

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Reactive Oxygen Species (ROS) Assay Kit

Catalog No.: BC00009

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale)	order@enkilife.com
✉ Email (Techsupport)	techsupport@enkilife.com
☎ Tel:	0086- 27-87002838
🌐 Website:	www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Reactive Oxygen Species (ROS) Assay Kit
Detection method	Fluorescent
Sample Type	Cells (including adherent cells and suspension cells), Tissues
Assay Type	Cell-based (quantitative)
Detection Instrument	Fluorescence microplate reader (measure the fluorescence value at 488 nm excitation wavelength and 525 nm emission wavelength.) Flow cytometer (set the excitation wavelength to 488 nm and the detection wavelength to 525 nm. The fluorescence spectrum of DCF is very similar to that of FITC. The parameters of FITC can be used to detect DCF. The number of cells can be selected to be 10^4 - 10^5 during detection.) Laser confocal microscopy

Product Introduction

Reactive oxygen species (ROS) are a class of highly reactive oxygen species, including oxygen anions (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH\cdot$), and nitric oxide, etc. Reactive oxygen species play a complex role in organisms, and are closely related to the regulation of normal physiological functions and the occurrence and development of diseases.

Product Features

- Low background, high sensitivity, wide linear range, and easy to use.

Principle

Reactive Oxygen Species (ROS) Assay Kit is a kit that uses the fluorescent probe DCFH-DA to detect reactive oxygen species. DA itself has no fluorescence and can freely pass through the cell membrane. After entering the cell, it can be hydrolyzed by the esterase in the cell to produce DCFH. However, DCFH cannot penetrate the cell membrane, making it easy for the probe to be loaded into the cell. The reactive oxygen species in the cell can oxidize the non-fluorescent DCFH to produce fluorescent DCF. By detecting the fluorescence of DCF, the level of reactive oxygen species in the cell can be known.

Components

Serial number	Components	Size (100T)	Storage
Reagent 1	DCFH-DA (10mM)	0.1ml	-20°C, avoid light, store at 4°C after opening, valid for 6 months.
Reagent 2	Reactive Oxygen Species Positive Control (Rosup, 50mg/ml)	1ml	-20°C, avoid light, store at 4°C after opening, valid for 6 months.

Storage

Unopened kit can be stored at -20°C for 6 months.

Operation process

1. Loading the probe

For cells with a short stimulation time (usually less than 2 hours), first load the probe, then stimulate the cells with the ROS positive control or the drug of interest. For cells with a long stimulation time (usually more than 6 hours), first stimulate the cells with the ROS positive control or the drug of interest, then load the probe.

(1) In situ probe loading (this method is only applicable to adherent cultured cells)

- A. DCFH-DA was diluted 1:1000 with serum-free culture medium to a final concentration of 10µmol/L.
- B. Remove the cell culture medium and add an appropriate volume of diluted DCFH-DA. The volume added should be sufficient to cover the cells. Usually, at least 1 ml of diluted DCFH -DA is added to one well of a six-well plate.
- C. Incubate in a 37°C cell culture incubator for 20 minutes.
- D. The cells were washed three times with serum-free cell culture medium to fully remove DCFH-DA that had not entered the cells.

(2) Collect cells and load probes

- A. DCFH -DA was diluted 1:1000 with serum-free culture medium to a final concentration of 10µmol/L.
- B. After the cells are collected, they are suspended in diluted DCFH-DA, with a cell concentration of 1 million to 20 million/ml.
- C. Incubate in a 37°C cell culture incubator for 20 minutes. Invert and mix every 3-5 minutes to ensure full contact between the probe and the cells.
- D. Wash the cells three times with serum-free cell culture medium to fully remove DCFH-DA that has not entered the cells.

(3) Instructions for use of positive control

- A. Directly stimulate the cells with active oxygen positive control or the drug you are interested in, or divide the cells into several parts and then stimulate the cells.
 - B. The positive control can be used at a ratio of 1:1000. For example, 1 μ l of positive control can be added to 1 ml of cells loaded with probes.
 - C. Typically, a very significant increase in reactive oxygen species levels can be observed within 20-30 minutes after stimulation. For different cells, the effect of active oxygen positive control may be quite different. If no increase in reactive oxygen species is observed within 30 minutes after stimulation, the concentration of the positive control of reactive oxygen species can be appropriately increased. If reactive oxygen species increase too quickly, the concentration of the active oxygen positive control can be appropriately reduced.
 - D. The reactive oxygen species positive control (Rosup) is only used for samples used as positive controls and does not need to be added to every sample.
- (4) This kit can also detect reactive oxygen species (ROS) in tissue samples, with the processing method as follows:
- A. Homogenize the tissue sample by adding 100-200 μ L of lysis buffer per 5-10 mg of tissue.
 - B. Centrifuge at approximately 12,000 \times g at 4°C for 3-5 minutes, then collect the supernatant for subsequent experiments. All the above operations must be performed at 4°C or on ice.
 - C. If the prepared tissue sample is not assayed immediately, it can be stored frozen at -20°C.
 - D. For probe loading: Add DCFH-DA to the supernatant at a ratio of 1:1000 or 1:10000 to achieve a final concentration of 10 μ mol/L or 1 μ mol/L (the probe concentration can also be adjusted based on preliminary experimental results).
 - E. After mixing well, incubate at 37°C for 20 minutes before detection (the incubation time can be adjusted according to preliminary experimental results).

2. Detection

(1) For samples loaded with probes in situ

The cells can be directly observed using a laser confocal microscope, or collected and detected using a fluorescence spectrophotometer, fluorescence microplate reader or flow cytometer.

(2) For samples loaded with probes after collecting cells

It can be detected using a fluorescence spectrophotometer, fluorescence microplate reader or flow cytometer, or directly observed using a laser confocal microscope.

3. Parameter settings

Use 488nm excitation wavelength and 525nm emission wavelength to detect the intensity of fluorescence before and after stimulation in real time or time point by time. The fluorescence spectrum of DCF is very similar to that of FITC, and DCF can be detected using the parameter settings of FITC.

4. Others

For some cells, if it is found that the fluorescence of the negative control cells without stimulation is also strong, DCFH-DA can be diluted at 1:2000-1:5000 so that the concentration of DCFH-DA is 2-5 μ mol/L when loading the probe.

The probe loading time can also be adjusted appropriately within 15-60 minutes according to the situation.

Notes

1. After probe loading, be sure to wash away the residual probe that has not entered the cells, otherwise it will result in a high background.
2. After the probe is loaded and the residual probe is washed off, the excitation wavelength and emission wavelength can be scanned to confirm whether the probe is loaded properly.
3. Try to shorten the time from probe loading to measurement (excluding stimulation time) to reduce various possible errors.
4. This product is for Research Use Only and shall not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residences.