Cell Meter™ Mitochondrial Hydroxyl Radical Detection Kit *Red Fluorescence*

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 16055 (200 assays)	Keep in freezer Avoid light	Fluorescence microscope Fluorescence microplate reader

Introduction

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 (HO·) 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. AAT Bioquest's Cell Meter™ Mitochondrial Hydroxyl Radical Detection Kit is optimized for detecting ROS in mitochondria. MitoROS™ OH580 is live-cell permeant probe and can rapidly and selectively target hydroxyl radical in live cells. It generates red fluorescence when it reacts with OH·, and can be easily read at Ex/Em= 540/590 nm. Cell Meter™ Mitochondrial Hydroxyl Radical Detection Kit provides a sensitive fluorimetric probe to detect OH· in live cells with one hour incubation. This kit can be used for fluorescence microplate readers and fluorescence microscopy applications.

Kit Components

Components	**Amount**
Component A: MitoROS™ OH580	1 vial
Component B: Assay Buffer	1 bottle (50 mL)
Component C: DMSO	100 µL

Assay Protocol for One 96-well Plate:

Brief Summary

Prepare cells → Incubate cells with MitoROS™ OH580 working solution at 37 °C for 60 minutes → Incubate cells with test compounds (to induce OH·) → Monitor the fluorescence increase at Ex/Em= 540/590 nm

Note: Thaw all the components at room temperature before use.

1. Prepare cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 10,000 to 40,000 cells/well/90 µL for a 96-well plate or 2,500 to 10,000 cells/well/20 µL for a 384-well plate.

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and suspend the cell pellets in culture medium at 100,000-200,000 cells/well/90 µL for a 96-well poly-D lysine plate or 25,000-50,000 cells/well/20 µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to your experiment.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

2. Prepare MitoROS™ OH580 working solution:

2.1 Prepare MitoROS™ OH580 stock solution (500X): Add 50 µL of DMSO (Component C) into the vial of MitoROS™ OH580 (Component A), and mix them well.
Note: 25 µL of reconstituted MitoROS™ OH580 stock solution is enough for 1 plate. Unused portion can be aliquoted and stored at ≤ -20ºC for more than one month if the tubes are sealed tightly and kept from light. Avoid repeated freeze-thaw cycles.

2.2 Prepare MitoROS™ OH580 working solution: Add 25 µL of 500X DMSO reconstituted MitoROS™ OH580 stock solution (from Step 2.1) into 10 mL of Assay Buffer (Component B), and mix them well. This working solution is stable for at least 2 hours at room temperature.

3. Run hydroxyl radical assay:

3.1 Remove medium, and add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of MitoROS™ OH580 working solution (from Step 2.2) into the cell plate. Incubate cells at 37 ºC for 1 hour.

3.2 To induce hydroxyl radical, treat cells with text compounds in your desired buffer (such as PBS or HHBS) at 37 ºC for a desired period of time, protected from light.
Note 1: We treated HeLa cells with Fenton reaction (10 µM $CuCl_2$ and 100 µM H_2O_2) at 37ºC for 1 hour to induce exogenous hydroxyl radical. See Figure 1 for details.

Note 2: We treated RAW 264.7 cells with PMA (phorbol 12-myristate 13-acetate) in growth medium at 37ºC for 4 hours to stimulate endogenous hydroxyl radical. See Figure 2 for details.

3.3 Wash cells 2-3 times with HHBS or DPBS, and add 100 µL Assay Buffer (Component B) to each well.

3.4 Monitor the fluorescence signal in cells using fluorescence microscope with a TRITC filter set, or measure fluorescence increase using fluorescence microplate reader at Ex/Em = 540/590 nm (cut off = 570 nm) with bottom read mode.

Data Analysis

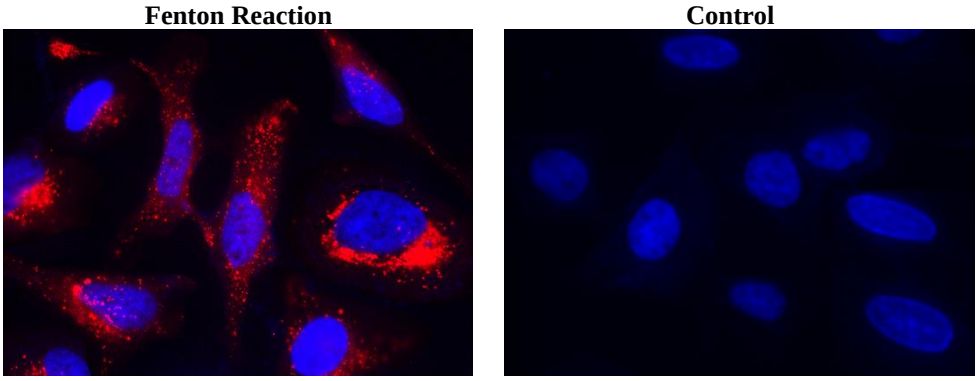

Figure 1. Fluorescence images of hydroxyl radical measurement in HeLa cells using MitoROS™ OH580 (Cat#16055). HeLa cells were incubated with MitoROS™ OH580 working solution at 37 ºC for 1 hour, then washed once with HHBS. Fenton Reaction: Cells were then treated with 10 µM $CuCl_2$ and 100 µM H_2O_2 in 1X HBSS buffer at 37 ºC for 1 hour. Control: HeLa cells were kept in 1X HBSS buffer without treatment. After washing 3 times with HHBS, HeLa cells were measured using a fluorescence microscope with a TRITC filter set (Red). Cell nuclei were stained with Hoechst 33342 (Cat#17530, Blue).

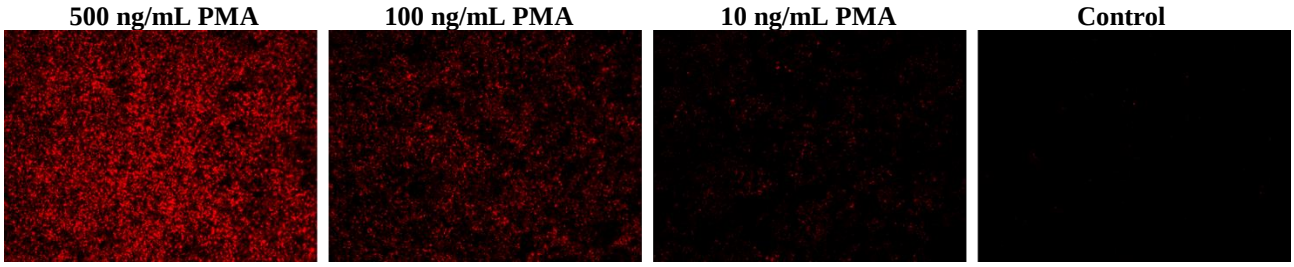

Figure 2. Detection of intracellular hydroxyl radical in RAW 264.7 cells using MitoROS™OH580 (Cat#16055). Cells were incubated with MitoROS™ OH580 working solution at 37 ºC for 1 hour, then washed once with HHBS. Cells were then incubated without or with PMA (phorbol 12-myristate 13-acetate, 10 to 500 ng/mL) in growth medium at 37ºC for 4 hours. After washing 3 times with HHBS, HeLa cells were measured using a fluorescence microscope with a TRITC filter set.

References

1. Livingstone DR. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar Pollut Bull. 2001; 42:656–66.
2. Cheng FC, Jen JF, Tsai TH. Hydroxyl radical in living systems and its separation methods. J Chromatogr B. 2002; 781:481–96.
3. Lipinski B. Hydroxyl radical and its scavengers in health and disease. Oxid Med Cell Longev. 2011; 1-9.