ROS Brite™ Dyes

Introduction

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen (such as 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. ROS are also generated by exogenous sources such as ionizing radiation. Under conditions of oxidative stress, ROS production is dramatically increased, resulting in subsequent alteration of membrane lipids, proteins, and nucleic acids. Oxidative damage of these biomolecules is associated with aging as well as with a variety of pathological events, including atherosclerosis, carcinogenesis, ischemic reperfusion injury, and neurodegenerative disorders. ROS Brite™ reagents are a series new fluorogenic probes to measure oxidative stress in cells. The cell-permeant ROS Brite™ reagents are nonfluorescent and produce bright fluorescence upon ROS oxidation. The resulting fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. ROS Brite™ 570, 670 and 700 reagents have good selectivity to both hydroxyl radical and supoeroxide. ROS Brite™ 700 is optimized for *in vivo* imaging.

Chemical and Physical Properties

Catalog Number	ROS Brite™ Dyes	Molecular Weight	Solvent	Excitation	Emission
16000	ROS Brite™ 570	732.81	DMSO	556 nm	566 nm
16002	ROS Brite™ 670	758.85	DMSO	650 nm	666 nm
16004	ROS Brite™ 700	1295.14	DMSO	680 nm	706 nm
16053	ROS Brite™ DHCF	701.50	DMSO	560 nm	579 nm

Assay Protocol with ROS Brite™ Dyes

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

1) Prepare a 10 to 20 mM ROS Brite™ stock solution in DMSO. Make 5 to 10 µM working solution by diluting the DMSO stock solution into Hanks solution with 20 mM Hepes buffer (HHBS).
2) Treat cells as desired (e.g., RASM cells are treated with 50-100 nM angiotensin II for 3-5 hours).
3) Incubate the cells with ROS Brite™ (5-10 µM, from Step #1) for 15 -30 minutes at 37 ºC.
   Note: The concentration of ROS Brite™ used varies with different cell lines, one will need test with different concentrations to get the optimal dose. For cat#16053, one might use less concentration such as 0.5-5 uM.
4) Replace the dye-loading solution with HHBS buffer.
5) Analyze the cells with a proper fluorescence instrument (e.g., a fluorescence microscope, flow cytometer or an *in vivo* imaging instrument).

References

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