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=$\Delta\Psi m$) 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	40 μL	200 μL	1 mL	-20℃, protected from light
CCCP (10 mM)	10 μL	50 μL	250 μL	-20℃
Assay Buffer (5×)	2 mL	10 mL	50 mL	4℃

Materials Required but Not Supplied

- Microcentrifuge, 24 well plate, Precision Pipettes, Disposable Pipette Tips

- Fluorescence Microscopy or Flow Cytometer
- Deionized Water, PBS

Reagent Preparation

JC-1 Stain: Warm to room temperature while using. Protect from light. Aliquot to avoid repeated freezing and thawing.

CCCP: Keep on ice while using.

1×Assay Buffer: Prepare 1×Assay Buffer by dilute 5× Assay Buffer with Deionized Water. Warm to 37℃ before use.

Staining Solution: Immediately before starting the assay, add 4 µL JC-1 Stain firstly, and then add 1 mL 1×Assay Buffer, vortex the solution immediately after adding into the buffer. Scale up accordingly for larger numbers of assays. Protect from light and pre-warm to 37℃ before use.

Assay Procedure

A. Quantification by Flow Cytometry

1. Treat cells with the desired method, and set up parallel control experiments.

For Negative Control: Treat cells with vehicle only.

For Positive Control: Treat cells with CCCP at 5-20 µM in a 37℃, 5% CO_2 incubator for 20 to 30 min.

Note: We recommend keeping unstained control cells (i.e. without JC-1 staining) suspended in Assay Buffer for both treated and untreated samples to set up the flow cytometer instrument.

2. For non-adherent cells, Collect 1×10^6 cells by centrifugation (4℃, 300 g, 5 min). Wash with ice-cold PBS twice and discard the PBS. For adherent cells, using Trypsin (EDTA free) to digest cells firstly and then centrifugation.

3. Resuspend the cells pellet in 500 uL Staining Solution.

4. Incubate the cells at 37℃ for 15-30 min in the dark.

5. Centrifuge cells at 500 g and discard supernatant.

6. Wash cell pellet with PBS and repeat step 5.

7. Resuspend cell pellet in 1 mL of the pre-warmed PBS and analyze cells 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.

B. Detection by Fluorescence Microscopy

1. For non-adherent cells: Follow the protocol for flow cytometry from step 1 to step 6 and place the cell suspension from Step A.6 on a glass slide. Cover the cells with a glass coverslip. Analyze cells by fluorescence microscopy using the appropriate filters as soon as possible.

2. For adherent cells: the suggested protocol is as below.

(1) Grow cells directly on a coverslip in 24 well dish. Incubate in a CO_2 Incubator at 37℃ for at least 24 h before treatment.

(2) Treat cells with the desired method and Prepare a positive control as mentioned in Step A.1.

(3) Wash cells with PBS twice and discard the PBS.

(4) Add 0.5 mL of Staining solution to cells and incubate at 37℃ for 15-30 min in the dark.

(5) Discard the supernatant and wash cells with PBS twice.

(6) Overlay the cells with the pre-warm growth medium and observe the cells under a fluorescence microscope.

Note: Phenol red does not interfere with JC-1 staining.

Recommended Products

Catalog No.	Product Name
KTA0002	Annexin V-AbFluor™ 488 Apoptosis Detection Kit

KTA2010	TUNEL Apoptosis Detection Kit (Green Fluorescence)
KTD102-EN	Apoptosis Assay Cocktail

Disclaimer

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