ab287839 – Reactive Oxygen Species (ROS) Detection Assay Kit

For the measurement of intracellular levels of ROS in suspension or adherent cell cultures.
For research use only - not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287839

Storage and Stability

The entire kit may be stored at -20°C protected from light. Do not to keep the vials open for long time periods. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay

Materials Supplied

Item	Quantity		Storage Condition
	100 Assay kit	250 Assay kti	
ROS Assay Buffer I/ROS Assay Buffer	25 mL	65 mL	4°C
1000X ROS Label I/ROS Label (1000X)	10 µL	25 µL	-20°C
250X ROS Inducer/ROS Inducer (250X)	20 µL	20 µL	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Tissue culture vessels and appropriate culturing media; flow cytometry vessels
- Phosphate Buffered Saline (PBS, pH 7.4)
- Fluorescence microscope, Flow cytometer (FL-1 channel) and Microplate reader capable of measuring Ex/Em 495/529 nm spectra
- 96-well microplates (black and/or clear)

Reagent Preparation

ROS Assay Buffer I/ROS Assay Buffer: Upon receipt place and store at 4°C. Always use sterile techniques for handling. Equilibrate to 37°C before use.

1000X ROS Label I/ROS Label (1000X): Due to light-induced auto-oxidation 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 ROS Assay Buffer I/Assay Buffer or culture media to a 1X final working concentration. Do not store the 1X reagent for future use. Stable for 6 months

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

Version 2a, Last updated Monday, January 15, 2024

Assay Procedure

- The following protocols, developed with Jurkat (suspension) and HeLa (adherent) cells, provide general guidelines and should be modified depending on application, cell line and sensitivity required. Growth conditions, cell density and other factors may affect ROS labeling; therefore, cells should not be overly dense during the experiment; do not exceed 5 X 10^5 cells/ml of complete growth medium.
- It is suggested to try several ROS Probe concentrations to find the best conditions for your cell type and experimental design. The assay volume is 100 µl in a 96-well tissue culture plate. Adjust volumes accordingly for other plate formats.
- Equilibrate all materials and prepared reagents to correct temperature prior to use.

1. Detection of ROS in Suspension and Adherent Cells by **Flow Cytometry**:
    a. Grow cells (adherent or suspension) in appropriate media to obtain at least of 3 x 10^4 cells per assayed conditions; positive, negative and experimental controls, and test compound(s). Ensure that adherent cells are sub-confluent. Account for cell loss during the processing.
        i. Negative control – unlabeled cells not exposed to ROS Inducer or treatment
        ii. Positive control – cells incubated with 1X ROS Label I/ROS Label only,
        iii. Experimental control – labeled cells treated with 1X ROS Inducer.
    b. 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. Re-suspend the cell pellets in culture media with 1X ROS Label I/ROS Label. Ensure a single cell suspension by gently pipetting up and down and incubate for 30 minutes at 37°C protected from light.
    c. 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 I/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.
    d. 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.

2. Detection of ROS in Suspension and Adherent Cells by Microplate Assay
    a. Seed 2.5 x104 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 I/ROS Assay Buffer. Collect suspension cells by centrifugation and wash once in PBS. Discard the wash.

b. Add 100 µl of 1X ROS Label I/ROS Label diluted in ROS Assay Buffer I/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.
c. For adherent cells: remove the ROS Label I/ROS Label, add 100 µl of ROS Assay Buffer I/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 I/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 I/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.
d. 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.

3. For Fluorescence microscope analysis:
   a. Seed the cells directly onto glass slides or tissue culture plates to ensure~50-70% confluency on the day of the experiment. Follow the kit protocol and upon completion observe the cells immediately using emission filter appropriate for fluorescein

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)