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Reactive Oxygen Species Assay Kit

Cat: AK0696 100T

Component

Solution A	DCFH-DA(10 mM)	0. 1 ml	-20℃
Solution B	Rosup(50mg/mL)	1 ml	-20℃

Introduction

The Reactive Oxygen Species Assay Kit is used to detect the reactive oxygen species(ROS) by a fluorescent probe DCFH-DA. DCFH-DA, which can freely penetrate the cell membrane without fluorescence, is hydrolyzed by intracellular esterase to DCFH after entering the cell. While the DCFH can't penetrate the cell membrane, it is easier to load the probe into cells. The DCFH can be oxidized to be fluorescence DCF by intracellular ROS, thus the fluorescence intensity is proportional to the ROS level in cells.

This kit provide a positive contrast reagent Rosup(5 0 mg/ ml) , in order to facilitate the detection of ROS.

The kit has the advantages of low background, high sensitivity, wide linear range and convenient use.

The kit can be used for the determination of 100 ~500 samples.

Protocol

I. Loading probe

For cells that are stimulated for a short time (usually within 2 hours) , cells are firstly loaded with probes and then activated with Solution B or interested drugs. For cells that are stimulated for longer time (usually more than 6 hours) , cells are firstly activated with Solution B or interested drugs, then loaded with probes.

Loading probe in situ: this method applies only to adherent cells. Dilute DCFH-DA by serum-free culture medium to the final concentration of 10μ mol/L. Remove the cell culture medium, add appropriate volume of DCFH-DA(10μ mol/ L) , fully covering cells, then incubate the cells at 37℃ for 20mins. After incubation, wash the cells using serum-free culture medium to fully remove DCFH-DA are not entered into cells. Usually ROS positive control in stimulated cells from 20 to 30 minutes can significantly improve the active oxygen level.

Loading probe after cells collection: Dilute DCFH-DA by serum-free culture medium to the final concentration of 10μ mol/L. Suspended the collected cells by DCFH-DA(10μ mol/L), adjust the cell concentration ranging from one million/ ml to twenty million/ ml, then incubate the cells at 37℃ for 20mins. After incubation, wash the cells using serum-free culture medium to fully remove DCFH-DA are not entered into cells. Cells are stimulated with Solution B or interested drugs directly, or divided into several parts before stimulation. Usually ROS positive control in stimulated cells from 20 to 30 minutes can significantly improve the active oxygen level.

II. Detection

For the samples of loading probe in situ, they could be observed by laser confocal microscopy directly or fluorescence spectrophotometer, fluorescence microplate or flow cytometry after cells collection. For the samples of loading probe after cells collection, they could be observed by fluorescence spectrophotometer, fluorescence microplate or flow cytometry. It can be observed directly with laser confocal microscope.

III. Parameter setting

DCF, has similar fluorescence spectrum with FITC, is detected in real time or time points before or after stimulation at 488nm excitation wavelength and 525nm emission wavelength. The DCF excitation spectra and emission spectra are shown in the following figure.

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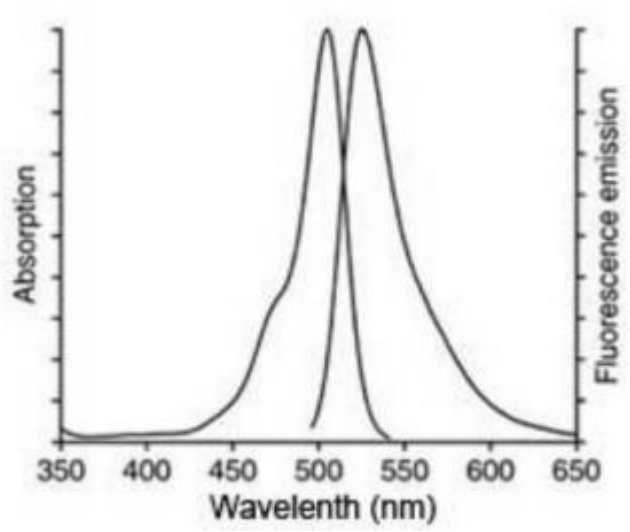

IV. Other introduction

Usually ROS could be observed high level within 20 to 30 minutes after stimulation by positive control diluted at 1:1000. While for different cells, the effect of positive control of ROS may be different. If the ROS level increase is not observed within 30 minutes after stimulation, the positive control concentration could be improved. If the ROS level increase too fast after stimulation, the positive control concentration could be reduced.

In addition, for some cells, if the negative control cell fluorescence is also relatively strong without stimulation, dilute DCFH-DA at the ratio of 1 ：2000 ~ 1 ：5000, making DCFH-DA concentration is 2 ~ 5μmol/L when loading probe. Adjust the probe loading time in 15-60 minutes according to the situation.

The ROS positive control(Rosup) only used for positive control samples, not for every sample.

Note

1. After loading probe, it is vital to wash out the probe that is not get into cells.
2. After probe loading and cleaning, the samples can be scanned with the excitation wavelength and the emission wavelength to confirm whether the loading probe is good or not.
3. Minimize the time required from the probe loading to measurement (except for the stimulation time) to reduce the possible errors.
4. For your safety and health, please wear the experimental clothes and wear disposable gloves

Storage: -20℃, away from light, valid for one year

For research use only, not for use for diagnostic procedures.