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

Enhanced Reactive Oxygen Species (ROS) Assay Kit

Catalog No.: BC00010

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

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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	Enhanced Reactive Oxygen Species (ROS) Assay Kit
Detection	Fluorescent
Sample Type	Cells (including adherent cells and suspension cells)
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^-), 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

● Compared with DCFH-DA used in the regular version of Reactive Oxygen Species (ROS) Assay Kit(BC00009), the product of H2DCF-DA after hydrolysis and oxidation has an additional negative charge, which hinders its infiltration out of cells, thus having lower background and better fluorescence signal.

Principle

The enhanced reactive oxygen species assay kit is a kit that uses the fluorescent probe H2DCF-DA to detect reactive oxygen species. The probe H2DCF-DA itself has no fluorescence and can freely pass through the cell membrane. After entering the cell, it can be hydrolyzed by esterases in the cell and oxidized by ROS, and converted into a green fluorescent form. The fluorescence of DCF can be used to determine the level of reactive oxygen species in the cell. This kit provides the reactive oxygen species positive control reagent Rosup to facilitate the detection of reactive oxygen species.

Components

Serial number	Components	Size (100T)	Storage
Reagent 1	H2DCF-DA (10mM)	0.1ml	-20℃, avoid light , store at 4℃ after opening, valid for 6 months.
Reagent 2	Reactive oxygen positive control (Rosup, 50mg/ml)	1ml	-20℃, avoid light , store at 4℃ 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. H2DCF-DA was diluted 1:1000 with serum-free culture medium to a final concentration of 10 μmol/L.

B. Remove the cell culture medium and add an appropriate volume of diluted H2DCF-DA. The volume added should be sufficient to cover the cells. Usually, at least 1 ml of diluted H2DCF-DA is added to one well of a six-well plate.

C. Incubate in a 37ºC cell culture incubator for 20 minutes.

D. The cells were washed three times with serum-free cell culture medium to fully remove H2DCF-DA that had not entered the cells.

(2) Collect cells and load probes

A. H2DCF-DA was diluted 1:1000 with serum-free culture medium to a final concentration of 10 μmol/L.

B. After the cells are collected, they are suspended in diluted H2DCF-DA, with a cell concentration of 1 million to 20 million/ml .

C. Incubate in a 37ºC 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 H2DCF-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. Positive controls can be used at a ratio of 1:1000. For example, if the probe-loaded cells total 1 ml, 1 μl of positive control can be added for stimulation.

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 .

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 the reaction product is very similar to FITC and can be detected using the FITC parameter settings.

4. Others

For some cells, if it is found that the fluorescence of the negative control cells without stimulation is also strong, H2DCF-DA can be diluted at 1:2000-1:5000 so that the concentration of H2DCF-DA is 2-5 μmol/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.