ROS Brite™ APF *Optimized for Detecting Reactive Oxygen Species (ROS)*

Catalog number: 16050
Unit size: 1 mg

Component	Storage	Amount
ROS Brite™ APF *Optimized for Detecting Reactive Oxygen Species (ROS)*	Freeze (<-15 °C), Minimize light exposure	1 mg

OVERVIEW

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. 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™ APF is a fluorogenic probe to measure hydroxyly radical in cells using conventional fluorescence microscopy, high-content imaging, microplate fluorometry, or flow cytometry. The cell-permeant ROS Brite™ APF reagent is nonfluorescent and produces bright green fluorescence upon reaction with hydroxyl radical. The resulting fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. In the presence of peroxidase, APF also reacts with hydrogen peroxide. APF has good selectivity to hydroxyl radical compared to other ROS. 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. APF will also react with the hypochlorite anion.

AT A GLANCE

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

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	FITC filter set
Emission:	FITC filter set
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	FITC filter set

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. ROS Brite™ APF stock solution:
Prepare a 10 to 20 mM ROS Brite™ APF stock solution in DMSO.

Note The stock solution can be stored at -20° C in single use aliquotes. Protect from light.

PREPARATION OF WORKING SOLUTION

ROS Brite™ APF working Solution:
Make 1 to 10 µM working solution by diluting the DMSO stock solution into Hanks solution with 20 mM Hepes buffer (HHBS).

Note The working solution should be made fresh before use.

SAMPLE EXPERIMENTAL PROTOCOL

1. Incubate the cells with ROS Brite™ APF (1-10 µM) for 20 - 60 minutes at 37°C.
2. Replace the dye-loading solution with HHBS buffer.
3. 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. APF can be used in solution assays or for intracellular measurements.

EXAMPLE DATA ANALYSIS AND FIGURES

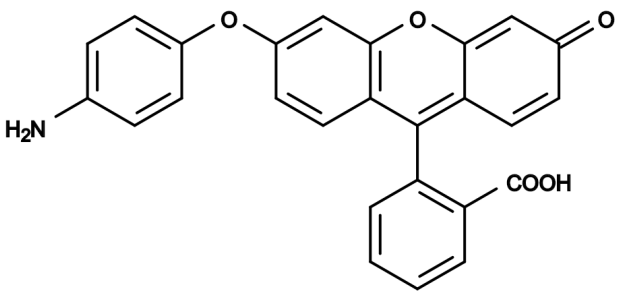

Figure 1. Chemical structure for ROS Brite™ APF *Optimized for Detecting Reactive Oxygen Species (ROS)*

DISCLAIMER