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CheKine™ Catalase (CAT) Activity Assay Kit (Ammonium Molybdate-Chromogenic Method)

Cat #: KTB1042 Size: 48 T/48 S 96 T/96 S

	Catalase (CAT) Activity Assay Kit (Ammonium Molybdate-Chromogenic Method)		
REF	**Cat #:** KTB1042	LOT	**Lot #:** Refer to product label
	Detection range: 3-195 mM		**Sensitivity:** 3 mM
	Applicable sample: Animal and Plant tissues, Cells or Bacteria, Serum (Plasma)		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Catalase (CAT, EC 1.11.1.6) is a common antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen. It is widely present in aerobic cells containing cytochrome systems. Hydrogen peroxide is highly toxic to cells; its accumulation can lead to oxidative damage of cellular targets such as DNA, proteins, and lipids, resulting in mutagenesis and cell death. By removing hydrogen peroxide (H_2O_2 from cells, catalase protects against oxidative damage. The role of catalase in oxidative stress-related diseases has been extensively studied. Catalase also exhibits peroxidatic activity, in which low-molecular-weight alcohols can serve as electron donors. Fatty alcohols are specific substrates for catalase, whereas other enzymes with peroxidatic activity do not utilize these substrates. CheKine™ Catalase (CAT) Activity Assay Kit (Ammonium Molybdate-Chromogenic Method) provides a simple, convenient, and rapid approach for measuring CAT Activity, suitable for animal and plant tissues, cells or bacteria, serum (plasma) samples. The assay is based on the oxidation of MoO_5^{2-} by hydrogen peroxide (H_2O_2) to form MoO_5^{2-}. MoO_5^{2-} then accepts electrons from hydroxide ions to form bonds, followed by immediate intermolecular dehydration and condensation, yielding a stable yellow complex $(H_2MoO_4 \cdot XH_2O)_n$. This product exhibits a strong absorption peak at 405 nm, and its absorbance is linearly correlated with the H_2O_2 concentration. By measuring the absorbance of residual H_2O_2 in the system at 405 nm, the catalytic activity of CAT (catalase) can be determined.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	70 mL	70 mL×2	4℃
Reagent Ⅰ	5 mL	10 mL	4℃, protected from light
Reagent Ⅱ	15 mL	30 mL	RT
Reagent Ⅲ	Powder×1 vial	Powder×2 vials	4℃, 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

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 405 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge, ice maker
- Deionized water, PBS (pH 7.4)
- Homogenizer (for tissue samples)

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℃, protected from light.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. If crystals precipitate, dissolve by heating and stirring at 37℃. Store at room temperature.

Working ReagentⅢ: Prepared before use. Add 40 mL of deionized water to one bottle of ReagentⅢ. If dissolution is difficult, heat and stir at 40℃ to facilitate solubilization. Store protected from light at 4℃ for up to one week.

Standard: Take 0.5 mL of Reagent Ⅰ as the standard stock solution, which gives a final concentration of 13.2 μmol/mL when added to the reaction system, and dilute the standard according to the table below:

Num.	Standard Volume (μL)	Deionized Water (μL)	Final Concentration (μmol/mL)
1	200 μL 13.2 μmol/mL	0	13.2
2	100 μL 13.2 μmol/mL	100	6.6
3	100 μL 6.6 μmol/mL	100	3.3
4	100 μL 3.3 μmol/mL	100	1.65
5	100 μL 1.65 μmol/mL	100	0.825
6	100 μL 0.825 μmol/mL	100	0.413
7	100 μL 0.413 μmol/mL	100	0.206
Blank	0	100	0

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Animal and plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize 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 or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, centrifuge at 800 g for 2 min, then discard the supernatant; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 30% 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.
3. Serum (Plasma): Test directly.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 405 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are operated in a 1.5 mL EP Tube):

Reagent	Standard Tube (μL)	Control Tube (μL)	Test Tube (μL)
Sample	0	0	50
Extraction Buffer	50	0	0
Reagent Ⅰ	0	30	30
Standard	30	0	0
Mix well and incubate at 37℃ for 10 min			
Reagent Ⅱ	100	100	100
Working Reagent Ⅲ	265	265	265
Sample	0	50	0

3. After mixing, transfer 200 μL to a 96-well plate and immediately measure the absorbance at 405 nm, and record the values as A_{Blank}, $A_{Standard}$, $A_{Controll}$ and A_{Test}. Calculate $\Delta A_{Test}=A_{Control}-A_{Test}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank and standard curve need only be run 1-2 times, a control tube must be set up for each assay tube. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.07, the sample volume can be appropriately increased. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor.

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. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is substituted into the equation to get y (μmol/mL).

2. Calculation of the CAT activity:

(1) Calculated by fresh weight of samples:

Definition of enzyme unit: One unit of enzyme activity is defined as the amount that catalyzes the degradation of 1 μmol of H_2O_2 per minute per g of tissue.

CAT (μg/g fresh weight)=$y\times V_{Total}\div(W\times V_{Sample}\div V_{Total\ Sample})\div T\times n$**$=0.89\times y\div W\times n$**

(2) Calculated by cell or bacteria number:

Definition of enzyme unit: One unit of enzyme activity is defined as the amount that catalyzes the degradation of 1 μmol of H_2O_2 per minute per 10^4 of cells or bacteria.

CAT ($U/10^4$)=$y\times V_{Total}\div(500\times V_{Sample}\div V_{Total\ Sample})\div T\times n$**$=0.00178\times y\times n$**

(3) Calculated by serum (plasma) volume:

Definition of enzyme unit: One unit of enzyme activity is defined as the amount that catalyzes the degradation of 1 μmol of H_2O_2 per minute per mL of serum (plasma).

CAT (U/mL)=$y\times V_{Total}\div V_{Sample}\div T\times n$**$=0.89\times y\times n$**

(4) Calculated by protein concentration

Definition of enzyme unit: One unit of enzyme activity is defined as the amount that catalyzes the degradation of 1 μmol of H_2O_2 per minute per mg of protein.

CAT (U/mg)=$y\times V_{Total}\div(V_{Sample}\times Cpr)\div T\times n$**$=0.89\times y\div Cpr\times n$**

V_{Total}: total reaction volume, 0.445 mL; V_{Sample}: sample volume added, 0.05 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 10 min; Cpr; sample protein concentration, mg/mL; W: sample weight, g; n: dilution factor; 500: Total number of cells or bacteria, 10^4; Cpr; sample protein concentration, mg/mL.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

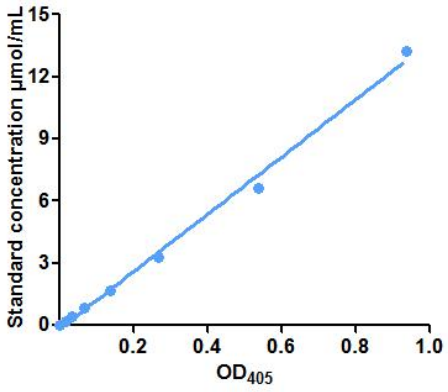

Figure 1. H_2O_2 standard curve

Recommended Products

Catalog No.	Product Name
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1510	CheKine™ Micro Uric Acid (UA) Assay Kit
KTB1400	CheKine™ Micro Nitric Oxide (NO) Assay Kit
KTB1690	CheKine™ Micro Gamma-Glutamyl Transpeptidase (GGT) Activity Assay Kit
KTB1100	CheKine™ Micro Lactate 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.