Reactive Oxygen Species Assay Kit

Cat : CA1410
Specification: 100T/500T
Storage: -20 ℃ avoid light, valid for 1 year.

Product contents:

Ingredients	100T	500T
Liquid A: DCFH-DA (10mM)	0.1mL	0.1mL×5
Liquid B: Reactive oxygen species positive control (Rosup, 50mg/mL)	1mL	1mL×5

Product description:

Reactive Oxygen Species Assay Kit (Reactive Oxygen Species Assay Kit) is a kit that uses fluorescent probe DCFH-DA to detect reactive oxygen species. Dcfh-da itself is not fluorescent and can freely cross the cell membrane, and after entering the cell, it can be hydrolyzed by the intracellular esterase to produce DCFH. However, DCFH cannot permeate the cell membrane, which makes the probe easy to be loaded into the cell. Intracellular reactive oxygen species can oxidize non-fluorescent DCFH to produce fluorescent DCF. The level of intracellular reactive oxygen species can be known by detecting the fluorescence of DCF.

This kit provides a positive control reagent Rosup to facilitate the detection of active oxygen species. Rosup is a mixture with a concentration of 50mg/mL.

This kit has low background, high sensitivity, wide linear range and is easy to use.

Protocols: (only for reference):

一、Load the probe:

For cells with short stimulation time (usually less than 2 hours), the probe is loaded first, and then the cells are stimulated with active oxygen positive controls or drugs of their interest. For cells that have been stimulated for a longer period of time (usually more than 6 hours), the cells are stimulated with an ROS positive control or a drug of interest before being loaded with a probe.

In situ loading of the probe: This method is only suitable for adherent culture cells. The final concentration of DCFH-DA was 10μmol/L by diluting DCFH-DA with serum-free medium at 1:1000. Remove the cell culture medium and add appropriate volume of diluted DCFH-DA. The volume should be sufficient to cover the cells. Usually, no less than 1mL of diluted DCFH-DA should be added to one well of the six-well plate. Incubate in a cell incubator at 37℃ for 20 min. The cells were washed three times with serum-free cell culture solution to fully remove DCFH-DA that did not enter the cells. Usually reactive oxygen species positive controls can significantly increase reactive oxygen species levels after stimulating cells for 20 to 30 minutes. (**Note: It is recommended to set the probe dilution gradient to determine the optimal working concentration)**

After cell collection, the cells were suspended in serum-free culture medium, the number of cells was adjusted to 1 million to 20 million /mL, and DCFH-DA was added in the ratio of 1∶1000

~ 1:10000, so that the final concentration was 1 ~10μmol/L, and the cells were incubated in the cell incubator at 37℃ for 15~60 minutes. Inversely mix every 3-5 minutes to make the probe fully contact with the cells. Wash the cells three times with a serum-free cell culture solution to adequately remove DCFH-DA that has not entered the cells. Directly stimulate the cells with active oxygen positive control or interested drugs, or divide the cells into several parts to stimulate the cells. Usually active oxygen positive control can significantly increase the ROS level after stimulating the cells for 20 to 30 minutes. **(Note: It is recommended to set the probe dilution gradient to determine the optimal working concentration)**

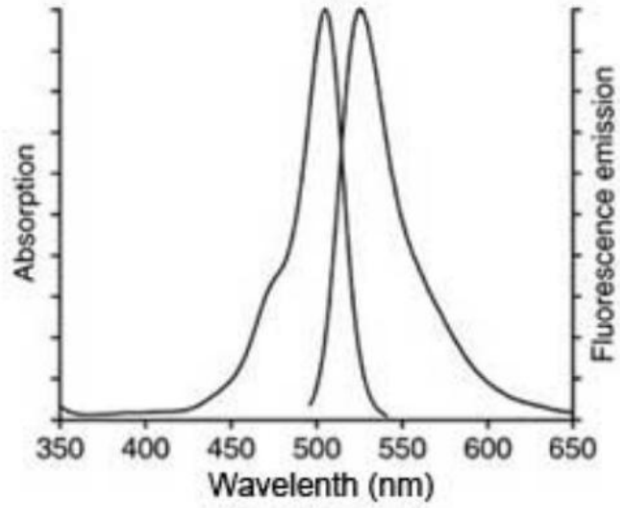
Note: Rosup is only added to the positive control hole as a positive control, and Rosup does not have to be added to the rest of the hole. Positive control can be used according to the ratio of 1∶1000. For example, 1mL of cells loaded with the probe can be stimulated with 1μL of positive control. A very significant increase in ROS levels can usually be observed within 20 to 30 minutes after stimulation. For different cells, the effect of positive ROS controls may vary considerably. If the elevation of reactive oxygen species is not observed within 30 minutes after stimulation, the concentration of reactive oxygen positive controls can be appropriately increased. If the rise of reactive oxygen species is too fast, the concentration of positive reactive oxygen species controls can be appropriately reduced

二、Detection:

Samples loaded with probes in situ can be observed directly with a laser confocal microscope, or cells collected can be examined with a fluorescence spectrophotometer, a fluorometer, or a flow cytometer. Samples loaded with probes after cell collection can be examined with a fluorescence spectrophotometer, fluorescent enzyme spectrometer, or flow cytometer, or can be observed directly with a laser confocal microscope.

三、Parameter setting

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



Note:

1. After the probe is loaded, be sure to wash the residual probe that has not entered the cell, otherwise it will lead to a high background.
2. After the probe is loaded and the residual probe is washed, the excitation wavelength scanning and emission wavelength scanning can be carried out to confirm whether the probe is well loaded. Please refer to the figure above for excitation spectrum and emission spectrum of DCF.
3. Try to shorten the time from probe loading to determination (except stimulation time) to reduce various possible errors.
4. For your safety and health, please wear a lab coat and disposable gloves.

Related literature:

[1] Jie Zhang, Shakil Ahmad, Lanying Wang, et al. Cell death induced by alpha -terthienyl via reactive oxygen species- mediated mitochondrial dysfunction and oxidative damage stress in the midgut of Aedes aegypti larvae. Free Radical Biology and Medicine. June 2019; 137:87-98. (IF 5.657)

[2] YingJuan Liu, Zhenzhen Deng, Lihua Geng, et al. In vitro evaluation of the neuroprotective effect of oligo-porphyran from Porphyra yezoensis in PC12 cells. Journal of Applied Phycology. January 2019. (IF 2.635)

Note: For more information about this product, please refer to the Solarbio website.