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

CheKine™ Pro Hydrogen Peroxide (H_2O_2) Fluorometric Assay Kit

Cat #: KTB9041 Size: 48 T/96 T

	Hydrogen Peroxide (H_2O_2) Fluorometric Assay Kit		
REF	**Cat #:** KTB9041	LOT	**Lot #:** Refer to product label
	Detection range: 0.02-10 μmol/L		**Sensitivity:** 0.02 μmol/L
	Applicable samples: Animal and Plant Tissues, Cells, Serum, Plasma or other Liquid samples		
	Fluorescence Excitation/Emission: Ex/Em=535/587 nm		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

CheKine™ Pro Hydrogen Peroxide (H_2O_2) Fluorometric Assay Kit can detect animal and plant tissues, cells, serum, plasma and other samples. The principle involves the reaction of H_2O_2 in the presence of an enzyme and a fluorophore, where the fluorescence intensity at an excitation wavelength of 535 nm and an emission wavelength of 587 nm is directly proportional to the concentration of H_2O_2.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Assay Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	25 μL	50 μL	-20℃, protected from light
Reagent Ⅱ	10 μL	20 μL	-20℃, protected from light
SOD	25 μL	50 μL	-20℃, protected from light
Standard (8.8 M)	100 μL	100 μL	-20℃, protected from light

Materials Required but Not Supplied

- Fluorescence microplate reader (the excitation wavelength is 535 nm, and the emission wavelength is 587 nm)
- Black 96-well plate, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, incubator, ice maker
- Deionized water, PBS (pH 7.0)
- Dounce homogenizer (for tissue samples)

Reagent Preparation

Assay 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; Unused reagents should be aliquoted and stored at -20℃ in the dark, avoiding repeated freezing and thawing.

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

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

Working Reagent: Prepare in the dark just before use; take 50 μL of Reagent Ⅰ and 20 μL of Reagent Ⅱ, and add them to 4.93 mL of Assay Buffer, mixing thoroughly. This volume is sufficient for 100 tests. Prepare as needed and use within the same day, ensuring protection from light during usage.

Standard (8.8 M): Before use, dilute the standard 1,000 times with deionized water to obtain a 8.8 mM solution, ensuring complete dissolution for later use. Any unused Standard (8.8 M) should be aliquoted and stored at -20°C in the dark, preventing multiple freeze-thaw cycles.

Standard setting: Prepare the standard solution as shown in the table below.

Num.	Volume of Standard	Volume of Assay Buffer (μL)	Standard Concentration (μM)
Std.1	11.5 μL 8.8 mM Standard	988.5	100
Std.2	500 μL of Std.1 (100 μM)	500	50
Std.3	500 μL of Std.2 (50 μM)	500	25
Std.4	500 μL of Std.3 (25 μM)	500	12.5
Std.5	500 μL of Std.4 (12.5 μM)	500	6.25
Std.6	500 μL of Std.5 (6.25 μM)	500	3.125
Std.7	500 μL of Std.6 (3.125 μM)	500	1.56
0 (Blank Well)	0	500	0

Note: The diluted standard solution is prepared and immediate used, and should not be stored for a long time.

Sample Preparation

Note: Fresh samples are recommended. If not assayed immediately, samples can be stored at -80℃.

1. Animal and plant tissues: Weigh 0.1 g tissue, add an appropriate amount of Assay Buffer and homogenize on ice. Centrifuge at 10,000 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 with cold PBS, discard the supernatant after centrifugation; add an appropriate amount of Assay Buffer to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma or other Liquid samples: Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: 1. It is recommended to perform a pilot experiment prior to the main assay by selecting 2-3 samples expected to have significant differences, and diluting them to various concentrations using Assay Buffer. Based on the results of this preliminary test and considering the linear range of this kit, which is 0.01-10 U/mL, please refer to the table below for guidance on dilutions (for reference only), if the test result is negative or if the sample contains NADH concentrations exceeding 10 μM and glutathione concentrations over 50 μM, it is recommended to add SOD to the reaction mixture, with 0.4 μL per well. .

Sample	Dilution Fold	Sample	Dilution Fold
10% Mouse Brain	1-3	10% Mouse Lung	1-3

FBS	1-2	10% Tobacco Leaf	1-2

2. Avoid using samples containing DTT or β-mercaptoethanol, as Resorufin is unstable in the presence of thiols at concentrations exceeding 10 μM.

3. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine catalog number: 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. The excitation wavelength is 535 nm, and the emission wavelength is 587 nm.

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

Reagent	Test Well (μL)	Standard Well (μL)
Sample	50	0
Standard	0	50
Working Reagent	50	50
Mix well and let stand at room temperature in the dark for 20 min. On the fluorescence microplate reader, set the excitation wavelength to 535 nm and the emission wavelength to 587 nm. Measure the fluorescence values of each well, recording them as RFU_{Test}, $RFU_{Standard}$, and RFU_{Blank}. Calculate $\Delta RFU_{Test}=RFU_{Test}-RFU_{Blank}$, and $\Delta RFU_{Standard}=RFU_{Standard}-RFU_{Blank}$.		

Data Analysis

1. Drawing of the standard curve

With the concentration of the standard solution as the x-axis and the $\Delta RFU_{Standard}$ as the y-axis, draw the standard curve, get the standard equation y=kx+b, and bring the ΔRFU_{Test} into the equation to get the x value (μM).

2.Calculation of Hydrogen Peroxide Content:

（1）Calculated by sample protein concentration:

H_2O_2(nmol/mg prot)**=x×f÷Cpr**

（2）Calculated by fresh weight of samples:

H_2O_2(nmol/mg prot)**=x×f÷Cpr**

（3）Calculated by cells:

H_2O_2(nmol/10^4 cell)**=x×f÷N**

（4）Calculated by volume of liquid samples:

H_2O_2(nmol/mL)**=x×f**

Cpr: Sample protein concentration, mg/mL; f: Sample dilution fold; W: Sample weight, g; N: The total number of cells, 10^4.

Typical Data

Typical standard curve-data:

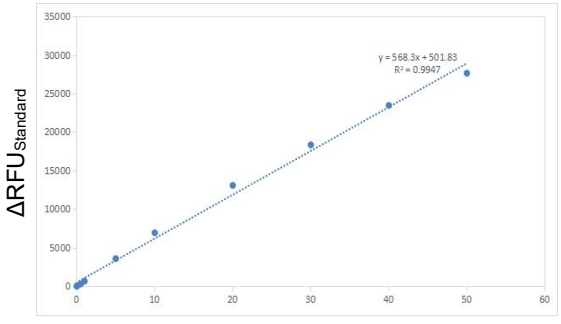

Figure 1. Standard Curve for H_2O_2.

Example:

1. Take 0.168 g of mouse brain tissue and add 1 mL of Assay Buffer to homogenize and grind it. After centrifugation, take the supernatant, dilute it 3 times, and proceed with the measurement steps. Using a full-black 96-well plate, the following readings were obtained: RFU_{Test} is 2,970, and RFU_{Blank} is 502. Thus, ΔRFU_{Test}=2,970-502=2,468. With the standard curve equation being y=1,790.1x+542.68, the H_2O_2 concentration calculated is 1.076 μM. Therefore, H_2O_2 (sample)=1.076×3÷0.168=19.21 nmol/g fresh weight.

2. Centrifuge the fetal bovine serum to obtain the supernatant and proceed directly with the assay procedures. Using a full-black 96-well plate, the following readings were obtained: RFU_{Test} is 1,172, and RFU_{Blank} is 502. Thus, ΔRFU_{Test}=1,172-502=670. With the standard curve equation being y=1,790.1x+542.68, the H_2O_2 concentration calculated is 0.071 μM. Therefore, CAT (sample)=0.071 nmol/mL.

Recommended Products

Catalog No.	Product Name
KTB9050	CheKine™ Pro Malondialdehyde (MDA) Fluorometric Assay Kit
KTB9300	CheKine™ Pro Glucose Fluorometric Activity Assay Kit
KTB9040	CheKine™ Pro Catalase (CAT) Fluorometric Activity Assay Kit

Disclaimer

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