ROS Brite™ APF and HPF

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. The cell-permeant ROS Brite™ APF and HPF reagents are nonfluorescent and produce bright green fluorescence (excitation/emission = 490/515 nm) upon reaction with hydroxyl radical or peroxynitrite anion, APF will also react with the hypochlorite anion (OCl^-). In addition, both APF and HPF also react with hydrogen peroxide in the presence of peroxidase. The resulting fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. APF and HPF show relatively high resistance to light-induced oxidation. APF and HPF are nonfluorescent until they react with the hydroxyl radical or peroxynitrite anion.

Chemical and Physical Properties

Catalog Number	ROS Brite™ Dyes	Molecular Weight	Solvent	Excitation	Emission
16050	ROS Brite™ APF	423.42	DMSO	490 nm	515 nm
16051	ROS Brite™ HPF	424.40	DMSO	490 nm	515 nm

Assay Protocol with ROS Brite™ Dyes

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

1) Prepare a 10 to 20 mM ROS Brite™ APF (or HPF) stock solution in DMSO. Make 1 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™ APF (or HPF) (1-10 µM, from Step #1) for 20 -60 minutes at 37 ºC.
4) Replace the dye-loading solution with HHBS buffer.
5) Analyze the cells with a proper fluorescence instrument at Ex/Em = 490/525 mm (cut off = 515 nM) with bottom read mode (e.g., FITC filter set for a fluorescence microscope, FL1 filter for a flow cytometer).
   Note: BSA and phenol red can affect the fluorescence and should be used with caution. Both APF and HPF can be used in solution assays or for intracellular measurements.

References

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3. Park WH, Kim SH. (2012) MG132, a proteasome inhibitor, induces human pulmonary fibroblast cell death via increasing ROS levels and GSH depletion. Oncol Rep, 27, 1284.
4. Wu YT, Lin CY, Tsai MY, Chen YH, Lu YF, Huang CJ, Cheng CM, Hwang SP. (2011) beta-Lapachone induces heart morphogenetic and functional defects by promoting the death of erythrocytes and the endocardium in zebrafish embryos. J Biomed Sci, 18, 70.
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