JC-10

Cat: IJ0310
Storage: Powder: -20℃, 2 years; Insolvent (mother liquid): -20℃, 6 months; -80℃, 1 year (protect from light)

Introduction

JC-10 is an ideal fluorescent probe widely used to detect mitochondrial membrane potential ΔΨm. It can detect the mitochondrial membrane potential of cells, tissues or purified mitochondria. When the mitochondrial membrane potential is high, JC-10 aggregates in the matrix of mitochondria and forms a polymer, which can produce red fluorescence; when the mitochondrial membrane potential is low, JC-10 can not be aggregated in the matrix of mitochondria, and at this time, JC-10 is a monomer, which can produce green fluorescence. In this way, it is very convenient to detect the change of mitochondrial membrane potential through the change of fluorescence color. The relative proportion of red and green fluorescence is commonly used to measure the proportion of mitochondrial depolarization.

Decrease in mitochondrial membrane potential is a hallmark event in the early stage of apoptosis. The decrease in membrane potential can be easily detected by the shift of JC-10 from red to green fluorescence, and the shift of JC-10 from red to green fluorescence can also be used as an indicator of the early stage of apoptosis.

The maximum excitation wavelength of JC-10 monomer is 515 nm and the maximum emission wavelength is 529 nm; the maximum excitation wavelength of JC-10 polymer is 585nm and the maximum emission wavelength is 590 nm. For actual observation, the conventional settings for observing red fluorescence and green fluorescence can be used.

Parameter

Purity: ≥90%
Appearance: Solid
Solubility: Soluble in DMSO

Protocols (*only for reference*)

Preparation of storage solution

Prepare a 1 mg/mL JC-10 stock solution in DMSO. For example, 1 mg of JC-10 powder was dissolved in 1 mL of DMSO.

Note:

a. Unused storage solution is recommended to be stored in portions at -20℃ to avoid repeated freezing and thawing.
b. Moisture-absorbing DMSO has a significant effect on the solubility of the product, please use freshly opened DMSO.

Preparation of working fluid

Dilute the reservoir solution with a suitable buffer (e.g., serum-free medium or PBS, etc.) to make

1~5 μg/mL of JC-10 working solution.

Note:

a. The final concentration of the working solution is recommended to be optimized according to different cell lines and experimental systems.
b. If it is found difficult to dissolve, it can be sonicated to promote dissolution.
c. Please adjust the concentration of the working solution according to the actual situation, and use it now.

Setting of positive controls

It is recommended to use 10 μM CCCP (Cat: IC2490) and treat the cells for 20 min. Subsequently, JC-10 was loaded as described below and the mitochondrial membrane potential was measured. For most cells, the mitochondrial membrane potential is usually completely lost after 20 min of treatment with 10 μM CCCP, and should fluoresce green after JC-10 staining; while normal cells should fluoresce red after JC-10 staining. For specific cells, the concentration and duration of action of CCCP may be different, please refer to the relevant literature to decide.

Suspension cells

(1) Take 100,000-600,000 cells and resuspend them in 0.5 mL cell culture medium, which can contain serum and phenol red.
(2) Add 0.5 mL of 1~5 μg/mL JC-10 working solution and mix by inverting several times. Incubate at 37℃ for 20 min in a cell culture incubator.
(3) During the incubation, place the buffer in an ice bath and pre-cool it in advance.
(4) After incubation at 37℃, centrifuge the cells at 600 g for 3-4 min at 4℃ to precipitate the cells. Discard the supernatant, taking care not to aspirate the cells as much as possible.
(5) Wash twice with buffer: add 1mL of buffer to resuspend the cells, centrifuge at 600 g for 3-4 minu at 4℃, precipitate the cells and discard the supernatant. Add 1 mL of buffer to resuspend the cells, centrifuge at 600 g for 3~4 min at 4℃, precipitate the cells and discard the supernatant.
(6) Resuspend the cells with appropriate amount of buffer and observe with fluorescence microscope or laser confocal microscope, or detect with fluorescence spectrophotometer or analyze with flow cytometer.

Adherent cells

Note: For adherent cells, if a fluorescence spectrophotometer or flow cytometer assay is desired, the cells can be collected first, resuspended, and then referred to the assay for suspended cells.

(1) One hole for a six-hole plate, aspirate the culture medium, wash the cells once with PBS or other appropriate solution if necessary according to the specific experiment, and add 1 mL of cell culture medium. The cell culture medium may contain serum and phenol red.
(2) Add 1mL of 1~5 μg/mL JC-10 working solution and mix thoroughly. Incubate in a cell incubator at 37℃ for 20 min.
(3) During the incubation, place the buffer in an ice bath and pre-cool it in advance.

(4) After incubation at 37℃, aspirate the supernatant and wash with buffer twice.
(5) Add 2 mL of cell culture medium, which may contain serum and phenol red.
(6) Observe under fluorescence microscope or laser confocal microscope.

Purified mitochondria

(1) 0.9 mL of 0.2~1 μg/mL JC-10 working solution was added with 0.1 mL of mitochondria purified with 10~100 μg of total protein.
(2) Detection by fluorescence spectrophotometer or fluorescence enzyme marker: after mixing, directly use a fluorescence spectrophotometer to perform a time scan (timescan) with Ex=485 nm and Em=590 nm . If the excitation wavelength cannot be set to 485 nm when using a fluorescence enzyme marker, the excitation wavelength can be set in the range of 475 to 520 nm.
(3) Observation by fluorescence microscope or laser confocal microscope.

Fluorescence observation and result analysis

When detecting JC-10 monomer, the excitation light can be set to 490 nm and the emission light to 530 nm; when detecting JC-10 polymer, the excitation light can be set to 525 nm and the emission light to 590 nm.

Note: It is not necessary to set the excitation and emission light at the maximum excitation and emission wavelengths for fluorescence measurement here. If you are using a fluorescence microscope, you can refer to the settings for other green fluorescence, such as GFP or FITC, for JC-10 monomer, and for other red fluorescence, such as propidium iodide or Cy3, for JC-10 polymer. The presence of green fluorescence indicates a decrease in mitochondrial membrane potential and that the cell is likely in the early stages of apoptosis. Red fluorescence indicates that the mitochondrial membrane potential is more normal and the cell is in a more normal state.

Note

1. JC-10 stock solution may solidify and stick to the bottom, wall or cap of the centrifuge tube at low temperature such as 4℃ or ice bath, so it can be used after warming in water bath at 20-25℃ for a few moments until it is all melted.
2. When washing with buffer after loading JC-10, keep the buffer at about 4℃, the washing effect is better at this time.
3. Try to complete subsequent assays within 30 min after the JC-10 probe has been loaded and washed. Store in an ice bath prior to testing.
4. CCCP is a mitochondrial electron transport chain inhibitor and is toxic.
5. All fluorescent dyes have quenching problems, please try to avoid light to slow down the fluorescence quenching.
6. For your safety and health, please wear lab coat and disposable gloves.
7. This product is for scientific research use only. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics. Please do not store in ordinary residential areas.

Related Products

IJ0300 JC-1

IF1770 Mitochondrial red fluorescent probes

IF1780 Mitochondrial green fluorescent probes