Mitochondrial Membrane Potential Detection Kit (JC-1 Method)

Cat. No. KITG-093 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit utilizes the JC-1 probe to detect changes in mitochondrial membrane potential. It can rapidly and sensitively detect mitochondrial membrane potential changes in cells, tissues, or purified mitochondria and can be used for early apoptosis detection. JC-1 is a widely used fluorescent probe for detecting mitochondrial membrane potential (ΔΨm). Compared with other cationic dyes such as $DiOC_6(3)$ and Rhodamine 123, JC-1 has higher specificity for mitochondrial membrane potential changes than for plasma membrane potential changes and provides better consistency in detecting mitochondrial depolarization. The red-to-green fluorescence intensity ratio is affected by changes in mitochondrial membrane potential rather than differences in mitochondrial size, shape, or density. The assay has strong detection sensitivity and can distinguish minor heterogeneity in cellular stress responses.
Description	JC-1 exists in two forms, monomer and aggregate, which have different emission spectra. The JC-1 monomer (J-monomer) has a maximum excitation wavelength of 514 nm and a maximum emission wavelength of 529 nm. The JC-1 aggregate (J-aggregates) has a maximum excitation wavelength of 585 nm and a maximum emission wavelength of 590 nm. JC-1 can penetrate the cell membrane and enter cells, where it exists as monomers free in the cytoplasm. In normal healthy cells, mitochondria have high membrane potential and polarity. JC-1 is rapidly taken up into the mitochondrial matrix depending on membrane-potential polarity and forms aggregates as its concentration increases. When excited at 488 nm, the aggregates have a maximum emission wavelength of 590 nm and emit orange-red fluorescence, appearing as FL1- and FL2-double-positive in flow cytometry. When cells undergo

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apoptosis, the mitochondrial transmembrane potential is depolarized and the mitochondrial membrane potential is low. JC-1 cannot aggregate in the mitochondrial matrix and is released from mitochondria into the cytoplasm. JC-1 is then present in monomer form, with a maximum emission wavelength of 527 nm when excited at 488 nm, producing green fluorescence and appearing as FL1-positive in flow cytometry. Apoptotic cells are mostly FL1-single-positive. A decrease in mitochondrial membrane potential is an early hallmark of apoptosis. The transition from red fluorescence to green fluorescence can be used to detect the decrease in mitochondrial membrane potential and as an early indicator of apoptosis. The relative red-to-green fluorescence ratio is commonly used to measure mitochondrial depolarization. Detection Methods Flow cytometry, fluorescence microscopy, and laser confocal microscopy. For JC-1 monomer detection, set excitation to 490 nm and emission to 530 nm. For JC-1 aggregate detection, set excitation to 525 nm, 568 nm, or 586 nm and emission to 590 nm. In flow cytometry, green fluorescence is detected through the FL1 channel and red fluorescence through the FL2 channel. FL-1+, FL-2+ cells are normal cells; FL-1+, FL-2- cells are apoptotic cells. For fluorescence microscopy or laser confocal microscopy, use settings suitable for green fluorescence, such as GFP or FITC, to detect JC-1 monomers and settings suitable for red fluorescence, such as PI or Cy3, to detect JC-1 aggregates. For fluorescence spectrophotometer or fluorescence microplate reader detection, use an excitation wavelength of 485 nm and an emission wavelength of 590 nm. If 485 nm cannot be selected on the fluorescence microplate reader, use an excitation wavelength within 475-520 nm. Compatible Sample Types Suspension cells, adherent cells, purified mitochondria, and tissue samples. Features and Benefits Rapid and sensitive detection of mitochondrial membrane potential changes in cells, tissues, or purified mitochondria. Suitable for early apoptosis detection. JC-1 has higher specificity for mitochondrial membrane potential changes than other cationic dyes such as $DiOC_6(3)$ and Rhodamine 123 and provides better consistency in detecting mitochondrial depolarization. The red-to-green fluorescence intensity ratio is affected by

	mitochondrial membrane potential changes rather than differences in mitochondrial size, shape, or density. The detection sensitivity is sufficient to distinguish minor heterogeneity in cellular stress responses. Size: 20T, 50T, 100T.
Applications	Detection of mitochondrial membrane potential changes; early apoptosis detection; cell analysis; flow cytometry analysis; fluorescence microscopy observation; laser confocal microscopy observation.
Notes	Quality Control Test Each batch of finished products is strictly tested to ensure product quality and consistency. Each batch of reagent raw materials is strictly tested to ensure product quality and consistency. Each batch of packaging consumables is strictly tested to ensure product quality and consistency. Important Notes 1. Before formal experiments, select several samples for preliminary experiments to optimize the experimental conditions and achieve the best results. 2. For reagents in screw-cap microtubes, briefly centrifuge before opening to collect liquid from the cap and inner wall at the bottom of the tube and avoid reagent loss. 3. Do not mix the kit reagents with reagents from other brands, because this will affect the results. 4. Bacterial or fungal contamination of samples or reagents, or cross-contamination among reagents, may produce incorrect results. 5. Disposable pipette tips, tubes, bottles, or glassware are recommended. Reusable glassware must be thoroughly cleaned and completely free of residual detergent before use. 6. After the experiment, dispose of all samples and contacted utensils according to the prescribed procedures. 7. The JC-1 staining solution is a DMSO solution. At low temperatures it may become solid and readily adhere to tube walls and pipette-tip walls. Warm it until dissolved, centrifuge the liquid to the bottom of the tube, and then open the tube. It may be warmed by hand, briefly in a 37°C water bath, or in a 20-25°C water bath until completely dissolved. Pipette tips should also be preheated in an incubator to prevent the solution from solidifying again on the inner wall of the tip and causing loss. 8. For long-term storage, the JC-1 staining solution may be aliquoted in small amounts according to usage and stored at -20°C. 9. JC-1 tends to form aggregates during

preparation of the staining working solution and should be added while shaking. If insoluble particles remain, centrifuge at 13,000 × g for 1 minute and use the supernatant. 10. Maintain a consistent pH in the balanced staining solution because pH changes affect membrane potential. 11. If a protein-containing cell suspension reaches equilibrium with the dye, proteins may bind part of the dye, reduce the dye concentration, and cause false depolarization. 12. Flow-cytometric detection of mitochondrial membrane potential is affected by multiple factors, including the inducer, cell-line type, and treatment time, and may produce different fluorescence-intensity ratios. There is no universal compensation and gating guide. Set negative- and positive-control groups in each experiment for fluorescence compensation and gating. 13. After JC-1 staining and washing, complete subsequent detection within 30 minutes whenever possible. Keep the samples on ice before detection. 14. If the JC-1 solution produces a precipitate, centrifuge it and use the supernatant. This does not affect the results. 15. Cell handling must be performed carefully to minimize artificial cell damage. Kit Components Component A: JC-1 staining solution - 100 µL for 20T; 250 µL for 50T; 500 µL for 100T. Component B: 10× incubation buffer - 4 mL for 20T; 10 mL for 50T; 20 mL for 100T. User manual: 1 copy for each kit. Materials Required but Not Supplied Instruments: Flow cytometer, laser confocal microscope, or fluorescence microscope; centrifuge; pipettes; refrigerator; ice box. Reagents: PBS buffer, pH 7.4, commonly used as 10 mM phosphate-buffered saline (1×), containing approximately 8 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, and 3 mM KCl. Alternatively, HBSS may be used: Hank's Balanced Salt Solution with calcium, magnesium, and glucose and without phenol red, containing $CaCl_2$, anhydrous, 140 mg/L (1.261 mM); $MgCl_2·6H_2O$, 100 mg/L (0.493 mM); $MgSO_4·7H_2O$, 100 mg/L (0.407 mM); KCl, 400 mg/L (5.333 mM); KH_2PO_4, 60 mg/L (0.441 mM); $NaHCO_3$, 350 mg/L (4.167 mM); NaCl, 8000 mg/L (137.931 mM); Na_2HPO_4, anhydrous, 48 mg/L (0.338 mM); and D-glucose, 1000 mg/L (5.556 mM). Consumables: Centrifuge tubes, pipette tips, and disposable gloves. Positive Control Setting Use carbonyl cyanide m-chlorophenylhydrazone (CCCP), a depolarizing agent, to

	prepare positive-control cells. Add CCCP to the cell-culture medium at a final concentration of 10 μM and treat the cells for 20 minutes, then perform mitochondrial membrane potential detection. For most cells, mitochondrial membrane potential is usually completely lost after treatment with 10 μM CCCP for 20 minutes; JC-1 staining should show green fluorescence, whereas normal cells should show red fluorescence. For specific cells, the required CCCP concentration and treatment time may vary. Safety Warning For research use only. Not for clinical, diagnostic, food, or cosmetic testing purposes. Wear an appropriate laboratory coat and disposable gloves. Avoid contact of reagents with skin or mucous membranes. If a reagent contacts the skin or eyes, rinse immediately with water.
Storage	Stability: Stable for one year when stored unopened under the required conditions. Use as soon as possible after opening. Store the kit at -20°C protected from light. Component A, JC-1 staining solution: Store at -20°C protected from light and avoid repeated freeze-thaw cycles. Component B, 10× incubation buffer: Store at 2-8°C.
Shipping	Ship on dry ice or with ice packs.
Reaction conditions	JC-1 Staining Working Solution Preparation Prepare the JC-1 staining working solution according to the number of samples. Reagent quantities may be adjusted proportionally. 1. Add 5 μL JC-1 stock solution to 450 μL pure water and shake vigorously until fully dissolved and mixed. 2. Add 50 μL 10× incubation buffer and mix well. 3. The resulting solution is the JC-1 staining working solution. Note: JC-1 must first be fully dissolved and mixed with ultrapure water before the 10× JC-1 incubation buffer is added. Do not first prepare 1× JC-1 incubation buffer and then add the JC-1 stock solution, because JC-1 will be difficult to dissolve completely and subsequent staining detection may be affected. Assay Protocol Suspension Cells 1. Collect the sample cells and the negative- and positive-control cells. Use 2-5 × 10^5 cells. 2. Wash the cells twice with PBS and collect them by centrifugation. 3. Resuspend the

	cells in 500 µL JC-1 staining working solution and incubate at 37°C in a 5% CO_2 incubator for 15-30 minutes. 4. Centrifuge at 4°C and 300-500 × g for 5 minutes. Discard the medium and collect the cells. Use the centrifugation speed normally applied to the specific cell type and take care not to aspirate the cells. 5. Wash the cells twice with cold PBS. Collect the cells by centrifugation at 500 × g and 2-8°C for 5 minutes. 6. Resuspend the cells in 500 µL PBS. 7. Analyze the results using a flow cytometer or fluorescence spectrophotometer, or observe the results using a fluorescence microscope or laser confocal microscope. Adherent Cells Collect and resuspend the adherent cells, then follow the detection procedure for suspension cells. When observation is performed with a confocal microscope, in situ staining may also be used. Use the staining conditions specified for suspension cells. Purified Mitochondria 1. Dilute the prepared JC-1 staining working solution 5-fold with 1× JC-1 incubation buffer. 2. Add 0.1 mL purified mitochondria containing 10-100 µg total protein to 0.9 mL of the 5-fold diluted JC-1 staining working solution. 3. Perform result detection. Tissue Samples Homogenize the tissue sample into a single-cell suspension using a glass homogenizer and detect it according to the cell-sample procedure. Alternatively, prepare the tissue using another cell-suspension preparation kit before detection. Assay Time Incubation: 15-30 minutes at 37°C in 5% CO_2. Complete detection within 30 minutes after staining and washing.
Unit definition	Analysis and Interpretation Flow Cytometry: Detect green fluorescence through the FL1 channel and red fluorescence through the FL2 channel. FL-1+, FL-2+ cells are normal cells; FL-1+, FL-2- cells are apoptotic cells. Fluorescence Microscopy or Laser Confocal Microscopy: Green fluorescence indicates decreased mitochondrial membrane potential and suggests that the cell is likely in early apoptosis. Red fluorescence indicates relatively normal mitochondrial membrane potential and normal cell status. Decreased mitochondrial membrane potential results in weakened fluorescence intensity. Fluorescence Spectrophotometer or Fluorescence Microplate Reader: Use an excitation wavelength of 485 nm and an emission wavelength of 590

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	nm. If the excitation wavelength cannot be set to 485 nm, use a wavelength within 475-520 nm. Judging Mitochondrial Membrane Potential Changes: Do not judge mitochondrial membrane potential from red or green fluorescence intensity alone. Use the relative change in red and green fluorescence, expressed as the red-to-green fluorescence intensity ratio, to assess membrane-potential changes. Calculate the ratio using the average red and green fluorescence intensities of the cell population and perform statistical analysis of the ratio using repeated experimental data. During flow-cytometry analysis, compared with the normal control, apoptosis-positive treated cell populations move toward the lower right; red fluorescence weakens and green fluorescence strengthens, indicating decreased mitochondrial membrane potential. Sensitivity: Strong detection sensitivity, capable of distinguishing minor heterogeneity in cellular stress responses.
Usage	For research use only. Not for use in diagnostic, food, or cosmetic testing procedures.

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