Product Name: **Reactive Oxygen Species (ROS) Assay Kit**

Product number: **RA20029**

Basic Information

Product name	Reactive Oxygen Species (ROS) Assay Kit
Size	1000T
Storage	-20℃, protected from light
Shipping	Shipped with ice pack
Validity	12 months

Product Introduction

DCFH-DA (dichlorodihydrofluorescein-acetoacetate) itself has no fluorescence, but can freely penetrate the living cell membrane into the cell, and is hydrolyzed by the esterase in the cell to form DCFH. DCFH has no fluorescence and cannot penetrate the cell membrane, so it is oxidized by the active oxygen in the cell to generate fluorescent DCF. Based on the generation of fluorescence in living cells, the content and changes of cellular active oxygen can be determined. It can be directly observed using a flow cytometer or a fluorescence microscope, which is a classic method for detecting active oxygen in tissues or living cells. Rosup is a positive inducing drug for active oxygen, and the true level of active oxygen can be analyzed based on the intensity of its fluorescence signal.

Experimental procedures

1. Loading ROS probe

(1) In situ loading of probes (only applicable to adherent cells)

a. Cell preparation: Cells should be plated one day before the test to ensure that the cell number is less than 5×10^5/mL during the test.

b. Drug induction: Remove the cell culture medium, add the drug diluted in serum-free medium, and incubate in a 37°C cell culture incubator away from light. The actual induction time is determined by the drug properties and cell type.

c. (Optional) Positive control: dilute the positive control (Rosup, 100 mM) to a common working concentration of 100 μM using serum-free medium, add to cells, and incubate at 37°C in the dark for 30 min-4 h to increase the level of reactive oxygen species, which varies for different cell types. For example, HeLa cells need to be incubated for 30-60 min, while MRC5 human embryonic fibroblasts need to be incubated for 90 min.

d. ROS probe preparation: Before probe loading, DCFH-DA was diluted 1:1000 with serum-free culture medium to a final concentration of 10 μM.

e. Loading ROS probe: Remove the treatment drug and add an appropriate volume of diluted DCFH-DA working solution. The added volume should be sufficient to cover the cells. For example: for a 6-well plate, it is usually not less than 1 mL, and for a 96-well plate, it is usually not less than 100 μL. Incubate in a 37℃ cell culture incubator in the dark for 30 min.

f. Cell washing: Wash the cells 1-2 times with serum-free culture medium to fully remove DCFH-DA that has not entered the cells.

(2) Collect cells and load probes (applicable to adherent cells and suspended cells)

a. Cell preparation: Cultivate cells according to standard methods to ensure that the cells are in the appropriate state for detection. Wash and collect sufficient cells according to appropriate methods.

b. Drug induction: Suspend the collected cells in an appropriately diluted drug and incubate in a 37°C cell culture incubator away from light. The actual induction time is determined by the characteristics of the drug and the cell type.

c. (Optional) Positive control: dilute the positive control (Rosup, 100 mM) with serum-free medium to a common working concentration of 100 μM, add to cells, and incubate at 37°C in the dark for 30 min-4 h to increase the level of reactive oxygen species, which varies for different cell types. For example: HeLa cells need to be incubated for 30-60 min, while MRC5 human embryonic fibroblasts need to be incubated for 90

min.
d. ROS probe preparation: Before probe loading, DCFH-DA was diluted 1:1000 with serum-free culture medium to a final concentration of 10 μM.
e. Probe loading: remove the intracellular drug, collect the cells by centrifugation, and add the diluted probe to make the cell density 1.0×10^6-2.0×10^7.
Note: The cell density needs to be adjusted according to the subsequent detection system, detection method, and total detection volume. For example, for flow cytometry analysis, the number of cells in a single tube should not be less than 10^4 and not more than 10^6.
f. Cell washing: Wash the cells 1-2 times with 1× PBS to fully remove DCFH-DA that has not entered the cells.

2. Fluorescence microscopy
(1) For adherent cells or living tissues, they can be observed directly under a fluorescence microscope. For suspended cells, drop 25-50 μL of the cell suspension onto a microscope slide and cover it with a coverslip.
(2) Under a fluorescence microscope, use a FITC filter to observe fluorescence and remove the background to observe changes in fluorescence.

3. Flow Cytometry Analysis
(1) For adherent cells, digest with trypsin to prepare a single-cell suspension; for suspended cells, collect the cells directly and resuspend the cells ($0.5\text{-}1\times10^5$/mL) in 0.5-1 mL PBS.
(2) Select the FL1 or BL1 channel of the flow cytometer, excite at 488 nm, and measure the emission at 530 nm. The cells should be divided into two subpopulations: ROS-negative cells have only very low fluorescence intensity, and ROS-positive cells have strong green fluorescence.

Excitation/emission wavelength: 504/529 nm

Precautions
1. All fluorescent dyes have quenching problems. Please try to avoid light to slow down fluorescence quenching.
2. To avoid repeated freezing and thawing, this product can be divided into small quantities.
3. The positive control Rosup is generally used at a concentration of 100 μM (recommended concentration 100-400 μM, depending on the cell type). Usually, a significant increase in the level of reactive oxygen species can be observed 30 min-4 h after stimulation. The effect of the reactive oxygen species positive control may vary greatly for different cells. If no increase in reactive oxygen species is observed within 30 min after stimulation, the induction time can be extended or the concentration of the reactive oxygen species positive control can be appropriately increased. If the reactive oxygen species increase too quickly, the induction time can be shortened or the concentration of the reactive oxygen species positive control can be appropriately reduced.
4. During the experiment, if the fluorescence of the negative control cells without stimulation is also strong, DCFH-DA can be diluted 1:2000-1:5000 to make the final concentration of DCFH-DA 2-5 μM. The probe loading time can also be adjusted appropriately within 15-60 min according to the situation.
5. The reactive oxygen species positive control (Rosup) is only used as a positive control sample and does not need to be added to every sample.
6. After the probe is loaded, be sure to wash away the residual probe that has not entered the cell, otherwise it will lead to a high background.

Note: This reagent is for scientific research use only!