Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

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

Cat #: KTB1910 Size: 50 T/100 T

	Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit		
REF	**Cat #:** KTB1910	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells		
	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 DCFH-DA. DCFH-DA is a cell-permeable sensitive probe used to detect intracellular reactive oxygen species (ROS). This probe could be hydrolyzed into DCFH by esterase in the cell while passing through the living cell membrane. DCFH has no fluorescence and cannot penetrate the cell membrane, which could be oxidized by intracellular ROS and produce the fluorescent DCF. And then the level of cellular reactive oxygen species can be analyzed via the fluorescent is detected by flow cytometry or fluorescence microscopy.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	50 T	100 T	
DCFH-DA (10 mM)	0.05 mL	0.1 mL	-20℃, protected from light

Materials Required but Not Supplied

- Flow cytometry or fluorescence microscopy
- Serum-free medium
- 37℃ carbon dioxide cell incubator
- Centrifuge tube
- Precision pipettes, Disposable pipette tips
- H_2O_2

• 6-well microplate

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 to ensure that the number of cells is less than 5×10^5/ml.

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 positive control H_2O_2 with serum-free medium, and dilute it from 100 mM to the normal working concentration of 100 μM. 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 30-60 min.

Note: Positive control is only needed in the positive control well, and no needed in other experimental groups.

d) Preparation of ROS probe: DCFH-DA is diluted with serum-free medium and the final concentration is 10 μM.

e) ROS probe loading: remove the treatment drugs, wash the cells with serum-free medium for 1-2 times, and add appropriate volume of diluted DCFH-DA 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 serum-free medium for 1-2 times to remove DCFH-DA that does not enter the cells.

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

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.

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 positive control H_2O_2 with serum-free medium, and dilute it from 100 mM to the normal working concentration of 100 μM. Cells are added and incubated at 37℃ for 30 min-4 h in dark. In order to improve the level of ROS, different cell types are different. For example, HeLa cells need to be treated for 30-60 min.

d) Preparation of ROS probe: DCFH-DA are diluted with serum-free medium and the final concentration is 10 μM.

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 diluted probes, and make the cell density 1.0×10^6-2.0×10^7. 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 serum-free medium to remove the DCFH-DA that does not enter the cells.

3. Fluorescence microscope photograph

a) The adherent cells (Step 1.f) can be observed directly under fluorescence microscope; For suspension cells (Step 1.f), drop 25-50 μL cell suspension onto a microscope slide and covered with a cover slide for observation.

b) Under the fluorescence microscope, FITC 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 ($0.5\text{-}1\times10^5$/mL).

b) 488 nm is the excitation wavelength and 525 nm is the emission wavelength in flow cytometry. It can detect the intensity of

fluorescence before and after stimulation in real time or time point.

Precautions

1. The concentration of H_2O_2 positive control is 100 μM (recommended concentration 50-200 μM, 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 DCFH-DA can be diluted according to 1:2,000-1:5,000 and the final concentration of DCFH-DA is 2-5 μM. 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. The ROS positive control (H_2O_2) 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.

Recommended Products

Catalog No.	Product Name
KTB1030	CheKine™ Superoxide Dismutases (SOD) Activity Colorimetric Assay Kit
KTB1040	CheKine™ Catalase (CAT) Activity Assay Kit
KTB1050	CheKine™ Lipid Peroxidation (MDA) Activity Colorimetric Assay Kit
KTB1640	CheKine™ Glutathione Peroxidase (GSH-Px) Activity Colorimetric Assay Kit
KTB1600	CheKine™ Reduced Glutathione (GSH) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.