

ROS Assay Kit (Red Fluorescence)

Cat. No.: R1520936 | Pack size: 200T | Storage: -20 °C, protected from light

Overview

Reactive oxygen species (ROS) include superoxide anion, hydrogen peroxide, and their downstream products such as peroxides and hydroxyl derivatives. ROS are involved in cell growth and proliferation, development and differentiation, senescence and apoptosis, as well as many physiological and pathological processes. The ROS Assay Kit is designed for ROS detection using a fluorescent probe. This kit employs a red fluorescent probe to detect ROS in various animal and plant samples. The maximum excitation/emission wavelengths of the red fluorescent ROS probe are 510/610 nm.

Intracellular ROS levels can be determined by detecting the fluorescence intensity. ROS content and changes in living cells can be evaluated based on fluorescence generation, which can be directly observed by flow cytometry or fluorescence microscopy. This is a classic method for ROS detection in tissues or living cells. The kit provides Rosup, a positive control reagent for ROS detection. Rosup is a mixture at a concentration of 50 mg/mL. As a positive inducer of ROS, it allows accurate analysis of actual ROS levels according to fluorescence signal intensity.

This kit is only suitable for ROS detection in cells and fresh tissue samples, and cannot be used for frozen tissue samples. It features low background, high sensitivity, wide linear range and ease of use.

Kit Contents & Storage

Item	Value
Storage Conditions	Protected from light; store at -20 °C
Shipped In	Ice chest + Ice pads
Stability and Storage	Each component has a shelf life of 1 year under corresponding storage conditions. R1520936A/B: Store at -20 °C long term (12 months). Store in the dark.

Item No.	Appearance	Component	200T	Storage
R1520936A	Liquid	Buffer A	200 mL	-20 °C

Item No.	Appearance	Component	200T	Storage
R1520936B	Liquid	Red Fluorescent Dye (5 mM)	2×0.2 mL	-20 °C. Store in the dark.
R1520936C	Liquid	Rosup positive control	2×1 mL	-20 °C

Precautions

1. After probe loading, residual unincorporated probe must be thoroughly washed away, otherwise high background will occur.
2. The typical working concentration of positive control Rosup is 100 μM (recommended range: 100-400 μM , depending on cell type). A significant increase in ROS levels can usually be observed 0.5-4 h after stimulation. The effect of the ROS positive control may vary considerably among different cell lines. If no ROS elevation is observed within 30 min of stimulation, extend the induction time or increase the Rosup concentration appropriately. If ROS increases too rapidly, shorten the induction time or reduce the concentration accordingly.
3. For some specific cell types, if high fluorescence is also observed in unstimulated negative control cells, dilute the probe at 1:2000 to 1:5000 to a final concentration of 2-5 μM . Probe loading time can also be adjusted from 15 to 60 min as needed.
4. The ROS positive control (Rosup) is only used for positive control samples and does not need to be added to every test sample.
5. After probe loading and washing away residual probe, excitation and emission scanning can be performed to verify proper probe loading.
6. Minimize the interval between probe loading and measurement (excluding stimulation time) to reduce potential errors.
7. For your safety and health, please wear a lab coat and disposable gloves during operation.
8. For quantitative analysis, a standard curve must be established. Measure fluorescence values oxidized by different concentrations of H_2O_2 to plot a standard curve with H_2O_2 concentration as the X-axis and fluorescence intensity as the Y-axis to obtain a regression equation. The corresponding X value from the sample fluorescence (Y value) is the ROS concentration.
9. Some cells tend to detach after probe loading, leading to cell loss during washing. To avoid this, seed cells at double the usual density so that cells form tight junctions and adhere firmly, resulting in higher fluorescence intensity in test groups. In addition, this probe is highly sensitive; a lower working concentration (1-2 μM) is sufficient. Higher concentrations may cause non-specific staining. The probe is unstable and its background fluorescence increases once oxidized, so the working solution should be prepared fresh immediately before use.

10. The staining solution is supplied in DMSO. It may solidify at low temperatures and adhere to tube or pipette tip walls. Ensure it is fully thawed to liquid form, centrifuged to the bottom of the tube, then opened before use.

Self-provided Equipment and Reagents

- Confocal laser scanning microscope, fluorescence spectrophotometer or fluorescence microplate reader (excitation: 488-535 nm, emission: 610 nm)
- Centrifuge
- Pipettes
- PBS buffer/HBSS (phenol red-free)
- Protein quantification assay kit

Protocol

1. Sample Preparation

- (1) Wash freshly isolated tissue samples with PBS.
- (2) Accurately weigh 50 mg of tissue, add 1 mL of Homogenization Buffer A, and homogenize thoroughly using a glass homogenizer.
- (3) Centrifuge at 100×g for 3 min at 4 °C. Discard the pellet and collect the supernatant.

2. Sample Assay

- (1) Add 200 µL of tissue homogenate supernatant and 2 µL of DHE probe to each well of a 96-well plate. Mix well by pipetting.
- (2) Incubate at 37 °C for 30 min in the dark.
- (3) Measure fluorescence intensity using a fluorescence microplate reader at excitation wavelength 488-535 nm and emission wavelength 610 nm.

3. Protein Quantification

- (1) Take another 50 µL of homogenate supernatant and dilute approximately 30-fold with PBS.
- (2) Transfer 100 µL for protein quantification.

4. Data Analysis

Express tissue ROS levels as fluorescence intensity per milligram of protein.

Recommended Applications

ROS detection in cells and fresh tissue samples from animal and plant samples using flow cytometry, fluorescence microscopy, fluorescence spectrophotometry, or fluorescence microplate reading.

Contact & Global Offices

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Limitations & Disclaimer

- For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, or household use.
- This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals for research and development purposes.
- Where any kit component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS) takes precedence over this document for handling, storage, and disposal information.
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