Reactive Oxygen Species (ROS) Assay Kit

Product #: G1706-100T
Quantity: 100 reactions

Product Description

- Reactive oxygen species (ROS) Assay Kit, also known as ROS Assay Kit, is designed for ROS detection based on the fluorescent probe DCFH-DA.
    - DCFH-DA, also known as 2',7' -dichlorofluorescein diacetate (molecular weight 487.29), does not have fluorescence itself and can freely cross the cell membrane. After entering cells, DCFH can be hydrolyzed by cellular esterases. DCFH is impermeable to the cell membrane and thus is trapped inside the cell. Non-fluorescent DCFH can be oxidized by intracellular ROS to generate fluorescent DCF, and the intensity of fluorescence signal generated is proportional to the level of intracellular ROS. The level of intracellular ROS can be indirectly evaluated by fluorescence microscopy, flow cytometry or laser confocal microscopy.
- Reactive oxygen species (ROS) Assay kit can quickly and effectively detect intracellular ROS with high sensitivity and easy to use. At the same time, ROS positive induction reagent is provided, which can induce ROS levels in multiple types of cells in a short time to provide positive samples. Taking the amount of sample added to each well of the 6-well plate as the standard calculation, this kit can be measured about 100 times.

Storage

- Store at -20℃ and away from light.

Kit Components

DCFH-DA probe	100 µL
Reactive Oxygen Positive Inducer (2×)	20 mL

Protocol

1. Preparation before experiment:

1.1. Preparation of DCFH-DA working solution: Prepare DCFH-DA working solution by diluting DCFH-DA probe with serum-free cell medium or Earle's balanced salt solution at a ratio of 1:1000.

1.2. (**Optional**) ROS positive inducer working solution preparation: prepare 1 × ROS positive inducer working solution by mixing and diluting the ROS positive inducer (2×) with serum-free cell medium or Earle's balanced salt solution in a 1:1 volume.

2. (Optional) Cell preparation for positive control group:

2.1. Remove the cell culture medium and wash with PBS buffer 2 times.

2.2. Cover cells with 1× reactive oxygen species positive inducer working solution and incubate in a CO_2 incubator at 37°C for 45 min.

- If an increase in reactive oxygen species is not observed after 45 minutes of stimulation, the induction time can be appropriately prolonged or the concentration of inducer can be increased; if the increase of reactive oxygen species is too fast, the induction time or the concentration of inducer can be appropriately decreased.

3. Reactive oxygen species detection of adherent cells:

3.1. **Plate cells:** plate cells to ensure that the cell confluence is 50-70% during the assay.

3.2. **Cell washing:** Remove the cell culture medium and wash with PBS buffer 2 times.

3.3. **Loading probe:** Remove the washing buffer by suction, add the corresponding volume of DCFH-DA working solution according to the table below, and incubate in a CO_2 incubator at 37°C for 30 min.

3.4. **Cell washing:** Remove the DCFH-DA working solution and wash with PBS buffer 3 times to fully remove the excess probe. Finally, add PBS to cells.

Cell Culture Plate	Reactive Oxygen Species Detection Working Solution/Well
6-well	1000 µL
12-well	500 µL
24-well	250 µL
48-well	200 µL
96-well	100 µL

3.5. **ROS detection:** Directly visualize the cells using a fluorescence microscope or confocal microscope. The cells can also be trypsinized, neutralized, and resuspended in PBS for detection by fluorescence spectrophotometer, microplate reader, flow cytometer and other instruments. Set the excitation wavelength at 488nm and emission wavelength at 525nm (the fluorescence spectrum of DCF is very similar to that of FITC, and the parameters of FITC can be set to detect DCF).