

Version 1b Last updated 10 March 2026

# **ab219931**

# **Mitochondrial**

# **Hydroxyl Radical**

# **Detection Assay Kit**

For the rapid, sensitive and accurate measurement of mitochondrial Hydroxyl Radical (OH $\cdot$ ) in live cells

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

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## 1. Overview

Mitochondrial Hydroxyl Radical Detection Assay Kit (ab219931) is a sensitive fluorometric one-step assay to detect intracellular hydroxyl radical ( $\text{OH}\cdot$ ) in live cells. The assay uses our OH580 probe: the probe is cell-permeable and selectively reacts with hydroxyl radical 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.

The detection of intracellular hydroxyl radical is of central importance to understanding proper cellular redox regulation and the impact of its dysregulation on various pathologies. The hydroxyl radical ( $\text{OH}\cdot$ ) is one of the reactive oxygen species (ROS) highly reactive with other molecules to achieve stability. In general, hydroxyl radical is considered to be a harmful by-product of oxidative metabolism, which can cause molecular damage in living system. It shows an average lifetime of  $10^{-9}$  s and can react with nearly every biomolecule such as nuclear DNA, mitochondrial DNA, proteins and membrane lipids.

## 2. Protocol Summary

Grow cells in appropriate medium



Stain cells with OH580 Working Solution



Incubate cells for 1 hour at 37°C



Treat cells to induce OH<sup>•</sup> formation



Monitor changes in fluorescence intensity (Ex/m = 5240/590 nm)



Determine OH<sup>•</sup> change as percentage of control

### 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.
- 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.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website [www.abcam.com](http://www.abcam.com).

### 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

<b>Item</b>	<b>Quantity</b>	<b>Storage temperature (before prep)</b>	<b>Storage temperature (after prep)</b>
Assay Buffer	50 mL	-20°C	-20°C
DMSO	100 µL	-20°C	-20°C
OH580 probe	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 = 540/590 nm
- HHBS or DPBS
- 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. 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 (50 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 OH580 probe (1 vial):

Reconstitute OH580 probe by adding 50 µL of DMSO to the vial of OH580 to prepare **250X OH580 Stain Stock Solution**. Mix well by pipetting up and down.

**Δ Note:** 25 µL of OH580 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 Adherent cells: plate cells overnight in growth medium at  $1-4 \times 10^4$  cells/90  $\mu$ L per well.

**Δ Note:** for 384 well plate, use  $2.5 \times 10^3-10^4$  cells/20  $\mu$ 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  $\mu$ 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  $\mu$ L per well.

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

### 10.2 Prepare OH580 Stain working solution:

10.2.1 Add 25  $\mu$ L of 250X OH580 Stain Stock Solution (Step 9.3) to 10 mL of Assay Buffer and mix well.

**Δ Note:** OH580 Stain working solution is stable for at least 2 hours at room temperature.

### 10.3 Run hydroxyl radical assay:

10.3.1 Remove medium from wells, being careful not to displace any cells.

10.3.2 Add 100  $\mu$ L/well of OH580 Stain Working Solution into the cell plate.

**Δ Note:** for 384 well plate, add 25  $\mu$ L/well.

10.3.3 Incubate cell plate at 37°C for one hour.

10.3.4 Induce hydroxyl radical by treating cells with the appropriate compounds in your desired buffer (PBS or HHBS) at 37°C for the required time, protected from light.

Suggested positive controls:

- HeLa cells treated with Fenton reaction (10  $\mu\text{M}$   $\text{CuCl}_2$  and 100  $\mu\text{M}$   $\text{H}_2\text{O}_2$ ) at 37°C for 1 hour (exogenous hydroxyl radical formation)
- RAW 264.7 cells treated with PMA (phorbol 12-myristate 13-acetate) at 37°C for 4 hours (endogenous hydroxyl radical formation)

10.3.5 Wash cells 2-3 times with HHBS or DPBS.

10.3.6 Add 100  $\mu\text{L}$  Assay Buffer to each well.

10.3.7 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

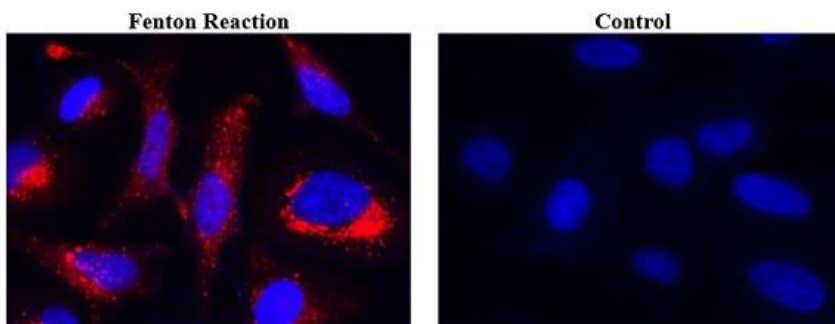
- Subtract blank reading (assay buffer only) from all measurements (control and treated).
- Using fluorescence intensity, determine fold change between control and treated cells.

### FOR FLUORESCENCE MICROSCOPY

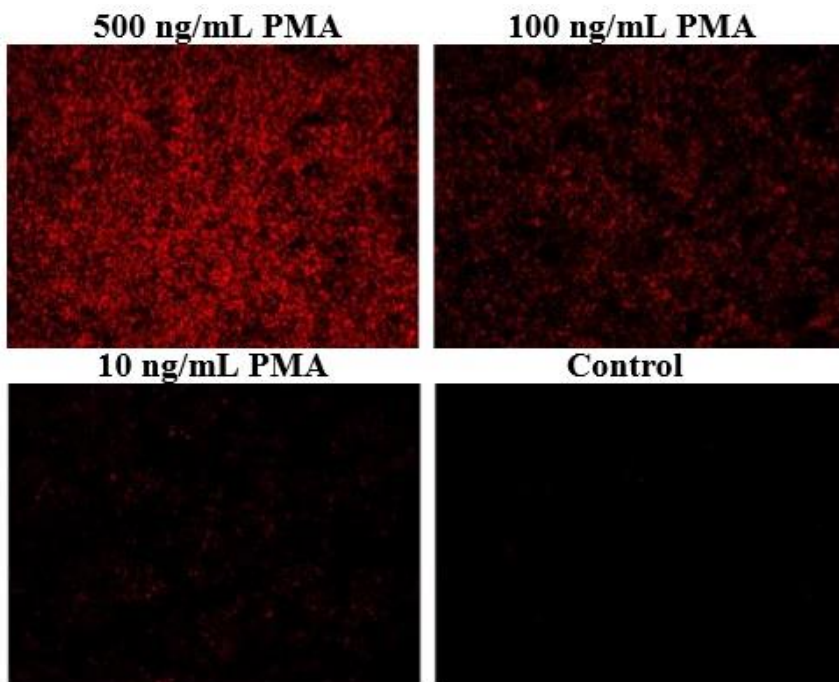
- We recommend acquiring several images per well.
- We recommend data analysis after coding and mixing images to ensure unbiased results.
- For manual analysis, if you do not have a specific software installed in your microscope, you can download ImageJ, an open source image processing designed for scientific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

## 12. Typical Data

Data provided for **demonstration purposes** only.



**Figure 1.** Hydroxyl radical production in HeLa cells. HeLa cells ( $10^5$  cells/well/100  $\mu$ L) were incubated with OH580 working solution at 37°C for 1 hour, then washed once with HHBS. Left: cells were treated with Fenton Reaction (10  $\mu$ M  $\text{CuCl}_2$  and 100  $\mu$ M  $\text{H}_2\text{O}_2$ ) in 1X HBSS buffer at 37°C for 1 hour. Right: control HeLa cells in 1X HBSS buffer without treatment. After 3 washes with HHBS, HeLa cells were measured using a fluorescence microscope with a TRITC filter set (Red). Cell nuclei were stained with Hoechst 33342.



**Figure 2.** Intracellular hydroxyl radical in RAW 264.7 cells. Cells were incubated with OH580 working solution at 37°C for 1 hour, then washed once with HHBS. Cells were then incubated without or with PMA (phorbol 12-myristate 13-acetate, 10-500 ng/mL) in growth medium at 37°C for 4 hours. After washing 3 times with HHBS, HeLa cells were measured using a fluorescence microscope with a TRITC filter set.

## 13. Notes

## Technical Support

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