Product Information

Reactive Oxygen Species Assay Kit

Catalog Number: R6033

Product Size: 1000 T

Contents:

Component	1000 T
A. 10 mM DCFH-DA	0.1 mL
B. 100 mM Positive control (Rosup)	1 mL

Storage

Store kit at 4°C in the dark immediately on receipt. For longer term storage of 12 months, store kit at -20°C.

Parameters

Fluorescence is measured at Ex/Em = 485/535 nm.

Description

Dichlorodihydrofluorescein-diacetate (DCFH-DA) is a fluorogenic dye that measures hydroxyl, peroxyl and other reactive oxygen species (ROS) activity within the cell. After diffusion in to the cell, the acetyl groups on DCFH-DA are cleaved by intracellular esterase to yield the non-fluorescent compound (DCFH) which is rapidly oxidized to highly fluorescent 2',7'-Dichlorodihydrofluorescein (DCF) by ROS. DCF can be detected by fluorescence spectroscopy with maximum excitation and emission spectra of 504 nm and 529 nm, respectively. The fluorescence intensity is proportional to the ROS levels within the cell cytosol. The ROS-inducing drug Rosup is used as a positive control. Each reactive oxygen species assay kit contains sufficient materials for approximately 1000 measurements in 96-well-plate format.

Protocol

1. ROS Detection Assay

1.1 ROS Detection in Situ Cells (adherent cells only)

a. Seed adherent cells per well in 96-well plate to obtain ~ 70-80% confluency on the day of experiment. Allow cells to adhere overnight. Grow suspension cells so that less than 5 x 10^4 cells per well are available.

b. Next day, if performing toxicity assays, remove the media and add serum-free media diluted drugs. Incubate at 37°C in the dark, the actual time depends on drug properties and cell types.

c. (Optional) Dilute 100 mM Rosup to 100 µM with serum-free media, add 100 µL per well in 96-well plate. Incubate for 30 min-4 h at 37°C in the dark to increase ROS levels, the actual time depends on cell types. i.e. HeLa cells require 30-60 min, MRC5 human embryonic fibroblasts require 90 min.

d. Dilute DCFH-DA to 10 µM with serum-free media.

e. Remove drugs, add appropriate volume of diluted DCFH-DA to cells. Added DCFH-DA needs to fully cover cells, i.e. more than 1000 µL per well in 6-well plate, more than 100 µL per well in 96-well plate. Incubate for 30 min at 37°C in the dark.

f. Wash cells twice in serum-free media to remove extracellular DCFH-DA.

1.2 ROS Detection in Collected Cells (suspension and adherent cells)

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a. Grow cells the day before the experiment, ensure cell status for detection. Wash and collect sufficient cells on the day of the experiment.

b. Suspend collected cells in diluted drugs, incubate at 37°C in the dark, the actual time depends on drug properties and cell types.

c. (Optional) Dilute 100 mM Rosup to 100 μM with serum-free media, add 100 μL per well in 96-well plate. Incubate for 30 min-4 h at 37°C in the dark to increase ROS levels, the actual time depends on cell types. i.e. HeLa cells require 30-60 min, MRC5 human embryonic fibroblasts require 90 min.

d. Dilute DCFH-DA to 10 μM with serum-free media.

e. Remove drugs, centrifuge to collect cells, add diluted DCFH-DA, and set cell density at 1.0×10^6-2.0×10^7.

P.S. Specific cell density depends on subsequent detection methods. i.e. for flow cytometry, cell number in one single tube should be controlled between 10^4 and 10^6.

f. Wash cells twice in serum-free media to remove extracellular DCFH-DA.

2. Fluorescence microscope

a. For adherent cells and live cells, directly image through fluorescence microscope.

b. For suspension cells, drop 25-50 μL cells onto a microscopic slide and cover it with a cover glass, and image it with fluorescence microscope.

c. Image cells with filter set appropriate for fluorescein (FITC).

3. Flow Cytometry Assay

a. For adherent cells, fully detach (e.g. trypsinize and quench with media) and harvest by centrifugation.

b. For suspension cells, directly harvest by centrifugation.

c. Re-suspend the cells with 0.5-1 mL PBS (0.5-1 x 10^5/ml).

d. Analyze on flow cytometer in FL1 or BL1 channel. Establish forward and side scatter gates from negative control cells to exclude debris and cellular aggregates. Mean fluorescence intensity in Ex/Em = 504/529 nm can be quantified and compared between untreated cells and cells treated with Rosup compounds, or between different cell types. Untreated cells barely show fluorescence, treated cells show bright green fluorescence.

Notes

1. Rosup is usually adopted at a concentration of 100μM (given different cell types, the recommended concentration is 100-400 μM). In general, a significant increase in ROS can be observed 30 min-4 h after stimulation. The effect of Rosup may vary greatly among different cell types. If the increase of ROS is not observed within 30 min after stimulation, the induction time can be prolonged or the concentration of Rosup can be appropriately increased. If ROS increase too quickly, the induction time can be shortened or the concentration of Rosup can be reduced appropriately.

2. If the negative control groups without stimulation have strong fluorescence, dilute DCFH-DA in accordance with 1:200-1:500 to set the final concentration of DCFH-DA at 2-5μM. The incubation time can also be adjusted within 15-60 min based on the situation.

3. Rosup is only applied in positive control samples, and it is not necessary to add Rosup in every sample.

4. After DCFH-DA incubation, clean the remaining probes that have not entered the cells which may cause a high noise.

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