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

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 component	Size		Storage conditions
	50 T	100 T	
DCFH-DA (10 mM)	55 μL	105 μL	-20℃, protected from light
RosUP（10,000×）	50 μL	100 μL	-20℃, protected from light

Materials Required but Not Supplied

- Flow cytometry , fluorescence microscopy or fluorescence microplate reader
- Serum-free medium, PBS
- 37℃ carbon dioxide cell incubator
- Centrifuge tube

• Precision pipettes, disposable pipette tips
• 6-well plate

Assay Procedure

Note: (1) The probe loading process requires the use of assay buffer, which can be serum-free and phenol red-free basic medium (serum-free medium with phenol red may increase the background), or commercial 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 with a relatively short stimulation time (usually within 2 hours), it is recommended to load the probe first and then stimulate the cells with a reactive oxygen species positive control or the drug of interest. For cells that require prolonged stimulation (typically over 6 hours), first stimulate the cells with an reactive oxygen species positive control or a drug of interest, then load the probe, and it is also recommended to use the KTB1913 kit to detection for longer stimulation experiment. (4) When loading the probes into cells and thereafter, it is important to protect from light.

1. Fluorescence microscopy (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) Pre-warm the assay buffer at 37℃ for 30 minutes .

c) ROS probe working solution: Dilute DCFH-DA in pre-warmed assay buffer at 37℃ to a final concentration of 10 μM.

d) ROS probe loading: Wash cells twice with pre-warmed assay buffer, then add an appropriate volume of the prepared DCFH-DA working solution and incubate in the dark at 37℃ for 30 minutes in a cell culture incubator. The probe solution should completely cover the cells.

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

e) Remove the probe working solution and wash the cells 1-2 times with 37℃ preheated assay buffer to thoroughly remove DCFH-DA that has not entered the cells.

f) Drug induction: Remove the wash solution, dilute the drug with 37℃ preheated assay buffer (10% FBS can be added as needed) to stimulate the cells, and incubate in the 37℃ cell incubator in the dark. The actual induction time is determined by the characteristics of the drug and the type of cells.

g) Positive control (optional): In step f, dilute RosUP (10,000×) to 1x with assay buffer and add it to the cells (24-well plate: no less than 500 μL, 12-well plate: no less than 1 mL, 6-well plate: no less than 2 mL, etc.), and incubate in the 37℃ cell incubator in the dark. To enhance the level of reactive oxygen species, the stimulation time varies among different types of cells. For instance, HeLa cells need to be treated for 15 to 30 minutes.

h) Select an appropriate time point and use a fluorescence microscope to detect. (It is recommended to conduct multiple time points of pre-experiment to explore the best detection point.)

Note: strong laser irradiation may increase the background signal in the NC group. Therefore, it is recommended to first find the appropriate field of view under the white light, then switch to the FITC channel for photography; if the background color of the induction group is relatively dark, the background can be subtracted and the cells can be washed for solution resolution by adjusting the instrument parameters.

2. Flow cytometry or fluorescence microplate reader Assay (for adherent cells and suspension cells)

a) Collect cells, prepare cell suspension, and recommend loading the probe and inducing with RosUP when the cell density is

approximately 1.0×10^6 cells/mL.

b) Preheat the assay buffer at 37℃ half an hour in advance.

c) ROS probe working solution: Dilute DCFH-DA with 37℃ preheated assay buffer to reach a final concentration of 10 μM.

d) Loading of ROS probe: Collect cells and wash them twice with 37℃ preheated assay buffer), centrifuge to collect cells, add the diluted probe, with a cell density of 1×10^6 - 2.0×10^7 cells/mL (the actual recommended cell density should not exceed 2.0×10^6 cells/mL to avoid cell aggregation), incubate in a 37℃ cell incubator with light shielding for 30 minutes.

e) Centrifuge, remove the supernatant, wash the cells 1-2 times with 37℃ preheated assay buffer to thoroughly remove DCFH-DA that has not entered the cells.

f) Drug induction: Remove the wash solution, dilute the drug with 37℃ preheated assay buffer (10%FBS can be added as needed) to stimulate the cells, and incubate in the 37℃ cell incubator in the dark. The actual induction time is determined by the characteristics of the drug and the type of cells.

g) Positive control (optional): In step f, dilute RosUP (10,000×) with assay buffer to 1x, add it to the cells (with a cell density of approximately 5×10^5 cells/mL), incubate in the 37℃ cell incubator in the dark for 30 min - 4 h. To increase the level of reactive oxygen species, there are differences in stimulation time for different types of cells. For example, the signal of jurkat cells is relatively stable after treatment for 2 h - 4 h.

Select an appropriate time point and perform flow cytometry or fluorescence microplate reader detection on the cells (microplate reader detection: take 100 μL of cell suspension from each well and add it to a 96-well black or black low-transparency plate for detection). Choose 488 nm as the excitation wavelength and 525 nm as the emission wavelength. (It is recommended to conduct preliminary experiments to try multiple time points to find the best detection point.)

Precautions

1. The positive control is recommended to be initially used at a concentration of 1:10,000 (it is recommended to dilute at a ratio of 1:10,000 - 1:100,000, depending on the type of cells). Usually, a significant increase in reactive oxygen species (ROS) levels can be observed 15 minutes to 4 hours after stimulation. The effect of the ROS positive control may vary significantly for different cells. If no increase in ROS is observed within 30 minutes after stimulation, the induction time can be extended or the concentration of the ROS positive control can be appropriately increased. If the fluorescence signal is too strong, the concentration of the ROS positive control 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. RosUP 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
KTB1914	CheKine™ Cell Assay Buffer
KTB1913	CheKine™ Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit (red Fluorescence)
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.