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

Cat #: KTB1912 Size: 50 T / 100 T

	Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit（Fluorescence Microplate Reader）		
REF	**Cat #:** KTB1912	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells		
	Fluorescence Excitation/ Emission: Ex/Em=488/525 nm		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Reactive OxygenSpecies (ROS) is a natural by-product of normal oxygen metabolism and plays an important role in cellular signaling and homeostasis. However, ROS levels can be greatly increased under oxidative stress-related states. The accumulation of ROS can seriously damage the cellular structure. Oxidative stress plays an important role in the research of cardiovascular diseases, diabetes, osteoporosis, stroke, inflammatory diseases, as well as neurodegenerative diseases and cancer. ROS assays will help determine how oxidative stress regulates various intracellular pathways. CheKine™ Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit（Fluorescence Microplate Reader） can be used to detect biological samples such as animal tissue, cells. The detection principle of this kit is to use the fluorescent probe DCFH-DA for reactive oxygen detection. DCFH-DA (2',7'-dichlorofluorescein diacetate) is hydrolyzed by esterase to form non-fluorescent DCFH. DCFH is oxidized by reactive oxygen species (ROS) to generate fluorescent DCF. The fluorescence intensity of DCF is proportional to the reactive oxygen level in the sample. The fluorescence intensity of DCF in the reaction solution can reflect the level of ROS in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	50 T	100 T	
Reagent Ⅰ	75 mL	75 mL×2	-20℃, protected from light
Reagent Ⅱ	10 μL	20 μL	-20℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Fluorescence microplate reader (Ex/Em=488/525 nm)
- 96-well black/white plate, precision pipettes, disposable pipette tips
- Incubator, ice maker, freezing centrifuge
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; The remaining reagent can also be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

Working Reagent II: Prepared before use. According to the dosage, dilute Reagent II with Reagent I for 50 times and then use it. The diluted Working Reagent II cannot be reused. The remaining Reagent II can also be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

Note: We recommend that you use fresh samples. It is recommended not to use long-term frozen tissue samples. Residual ROS in frozen tissue samples can also be detected using this kit.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Reagent I and homogenize or mortar on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells or bacteria with cold PBS, centrifuge at 800 g for 2 min and discard the supernatant. Add 1 mL Reagent I to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the fluorescence microplate reader for more than 30 min, and adjust the excitation wavelength to 488 nm and the emission wavelength to 525 nm.

2. Sample measurement. (The following operations are operated in the 96-well black/white plate)

Reagent	Test Well (μL)	Blank Well (μL)
Sample	195	0
Reagent I	0	195
Working Reagent II	5	5

3. Mix well, incubate in dark for 20 min at 37℃. Detect the fluorescence value RFU at Ex/Em=488/525 nm. The Blank Well is recorded as RFU_{Blank}, the Test Well is marked as RFU_{Test}. Finally calculate $\Delta RFU=RFU_{Test}-RFU_{Blank}$.

Note: The Blank Well only need to be done 1-2 times. If ΔRFU is too high, the sample can be appropriately diluted with Reagent I , the calculated result multiplied by the dilution factor, or decrease the incubation time (not less than 15 min). If ΔRFU is too low, increase the sample quantity appropriately, or increase the incubation time (not more than 25 min).

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculation of the ROS content

(1) Calculated by protein concentration

ROS (RFU/ mg prot)=$\Delta RFU\div(V_{Sample}\times Cpr)\times n$**$=5.13\times\Delta RFU\div Cpr\times n$**

(2) Calculated by fresh weight of samples

ROS (RFU/g)=$\Delta RFU\div(V_{Sample}\div V_{Extract}\times W)\times n$**$=5.13\times\Delta RFU\div W\times n$**

(3) Calculated by cells

ROS (RFU/10^4)=$\Delta RFU\div(V_{Sample}\div V_{Extract}\times 500)\times n$**$=0.0103\times\Delta RFU\times n$**

$V_{Extract}$: Added the Reagent I volume, 1 mL; V_{Sample}: Added the sample volume, 0.195 mL; Cpr: sample protein concentration,

mg/mL; W: Sample weight, g; 500: Number of cells, calculated in units of 10^4; n: sample dilution multiple.

Typical Data

Example-1: Take 0.1 g of mouse brain, follow the measurement steps, and use a 96-well black plate for detection. Measured $\Delta RFU=RFU_{Test}-RFU_{Blank}=6,193-26=6,167$. Calculated according to the sample quality, ROS (RFU/g)=5.13×ΔRFU÷W×n=316,367 RFU/g

Recommended Products

Catalog No.	Product Name
KTB9300	CheKine™ Pro Glucose Fluorometric Activity Assay Kit
KTB9041	CheKine™ Pro Hydrogen Peroxide (H_2O_2) Fluorometric Assay Kit
KTB1910	CheKine™ Reactive Oxygen Species (ROS) Detection 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.