CheKine™ Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit (Red Fluorescence)

Cat #: KTB1913 Size: 50 T/100 T

	Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit		
REF	**Cat #:** KTB1913	LOT	**Lot #:** Refer to product label
	Applicable samples: Living cells, fresh tissues **Detection instruments:** Fluorescence microscope / Flow cytometer / Fluorescence plate reader		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Reactive oxygen species (ROS) are natural byproducts of normal oxygen metabolism and play important roles in cellular signaling and homeostasis. However, under conditions associated with oxidative stress, ROS levels can increase significantly. Accumulation of ROS severely damages cellular structures. Oxidative stress plays a crucial role in the study of cardiovascular diseases, diabetes, osteoporosis, stroke, inflammatory disorders, neurodegenerative diseases, and cancer. Detection of ROS will help elucidate how oxidative stress regulates various intracellular pathways.

The detection principle of this kit relies on dihydroethidium (DHE), a probe that is taken up by living cells. Inside the cells, DHE undergoes dehydrogenation upon oxidation by superoxide anion, a reactive oxygen species, generating ethidium. Ethidium binds to RNA or DNA and emits red fluorescence. DHE itself exhibits blue fluorescence with an excitation maximum at 370 nm and an emission maximum at 420 nm. After dehydrogenation and binding to RNA or DNA, it produces red fluorescence with excitation wavelengths at either 300 nm or 518 nm and an emission wavelength at 610 nm.

Materials Supplied and Storage Conditions

Kit component	Size		Storage conditions
	50 T	100 T	
DHE (1000×)	60 μL	120 μL	-20℃, protected from light
RosUP（1000×）	60 μL	120 μL	-20℃, protected from light

***Please check the quantity of each component before the experiment.**

***Each component is provided with an additional 10% quantity beyond the specification for the preparation of standard curves or preliminary experiments.**

Materials Required but Not Supplied

- Flow cytometry , fluorescence microscopy or fluorescence microplate reader
- Serum-free medium, PBS,trypsin

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- 37℃ carbon dioxide cell incubator
- Centrifuge tube
- Precision pipettes, disposable pipette tips
- 6-well plate/24-well plate/96-well plate (fully black or fully black with transparent bottom)

Assay Procedure

Note:(1) The probe loading process requires the use of detection buffer, which can be prepared using serum-free phenol red-free basic medium (serum-free medium with phenol red may increase background noise), or commercially available PBS, DPBS, or HBSS. It is recommended to use Abbkine's CheKine™ cell assay buffer (*Cat #: KTB1914*). Compared to PBS and other buffers, this buffer has less impact on the morphology of adherent cells and provides better signal-to-noise ratio. During probe loading, these assay buffers must not contain serum. After loading the probe, the cells can be treated with drugs or positive control reagents RosUP using assay buffer without or with serum. (2) RosUP was only added to the positive control Wells (optional), and no positive control was needed in the other experimental groups. (3) For cells subjected to short-term stimulation (typically within 2 hours), it is recommended to first load the probe, followed by stimulation of the cells with a positive control or the target drug; for cells requiring prolonged stimulation (typically for 6 hours or longer), it is recommended to first stimulate the cells with a positive control or the target drug, then load the probe; additionally, the detection buffer intended for use may be preheated in advance before cell treatment. (4) When loading the probes into cells and thereafter, it is important to protect from light.

This section provides only the latter experimental protocol, with specific steps as follows:

1. Loading probe in situ (only for adherent cells)

a) Seed cells the day before the assay to ensure a cell density of approximately 70% at the time of detection.

b) Remove the cell culture medium, add the drug to the cells diluted in serum-free culture medium, and incubate the cells under light-protected conditions in a 37℃ cell culture incubator; the actual induction duration should be determined based on the properties of the drug and the cell type.

c) (Optional) Dilute RosUP in serum-free culture medium (1000×) to a concentration of 1×, add the cells, and incubate at 37℃ in the dark for 30 min-4 h. To increase the levels of reactive oxygen species, the stimulation duration varies depending on the cell type; for example, HeLa cells require a treatment time of 2 h.

d) Dilute DHE (1000×) with PBS or serum-free culture medium to 1× concentration.

Note: The recommended final concentration of DHE is 1×, which is applicable to most cell types; however, to achieve more optimal results, you may need to conduct some preliminary experimentation for different cell types. The typical final concentration of DHE ranges from 0.5–2×.

e) Remove the treatment reagent, wash the cells 1-2 times with PBS or serum-free culture medium, add an appropriate volume of diluted DHE working solution, and incubate at 37℃ in a dark cell culture incubator for 30 minutes. The probe working solution must fully cover the cells, e.g:

Reagents	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate
Probe working solution (μL)	100	150	200	300	500

f) Wash cells 1-2 times with PBS or serum-free culture medium to fully remove DHE that has not entered the cells.

2. After collecting cells, load the probes (suitable for adherent cells, suspension cells, and fresh tissues.)

a) Wash and collect an adequate number of cells in good growth condition; prepare tissue samples into a monolayer suspension using trypsin or a mesh sieve.

b) Resuspend the collected cells in an appropriate volume of appropriately diluted drug solution, and incubate them under light-protected conditions in a 37℃ cell culture incubator; the actual induction duration can be determined based on the properties of the drug and the cell type.

c) (Optional) Dilute RosUP in serum-free culture medium (1000×) to a concentration of 1×, add the cells, and incubate at 37℃ in the dark for 30 min-4 h. To increase the levels of reactive oxygen species, the stimulation duration varies depending on the cell

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type; for example, HeLa cells require a treatment time of 2 h.

d) Dilute DHE (1000×) with PBS or serum-free culture medium to 1× concentration.

e) Centrifuge to collect cells and remove the treatment reagent; wash the cells 1-2 times with PBS or serum-free culture medium; centrifuge again to collect the cells; add the diluted DHE working solution to achieve a cell density of 1×10^6 cells/mL; then incubate at 37℃ in an opaque cell culture incubator for 30 minutes.

f) Wash cells 1-2 times with PBS or serum-free culture medium to fully remove DHE that has not entered the cells.

Fluorescence microscopy detection

For adherent cells: After probe loading is complete and cells have been washed, add an appropriate volume of serum-free cell culture medium or PBS (to prevent cell dehydration) to the cell culture wells, then observe under a fluorescence microscope; for suspension-cultured cells: after probe loading is complete and cells have been washed, place 25-50 μL of cell suspension onto a microscope slide, cover with a coverslip, and observe the fluorescence using a PE filter on a fluorescence microscope; additionally, remove the background to visualize the fluorescence signal changes.

Fluorometer and flow cytometry assays

Fluorometer-based detection: After probe loading is complete and cells have been washed, take 100 μL of cell suspension from each well and add it to a 96-well fully black or fully black-bottom plate for detection; set the excitation wavelength at 300 nm and the emission wavelength at 610 nm, or set the excitation wavelength at 518 nm and the emission wavelength at 610 nm.

Flow cytometry analysis: Select the PE channel; perform real-time detection or set specific time points to measure the intensity of fluorescence before and after stimulation.

Precautions

1. Before conducting formal testing, it is recommended to select 2-3 samples with expected significant differences for a preliminary experiment.
2. For positive controls, the recommended initial concentration is 1× (the recommended range is 0.5-2×; the specific value may vary depending on the cell type). Typically, a significant increase in reactive oxygen species (ROS) levels can be observed within 30 minutes to 4 hours after stimulation. The efficacy of positive controls may vary considerably across different cell types. If no increase in ROS levels is observed within 30 minutes post-stimulation, the induction time may be extended or the concentration of the positive control appropriately increased. Conversely, if the fluorescence signal is excessively strong, the induction time may be shortened or the concentration of the -positive control appropriately reduced.
3. During the experiment, if the fluorescence intensity of the negative control cells is also relatively strong, dilute the DHE fluorescent probe at a ratio of 1:2000 to achieve a final DHE concentration of 0.5×. The probe loading time can be adjusted appropriately within the range of 15-60 minutes; strive to minimize the exposure time during image acquisition, and ensure that the exposure times for both the experimental group and the control group remain consistent.
4. RosUP (1000×) is intended solely for use as a positive control sample; it is not required to add a reactive oxygen species positive control to all test group samples.
5. After probe loading, it is essential to thoroughly wash out any probes that have not entered the cells; otherwise, this may result in elevated background signals.
6. Strive to minimize the time between probe loading and detection (excluding the stimulation duration) to reduce any potential errors.
7. DHE is prone to oxidation in air; therefore, it should be kept away from exposure to air as much as possible. Upon dehydrogenation, DHE yields ethidium, which possesses certain toxicity; appropriate protective measures should be taken.
8. Most biochemical testing reagents are generally irritating or possess biological toxicity; to ensure your safety and health, please implement comprehensive biosafety precautions throughout the entire experiment: wear appropriate protective gear-including laboratory coats, masks, gloves, and headgear-and conduct experiments within fume hoods or biosafety cabinets.
9. This product is for scientific research purposes only and is not intended for clinical diagnosis.

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Recommended Products

Catalog No.	Product Name
KTB1910	Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit (Green Fluorescence)
KTB1914	CheKine™ Cell Assay BufferCheKine™
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1050	CheKine™ Micro Lipid Peroxidation (MDA) Assay Kit
KTB1640	CheKine™ Micro Glutathione Peroxidase (GSH-Px) Activity Assay Kit
KTB1600	CheKine™ Micro Reduced Glutathione (GSH) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.

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