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CheKine™ Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit (Red, Flow Cytometry, Fluorescence Microscope)

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		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Reactive Oxygen Species (ROS) is a natural by-products of oxygen normal metabolism, plays an important role in cell signaling and homeostasis. Under conditions associated with oxidative stress, ROS levels can be significantly increased. The accumulation of ROS could seriously damage the cell structure. Oxidative stress plays an important role in the research of cardiovascular disease, diabetes, osteoporosis, stroke, inflammatory disease, and neurodegenerative diseases and cancer, etc. ROS detection could help determine how oxidative stress adjusts various intracellular pathways. CheKine™ Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit provides a simple, sensitive, rapid ROS detection method. Its principle is based on the fluorescent probe Dihydroethidium (DHE). Dihydroethium (DHE) can be dehydrogenated by intracellular superoxide anion reactive oxygen species after being ingested by living cells, producing Ethidium. Ethidium can bind to RNA or DNA to produce red fluorescence. Dihydroethidium itself emits blue fluorescence with a maximum excitation wavelength of 370 nm and a maximum emission wavelength of 420 nm. After dehydrogenation, it binds to RNA or DNA to produce red fluorescence with an excitation wavelength of 300 nm or 518 nm and an emission wavelength of 610 nm.

Materials Supplied and Storage Conditions

Kit component	Size		Storage conditions
	50 T	100 T	
DHE (1,000×)	60 μL	120 μL	-20℃, protected from light
ROS Positive Control (1,000×)	60 μL	120 μL	-20℃

Materials Required but Not Supplied

- Flow cytometry or fluorescence microscopy
- Serum-free medium, PBS
- 37℃ carbon dioxide cell incubator
- Centrifuge tube
- Precision pipettes, disposable pipette tips

• Cell culture plate

Assay Procedure

For the cells with short stimulation time (usually less than 2 h), it is recommended to load the probe first, and then stimulate the cells with ROS positive control or interested drugs; For the cells that need to be stimulated for a long time (usually more than 6 h), it is suggested that the cells should be stimulated with ROS positive control or interested drugs before loading the probe, only the latter experimental method is provided here, the steps are as follows:

1. In situ loading probe (only for adherent cells)

a) Cell preparation: lay the cell board one day before the test, the number of cells seeded in each well of the 96 well plate should be controlled between 100-10,000. It is recommended to inoculate 2,000-5,000 cells per well of the 96 well plate.

b) Drug induction: remove the cell culture medium, treat the cells by adding serum-free diluted drugs, and incubated in 37℃ cell incubator in dark. The actual induction time depends on the drug characteristics and cell type.

c) (Optional) Positive control: dilute the ROS Positive Control (1,000×) with serum-free medium to the normal working concentration of 1×. Cells were added and incubated at 37℃ for 30 min-4 h in dark. In order to improve the level of ROS, the actual time of different cell types was different. For example, HeLa cells need to be treated for 1 h.

Note: (1) Positive Control is only needed in the positive control well, and no needed in other experimental groups. (2) The recommended initial concentration for Positive Control is 1×, the working solution should be used freshly each time, do not store the diluted solution, and Positive Control can also be diluted with 10% FBS completely without phenol red.

d) Preparation of DHE working solution: DHE (1,000×) is diluted with PBS or serum-free medium and the final concentration is 1×.

Note: The recommended final concentration for DHE is 1×, which is suitable for most cells. However, in order to obtain more ideal results, please explore different types of cells on your own. The final concentration for DHE is usually 0.5-2×.

e) ROS probe loading: remove the treatment drugs, wash the cells with PBS or serum-free medium for 1-2 times, and add appropriate volume of DHE working solution, the cells need to be fully covered, for example, 6-well plate is usually added not less than 1 mL/well, 96-well plate is usually added not less than 100 μL/well. The cells are incubated in 37℃ incubator for 30 min in dark.

f) Cell cleaning: cells are washed with PBS or serum-free medium for 1-2 times to remove DHE that does not enter the cells.

2. Collect cells and load probes (for adherent cells, suspension cells and fresh tissues)

a) Cell preparation: cells cultured according to the standard method. It is necessary to ensure that the cells used for test are in good condition. Clean and collect enough cells according to appropriate methods. Prepare single-cell suspension of tissue samples using trypsin or mesh sieve.

b) Drug induction: the collected cells are suspended in an appropriate amount of diluted drug and incubated in 37℃ incubator in dark. The actual induction time could be determined according to the drug characteristics and cell types.

c) (Optional) Positive control: dilute the ROS Positive Control (1,000×) with serum-free medium to the normal working concentration of 1×. Cells were added and incubated at 37℃ for 30 min-4 h in dark. In order to improve the level of ROS, the actual time of different cell types was different. For example, HeLa cells need to be treated for 1 h.

Note: (1) Positive Control is only needed in the positive control well, and no needed in other experimental groups. (2) The recommended initial concentration for Positive Control is 1×, the working solution should be used freshly each time, do not store the diluted solution, and Positive Control can also be diluted with 10% FBS completely without phenol red.

d) Preparation of DHE working solution: DHE (1,000×) is diluted with PBS or serum-free medium and the final concentration is 1×.

e) Probe loading: collect cells by centrifugation, remove the treatment drugs, wash cells with serum-free medium for 1-2 times, collect cells by centrifugation, add DHE working solution, and make the cell density 1.0×10^6/mL. The cells are incubated in 37℃ incubator for 30 min in dark.

Note: the cell density should be adjusted according to the subsequent detection system, detection method and total detection amount. For example, for flow cytometry, the number of cells in a single tube should not be less than 10^4 and more than 10^6.

f) Cell cleaning: the cells are washed 1-2 times with PBS or serum-free medium to remove the DHE that does not enter the cells.

3．Fluorescence microscope photograph

a) For adherent cells (Step 1.f), after adherent cell cleaning, appropriate amount of serum-free medium or PBS was added and observed under fluorescence microscope; For suspension cells (Step 2.f), drop 25-50 μL cell suspension onto a microscope slide and covered with a cover slide for observation.

b) Under the fluorescence microscope, PE filter is used to observe the fluorescence, and the background is removed to observe the change of fluorescence.

4．Operation and analysis method of flow cytometry

a) The adherent cells (Step 1.f) are digested with trypsin to prepare single cell suspension; For suspension cells (Step 2.f), the cells are collected directly. Cells are resuspended with 0.5-1 mL PBS (0.5-1×10^5/mL).

b) Select the PE channel of the flow cytometer. It can detect the intensity of fluorescence before and after stimulation in real time or time point.

Precautions

1. The recommended initial concentration for positive control is 1× (The recommended concentration is 0.5×-2×, it depends on the cell type). In general, a significant increase in ROS could be observed 30 min-4 h after stimulation. For different cells, the effect of ROS positive control may be significantly different. If the increase of ROS was not observed within 30 min after stimulation, the induction time could be prolonged or the concentration of ROS positive control could be increased appropriately. If the fluorescence signal is too strong, the induction time can be shortened or the concentration of ROS can be appropriately reduced.
2. During the experiment, if the fluorescence of negative control cells is also relatively strong, the fluorescent probe DHE (1,000×) can be diluted according to 1:2,000 and the final concentration of DHE is 0.5×. The loading time of the probe can be adjusted within 15-60 min to shorten the exposure and reading time as much as possible, the exposure time of the experimental group and the control group should be consistent.
3. ROS Positive Control (1,000×) is only used as positive control sample, and not all the samples in the test group need to be added ROS positive control.
4. After probes were loaded, the probes that does not enter the cells must be cleaned, which would cause higher background.
5. The time from probe loading to detection (except stimulation time) should be shortened as much as possible to reduce various possible errors.
6. DHE is prone to oxidation in air, please try to avoid exposure to air as much as possible. After DHE dehydrogenation, Ethidium with certain toxicity is produced. Please pay attention to protection.

Recommended Products

Catalog No.	Product Name
KTB1050	CheKine™ Micro Lipid Peroxidation (MDA) Assay Kit
KTB1910	CheKine™ Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit
KTB1911	CheKine™ Mitochondrial Reactive Oxygen Species (ROS) Production Rate Fluorometric 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.