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Mitochondrial Membrane Potential Assay Kit (JC-1)

Cat #: KTA4001 Size: 20 T/100 T/500 T

	Mitochondrial Membrane Potential Assay Kit (JC-1)		
REF	**Cat #:** KTA4001	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Mitochondria, the site of most energy production in eukaryotic cells, have a double membrane structure: an outer membrane and a folded inner membrane. Across the inner membrane of intact mitochondria there is a voltage gradient (membrane potential=△Ψm) with the inside negative and the outside positive. Disruption of the mitochondrial transmembrane potential is one of the earliest intracellular events that occur following induction of apoptosis. Mitochondrial Membrane Potential Assay Kit (JC-1) provides a simple method for distinguishing between healthy and apoptotic cells by detecting the changes in the mitochondrial transmembrane potential, based on a carbocyanine dye, JC-1. In healthy cells, this dye accumulates and aggregates in the mitochondria, where it forms bright red fluorescent agglomerates. Any event that dissipates the mitochondrial membrane potential prevents the accumulation of the JC-1 dye in the mitochondria and thus, the dye remains in the cytoplasm in its monomer form, leading to a shift from red (agglomerated JC-1, Ex/Em=585/590 nm) to green fluorescence (JC-1 monomers, Ex/Em=510/527 nm). JC-1 can be used as an indicator of mitochondrial potential in a variety of cell types, including myo-cytes and neurons, as well as in intact tissues and isolated mitochondria. This kit contains CCCP that causes an uncoupling of the proton gradient, which established during the normal activity of electron carriers in the electron transport chain, and thus, dissipates the mitochondrial electrochemical potential and may be used as a control that prevents JC-1 aggregation.

Materials Supplied and Storage Conditions

Kit components	Size			Storage conditions
	20 T	100 T	500 T	
JC-1 Stain (100×)	220 μL	1.1 mL	1.1 mL×5	-20℃, protected from light
CCCP (50 mM)	25 μL	125 μL	625 μL	-20℃
PBS (10×)	5 mL	25 mL	125 mL	4℃

Materials Required but Not Supplied

- Culture plate, precision pipettes, disposable pipette tips
- Centrifuge, 37℃, 5% CO_2 cell incubator

- Fluorescence microscopy or flow cytometer
- Deionized water

Reagent Preparation

JC-1 Stain (100×): Equilibrate to room temperature before use; Store at -20℃, protected from light. Aliquot to avoid repeated freezing and thawing.

CCCP (50 mM): Equilibrate to room temperature before use; Store at -20℃. Aliquot to avoid repeated freezing and thawing.

PBS (10×): Prepare 1×PBS by dilute PBS (10×) with deionized water; Store at 4℃.

Assay Procedure

A. Quantification by Flow Cytometry

1. For non-adherent cells, the cells were collected by centrifugation at 300 g for 5 min and washed twice with 1×PBS. For adherent cells, the cells were first digested with trypsin solution, then centrifuged at 300 g for 5 min to collect the cells, and washed twice with 1×PBS. 1×PBS was discarded and 1 mL of cell medium was added to re-suspend the cells, and the cell density was 1×10^6 cells /mL.

2. Setting of positive control: 1 μL CCCP (50 mM) was added into the medium of positive control well, and its final concentration was 50 μM, thoroughly mixed, and incubated in a 5% CO_2 cell incubator at 37℃ for 5 min.

Note: (1) The concentration and duration of action of CCCP may be different for specific cells, which should be determined by reference to relevant literature. (2) It is recommended to retain unstained control cells (without JC-1 staining), suspended in the assay buffer, so that both treated and untreated samples can pass through flow cytometry.

3. Add 10 μL JC-1 (100×) to each well of the medium, mix thoroughly, and incubate at 37℃ in 5% CO_2 cell incubator for 15-30 min.

Note: 1) Different cells have different staining times. Before the formal experiment, do pre-experiment to determine the best incubation time. 2) Select cells with good state for staining to obtain the best experimental results.

4. After incubation at 37℃, the cells were precipitated by centrifugation at 4℃ for 3-4 min at 400 g. Discard the supernatant and take care not to remove the cells as much as possible.

5. Add 1 mL of precooled 1×PBS suspension cells, 400 g, centrifuge at 4℃ for 3-4 min, precipitate the cells, discard the supernatant, and repeat washing once.

6. The cells were re-suspended in 500 μL pre-cooled 1×PBS, and the cells were analyzed immediately by flow cytometry. JC-1 monomers in apoptotic cells are detectable in the FITC channel (usually FL1) showing diffused green fluorescence. JC-1 aggregates in healthy cells are detectable in the PI channel (usually FL2) showing punctate red fluorescence.

Note: After dyeing, try to complete the machine test within 30-60 min.

B. Detection by Fluorescence Microscopy

1. For non-adherent cells, place the cell suspension collected in step A6 on a slide and cover the cells with a cover slide following the procedure A1-6 of flow cytometry. The cells were analyzed by fluorescence microscopy with suitable filters.

2. For adherent cells: The recommended scheme is as follows:

(1) Taking 6-well plate as an example, 1 mL cell medium was added to each well. The same goes for other culture vessels.

(2) Setting of positive control: 1 μL CCCP (50 mM) was added into the medium of positive control well, and its final concentration was 50 μM, thoroughly mixed, and incubated in a 5% CO_2 cell incubator at 37℃ for 5 min.

Note: The concentration and duration of action of CCCP may be different for specific cells, which should be determined by reference to relevant literature.

(3) Add 10 μL JC-1 (100×) to each well of the medium, mix thoroughly, and incubate at 37℃ in 5% CO_2 cell incubator for 15-30 min.

Note: 1) Different cells have different staining times. Before the formal experiment, do pre-experiment to determine the best incubation time. 2) Select cells with good state for staining to obtain the best experimental results.

(4) After incubation at 37℃, the supernatant was removed and washed twice with 1×PBS.

(5) Add 500 μL of 1×PBS at room temperature and observe the cells under fluorescence microscope.

Note: Phenol red does not interfere with JC-1 staining. Try to take pictures within 30 minutes after dyeing.

Precautions

1.JC-1 (100×) will solidify and stick to the bottom, wall or cap of the centrifugal tube at low temperatures such as 4℃ and ice bath, and can be used after a short time in a water bath of 20-25℃ until completely melted.

2. After the JC-1 probe is loaded and washed, the follow-up test should be completed within 30 min as far as possible. Store in an ice bath before testing.

3.CCCP is a mitochondrial electron transport chain inhibitor, which is harmful to human body. Please be careful when operating and pay attention to effective protection to avoid direct contact with human body or inhalation.

Recommended Products

Catalog No.	Product Name
KTA0002	Annexin V-AbFluor™ 488 Apoptosis Detection Kit
KTA2010	TUNEL Apoptosis Detection Kit (Green Fluorescence)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.