TECHNICAL MANUAL

Mitochondrial Membrane Potential Assay Kit (with JC-1)

- **SKU CODE:** AKES067
- **SIZE:** 20 Assays / 50 Assays / 100 Assays
- **DETECTION PRINCIPLE:** Fluorescence (JC-1)
- **RUO:** Research-Use-Only

Revised: 09/26

Mitochondrial Membrane Potential Assay Kit (with JC-1)

Please read entire manual carefully before starting experiment.

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1. Key Features

Specification:

20 Assays / 50 Assays / 100 Assays

Measuring instrument:

Fluorescence microscope, laser scanning confocal microscope or flow cytometer.

Detection wavelengths:

JC-1 monomer: excitation 514 nm, emission 529 nm. JC-1 polymer (aggregate): excitation 585 nm, emission 590 nm.

Sample type:

Suspension cells and adherent cells.

2. Storage & Expiry

JC-1 Assay Buffer (10×) should be stored 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.

For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

3. Product Description

The Assay Genie 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) 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.

4. Kit Contents

Component Name	20 Assays	50 Assays	100 Assays	Storage
JC-1 (500×)	25 µL	25 µL×2	100 µL	-20°C, shading light
JC-1 Assay Buffer (10×)	10 mL	10 mL	10 mL×2	2-8°C
10 mM CCCP	40 µL	40 µL	40 µL	-20°C, shading light
Manual	One Copy			

Note: All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.

Additional materials required:

- Ultrapure water.
- Vortex mixer.
- Fluorescence microscope, laser scanning confocal microscope or flow cytometer.
- Centrifuge, pipettes and disposable pipette tips.

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves. 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.

4. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
5. JC-1 may coagulate or precipitate at lower temperatures. Please heat at 20-25°C water bath until it is completely dissolved.
6. 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.
7. Excessive acceleration and deceleration of the centrifuge may cause cell loss. It is suggested to adjust the acceleration to no more than 3 and the deceleration to no more than 2, that is, Acc ≦ 3, Dec ≦ 2.
8. This kit has a validity period of one year. To ensure optimal performance, it is recommended to use it within six months.
9. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

6. 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. After thawing completely at room temperature, vortex each reagent to ensure homogeneity. 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

Note:

- The JC-1 working solution must be freshly prepared and used immediately. If both the 1× JC-1 Assay Buffer and the JC-1 working solution are prepared simultaneously, two thorough mixing steps are essential: first, after diluting the JC-1 Assay Buffer (10×) 10-fold to prepare the 1× JC-1 Assay Buffer, mix it thoroughly; then, add JC-1 (500×) to the well-mixed 1× JC-1 Assay Buffer and mix thoroughly again before use. (Insufficient mixing may cause aggregation and precipitation due to the interaction between high concentrations of salt ions and the JC-1 probe).
- 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.
- For cell suspension, the volume of JC-1 working solution required for every 5×10^5~1×10^6 cells is 0.5 mL.

7. Sample Preparation

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.

8. Assay Procedure

A. Operation for suspension cells

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.
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:

- 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.

- 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.
- 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.
- 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.

B. Operation for adherent cells

Volume of JC-1 working solution required for each cultureware format (referred to in step 2 below):

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

1. Discard the culture supernatant of the adherent cells, and wash the cells once with 1× JC-1 Assay Buffer (rewarmed at 37°C 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 above, and then incubate at 37°C for 20 min.
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.
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

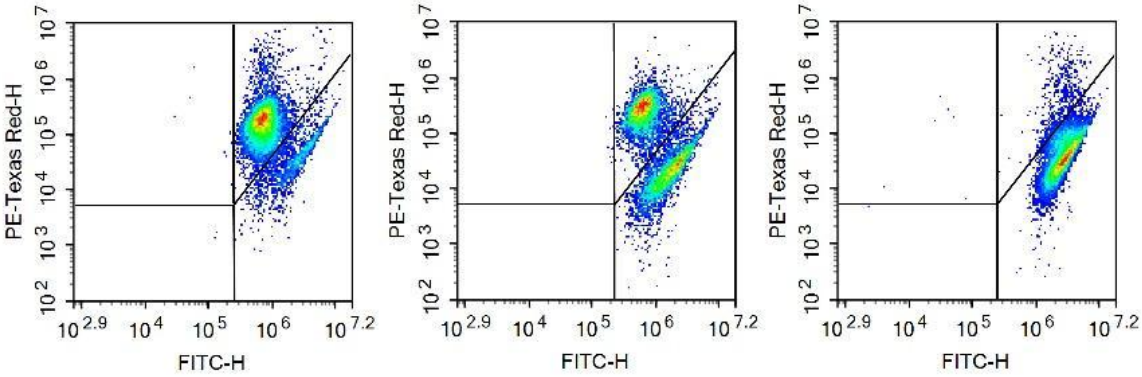
trypsin digestion and pipetting may cause cell necrosis or apoptosis, which may affect the experimental results.

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.
- 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.
- 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.
- 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.
- 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.

9. Representative Data

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 camptothecin-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.



The data shown above are for reference only and will vary with cell type, treatment conditions and instrument settings.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.