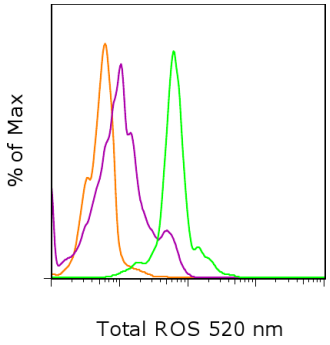
Total Reactive Oxygen Species (ROS) Assay Kit 520 nm

Catalog Number: 88-5930

RUO: For Research Use Only. Not for use in diagnostic procedures.



Staining of total reactive oxygen species (ROS) in unstimulated (orange histogram), 60-minute LPS-activated (purple histogram), or hydorgen peroxide-treated (green histogram) mouse peritoneal exudate cells using the Total Reactive Oxygen Species (ROS) Assay Kit 520 nm. The assay was analyzed off the 488 nm (blue laser) in the FITC channel.

Product Information

Contents: Total Reactive Oxygen Species (ROS) Assay Kit 520 nm

REF **Catalog Number:** 88-5930

Temperature Limitation: Store at less than or equal to -20°C.

LOT **Batch Code:** Refer to vial

Use By: Refer to vial

Description

The Total Reactive Oxygen Species (ROS) Assay Kit 520 nm contains the necessary regent and buffer for identifying ROS in cells by flow cytometry in the FITC channel. Reactive oxygen species are chemically reactive oxygen-containing molecules that are generated as a natural byproduct of the oxygen metabolism. ROS are constantly generated and eliminated under normal physiologic conditions and have important functions in cell signaling, homeostasis, and clearance of microbial infections. During times of environmental stress such as exposure to UV or heat, or during infection, ROS levels can increase dramatically leading to oxidative stress. Oxidative stress can result in damage to cellular proteins, lipids, and DNA, and has been linked to cardiovascular disease, cancer, diabetes, inflammation, and aging. Examples of ROS include superoxide ions and peroxides.

Components

ROS Assay Stain Concentrate (lyophilzed powder): 1 vial, store at less than or equal to -20°C.
ROS Assay Buffer: 20 mL, store at less than or equal to -20°C.

Applications Reported

The Total Reactive Oxygen Species (ROS) Assay Kit 520 nm has been reported for use in flow cytometric analysis and in plate-based assays using a fluorescent microplate reader.

Applications Tested

The Total Reactive Oxygen Species (ROS) Assay Kit 520 nm has been tested by flow cytometric analysis of stimulated and unstimulated mouse peritoneal exudate cells using the attached protocol. Cell number should be determined empirically but can range from 10^5 to 10^8 cells/test.

References

Freemerman AJ, Johnson AR, Sacks GN, Milner JJ, Kirk EL, Troester MA, Macintyre AN, Goraksha-Hicks P, Rathmell JC, Makowski L. Metabolic reprogramming of macrophages: glucose transporter (GLUT1)-mediated glucosemetabolism drives a pro- inflammatory phenotype. J Biol Chem. 2014 Feb 3.

Kramer PA, Ravi S, Chacko B, Johnson MS, Darley-Usmar VM. A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: Implications for their use as bioenergetic biomarkers. Redox Biol. 2014 Jan 10;2:206-210.

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Miletic AV, Graham DB, Montgrain V, Fujikawa K, Kloeppel T, Brim K, Weaver B, Schreiber R, Xavier R, Swat W. Vav proteins control MyD88-dependent oxidative burst. Blood. 2007 Apr 15;109(8):3360-8.

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Total Reactive Oxygen Species (ROS) Assay Kit

Protocol

Materials Provided

- Please refer to the components section of the datasheet.

Other Materials Needed

- 12x75 mm round bottom test tubes
- Refrigerator and frost-free ≤-20°C freezer
- Dimethyl sulfoxide (DMSO) (freshly opened)

Time Requirements

- Thawing of components to room temperature, 30-60 minutes
- Reconstitution of ROS Assay Stain Concentrate with DMSO, 5 minutes
- Incubation of cells with ROS Assay Stain, 60 minutes
- Cell stimulation, variable depending on cell type and treatment

Experimental Procedure

1. Make a 500X stock solution of the ROS Assay Stain by adding 40 µL of DMSO into the vial of ROS Assay Stain Concentrate and mix well.

Note: Unused ROS Assay Stain can be aliquoted and stored at ≤-20°C, protected from light.

2. The 500X ROS Assay Stain stock solution should be used at 1X to label cells. It may be added directly to cells in culture media at a final concentration of 1X by adding 2 µL of the 500X ROS Assay Stain stock solution for every 1 mL of cells; mix well. Alternatively, the 500X ROS Assay Stain stock solution may be diluted to 1X using the ROS Assay Buffer. For each sample, you will need 100 µL of ROS Assay Stain Solution. For example, for 10 experimental samples, you will need 1 mL of 1X ROS Assay Stain, therefore, add 2 µL of 500X ROS Assay Stain to 1 mL of ROS Assay Buffer. Then use 100 µL of 1X ROS Assay Stain to resuspend the cells.

Note: Cells can be in media or in PBS.

3. Incubate for 60 minutes in a 37°C incubator with 5% CO_2.
4. Treat cells with the desired reagents to induce production of ROS.
5. Analyze on a flow cytometer at the desired time points.

Note: It is not necessary to wash cells before analysis.

Note: Cells can be fixed with IC Fixation Buffer (cat. 00-8222) after staining and stored at 2-8°C, and protected from light until ready to analyze on a flow cytometer. Some loss of signal will occur following fixation. Signal is not retained following permeabilization of cells.

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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