ROS-100 Reactive Oxygen Species Detection Kit

Size: 100 tests

Kit Contents

	Description	Package
Component A	H2DCFDA (10mM) in DMSO	0.1mL
Component B	Reactive oxygen control (Rosup, 50mg/mL)	1 mL

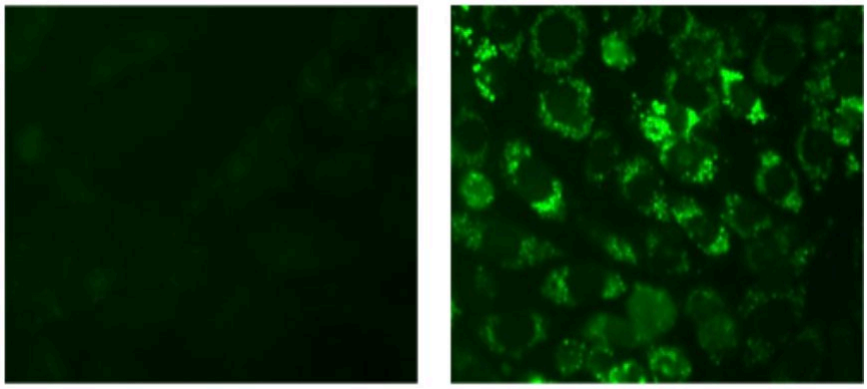
Introduction： The Reactive Oxygen Species (ROS) Detection Kit provides the key reagents necessary for the detection of ROS in live cells. The assay is based on H2DCFDA, a reliable fluorogenic marker for ROS in live cells. We also provide the common inducer of ROS production Reactive oxygen control (Component B, Rosup), as a positive control. Using this combination of dyes according to the optimized protocol provided here, oxidatively stressed and nonstressed cells are reliably distinguished by fluorescence microscopy. Generation of ROS is inevitable for aerobic organisms, and, in healthy cells, occurs at a controlled rate. Under conditions of oxidative stress, ROS production is dramatically increased, resulting in subsequent alteration of membrane lipids, proteins, and nucleic acids. Oxidative damage of these biomolecules is associated with a variety of pathological events including atherosclerosis, carcinogenesis, ischemic reperfusion injury, neurodegenerative disorders and with aging.

We utilize H2DCFDA, a unique cell-permeable fluorogenic probe, compatible with phenol red, FBS and BSA to detect reactive oxygen species in live cells. Upon the cell entry, H2DCFDA is modified by cellular esterases to form a non-fluorescent H2DCF. Oxidation of H2DCF by intracellular ROS yields highly a fluorescent product that can be detected by FACS, microplate reader, or fluorescence microscope (Ex/Em 495/529 nm). The fluorescence intensity is proportional to the ROS levels. Our kit provides a simple and specific assay for the real-time measurement of global levels of ROS in living cells. We include sufficient reagents to perform 100 assays and a common ROS inducer as a control for measurement of ROS levels or antioxidant activity with high sensitivity, specificity and accuracy.

Materials Recommended but Not Provided：
Fluorescence microscope, Flow cytometer (FL-1 channel) and Microplate reader capable of measuring Ex/Em 495/529 nm spectra Most live-cell buffering systems are suitable for the kit, the Hank's balanced salt solution (HBSS/Ca/Mg) is recommend.

Storage:
Component A: Store at -20°C protected from light, avoid multiple freeze/thaw cycles. Stable for12 months after received.
Component B: Store at -20°C protected from light. Warm to room temperature before use.

ROS Detection Assay Protocol:
The protocol was developed using live bovine pulmonary artery endothelial cells (BPAEC) and MRC5 human lung fibroblasts adhering to coverslips, but is amenable for use with other cell types. An additional protocol is provided for the use of (Component B, Rosup) as a positive control for the induction of ROS, which, if desired, must be performed before labeling with H2DCFDA.
1. Labeling with H2DCFDA
1.1 The Component A (H2DCFDA（10mM）in DMSO) is thoroughly thawed at room temperature (about 25°C)
1.2 Prepare 25 µM carboxy-H2DCFDA working solution. Add 5.0 µL of the 10 mM H2DCFDA stock solution (prepared in step 1.1) to 2.0 mL of warm HBSS/Ca/Mg or other suitable buffer.
1.3 Wash cells. Gently wash cells once with warm HBSS/Ca/Mg or other suitable buffer.
1.4 Label cells. Apply a sufficient amount of the 25 µM H2DCFDA working solution (prepared in step 1.2) to cover the cells adhering to the coverslip(s). Incubate for 30 minutes at 37°C, protected from light.
1.5 Wash cells. Gently wash the coverslips three times in warm HBSS/Ca/Mg or other suitable buffer.
1.6 Mount in warm buffer and image immediately. Best results are obtained when imaging takes place immediately after washing and mounting the sample.
2. Induction of Cellular ROS Production with Component B(Rosup):
2.1 Make 100 µM working solution of Rosup.
Dilute the Component B 1:5000 in appropriate complete growth media to produce a 100 µM working solution. For example, add 1µL Component B to 5 mL of complete media. to make 5.0 mL of 100 µM Rosup working solution,
2.2 Induce ROS production in cells. Apply a sufficient amount of the 100 µM Rosup working solution (prepared in step 2.1) to the cells adhering to the coverslip(s). Incubate the coverslip(s)at 37°C and 5% CO2. During development of the product using BPAE and MRC5 cells, a 60–90 minute incubation period was required. Appropriate incubation periods for ROS production in other cell lines should be determined empirically. After induction, label the cells with H2DCFDA starting with step 1.1, above
2.3 Wash cells. Gently wash the coverslips twice in war warm HBSS/ Ca/Mg or other suitable buffer. After washing, label the cells with H2DCFDA starting with step 1.1, above.



Immunofluorescence of Reactive Oxygen Species Detection Kit

PROTOCOL FOR ADHERENT CELLS

NOTE: *To create positive controls, oxidative activity is stimulated with Component B prior to analysis*

1. Seed adherent cells at 25 x 10^3 per well one day before performing the assay.
2. Remove the media and add 100 µL/well of diluted Component A.
3. Remove Component A and stain cells by adding 100 µL/well of Probe Working Solution.
4. Incubate at cells' optimal temperature in dark conditions. An incubation time of 15～60 minutes is enough.
5. Remove media and add at least 100µL of PBS. Measure fluorescence immediately.

PROTOCOL FOR SUSPENSION CELLS

NOTE: *To create positive controls, oxidative activity is stimulated with Component B prior to analysis*

1. Grow suspension cells in sufficient amount. (In the step 5 you will need 100 x 10^3 cells per group).
2. Collect and wash cells with PBS using centrifugation.
3. Resuspend cells at a density of 1x10^6 cells/mL. Stain the cells with the desired volume of Probe Working Solution.
4. Incubate at cells' optimal temperature in dark conditions. An incubation time of 15–60 minutes is enough.
5. Wash cells by centrifugation. Resuspend cells in PBS, seed in 96-well microplate or fluorimeter with 100,000 stained cells/well and measure fluorescence immediately.

PROTOCOL FORFLOW CYTOMETER

NOTE: *To create positive controls, oxidative activity is stimulated with Component B prior to analysis*

1. Grow cells (adherent or suspension) so that on the day of the experiment there are at least 15 x 10_3 cells per assayed condition (treatment, dose, time). Include in the calculation enough cells for a control
2. Harvest cells and ensure a single cell suspension by gently pipetting up and down suspension cells or by fully detaching adherent cells (e.g. trypsinize and quench with media).
3. Stain cells in culture media with 10-25 µM DCFH-DA and incubate for 30 minutes at 37°C. Once the incubation is completed, DO NOT wash the cells
4. After staining, treat the cells with compound(s) of interest and ensure that appropriate controls are included. If using Rosup as positive control, optimal signal is obtained after 4 hours of treatment.
5. Analyze on flow cytometer. Establish forward and side scatter gates to exclude debris and cellular aggregates from analysis. DCF should be excited by the 488 nm laser and should be detected at 535 nm.