Product Information

JC-1 Mitochondrial Membrane Potential Detection Kit

Catalog Number: J6004

Product Size: 20 T, 100 T

Contents:

Component	20 T	100 T
A. JC-1,100× in DMSO	100 µL	500 µL
B. 10× Assay Buffer	2 mL	10 mL
C. CCCP, 50 mM	10 µL	50 µL

Storage

Component A, B: Store at 4℃ and protect from light, avoid freeze-thawing; Component C: Store at -20℃. When stored as directed, product is stable for at least 12 months.

Parameters

Ex/ Em: 510/527 nm (Monomer, green fluorescence)

585/590 nm (Aggregation, red fluorescence)

Description

JC-1 is a membrane permeable dye widely used for determining mitochondrial membrance potential with flow cytometry or fluorescent microscopy. This dye can selectively enter the mitochondria where it reversibly changes color as membrane potentials increase. This property is due to the reversible formation of JC-1 aggregates upon membrane polarization that causes shifts in emitted light from 530 nm to 590 nm when excited at 488 nm. Both colors can be detected using FITC and PE filter, respectively. JC-1 is qualitative in regards to the shift from green to red fluorescence emission, and and can be quantitatived as measured by fluorescence intensity in both filter sets. JC-1 can be used to indicate the initiation of apoptosis.

Protocol

1. Reagent preparation:

Prepare JC-1 working solution

Prepare 1mL 1×JC-1 dye working solution according to the following scheme: add 10 µL 100 × JC-1 dye solution to 890 µL sterilized diH_2O, mix evenly with vortex, then add 100 µL 10 × Assay Buffer into the above mixture, mix evenly by vortex.

Note: Configuration volume can be expanded or reduced in the same proportion; It is not recommended to directly dilute 100 × JC-1 dye solution with 1 × Assay Buffer, which may lead to precipitation.

Prepare 1 × Assay Buffer

Dilute 10 × Assay Buffer with diH_2O for 10 times, such as 1 mL 10 × Assay Buffer + 9 mL diH_2O.

2. Protocol for flow cytometry detection

Cell staining:

Before starting the experiment, make sure that JC-1 and CCCP solutions have returned to room temperature.

1). Inoculate cells in culture plate with experiment requires. (For suspension cell, inoculate no more than 10^6 cells/mL).

2). Positive treatment group: add 50 mM CCCP solution of corresponding volume according to the amount of medium (for

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example, add 1 μL 50 mM CCCP solution into 1 mL medium if the final concentration is 50 μM), and incubate at 37 ℃ for 20 min. For specific cells, the concentration and incubate time of CCCP treatment may be different, which should be determined by referring to relevant literature.

3). Adherent cells should be digested before staining to prepare cell suspension, then put 0.5 mL into centrifuge tube.

4). Centrifuge for 5 minutes at 400 xg and remove supernatant.

5). Resuspend cells with 0.5 mL JC-1 working solution.

6). Incubate for 15 min at 37℃.

7). Centrifuge for 5 minutes at 400 xg and remove supernatant.

8). Resuspend cells with 2 mL PBS, culture medium or 1 × Assay Buffer. Centrifuge and remove supernatant. Repeated once.

9). Resuspend cells with 0.5 mL PBS, culture medium or 1 × Assay Buffer, analyzed by flow cytometry.

Quantitative analysis by flow cytometry:

For normal cells, JC-1 aggregation(red) in mitochondria can be detected in PE or PI (FL2) channels, and for apoptotic cells, JC-1 monomer(green) can be detected in FITC (FL1) channels.

3. Protocol for fluorescence microscopy detection

Staining of suspension cell:

1). Cells were stained according to the flow cytometry staining protocol.

2). Resuspend cells with 0.3 mL PBS or medium.

Staining of adherent cells:

1). Inoculate cells on a culture dish or culture plate.

2.) Positive treatment group: add 50 mM CCCP solution of corresponding volume according to the amount of medium (for example, add 1 μL 50 mM CCCP solution into 1 mL medium if the final concentration is 50 μM), and incubate at 37 ℃ for 20 min. For specific cells, the concentration and incubate time of CCCP treatment may be different, which should be determined by referring to relevant literature.

3). Remove the medium and add JC-1 working solution to cover cell surface.

4). Incubate for 15 min at 37℃.

5). Remove staining solution and wash cells with PBS, culture medium or 1×Assay Buffer.

6). Analysis with fluorescence microscope.

Fluorescence microscopy imaging

Observe by fluorescence microscopy with dual channel filter which can detect fluorescein and Rhodamine, or fluorescein and Texas Red simultaneously.

For normal cells, the dye is aggregation and emits red fluorescence; for apoptotic or necrotic cells, the dye is monomer and emits green fluorescence.

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