C. 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.

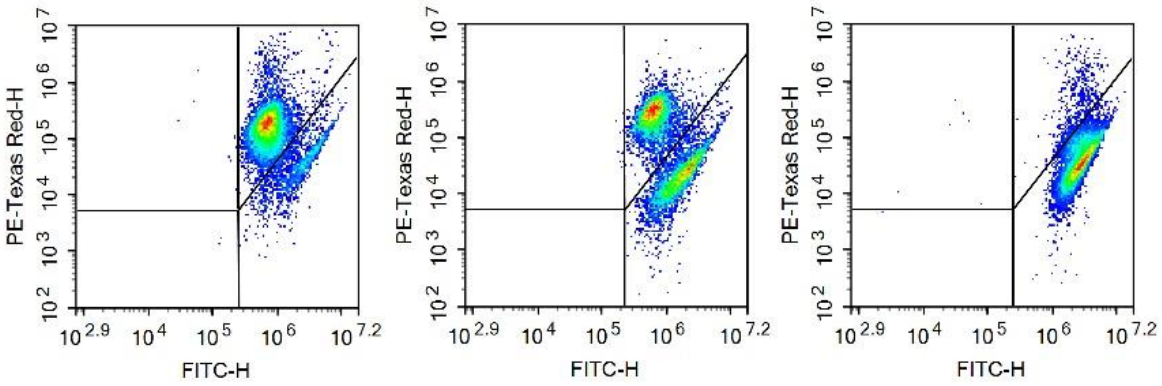
JC-1 is an ideal fluorescent probe widely used to detect mitochondrial membrane potential ΔΨm. In normal cells, the mitochondrial membrane potential is high, and JC-1 exists in the mitochondrial matrix in the form of multimers, producing red fluorescence; in the early stage of apoptosis, the mitochondrial 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 to detect apoptosis induced by camptothecin in Jurkat cells 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. 1× JC-1 Assay Buffer Preparation

Dilute the JC-1 Assay Buffer (10×) 10-fold with ultrapure water and mix thoroughly to prepare the 1× JC-1 Assay Buffer. The prepared 1× JC-1 Assay Buffer should be sealed and stored at 2-8°C, and used within one week.

2. Preparation of JC-1 Working Solution

Retrieve the aliquoted JC-1 (500×) and the prepared 1× JC-1 Assay Buffer. Prepare a sufficient amount of staining working solution by mixing JC-1 (500×) and 1× JC-1 Assay Buffer according to the ratio specified in the table below:

Component	Volume of JC-1 Working Solution		
	1 mL	5 mL	10 mL
JC-1 (500×)	2 μL	10 μL	20 μL
1× JC-1 Assay Buffer	998 μL	4990 μL	9980 μL

The mixing procedure is as follows (The JC-1 working solution must be freshly prepared and used immediately.):

① First, add the required amount of JC-1 (500×) to the bottom of the EP tube;

② Then, add the entire corresponding volume of 1× JC-1 Assay Buffer into the tube;

③ Mix thoroughly by pipetting up and down with a 1 mL pipette (if mixing is insufficient, the high-concentration salt ions and the concentrated JC-1 probe will aggregate and precipitate easily; do not

vortex, as it may compromise the dissolution).

Note:

a) For 6-well plates, apply 1 mL JC-1 working solution per well. Scale volumes proportionally for other culture vessels based on their growth surface area.

b) For cell suspension, the volume of JC-1 working solution required for every 5×10^5~1×10^6 cells is 0.5 mL.

3. **1× JC-1 Assay Buffer**

   Dilute the JC-1 Assay Buffer (10×) with ultrapure water for 10 times,the prepared 1× JC-1 Assay Buffer should be sealed and stored at 2-8°C and used within one week.

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 5×10^5~1×10^6 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°C for 20 min.

Note: The incubation temperature varies with different cell types. Generally, the temperature for mammalian cells is 37°C. 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 1×JC- 1 Assay Buffer (300×g, 5 min), discard the supernatant.
4. Resuspend the cells with an appropriate amount of 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°C 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.

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➢ **Operation for adherent cells**

1. Discard the culture supernatant of the adherent cells, and wash the cells once with 1×JC-1 Assay Buffer (rewarmed at 37℃ before use to avoid low temperature affecting the state of the cells).
2. Add the appropriate volume of JC-1 working solution according to the table below, and then incubate at 37°C for 20 min.

Cultureware	Volume of JC-1 working solution
96-well plate	100 µL/well
24-well plate	300~500 µL/well
12-well plate	0.5~1 mL/well
6-well plate	1 mL/well

Note:

a) The incubation temperature varies with different cell types. Generally, the temperature for mammalian cells is 37°C. For other species, select the appropriate temperature according to the cell culture conditions.

b) 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°C 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.

5. 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.

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°C 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.
5. Excessive acceleration and deceleration of centrifuge may cause cell loss. It is suggested to adjust theacceleration no more than 3 and deceleration no more than 2, that is, Acc≦3, Dec≦2.
6. This kit has a validity period of one year. To ensure optimal performance, it is recommended to use it within six months.

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