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

CheKine™ Mitochondrial Reactive Oxygen Species (ROS) Production Rate Fluorometric Assay Kit

Cat #: KTB1911 Size: 96 T

	Mitochondrial Reactive Oxygen Species (ROS) Production Rate Fluorometric Assay Kit		
REF	**Cat #:** KTB1911	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Fungus		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Reactive oxygen species (ROS) are natural by-products of normal oxygen metabolism, including superoxide radicals, hydrogen peroxide, and its downstream products peroxides and hydroxides. Studies have shown that more than 95% of ROS in the body come from mitochondria, and the oxidative stress caused by its imbalance is related to cell growth and proliferation, developmental differentiation, aging and apoptosis, as well as many physiological and pathological processes. Under normal conditions, the intracellular antioxidant defense system is in balance with oxygen free radicals, and the level of intracellular ROS is maintained in a low physiological range. In pathological conditions, the balance between the intracellular antioxidant system and oxygen free radicals is disrupted. Excessive levels of ROS in cells can disrupt mitochondrial enzymes, lipids, and nucleic acids, leading to oxidative stress in the body. Meanwhile, ROS can also attack mitochondrial DNA and cause oxidative damage, resulting in structural and functional changes such as reduced mitochondrial ATP synthesis and mitochondrial membrane potential destruction. CheKine™ provides a simple, sensitive, and rapid method for detecting the rate of mitochondrial ROS production. The principle is to use fluorescent probe DCFH-DA for reactive oxygen species detection. DCFH-DA (2',7'-Dichlorofluorescin Diacetate) can diffuse through the mitochondrial membrane and be hydrolyzed by esterase in mitochondria to form non fluorescent DCFH. DCFH is oxidized by ROS to produce fluorescent DCF, and the rate of increase in fluorescence intensity of DCF is proportional to the rate of ROS production.

Materials Supplied and Storage Conditions

Kit components	Size (96 T)	Storage conditions
Extraction Buffer	100 mL	4℃
Reagent Ⅰ	50 mL	4℃
Reagent Ⅱ	1.5 mL	-20℃, protected from light
ReagentⅢ	1	4℃, protected from light
ReagentⅣ	1	4℃, protected from light

Reagent Ⅴ	1	4℃, protected from light
Reagent Ⅵ	20 μL	-20℃, protected from light

Materials Required but Not Supplied

- Precision pipettes, disposable pipette tips
- Homogenizer, refrigerated centrifuge, 96-well black plate or 96-well white plate
- Incubator, multimode reader

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅱ: Ready to use as supplied. Store at -20℃, protected from light.

Reagent Ⅲ: Prepare before use, add 6 mL Reagent Ⅰ to dissolve it, The unused dissolved Reagent Ⅲ can be stored at 4℃ for 1 month, protected from light.

Reagent Ⅳ: Prepare before use, add 6 mL Reagent Ⅰ to dissolve it, The unused dissolved Reagent Ⅳ can be stored at 4℃ for 1 month, protected from light.

Reagent Ⅴ: Prepare before use, add 6 mL Reagent Ⅰ to dissolve it, The unused dissolved Reagent Ⅴ can be stored at 4℃ for 1 month, protected from light.

Working Reagent Ⅵ: Prepare before use, Reagent Ⅵ diluted 300 times with Reagent Ⅰ according to the dosage before use, The diluted Working Reagent Ⅵ can not be reused.

Sample Preparation

Extraction of mitochondria from tissues and cells

1. Weigh 0.1 g tissue or collect 5×10^6 cells, add 1 mL Extraction Buffer and 10 μL Reagent Ⅱ, homogenize on ice. Centrifuge at 600 g for 5 min at 4℃. Collect the supernatant to a new centrifuge tube and discard the precipitation.
2. Centrifuge the supernatant again at 11,000 g for 10 min at 4℃, precipitation is the extracted mitochondria.
3. Discard the supernatant, add 200 μL Reagent Ⅰ to resuspend precipitation.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine catalog number: KTD3002 Protein Quantification Kit (Bradford Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the multimode reader to 37℃. The excitation wavelength is 488 nm, and the emission wavelength is 525 nm.
2. Add samples to the 96-well black plate or 96-well white plate as follows:

Regent	Blank well (μL)	Test well (μL)
Sample	0	20
Reagent Ⅰ	20	0
Reagent Ⅲ	50	50
Reagent Ⅳ	50	50
Reagent Ⅴ	50	50
Working Reagent Ⅵ	30	30

3. Mix well and incubate at 37℃ for 15 min, protected from light. After incubation, the fluorescence value within 10 min was

measured with a multimode reader, the excitation wavelength was 488 nm, the emission wavelength was 525 nm, the instrument temperature was kept at 37℃, and the fluorescence value changes within 10 min were recorded.

Precautions

1. It is necessary to measure the fluorescence intensity change within 10 min at a constant temperature of 37℃.
2. When mixing with a pipette, be careful to absorb and beat to avoid bubbles.
3. During testing, a 96-well black or white plate should be used to prevent interference from adjacent wells.

Data Analysis

1. The linear regression fitting process was carried out on the change of the sampled data point, that is, the fluorescence intensity over time, and the regression coefficient, that is, the linear slope (k), was calculated. The actual mitochondrial ROS production rate is equal to the linear regression line slope of data points with sample fluorescence intensity changing over time (k_{Test}) minus the linear regression line slope of data points with background fluorescence intensity changing over time (k_{Blank}), $k=(RFU_{10min}-RFU_{0min})/600$.
2. Calculation of results

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) Calculated by weight of samples: Change of fluorescence unit per second of mitochondria per g tissue, u/s/g fresh weight.

ROS production rate (u/s/g fresh weight) $=(k_{Test}-k_{Blank})\div(V_{Sample}\div V_{Sample\ Total}\times W)$**$=100\times(k_{Test}-k_{Blank})$**

(2) Calculated by protein concentration: Changes in fluorescence units per second per mg protein mitochondria, u/s/mg prot.

ROS production rate (u/s/mg prot)$=(k_{Test}-k_{Blank})\div(V_{Sample}\div V_{Sample\ Total}\times Cpr)$**$=10\times(k_{Test}-k_{Blank})\div Cpr$**

(3) Calculated by cell number: Changes in fluorescence units per second of mitochondria per 10^4 Cells, u/s/10^4 Cells.

ROS production rate(u/s/10^4 Cells)$=(k_{Test}-k_{Blank})\div(500\times V_{Sample}\div V_{Sample\ Total})$**$=(k_{Test}-k_{Blank})\div 50$**

Where: V_{Sample}: Sample volume added, 0.02 mL;2; $V_{Sample\ Total}$: Resuspension of sample volume, 0.2 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, 0.1 g ; 500: Total number of cells, 5×10^6.

Recommended Products

Catalog No.	Product Name
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1500	CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit
KTB1501	CheKine™ Oxygen Radical Antioxidant Capacity (ORAC) Fluorometric Assay Kit
KTB1502	CheKine™ Hydroxyl Radical Antioxidant Capacity (HORAC) 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.