Reactive Oxygen Species Assay Kit

Description

TargetMol's **Reactive Oxygen Species (ROS) Assay Kit** utilizes DCFH-DA as a fluorescent probe to detect intracellular ROS levels. Reactive oxygen species (ROS) are oxygen-derived molecules or ions with high chemical reactivity, including hydrogen peroxide (H_2O_2), superoxide anions, and hydroxyl radicals. At physiological levels, ROS act as important signaling molecules involved in various biological processes such as immune defense. However, when ROS production exceeds the capacity of the antioxidant system, oxidative stress occurs, leading to DNA and protein damage and potentially triggering various diseases.

DCFH-DA (2',7'-Dichlorodihydrofluorescein diacetate) is a cell-permeable fluorescent probe. The presence of two acetate groups in its structure allows DCFH-DA to freely cross cell membranes. Inside the cell, DCFH-DA is hydrolyzed by non-specific esterases to remove the acetate groups, generating non-fluorescent 2',7'-dichlorodihydrofluorescein (DCFH), which becomes trapped within the cell. When ROS are present, DCFH is oxidized to 2',7'-dichlorofluorescein (DCF), which emits a bright green fluorescence (excitation: 488 nm, emission: 525 nm). The fluorescence intensity is directly proportional to the intracellular ROS levels, enabling quantitative analysis of ROS generation.

This kit is compatible with multiple detection instruments, including fluorescence microscopes, confocal microscopes, fluorescence spectrophotometers, and flow cytometers.

For use in 6-well plates, each well requires 1 mL of working solution, providing approximately 100 assays.

For use in 96-well plates, each well requires 100 µL of working solution, providing approximately 1,000 assays.

Product Information

E.G., Taking 100 T packing for example

Catalog No.	Product Name	Packing
C0177-1	DCFH-DA（1000×）	100 µL
C0177-2	Rosup（125×）	800 µL

Features

1. High sensitivity;
2. Well-established control system;
3. Applicable to various sample types;
4. Compatible with multiple detection instruments;
5. Excellent experimental reproducibility;
6. Easy to operate.

Application

Research on oxidative stress signaling pathways, cellular metabolism regulation, antioxidant drug screening, and drug toxicity testing.

Instructions

I. Probe Loading

Select the appropriate probe loading method based on the stimulation duration:

Short-term stimulation (usually≤ 2 h): It is recommended to load the probe first, then stimulate the cells with the reactive oxygen species (ROS) positive control (Rosup) or the experimental compound.

Long-term stimulation (usually ≥ 6 h): It is recommended to treat the cells with the compound or Rosup first, and then load the probe.

A. In Situ Loading Method (for Adherent Cells)

1. Seed the cells into an appropriate culture plate one day before the experiment to ensure that cell confluence reaches 50-70% at the time of detection.

2. Remove the culture medium and add the drug at the desired concentration according to the experimental design. Incubate at 37°C in the dark. The stimulation time depends on the drug properties and cell type. Positive control (optional): Dilute Rosup (125×) at 1:125 to achieve a final concentration of 1×. Add to the cells and incubate at 37°C in the dark for 0.5-4 hours.
3. Dilute DCFH-DA 1:1000 with an appropriate diluent (serum-free medium, PBS, or HBSS) to prepare a 1× working solution.
4. Remove the culture medium and add a sufficient amount of the diluted DCFH-DA working solution to completely cover the cells. For example, in a six-well plate, add at least 1 mL per well. Incubate at 37°C for 20-30 minutes.
5. After incubation, wash the cells three times with an appropriate diluent (serum-free medium, PBS, or HBSS) to remove excess probe that has not entered the cells.

B. Cell Loading Method after Collection (Applicable to Adherent and Suspension Cells)

1. Culture cells using standard procedures. Collect and wash the cells to obtain an adequate cell suspension.
2. Add the appropriate concentration of the drug according to your experimental design. Incubate at 37°C in the dark; the stimulation time depends on the properties of the drug and the cell type. Positive control (optional): Dilute Rosup 1:125 (125×), giving a final concentration of 1×. Add to cells and incubate at 37°C in the dark for 0.5–4 h.
3. Dilute DCFH-DA at 1:1000 with a suitable diluent (serum-free medium, PBS, or HBSS) to a final concentration of 1×.
4. After collection, resuspend the cells in the diluted DCFH-DA solution and adjust the cell density to 1×10^6 – 2×10^7 cells/mL. Incubate in a 37°C incubator for 20–30 min, gently inverting the tube every 3–5 min to facilitate probe uptake by the cells.
5. After incubation, wash the cells three times with a suitable diluent (serum-free medium, PBS, or HBSS) to remove excess probe that has not entered the cells.
6. After washing, cells can be directly stimulated with the drug or positive control, or grouped for subsequent stimulation treatments.

II. Fluorescence Detection

A. In situ loaded samples: Can be directly observed using a laser confocal microscope, or cells can be collected and measured using a fluorescence spectrophotometer, a fluorescence microplate reader, or a flow cytometer.
B. Post-collection loaded samples: Suitable for measurement with a fluorescence spectrophotometer, a fluorescence microplate reader, or a flow cytometer, and fluorescence microscopy images can also be directly observed.
Note: Excitation wavelength (Ex): 488 nm; Emission wavelength (Em): 525 nm. Real-time monitoring or multi-time sampling can be performed to compare fluorescence intensity changes before and after stimulation.

Storage

Store at -20 °C, protected from light. Valid for one year.

Precautions

1. The effect of Rosup may vary significantly among different cell types. Please adjust the concentration of the positive control based on pre-experiment results.
2. Since the sample type and experimental conditions can affect staining efficiency, it is recommended to optimize the concentration of probe working solution and staining time through preliminary experiments.
3. If the negative control cells without stimulation show relatively strong fluorescence, the probe concentration can be appropriately reduced.
4. Before detection, the cell density for resuspension should be determined according to the fluorescence intensity. If fluorescence is strong, decrease cell density by reducing the volume of serum-free medium used for resuspension, and vice versa. At the same time, ensure that the cell density is consistent across all treatment groups.
5. DCF fluorescence is prone to quenching. Samples should ideally be detected within 2 hours after staining. During the procedure, avoid exposure to light.
6. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
7. Please wear a lab coat and disposable gloves.