3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Fluorometric Intracellular ROS Kit
Red Fluorescence

Catalog Number **MAK145**
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 Assay Kit provides a sensitive, one-step fluorometric assay to detect intracellular ROS (especially superoxide and hydroxyl radicals) in live cells within 1 hour incubation. ROS react with a cell-permeable sensor, resulting in a fluorometric product (λ_{ex} = 520/λ_{em} = 605 nm) proportional to the amount of ROS present The assay can be performed in either a 96 or 384 multiwell plate format using a fluorescence microplate reader.

Components

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

ROS Detection Reagent, Red Catalog Number MAK145A	1 vl
Assay Buffer Catalog Number MAK145B	20 mL
DMSO Catalog Number MAK145C	0.2 mL

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.
- Fluorescence multiwell plate reader

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.

Preparation Instructions

Allow reagents to come to room temperature before use. Briefly centrifuge vials before opening.

ROS Detection Reagent – Reconstitute with 40 µL of DMSO to generate the ROS Detection Reagent Stock Solution. Mix well by pipetting, aliquot, and store at –20 °C any remaining Stock Solution. Stable for 1 month when stored at –20 °C.

Procedure
All samples and standards should be run in duplicate.

Sample Preparation
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.

Assay Reaction for One 96 well Plate
1. Set up the Master Reaction Mix according to the scheme in Table 1.

Table 1.
Master Reaction Mix

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

Note: The Master Reaction Mix is enough for one plate. If necessary, the Master Reaction Mix can be scaled down. The Master Reaction Mix is best used within 2 hours.

2. 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% CO_2, 37 °C incubator for one hour.

3. Treat cells with 20 µL/well of 11× test compounds (96 well plate) or 10 µL/well of 6× test compounds (384 well plate) in suitable buffer (such as PBS or HHBS). For control wells (untreated cells), add the corresponding amount of compound buffer.

4. 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.

5. Measure the fluorescence intensity at λ_{ex} = 520/ λ_{em} = 605 nm.

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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