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ab219943 Mitochondrial Superoxide Detection Kit (Fluorometric)

For quantification of superoxide levels in live cells

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

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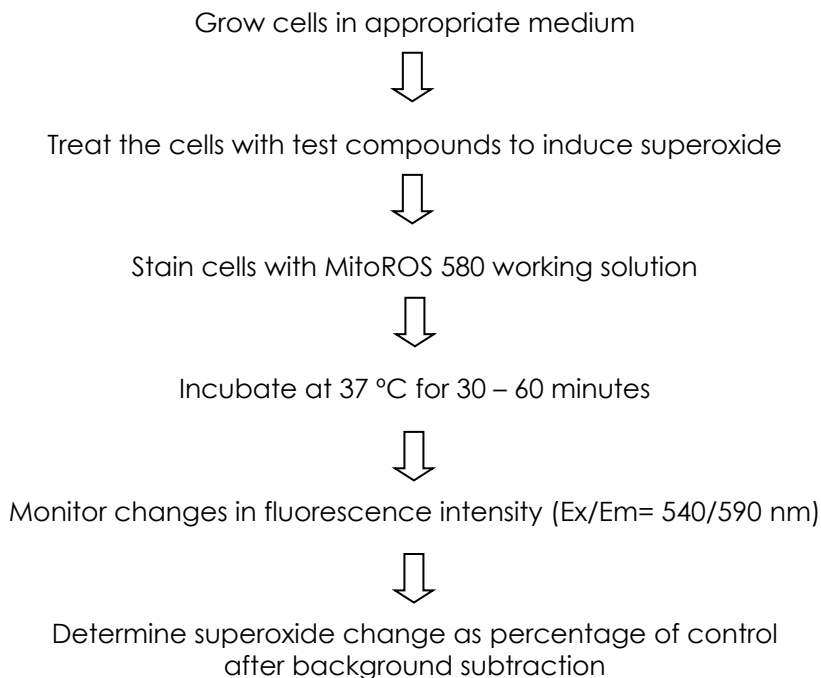
1. Overview

Mitochondrial Superoxide Detection Kit (Fluorometric) (ab219943) is a sensitive fluorometric one-step assay to detect intracellular superoxide radical in live cells. The assay uses our MitoROS 580 dye: the dye is cell-permeable and selectively reacts with mitochondrial superoxide present in live cells to generate a red fluorescence signal that can be easily read at Ex/Em = 540/590 nm.

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

Mitochondria are major producers of cellular superoxide. The production of low to moderate levels of superoxide is critical for the proper regulation of many essential cellular processes including gene expression, signal transduction, and muscle adaptation to endurance exercise training. Uncontrolled mitochondrial superoxide production can trigger cellular oxidative damage that contributes to the pathogenesis of a wide variety of disorders including cancer, cardiovascular diseases, neurodegenerative diseases and aging. The detection of intracellular mitochondrial superoxide is of central importance to understanding proper cellular redox regulation and the impact of its dysregulation on various pathologies.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- 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 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 temperature (before prep)	Storage temperature (after prep)
Assay Buffer	20 mL	-20°C	-20°C
MitoROS 580 dye	1 vial	-20°C	-20°C
DMSO	100 µL	-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 = 540/590 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 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 (20 mL):

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

9.2 DMSO (100 µL):

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

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time probe is needed. Store at -20°C.

9.3 MitoROS 580 dye (1 vial):

Reconstitute MitoROS 580 dye by adding 50 µL of DMSO to the vial of OH580 to prepare **500X MitoROS580 Stain Stock Solution**. Mix well by pipetting up and down.

Δ Note: 25 µL of MitoROS 580 Stain stock solution is enough for 1 x 96 well plate.

Aliquot unused stock solution so that you have enough volume to perform the desired number of assays. Store at -20°C with the cap sealed tightly away from light. Avoid repeated 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.
- 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:

10.1.1.1 Adherent cells: plate cells overnight in growth medium at $1-4 \times 10^4$ cells/90 μ L per well.

Δ Note: for 384 well plate, use 2.5×10^3 - 10^4 cells/20 μ L 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 $1-2 \times 10^5$ cells/90 μ L per cell in a poly-D-lysine coated plate.

Δ Note: for 384 well plate, use $2.5-5 \times 10^4$ cells/20 μ L per well.

10.1.3 Centrifuge plate at 800 rpm for 2 minutes with brake off.

10.2 Prepare MitoROS 580 Stain working solution:

10.2.1 Add 25 μ L of 500X MitoROS 580 Stain Stock Solution (Step 9.3) to 10 mL of Assay Buffer and mix well.

Δ Note: MitoROS 580 Stain working solution is stable for at least 2 hours at room temperature.

10.3 Run superoxide assay:

10.3.1 Treat cells with 10 μ L of 10X test compounds in PBS or HHBS. For untreated cells, add 10 μ L of compound buffer.

Δ Note: for 384 well plate, add 5 μ L of 5X test compounds.

10.3.2 Induce superoxide production by incubating cell plate in a 37°C/5% CO₂ incubator, protected from light, for the required time for your compound.

Δ Note: for example, incubate HeLa cells for 30 minutes when treated with 50 μ M Antimycin A (AMA).

10.3.3 Add 100 μ L/well of MitoROS 580 Stain Working Solution into the cell plate.

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

- 10.3.4 Incubate cell plate at 37°C for 30-60 minutes.
- 10.3.5 Monitor fluorescence increase at Ex/Em = 540/590 nm (cut off 570 nm) with bottom read mode. Alternatively, monitor the fluorescence signal using a fluorescence increase with a TRITC filter set.

11. Data Analysis

FOR MICROPLATE READER

- Using fluorescence intensity, determine fold change between control and treated cells.

FOR FLUORESCENCE MICROSCOPY

- We recommend acquiring several images per well.
- We recommend data analysis after coding and mixing images to ensure unbiased results.
- For manual analysis, if you do not have a specific software installed in your microscope, you can download ImageJ, an open source image processing designed for scientific multidimensional images by the National Institute of Health (NIH).

12. Typical Data

Data provided for demonstration purposes only.

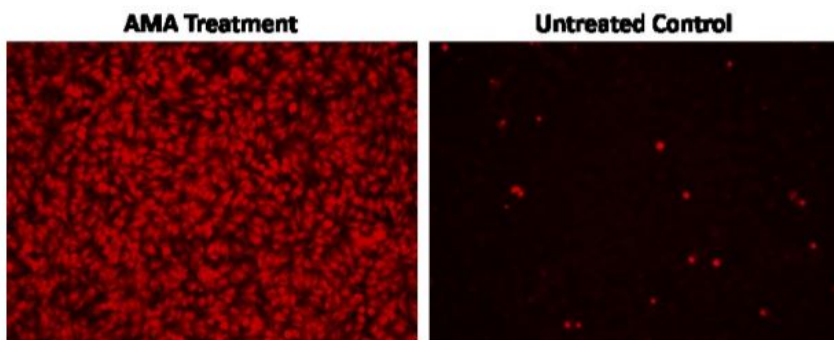


Figure 1. Superoxide production in HeLa cells. HeLa cells were seeded overnight (10^5 cells/well/100 μ L) in a 96 well black wall/clear bottom plate. Left: cells were treated with 50 μ M Antimycin A (AMA) at 37°C for 30 minutes, then incubated with MitoROS 580 for 1 hour. Right: control HeLa cells were incubated with MitoROS580 at 37 °C for 1 hour without treatment. The fluorescence signal was measured using fluorescence microscope with a TRITC filter.

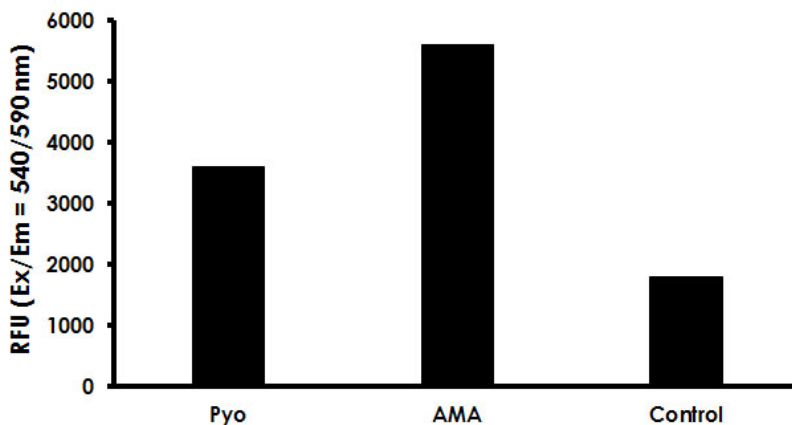


Figure 2. Quantification of superoxide production in HeLa cells. HeLa cells were seeded overnight (10^5 cells/well/100 μ L) in a 96 well black wall/clear bottom plate. Cells were left untreated (control) or treated with either pyocyanin (Pyo, 50 μ M Pyocyanin) or antimycin A (AMA, 50 μ M Antimycin A) at 37 $^{\circ}$ C for 30 minutes. Cells were then incubated with MitoROS 580 at 37 $^{\circ}$ C for 1 hour. The fluorescence signal was monitored at Ex/Em = 540/590 nm (cut off = 570 nm) with bottom read mode using a microplate reader.

13. Notes

Technical Support

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