Reactive Oxygen Species Assay Kit

User Manual

Catalog # CAK1275

(Version 1.1A)

Detection and Quantification Intracellular Levels of Reactive Oxygen Species (ROS) Content in Suspension or Adherent Cells Cultures Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Constant generation of low levels of reactive oxygen species (ROS) and free radicals is a basic feature of all living cells. Low levels of ROS play an essential role in signaling pathways, whereas increased under oxidative stress, ROS activity result in damage to nucleic acids, proteins and membrane lipids. Accumulation of ROS during oxidative stress is also associated with aging, apoptosis or necrosis, and is implicated in pathological conditions such as; vascular diseases, diabetes, renal ischemia, arteriosclerosis, pulmonary disorders, inflammatory diseases, and cancer. Cellular activity of ROS is offset by antioxidants, numerous repair systems, and replacement of damaged DNA. Probes for measuring intracellular ROS levels provide important tools to study oxidative stress inducers and effects of antioxidant therapies. Reactive Oxygen Species Microplate Assay Kit provides a simple and direct procedure for measuring ROS levels in suspension or adherent cells cultures samples. Upon the cell entry, DCFH-DA is modified by cellular esterases to form a non-fluorescent DCFH. Oxidation of DCF 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.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 1	4 °C
ROS Label (1000X)	Powder x 1	-20 °C
ROS Label Buffer	0.1 ml x 1	4 °C
ROS Inducer (250X)	1 ml x 1	4 °C
Positive Control (10 mM)	1 ml x 1	4 °C
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Note:

ROS Label (1000X): add 0.1 ml ROS Label Buffer to dissolve before use. Always store at -20°C protected from light, avoid multiple freeze/thaw cycles. Prior to labeling, dilute the stock solution at 1:1000 in pre-warmed Assay Buffer or culture media to a 1X final working concentration. Do not store the 1X reagent for future use.

ROS Inducer (250X): Store at 4°C protected from light. Warm to room temperature before use. Prepare fresh 1X working solution in pre-warmed Assay Buffer or culture media prior to experiment. Do not store the 1X reagent for future use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader capable of measuring Ex/Em 495/529 nm spectra
2. Tissue culture vessels and appropriate culturing media
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer

IV. ASSAY PROCEDURE

Detection by Microplate Assay:

1. Seed 2.5 x10^4 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 approximately 1.5 x 10^5 cells per well are available. Next day, remove the media and wash the adherent cells in 100 µl of ROS Assay Buffer. Collect suspension cells by centrifugation and wash once in PBS. Discard the wash.

2. Add 100 µl of 1X ROS Label diluted in ROS Assay Buffer per well into adherent cells or re-suspend the pelleted cells at 1.5 x 10^6 cells/ml. Incubate for 45 min at 37°C in the dark.

3. For adherent cells: remove the ROS Label, add 100 µl of ROS Assay Buffer or PBS and measure fluorescence immediately, or treat the cells with 100 µl of diluted test compound(s) for desired period of time. Include appropriate controls as well as blank wells (media or buffer only).

For suspension cells: wash the cells by centrifugation in ROS Assay Buffer, maintain the same cell concentration. Seed 100,000 labeled cells per well in 100 µl volume and measure the ROS or treat the cells with test compound(s) in ROS Assay Buffer supplemented with 10% FBS or media without phenol red for appropriate time.

If using ROS Inducer as an experimental control, dilute the ROS inducer stock to 1X and treat the cells for 1 hour prior to analyses.

Positive control (Optional): dilute the positive control to the common working concentration of 100 µM with serum-free medium. Cells were added and incubated at 37°C for 30 min - 4 hours to improve the level of reactive oxygen species. There were differences among different cell types.

4. Measure fluorescence at Ex/Em= 495/529 nm in end point mode in presence of compounds and controls. Determine change in fluorescence after background subtraction.

Detection by Flow Cytometry:

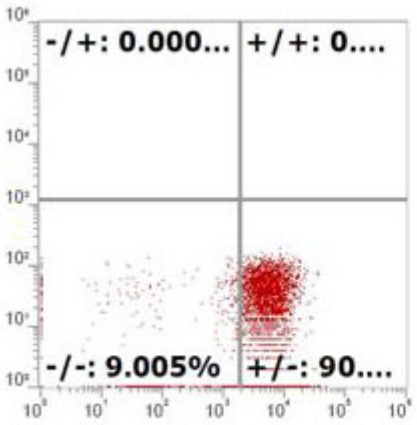
1. Grow cells (adherent or suspension) in appropriate media to obtain at least of 3 x 10^4 cells per assayed conditions. Ensure that adherent cells are sub-confluent. Account for cell loss during the processing.

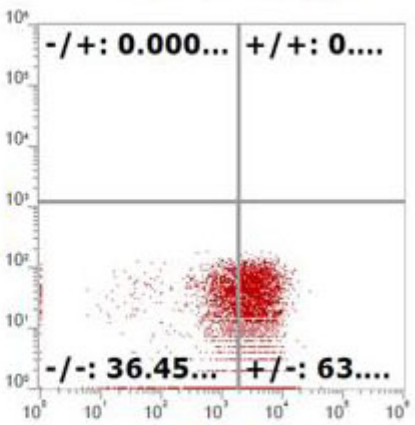
2. Harvest the suspension cells by centrifugation at 300 x g for 5 min at room temperature. Use these setting throughout the entire protocol for both cell types. Fully detach adherent cells (e.g. trypsinize and quench with media) and harvest by centrifugation. Resuspend the cell pellets in culture media with 1X ROS Label. Ensure a single cell suspension by gently pipetting up and down and incubate for 30 minutes at 37°C protected from light.

3. Upon completion, spin down the cells and remove the media. DO NOT wash the cells. Treat the cells with compound(s) of interest for desired time period directly in culture media, ROS Assay Buffer supplemented with 10% FBS, or culture media without phenol red. Include appropriate controls. If using ROS Inducer as an experimental control, dilute the stock to 1X and treat the cells for 1 hour prior to analyses.

4. Adjust the cell concentration so at least 1 x10^4 cells should be analyzed per experimental condition. Gently pipette cells up/down to ensure single cell suspension and analyze on flow cytometer in FL-1 channel. Establish forward and side scatter gates from negative control cells to exclude debris and cellular aggregates. Mean fluorescence intensity in Ex/Em = 495/529 nm can be quantified and compared between untreated cells and cells treated with test compounds, or between different cell types.

V. TYPICAL DATA





VI. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VII. NOTES