SO Green™ 520AM Singlet Oxygen Sensor *Cell-Permeable*

Catalog number: 16062
Unit size: 10x100 ug

Component	Storage	Amount (Cat No. 16062)
SO Green™ 520AM Singlet Oxygen Sensor *Cell-Permeable*	Freeze (< -15 °C), Minimize light exposure	10x100 ug

OVERVIEW

SO Green™ 520AM Singlet Oxygen Sensor has been developed for detecting intracellular singlet oxygen, It is cell-permeant. It is highly selective for singlet oxygen. Unlike other available fluorescent and chemiluminescent singlet oxygen detection reagents, SO Green™ 520AM Singlet Oxygen Sensor does not show any appreciable response to hydroxyl radical, superoxide, or other reactive oxygen species (ROS). This indicator only exhibits weak blue fluorescence. It emits a strong green fluorescence (excitation/emission maxima 504/525 nm) upon reaction with singlet oxygen. The PET quencher of SO Green™ 520AM Singlet Oxygen Sensor is eliminated (by singlet oxygen reaction) to recover its fluorescence. Singlet oxygen can be produced from many different sources such as dye photosensitizations. In mammalian biology, singlet oxygen is one of the ROS, which is linked to oxidation of LDL cholesterol and resultant cardiovascular effects. Polyphenol antioxidants can scavenge and reduce concentrations of reactive oxygen species and may prevent such deleterious oxidative effects. Ingestion of pigments capable of producing singlet oxygen with activation by light can produce severe photosensitivity of skin. This is especially a concern in herbivorous animals. Singlet oxygen is the active species in photodynamic therapy.

AT A GLANCE

1. Plate cells in growth medium
2. Add SO Green™ 520AM Singlet Oxygen Sensor working solution
3. Incubate at 37 °C for 30 to 60 minutes
4. Wash cells and add Singlet Oxygen inducer such as through photodynamic therapy (PDT, for example methylene blue), activated by specific light wavelengths (UVA/visible) or via chemical reactions involving inflammatory responses
5. Image under FITC filter set

Note: Thaw the dye at room temperature before opening.

KEY PARAMETERS

Fluorescence microscope

Emission	FITC filter
Excitation	FITC filter
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

Prepare a 2 to 5 mM stock solution of SO Green™ 520AM Singlet Oxygen Sensor in high-quality anhydrous DMSO. E.g. to make 5 mM stock solution, add 26.4 µL of DMSO to the vial and mix well.

PREPARATION OF WORKING SOLUTION

Prepare a 2 to 20 µM SO Green™ 520AM Singlet Oxygen Sensor working solution in HHBS (AAT cat#20011) or buffer of your choice with 0.04% Pluronic® F-127. For most cell lines, SO Green™ 520AM Singlet Oxygen Sensor at a final concentration of 10 µM is recommended. The exact concentration of indicators required for cell loading must be determined empirically.

Notes:

1. The nonionic detergent Pluronic® F-127 is sometimes used to increase the aqueous solubility of SO Green™ 520AM Singlet Oxygen Sensor. A variety of Pluronic® F-127 solutions can be purchased from AAT Bioquest.
2. If your cells contain organic anion-transporters, probenecid (1-2 mM) may be added to the dye working solution (final in well concentration will be 0.5-1 mM) to reduce leakage of the de-esterified indicators. A variety of ReadiUse™ Probenecid products, including water-soluble, sodium salt, and stabilized solutions, can be purchased from AAT Bioquest.
3. For best results, this solution should be used within a few hours of its preparation.

SAMPLE EXPERIMENTAL PROTOCOL

Following is our recommended protocol for loading AM esters into live cells. This protocol only provides a guideline and should be modified according to your specific needs.

1. Prepare cells in growth medium overnight.
2. On the next day, remove the medium and add 100 µL of SO Green™ 520AM Singlet Oxygen Sensor working solution.
3. Incubate the dye-loaded plate in a cell incubator at 37 °C for 30 to 60 minutes.
   Note: Incubating the dye for longer than 2 hours can improve signal intensities in certain cell lines.
4. Remove the dye working solution and wash cells with HHBS or buffer of your choice to remove any excess probes.
5. Add Methylene Blue containing probenecid.
6. Expose the cells to flash Cy5 light or expose longer for 10 minutes.
7. Image cells with fluorescence microscope using FITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

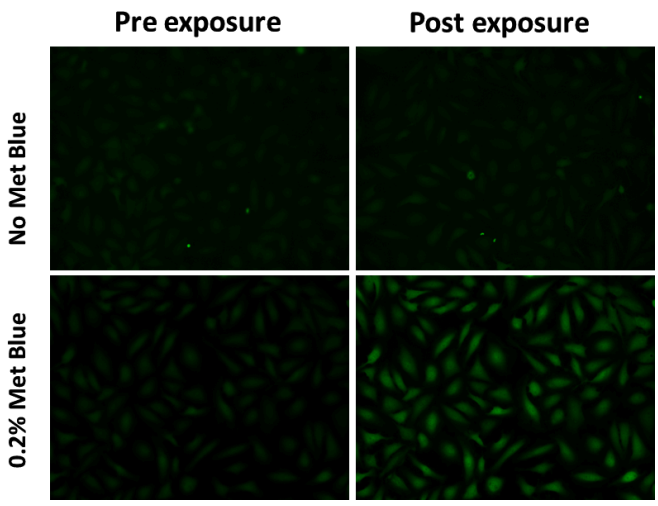

Figure 1. Fluorescence response of SO Green™ 520 AM (20 µM) with and without Methylene Blue, before and after a 10-second exposure to a 650 nm laser.

DISCLAIMER

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