Reactive oxygen species (ROS) measurement kit

(Cat N.: BC005 Size:100T-500T)

1. Measuring principle

The DCFH-DA (2, 7-Dichlorofuorescin Diacetate) probe used in this kit is by far the most commonly used and most sensitive probe for detecting intracellular reactive oxygen species. DCFH-DA itself has no fluorescence and can freely pass through the cell membrane. When it enters the cell, it will be hydrolyzed into DCFH (Dichlorofluorescin) by the relevant esterase in the cell. DCFH cannot penetrate the cell membrane, so that the probe can be easily labeled into the cell. When reactive oxygen species exist in the cell, DCFH is oxidized to a strong green fluorescent substance called DCF (Dichlorofluorescein). Its fluorescence has a maximum peak near the excitation wavelength of 502nm and emission wavelength of 530nm, and its fluorescence intensity is proportional to the level of reactive oxygen species in the cell.

2. Reagent composition and storage

1. 0.1ml 10mM DCFH-DA in DMSO, store at -20°C.
2. 1ml of active oxygen hydrogen donor, stored at 4-8°C.

3. Tissue sample operation steps

(It can be observed by laser confocal microscope, it can also be used for flow cytometer, fluorescence microplate reader, fluorescence Spectrophotometer measurement)

1. Single cell suspension preparation:

Method 1. A single cell suspension preparation apparatus was used to prepare a single cell suspension.

Method 2. Enzymatic digestion method:

Method 3. Mechanical method (net rubbing method):

2. Add fluorescent probe:

① Take the cells without any treatment and resuspend them in 0.01MPBS and set them as negative control tubes. Positive control tube: use dilution

A good DCFH-DA resuspends the cell pellet, and at the same time adds reactive oxygen species hydrogen donor to induce cells. The recommended working concentration of the reagent is 20-100 μM.

② Sample tube: Resuspend the cell pellet with diluted DCFH-DA, the cell density generally requires 1 x10^6-2x10^7/mL

③ Incubate the cells at 37°C for 30 minutes to several hours. Usually, it is 30-60 minutes.

④ Collect the single cell suspension after incubation (probe labeling), centrifuge at 1000g for 5-10 minutes, remove the supernatant to collect the cell sediment

Dehydrate and wash with PBS 1 to 2 times. Centrifugation to collect cell pellets for fluorescence detection;

3. Fluorescence detection:

①. Resuspend the collected cell pellet in PBS and use it for detection;

② Wavelength setting: the best excitation wavelength is 500 (500±15nm), the best emission wavelength is 525 (530±20 nm). You can also press

According to FITC fluorescence detection conditions.

③. The result is expressed by fluorescence value.

4. Cell sample operation steps

(It can be observed with a laser confocal microscope, and can also be used for flow cytometry, fluorescence microplate reader,

fluorescence spectrophotometer)

1. Add the probe directly to the culture medium:

①. Directly add DCFH-DA probe to serum-free medium: generally dilute with serum-free medium according to 1:1000

DCFH-DA (final concentration is 10 JM). After removing the culture medium, add

DCFH-DA diluted in an appropriate volume. The volume to be added should be

sufficient to cover the cells. Usually, the diluted DCFH-DA is added to one well of the 6-well plate not less than Imlo

②. Take a portion of the cells without probe and only medium as a negative control tube. Positive control tube: Take a portion of the cells to which the probe has been added, and at the same time add active oxygen donors to induce the cells. The

recommended working concentration of the reagent is 20-100 μM.

③. Incubate the cells at 37°C for 30min~a few hours, usually 30~60min. The length of the incubation time depends on the cell type and stimulation strip.

It is related to the concentration of DCFH-DA. Generally, after the positive control stimulates the cells for about 30 minutes, obvious green fluorescence can be

observed.

④. Aspirate the culture medium, use serum-free culture medium or 0.01MPBS to repeatedly pipette, visually observe that the bottom of the bottle is translucent (cell single

The layers are connected into a sheet) to become transparent, and almost all the cell layers are pipetted into PBS.

⑤ Collect all the cell suspension into a 1.5ml centrifuge tube. Wash twice with serum-free culture medium or PBS to fully remove

DCFH-DAo that did not enter the cells was 1000rpm/min, 5min, aspirate the supernatant and add PBS to resuspend the cells for determination.

⑥ Wavelength setting: the best excitation wavelength is 500 (500±15nm), the best emission wavelength is 525 (530±20 nm). Also available in accordance with FITC

Fluorescence detection condition detection.

⑦. The result is expressed by fluorescence value.

2. Collect the cells first

prepare a cell suspension and then measure

①Cell collection:

a. For adherent cells, aspirate the culture medium, use serum-free culture medium or 0.01MPBS to repeatedly pipette, visually observe the bottom of the orifice plate (bottom of the bottle) from translucent (cell monolayers connected into pieces) to transparent, almost all of the cell layer Pipette into PBS.

b. Collect all the cell suspension into a 1.5ml centrifuge tube. Wash twice with serum-free culture medium or 0.01MPBS, 1000 rpm/min, centrifuge for 5 min, aspirate the supernatant, and save the cell pellet for determination.

c. Centrifuge the suspended cells according to the conventional method
(2000rpm/min, 5min), collect the cell pellet, wash twice with serum-free culture
medium or 0.01MPBS, 1000rpm/min, centrifuge for 5min, aspirate the supernatant, and save the cell pellet for use Determination.

② Cell resuspension: The cell density generally requires 1x10-2x10/ml, and there are generally two methods: a. First add serum-free culture medium or PBS to
resuspend the cells, and then according to the volume of the added culture medium or PBS, according to 10 Jie M Add the probe to the initial concentration (it is best to do a preliminary experiment to determine the appropriate concentration of your
sample),

b. First dilute the probe with serum-free culture medium or PBS at a concentration of 10 M, and then resuspend the above cell pellet with the diluted probe to prepare a cell suspension.

③. Take a portion of the cells that do not add the probe and only add the culture medium or PBS as the negative control tube. Positive control tube: take a copy
Into the cell suspension of the probe, and at the same time add the reactive oxygen
hydrogen donor to induce the cells, the recommended working concentration of the reagent is 20~100gMo

④. Incubate the cells at 37 bar for 30min~a few hours, usually 30~60min. The length of the incubation time depends on the cell type and stimulation strip.
It is related to the concentration of DCFH-DA; mix upside down every 3-5 minutes to make the probe fully contact the cells.

⑤ Collect the single cell suspension after incubation (probe labeling), centrifuge at
1000 rpm/min for 5 min, aspirate the supernatant, and wash with PBS 1~2 times, centrifuge to collect the cell pellet for fluorescence detection.

⑥. Resuspend the collected cell pellet in PBS and use it for detection.

⑦Wavelength setting: the best excitation wavelength is 500 (500±15nm), the best emission wavelength is 525 (530±20 nm). Also available in accordance with FITC
Fluorescence detection condition detection.

⑧. The result is expressed by fluorescence value.