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ab186027 Cellular Reactive Oxygen Species Detection Assay Kit (Red Fluorescence)

For the detection of Reactive Oxygen Species (ROS) in live cells.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Cellular Reactive Oxygen Species Detection Assay Kit (Red Fluorescence) (ab186027) is a sensitive fluorometric one-step assay to detect intracellular reactive oxygen species (ROS) such as superoxide and hydroxyl radical in live cells. The assay uses our proprietary ROS Red dye to quantify ROS: the dye is cell-permeable and reacts with ROS present in the cell to generate a red fluorescent signal (Ex/Em = 520/605 nm).

The assay can be performed in 1 hour and can be detected by fluorescence microscopy, microplate fluorometry or high-content imaging. It can be easily adapted to use in 384-well microplate format.

Reactive oxygen species (ROS) are natural by-products of the normal metabolism of oxygen. The two major sources of cellular ROS are Complex I (NADH dehydrogenase ubiquinone-ubiquinol reductase) and Complex III (ubiquinol cytochrome c reductase), both part of the mitochondrial electron transport chain. These two complexes generate ROS particularly when electron transport is slowed by high mitochondrial membrane potential ($\Delta\psi m$). The major product of ROS in mitochondrial is in the form of superoxide and hydroperoxyl radical. Superoxide generated in complex III occurs in the presence of slow electron transport which allows for the ubisemiquinone anion radical to react with oxygen dissolved in the membrane. The exact source of superoxide generated by complex I is less known and it is believed to be due to electron leakage from its iron-sulfur clusters.

Low levels (or optimum levels) of ROS play an important role in signaling pathways. However, when ROS production increases and overwhelms the cellular antioxidant capacity, it can induce macromolecular damage (by reacting with DNA, proteins and lipids) and disrupt thiol redox circuits. In the first instance, damage can lead to apoptosis or necrosis. Disruption of thiol redox circuits can lead to aberrant cell signaling and dysfunctional redox control.

2. Protocol Summary

Grow cells in appropriate medium

Stain cells with ROS Red Working Solution

Incubate cells for 30 – 60 minutes at 37°C

Treat cells to induce ROS

Monitor changes in fluorescence intensity (Ex/m = 520/605 nm)

Determine ROS change as percentage of control
after background subtraction

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition (Before prep)	Storage Condition (After prep)
Assay Buffer	20 mL	-20°C	-20°C
DMSO	200 µL	-20°C	-20°C
ROS Red Dye (lyophilized)	1 vial	-20°C	-20°C

7. Materials Required, Not Supplied

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

- Fluorometric microplate reader (bottom read mode) or fluorescence microscopy capable of measuring fluorescence at Ex/Em = 520/605 nm
- PBS or HHBS buffer
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- General tissue culture supplies
- Sterile 96 well plate with clear flat bottom, preferably black (if performing assay in microplate format). Use a poly-D-lysine coated plate for suspension cells

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 DMSO:

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 min to thaw the DMSO solution before use.

9.3 ROS Red Dye:

Reconstitute dye by adding 40 µL of DMSO (step 9.2) to the vial to prepare **ROS Red Stain Stock Solution**. Mix well by pipetting up and down. Equilibrate Stock Solution to room temperature before use.

Reconstituted probe may appear as a clear to pink/red solution.

Aliquot unused Stock Solution so that you have enough volume to perform the desired number of assay. Store at -20°C with the cap sealed tightly away from light. Avoid freeze-thaw cycles. Use within 2 months.

10. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- Prepare all reagents and samples as directed in the previous sections.
- Each cell line should be evaluated on an individual basis to determine the optimal cell density.
- The protocol described here is for 96-well microplate format. You can adapt the protocol for 384-well format by dividing working volumes by half.

10.1 Grow cells:

10.1.1 Adherent cells: plate cells overnight in growth medium at 10^4 – 4 x 10^4 cells/100 µL per well.

Δ Note: for 384 well plate, use 2,5 x 10^3 – 10^4 cells/25 µL per well.

10.1.2 Suspension cells: on the day of the assay, centrifuge cells from the culture medium and resuspend the cell pellet in culture medium at 5 x 10^4 – 10^5 cells/100 µL per cell in a poly-D-lysine coated plate. Centrifuge plate at 800 rpm for 2 minutes with brake off.

Δ Note: for 384 well plate, use 10^4 – 2.5 x 10^4 cells/20 µL per well.

10.2 Prepare ROS Red Stain working solution:

10.2.1 Add 20 µL of ROS Red Stock Solution (Step 9.3) to 10 mL of Assay Buffer and mix well.

Δ Note: ROS Red Stain working solution is stable for at least 2 hours at room temperature.

10.3 Run ROS assay:

10.3.1 Add 100 µL/well of ROS Red Working Solution into the cell plate.

Δ Note: for 384 well plate, add 25 µL/well.

10.3.2 Incubate cell plate at room temperature or in a 37°C/5% CO_2 incubator for one hour.

10.3.3 Treat cells with 20 µL of 11X test compounds in PBS or HHBS. For untreated cells, add 10 µL of compound buffer.

Δ Note: for 384 well plate, add 10 µL of 6X test compounds.

10.3.4 Induce ROS production by incubating cell plate at room temperature or in a 37°C/5% CO_2 incubator for at least 15 minutes, or required time for your compound.

Δ Note: for example, incubate HeLa cells for 30 minutes when treated with 1 mM H_2O_2.

10.3.5 Monitor fluorescence increase at Ex/Em = 520/605 nm (cut off 590 nm) with bottom read mode.

11. Data Analysis

11.1 Subtract blank readings from all measurements (control and treated)

11.2 Using fluorescent intensity, determine fold change between control and treated cells.

12. Typical data

Data provided for **demonstration purposes** only.

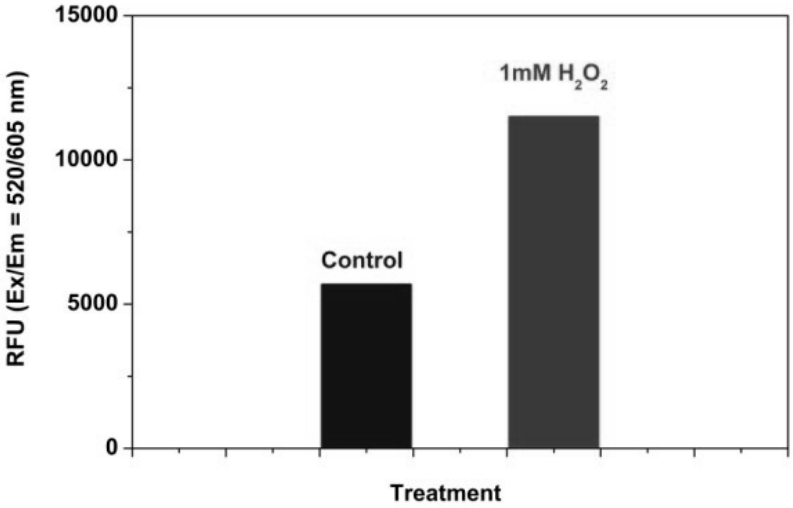

Figure 1. Detection of ROS in Jurkat cells. Jurkat cells were seeded on the same day at 3 x 10^5 cells/100 µL per well in a Costar black wall/clear bottom 96-well plate. The ROS Red Stain solution (100 µL) was added and incubated in a 37°C/5% CO_2 incubator for 1 hour. Cells were then treated with or without 1 mM H_2O_2 for 2 hours. The fluorescence signal was monitored at Ex/Em = 520/605 nm (cut off 590 nm) with bottom read mode using FlexStation (Molecular Devices).

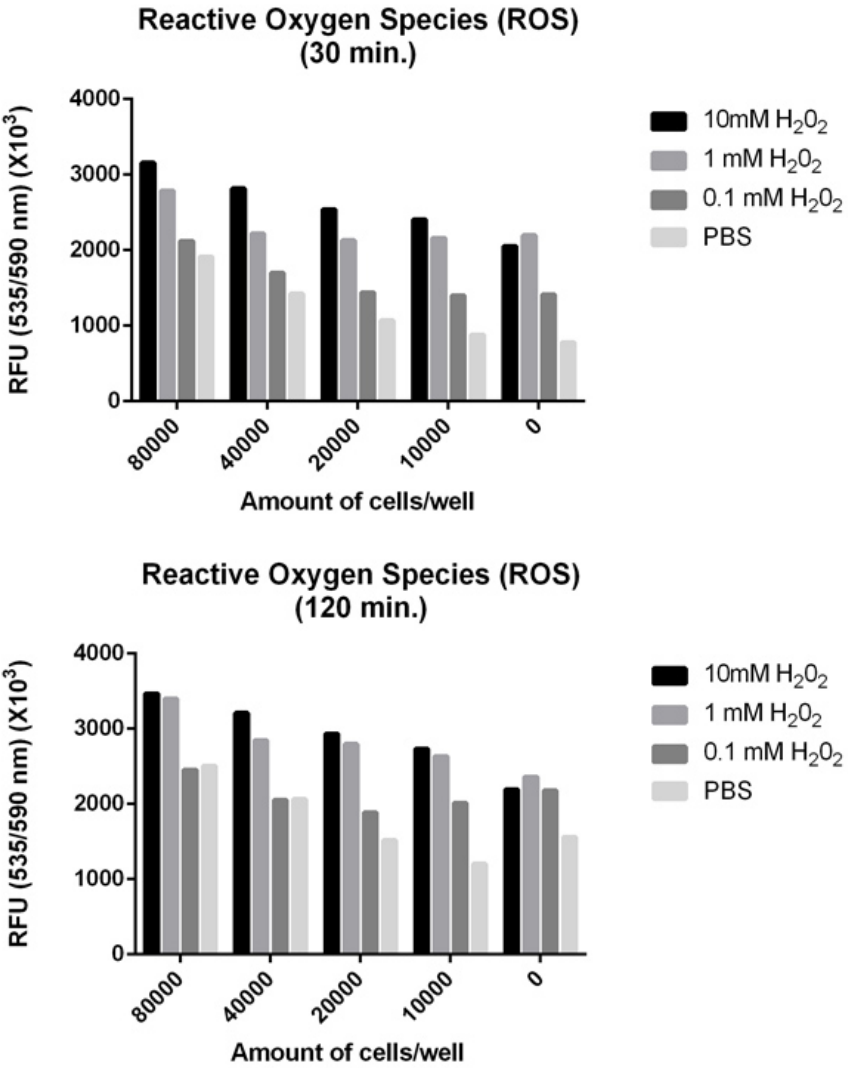

Figure 2. Graph showing relative fluorescence of ROS induced in various quantities of HeLa cells at various concentrations of H_2O_2 after 30 minutes exposure time (top) and after 120 minutes (bottom).

13. FAQs

Q. Do any I to do any washing steps?

A. It is possible to wash cells between staining and treatment steps, but be aware the signal might decrease a bit.

Q. Can I use phenol red free media instead of the assay buffer?

A. Yes, it should be ok.

14. Notes

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)