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CheKine™ Pro Catalase (CAT) Fluorometric Activity Assay Kit

Cat #: KTB9040 Size: 48 T/96 T

	Catalase (CAT) Fluorometric Activity Assay Kit		
REF	**Cat #:** KTB9040	LOT	**Lot #:** Refer to product label
	Detection range: 0.01-10 U/mL		**Sensitivity:** 0.01 U/mL
	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 Catalase (CAT) Fluorometric Activity Assay Kit can detect animal and plant tissues, cells, serum, plasma and other samples. The principle involves the catalase (CAT) in the detection system breaking down H_2O_2 into water and oxygen. The residual hydrogen peroxide, in the presence of an enzyme and a fluorescent substance, undergoes a reaction where its fluorescence intensity at an excitation wavelength of 535 nm and an emission wavelength of 587 nm is directly proportional to the concentration of hydrogen peroxide.

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	15 μL	30 μL	-20℃, protected from light
Reagent Ⅲ	20 μL	40 μL	-20℃, protected from light
Standard (1 M)	0.4 mL	0.4 mL	-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.

H_2O_2 ReagentⅢ: Prepared before use; take 5 μL of Reagent Ⅲ and add it to 4.995 mL of deionized water, mix well. Then, take 10 μL of the diluted Reagent Ⅲ and combine it with 2.93 mL of Assay Buffer, mixing thoroughly. This solution should be prepared freshly as needed and in the required amount, should be protected from light during use.

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 (1 M): Before use, dilute the standard 10,000 times with deionized water to obtain a 0.1 mM solution, ensuring complete dissolution for later use. Any unused Standard (1 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 0.1 mM Standard (μL)	Volume of 1×Assay Buffer (μL)	Standard Concentration (μM)
Std.1	100	100	50
Std.2	80	120	40
Std.3	40	160	20
Std.4	20	180	10
Std.5	10	190	5
Std.6	5	195	2.5
Std.7	2.5	197.5	1.25
0 (Blank Well)	0	200	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).

Sample	Dilution Fold	Sample	Dilution Fold

10% Mouse Brain	30-60	10% Mouse Lung	150-300
FBS	2-10	293 cells	4-10
Human Saliva	30-60	L929 Cell Supernatan	Undiluted
Human Urine	30-50	10% Tobacco Leaf	2-6

2. 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 to 37℃. 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)	Control Well (μL)	Standard Well (μL)
Sample	25	0	0
Standard	0	0	25
Deionized water	0	0	25
H_2O_2 Reagent III	25	25	0
On the microplate shaker, shake for 10 s, then allow the reaction to proceed at 37℃ for 5 min.			
Working Reagent	50	50	50
Sample	0	25	0
Mix well and let stand at room temperature in the dark for 10 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}, $RFU_{Control}$, $RFU_{Standard}$, and RFU_{Blank}. Calculate $\Delta RFU_{Test}=RFU_{Control}-RFU_{Test}$, 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 CAT activity:

（1）Calculated by sample protein concentration:

Active unit definition: Under 37℃ conditions, one unit of activity is defined as the amount that decomposes 1 nmoL of H_2O_2 per min per mg of protein.

CAT(U/mg prot)**=x×f÷5÷Cpr**

（2）Calculated by fresh weight of samples:

Active unit definition: Under 37℃ conditions, one unit of activity is defined as the amount that decomposes 1 nmoL of H_2O_2 per min per g of tissue.

CAT(U/g fresh weight)**=x×f÷5÷W**

（3）Calculated by cells:

Active unit definition: Under 37℃ conditions, one unit of activity is defined as the amount that decomposes 1 nmoL of H_2O_2 per min per 10^4 of cells.

CAT(U/10^4 cell)**=x×f÷5÷N**

（4）Calculated by volume of liquid samples:

Active unit definition: Under 37℃ conditions, one unit of activity is defined as the amount that decomposes 1 nmoL of H_2O_2 per min per mL of sample.

CAT(U/mL)**=x×f÷5**

Cpr: Sample protein concentration, mg/mL; 5: Reaction time, 5 min; f: Sample dilution fold; W: Sample weight, g; N: The total number of cells, 10^4.

Typical Data

Typical standard curve-data:

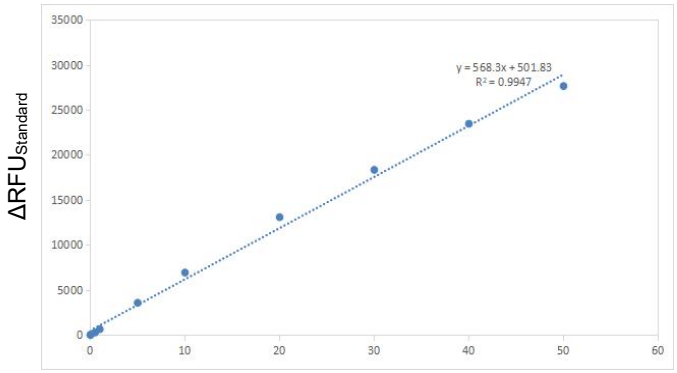

Figure 1. Standard Curve for CAT.

Example:

1. Take 0.16 g of mouse brain tissue and add 1 mL of Assay Buffer to homogenize and grind it. After centrifugation, take the supernatant, dilute it 50 times, and proceed with the measurement steps. Using a full-black 96-well plate, the following readings were obtained: RFU_{Test} is 10,395, $RFU_{Control}$ is 16,600, and RFU_{Blank} is 305. Thus, ΔRFU_{Test} =16,600-10,395=6,205. With the standard curve equation being y=568.3x+501.83, the H_2O_2 concentration calculated is 10.04 μM. Therefore, CAT (sample)=10.04 ×50÷5÷0.16=627.5 U/g fresh weight.

2. For fetal bovine serum, after centrifugation and taking the supernatant, dilute it 5 times before continuing with the assay procedures. Using a full-black 96-well plate, the following readings were obtained: RFU_{Test} is 6,380, $RFU_{Control}$ is 13,390, and RFU_{Blank} is 305. Thus, ΔRFU_{Test}=13,390-6,380=7,010. With the standard curve equation being y=568.3x+501.83, the H_2O_2 concentration calculated is 11.45 μM. Therefore, CAT (sample)=11.45×5÷5=11.45 U/mL.

Recommended Products

Catalog No.	Product Name
KTB9050	CheKine™ Pro Malondialdehyde (MDA) Fluorometric Assay Kit
KTB9300	CheKine™ Pro Glucose Fluorometric Activity Assay Kit
KTB9041	CheKine™ Pro Hydrogen Peroxide (H_2O_2) Fluorometric Assay Kit

Disclaimer

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