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Product Information

Fluorometric Intracellular ROS Kit
Deep Red Fluorescence

Catalog Number **MAK142**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Reactive oxygen species (ROS) are generated as a result of the reduction of oxygen during aerobic respiration and by various enzymatic systems within the cell. At physiological levels, ROS contribute to cell signaling and host defense. Increased ROS generation, above the detoxification capacity of the biological system, results in oxidative stress and cellular damage. The main damage to cells results from the ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential proteins, and DNA. ROS has been implicated in disease states, such as Alzheimer's disease, Parkinson's disease, cancer, and aging.

The Fluorometric Intracellular ROS Kit provides a sensitive, one-step fluorometric assay to detect intracellular ROS (especially superoxide and hydroxyl radicals) in live cells after a 1 hour incubation. ROS react with a fluorogenic sensor localized to the cytoplasm, resulting in a fluorometric product proportional to the amount of ROS present The assay can be performed in either a 96 or 384 multiwell plate format and with detection at $\lambda_{ex} = 640/\lambda_{em} = 675$ nm using either a fluorescence microplate reader, flow cytometer, or a fluorescent microscope with Cy®5 filter.

Components

The kit is sufficient for 200 assays in 96 well plates.

ROS Detection Reagent, Deep Red — 1 vl
Catalog Number MAK142A

Assay Buffer — 20 mL
Catalog Number MAK142B

DMSO — 0.1 mL
Catalog Number MAK142C

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorometric assays in multiwall plate readers.
- Fluorescence multiwell plate reader, Microscope, or Flow Cytometer.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped under ambient conditions and storage at –20 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

Cell Preparation for Multiwell Plate Reader

Adherent cells: Plate cells overnight in growth medium at 10,000–40,000 cells/well/90 μL for a 96 well plate or 2,500–10,000 cells/well/20 μL for a 384 well plate.

Non-adherent cells: Centrifuge the cells from the culture medium and suspend the cell pellets in culture medium at 50,000–100,000 cells/well/90 μL for a 96 well poly-D lysine plate or 10,000–25,000 cells/well/20 μL for a 384 well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiment.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

Procedure for Assay with Multiwell Plate Reader

Note: Allow all reagents to come to room temperature before starting experiment.

1. Reconstitute ROS Detection Reagent with 40 µL of DMSO to generate the 500× ROS Detection Reagent Stock Solution. Mix well by pipetting. Remaining stock solution can be aliquoted and stored at –20 °C protected from light and moisture. Stable for 1 month when stored at –20 °C.

2. Treat cells with 10 µL of 10× test compound solution (96 well plate) or 5 µL of 5× test compound solution (384 well plate) in suitable buffer (such as PBS or HHBS). For control wells (untreated cells), add the corresponding amount of buffer.

3. To induce ROS, incubate the cell plate at room temperature or in a 5% CO_2, 37 °C incubator for a desired period of time [for example, 30 minutes treatment for Hela cells with 100 µM *tert*-butyl hydroperoxide (TBHP)].

4. Set up the Master Reaction Mix according to the scheme in Table 1.

Table 1.
Master Reaction Mix

Reagent	Volume
500× ROS Detection Reagent Stock Solution	20 µL
Assay Buffer	10 mL

Note: The Master Reaction Mix is enough for one plate. The Master Reaction Mix is best used within 2 hours.

5. Add 100 µL/well (96 well plate) or 25 µL/well (384 well plate) of Master Reaction Mix into the cell plate. Incubate the cells in a 5% CO2, 37 °C incubator for 30 minutes to one hour.

6. Measure the fluorescence intensity (λ_{ex} = 640/ λ_{em} = 675 nm) or take images with Cy5 filter set.

Cell Preparation for Flow Cytometry Analysis

Prepare cells at a density from 5×10^5 to 1×10^6 cells/mL.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

Procedure for Assay with Flow Cytometry

1. Reconstitute ROS Detection Reagent with 40 µL of DMSO to generate the 500× ROS Detection Reagent Stock Solution. Mix well by pipetting. Remaining stock solution can be aliquoted and stored at –20 °C protected from light and moisture. Stable for 1 month when stored at –20 °C.

2. Treat cells with test compounds in suitable buffer (such as PBS or HHBS). For control wells (untreated cells), add the corresponding amount of buffer.

3. To induce ROS, incubate the cell plate at room temperature or in a 5% CO_2, 37 °C incubator for a desired period of time [for example: 30 minutes treatment for Hela cells with 100 µM *tert*-butyl hydroperoxide (TBHP)].

4. Add 1 µL of 500× ROS Detection Reagent Stock Solution per mL of cells. Incubate the cells in a 5% CO_2, 37 °C incubator for 30 minutes to one hour.

5. Measure the fluorescence intensity on a flow cytometer.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorometric assays, use black plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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