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

Catalog number: 16055
Unit size: 200 Tests

Component	Storage	Amount
Component A: MitoROS™ OH580	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (50 mL)
Component C: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

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 ('OH) 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 nano seconds 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 hydroxyl radical 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. 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.

AT A GLANCE

Protocol summary

1. Prepare cells
2. Incubate cells with MitoROS™ OH580 working solution at 37°C for 60 minutes
3. Incubate cells with test compounds (to induce OH^-)
4. Monitor the fluorescence increase at Ex/Em= 540/590 nm

Important Thaw all the components at room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Instrument specification(s):	Bottom read mode
Recommended plate:	Black wall/clear bottom

Instrument:	Fluorescence microscope
Excitation:	Cy3/TRITC filter set
Emission:	Cy3/TRITC filter set
Recommended plate:	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. 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 uL of stock solution is enough for 1 plate.

Note 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.

PREPARATION OF WORKING SOLUTION

Add 25 µL of 500X DMSO reconstituted MitoROS™ OH580 stock solution into 10 mL of Assay Buffer (Component B). Mix well.

Note This working solution is stable for at least 2 hours at room temperature.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Remove medium, and add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of MitoROS™ OH580 working solution into the cell plate. Incubate cells at 37°C for 60 minutes.

2. To induce hydroxyl radical, treat cells with test compounds in your desired buffer (such as PBS or HHBS) at 37°C for a desired period of time, protected from light.

   Note We treated HeLa cells with Fenton reaction (10 µM $CuCl_2$ and 100 µM H_2O_2) at 37°C for 1 hour to induce exogenous hydroxyl radical. See Figure 1 for details. We treated RAW 264.7 cells with PMA (phorbol 12-myristate 13-acetate) in growth medium at 37°C for 4 hours to stimulate endogenous hydroxyl radical.

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

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.

EXAMPLE DATA ANALYSIS AND FIGURES

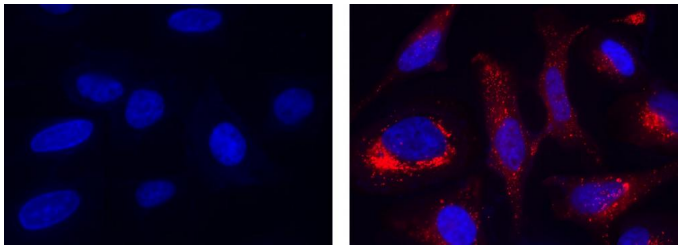

Figure 1. Fluorescence images of hydroxyl radical measurement in HeLa cells using MitoROS™ OH580 (Cat#16055). HeLa cells were incubated with MitoROS™ OH580 working solution at 37 °C for 1 hour, then washed once with HHBS. Fenton Reaction: Cells were then treated with 10 µM CuCl2 and 100 µM H_2O_2 in 1X HBSS buffer at 37 °C 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).

DISCLAIMER