MitoROS™ 580

Ordering Information	*Storage Conditions*
Product Number: 16052 (500 tests, 5X 100 tests/vial)	Keep at -20 °C and avoid exposure to light

Spectral Properties

Ex/Em = 510/580 nm

Biological Applications

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Examples include superoxide, hydroxyl radical, singlet oxygen and peroxides. ROS is highly reactive due to the presence of unpaired valence shell electrons. ROS forms as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, during times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically. This may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress. MitoROS™ 580 is a superoxide-sensitive dye that is localized in mitochondria upon loading into live cells. Oxidation of MitoROS™ 580 by superoxide generates red fluorescence. MitoROS™ 580 can be used for monitoring superoxide in mitochondria either with a fluorescence microscope or a fluorescence flow cytometer. MitoROS™ 580 reagent permeates live cells where it selectively targets mitochondria. It is rapidly oxidized by superoxide. It is less likely to be oxidized by other reactive oxygen species (ROS) and reactive nitrogen species (RNS). The oxidized product is highly fluorescent in cells. MitoROS™ 580 provides a valuable tool for investigating oxidative stress in various pathologies.

Assay Protocol with MitoROS™ 580

This protocol only provides a guideline, and should be modified according to your specific needs. Treat cells as desired before making the MitoROS™ 580 working solution.

1) **Prepare 1000X MitoROS™ 580 DMSO stock solution** by dissolve the contents of one vial of MitoROS™ 580 mitochondrial superoxide indicator in 13 µL of DMSO.
2) **Prepare 2X MitoROS™ 580 working solution** by diluting the DMSO stock solution into Hanks solution with 20 mM Hepes buffer (HHBS).
   Note: The 2X MitoROS™ 580 working solution is not stable, use it promptly.
3) Treat cells as desired.
4) Incubate the cells (such as 100 µL/well in 96-well plate) with equal volume of 2X MitoROS™ 580 working solution for 10-30 minutes at 37 °C, protected from light.
   Note 1: The final in cell concentration of the MitoROS™ 580 should not exceed 1 X. Concentrations exceeding 1 X can produce cytotoxic effects, including altered mitochondrial morphology and redistribution of fluorescence to nuclei and the cytosol.
   Note: Different cells react to MitoROS™ 580 differently, adjust the working concentration accordingly.
5) Wash cells gently three times and replace it with HHBS buffer.
6) Analyze the cells with a proper fluorescence instrument (e.g., a fluorescence microscope, flow cytometer) with Ex/Em = 510/580 nm.

References

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2. Yuanbin Liu, Gary Fiskum and David Schubert (2002). Generation of reactive oxygen species by the mitochondrial electron transport chain. J Neurochem 80, 780.
3. David X Zhang, Ai-Ping Zou, Pin-Lan Li. (2003). Hossain MZ, Kleve MG. (2011) Ceramide-induced activation of NADPH oxidase and endothelial dysfunction in small coronary arteries. Am J Physiol Heart Circ Physiol 284, H605.
4. Fabienne de Bilbao, Denis Arsenijevic, Philippe Vallet, Ole Petter Hjelle, Ole Petter Ottersen, Constantin Bouras, Yvette Raffin, Karin Abou, Wolfgang Langhans, Sheila Collins, Julie Plamondon, Marie-Clotilde Alves-Guerra, Anne Haguenauer, Irene Garcia, Denis Richard, Daniel Ricquier and Panteleimon Giannakopoulos (2004). Resistance to cerebral ischemic injury in UCP2 knockout mice: evidence for a role of UCP2 as a regulator of mitochondrial glutathione levels. J Neurochem 89, 1283

Disclaimer: *This product is for research use only and is not intended for therapeutic or diagnostic applications.*

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