ROS-ID® Total ROS/Superoxide detection kit

Widely cited kit to detect total ROS and Superoxide in live cells by microscopy and flow cytometry applications

Enzo Life Sciences ROS-ID® Total ROS/Superoxide detection kit includes dyes to measure Reactive Oxygen Species (ROS) and Superoxide production in live cells. The non-fluorescent, cell-permeable Oxidative Stress Detection Reagent (Green, Ex/Em 490/525 nm) reacts directly with a wide range of reactive species (hydrogen peroxide, peroxynitrite and hydroxyl radicals) yielding a green fluorescent product. The Superoxide Detection Reagent (Orange, Ex/Em 550/620 nm) is a cell-permeable probe that reacts specifically with superoxide, generating an orange fluorescent product. Pyocyanin (ROS inducer) and NAC (ROS inhibitor) are also provided with the kit.

Citations: 133 View Online »

Ordering Information Order Online »

ENZ-51010	1Kit

Manuals, SDS & CofA View Online »

- Specifically designed to measure Superoxide production in live cells
- Compatible with major components of tissue culture media (phenol red, FBS and BSA)
- Validated on microscopy, flow cytometry, and microplates

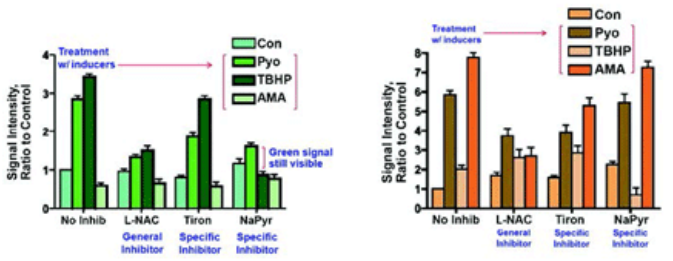

Figure 6. ROS-ID® Total ROS/Superoxide detection Kit and a set of ROS scavengers/inhibitors were used to profile ROS production in HeLa cells treated with antimycin A, (AMA, specific superoxide inducer), t-butyl-peroxide (TBHP, specific peroxide inducer), and pyocyanin (general ROS inducer). Similar results were obtained using U-2 OS and CHO K1 cells.

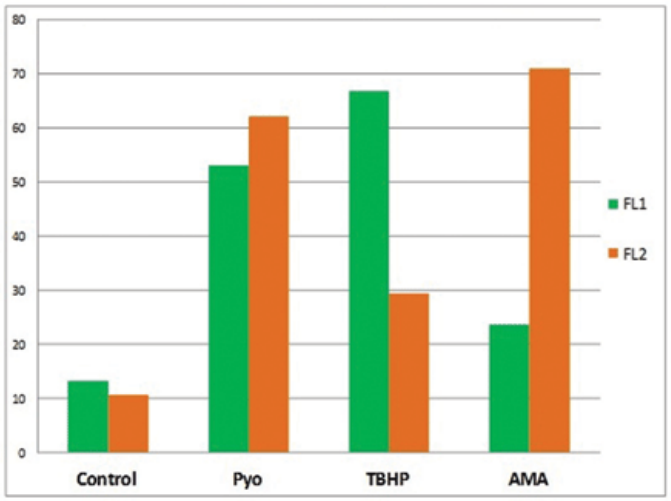

Figure 4. Profiling of ROS formation by flow cytometry in Hela cells. Data represents % positive following treatment with Pyocyanin (ROS/SO inducer), TBHP (ROS inducer), and AMA (superoxide inducer). Green columns indicate % positive for ROS and orange columns represent % positive for superoxide.

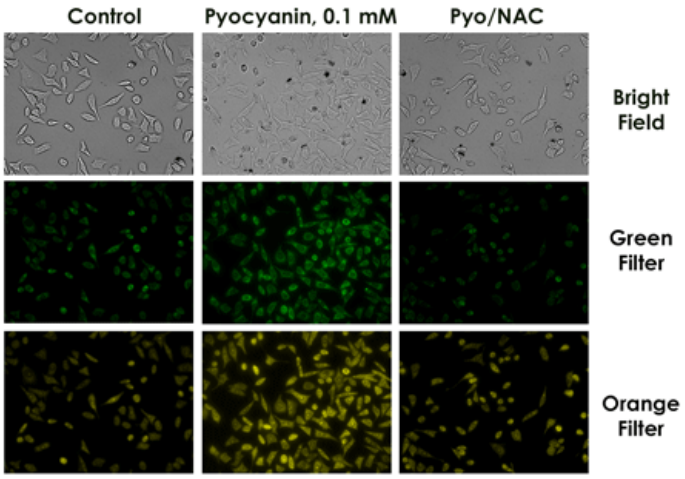

Figure 1. Profiling of reactive oxygen species formation by fluorescence microscopy was achieved in HeLa cells loaded with ROS/Superoxide detection reagents and treated with pyocyanin. General oxidative stress levels were monitored in the green channel, while superoxide production was detected in the orange channel. Pretreatment with NAC, a general ROS inhibitor, prevents formation of ROS.

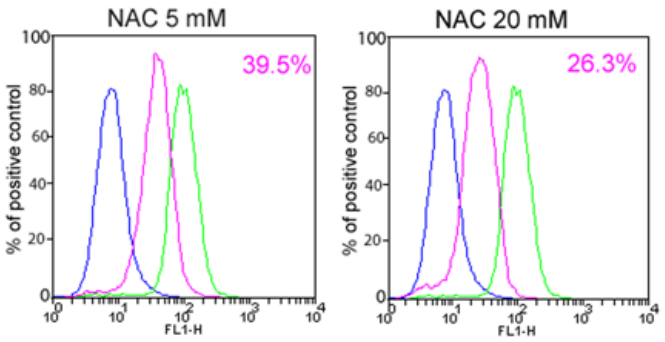

Figure 3. Flow cytometry histograms of ROS inhibition by N-acetyl-L-cysteine (NAC, ROS inhibitor). Jurkat cells were pretreated with 5 mM (pink, left) or 10 mM (pink, right), or without NAC (green, positive control) for one hour. Cells were then incubated with 100 µM pyocyanin (ROS inducer) and stained with 1 µM oxidative stress detection reagent. Untreated cells (blue) were used as a negative control. The numbers in the upper right corners indicate the percentage of median fluorescence intensity of NAC-treated cells as compared with positive control cells.

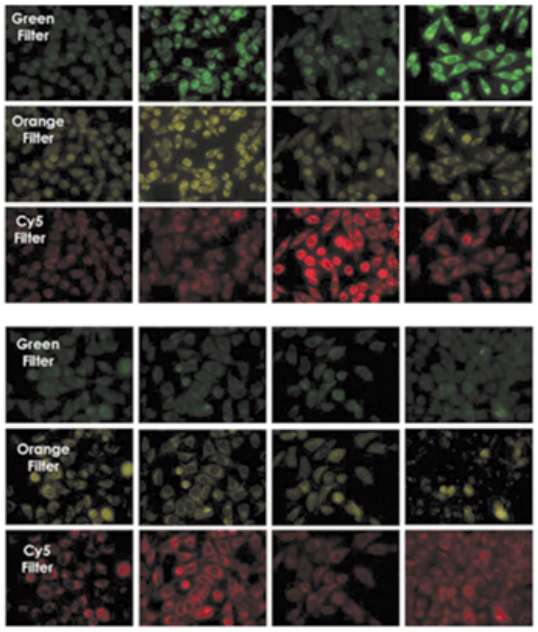

Figure 5. (Top) HeLa Cells Loaded with ROS/RNS 3-Plex Detection Reagent for 2 h, 37°C and Induced for 20 min, 37°C.
Column 1: Control
Column 2: Pyocyanin, 0.1 mM
Column 3: L-Arginine, 1mM
Column 4: Pyo/Arg

(Bottom) Pretreatment with 5 mM NAC
Column 1: Control
Column 2: L-Arginine, 1mM
Column 3: Pyocyanin, 0.1 mM
Column 4: Pyo/Arg

Specific profiling of reactive oxygen/nitrogen species formation by fluorescence microscopy in HeLa cells loaded with dyes from ROS-ID® ROS/RNS Detection Kit (ENZ-51001). (Top) – Combined treatment of Pyocyanin and L-Arg generates peroxynitrite (green fluorescence) due to the reaction of NO with superoxide, bur little NO (red fluorescence). (Bottom) – Pretreatment with NAC inhibits peroxynitrite and superoxide formation, but not NO.

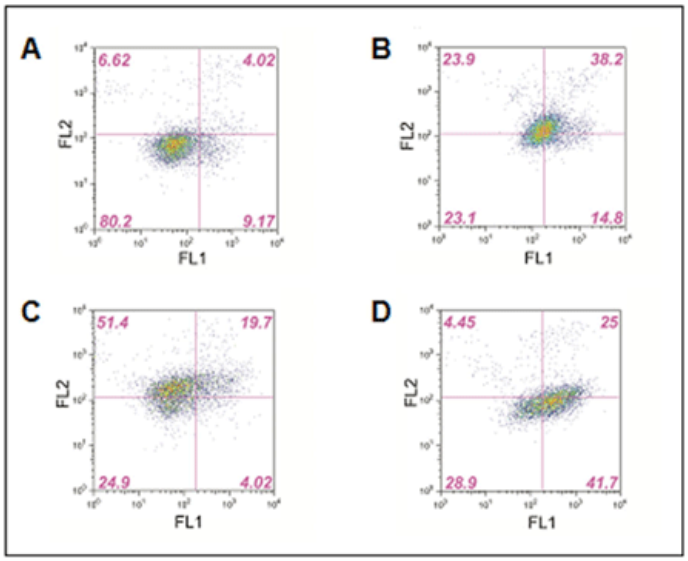

Figure 2. Jurkat cells were induced with 100 &microM pyocyanin (general ROS inducer, panel B), 200 &microM antimycin A (superoxide inducer, panel C) or 1 &microM of t-butyl-hydroperoxide (peroxide inducer, panel D), stained with two color ROS Detection Kit and analyzed using flow cytometry. Untreated cells (panel A) were used as a control. Cell debris were ungated and compensation was performed using single stained pyocyanin-treated samples. Red numbers reflect the percentage of the cells in each quadrant.


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Wash Buffer Salts
CONTENTS: Phosphate buffered saline for in situ applications using a peroxidase detection system.
Dissolve entire contents of packet in 1 liter of distilled or deionized water.
Store reconstituted buffer at 2-8°C.
LOT 24KJN0

Handling & Storage

Use/Stability	With proper storage, the kit components are stable up to the date noted on the product label. Store kit at -20°C in a non-frost free freezer, or -80°C for longer term storage.
Handling	Protect from light. Avoid freeze/thaw cycles.
Short Term Storage	-20°C
Long Term Storage	-80°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	Reactive oxygen species / Superoxide
Application	Flow Cytometry, Fluorescence microscopy, Fluorescent detection, HTS
Application Notes	This kit is designed to directly monitor real time reactive oxygen and/or nitrogen species (ROS/RNS) production in live cells using fluorescence microscopy and/or flow cytometry.
Contents	Oxidative Stress Detection Reagent (Green), 300 nmoles Superoxide Detection Reagent (Orange), 300 nmoles ROS Inducer (Pyocyanin), 1 µmole ROS Inhibitor (N-acetyl-L-cysteine), 2 x 10 mg Wash Buffer Salts, 1 pack
Quality Control	A sample from each lot of ROS-ID® Total ROS/Superoxide detection kit is used to stain HeLa cells using the procedures described in the user manual. The stained cells are analyzed using a wide-field fluorescence microscope equipped with standard green (490/525 nm) and orange (550/620 nm) filter set. The following results are obtained: ROS positive control samples induced with Pyocyanin exhibit bright orange signal in nucleus as well as bright green fluorescence in the cytoplasm. Cells pretreated with the ROS inhibitor don’t demonstrate any green or orange fluorescence signal upon induction.
Quantity	200 fluorescence microscopy assays or 50 flow cytometry assays or 2×96 microplate assays.

Technical Info / Product Notes

The ROS-ID® Total ROS/Superoxide detection kit is a member of the CELLESTIAL® product line, reagents and assay kits comprising fluorescent molecular probes that have been extensively benchmarked for live cell analysis applications. CELLELSTIAL® reagents and kits are optimal for use in demanding imaging applications, such as confocal microscopy, flow cytometry and HCS, where consistency and reproducibility are required.

Toxicology Application Note:

Use of 3D Cultured Human iPSC-Derived Hepatocytes for Long-Term Hepatotoxicity Studies

Screening Reactive Oxygen Species (ROS) on IQUE® Screener

Cited samples:

For an overview on cited samples please click here.

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