（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K031-M
Specification：48T(16 samples)/96T(40 samples)/500 assays(242 samples)
Measuring instrument：Microplate reader(400-410 nm)
Detection range：1.12-150 U/mL

Elabscience® Catalase (CAT) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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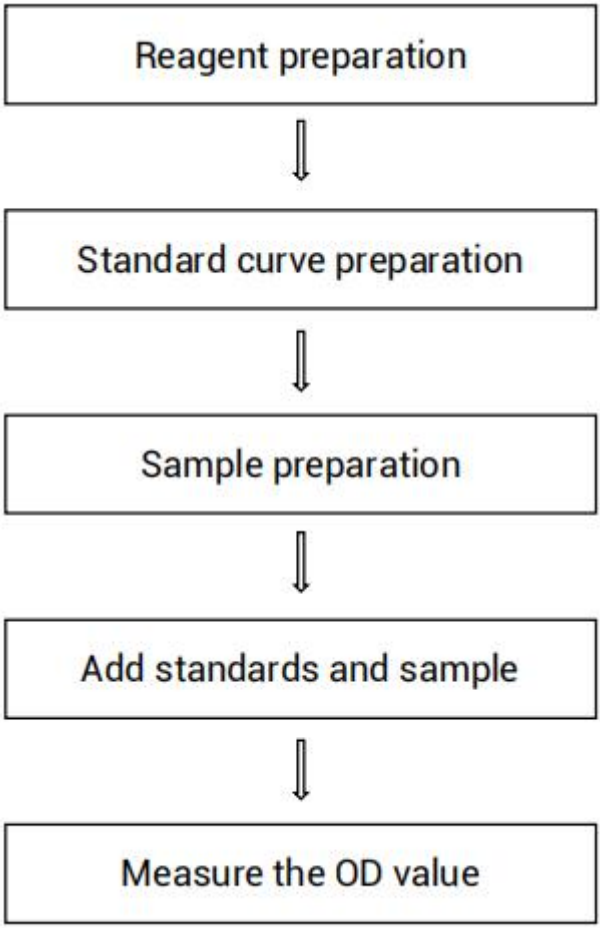
Assay summary



Intended use

This kit can be used to measure catalase (CAT) activity in serum, plasma, cells, cell culture supernatant and tissue homogenate samples.

Detection principle

Catalase (CAT) can catalyze the decomposition of H_2O_2 into oxygen and water, and this reaction is quickly stopped upon the addition of ammonium molybdate. The residual H_2O_2 reacts with ammonium molybdate to generate a yellowish complex. CAT activity can be calculated by production of the yellowish complex at 405 nm.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 assays)	Storage
Reagent 1	Buffer Solution	12 mL × 1 vial	24 mL × 1 vial	60 mL × 2 vials	2-8℃, 12 months
Reagent 2	Substrate	1.5 mL × 1 vial	1.5 mL × 2 vials	15 mL × 1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent	Powder ×1 vial	Powder ×1 vial	Powder ×5 vials	2-8℃, 12 months
Reagent 4	Clarificant	1.5 mL × 1 vial	1.5 mL × 2 vials	15 mL × 1 vial	2-8℃, 12 months
Reagent 5	9.6 mol/L H_2O_2 Standard Solution	1.5 mL × 1 vial	1.5 mL × 2 vials	15 mL × 1 vial	2-8℃, 12 months shading light
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces			
	Sample Layout Sheet	1 piece			

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (400-410 nm), Micropipettor, Vortex Mixer, Incubator

Reagents:

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

Reagent preparation

① Incubate buffer solution and substrate at 37℃ for 10 min before use.

② The preparation of chromogenic application solution：
Dissolve one vial of chromogenic agent with 24 mL of double distilled water. (If there is sediment in the bottom, please directly take the supernatant for test, it will not affect the result). Store at 4°C for 3 months.

③ Clarificant will be frozen when cold, please warm it in 37℃ water-bath until clear.

④ The preparation of 1 mmol/mL H_2O_2 standard solution：
Before testing, please prepare sufficient 1 mmol/mL H_2O_2 standard solution according to the test wells. For example, prepare 384 μL of 1 mmol/mL H_2O_2 standard solution (mix well 40 μL 9.6 mol/L H_2O_2 standard solution with 344 μL double distilled water).

⑤ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 1 mmol/mL H_2O_2 standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 50, 60, 100 μmol/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/mL)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**100**
1 mmol/mL H2O2 standard(μL)	0	10	20	30	40	50	60	100
Double distilled water (μL)	1000	990	980	970	960	950	940	900

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M is recommended for animal tissue samples. E-BC-K168-M is recommended for plant tissue samples).

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
293T supernatant	1
10% Rat heart tissue homogenization	50-100
10% Rat liver tissue homogenization	100-200
10% Rat spleen tissue homogenization	50-100
Mouse serum	1
10% Epipremnum aureum tissue homogenization	1-2
10% Rat lung tissue homogenization	50-100
10% Rat kidney tissue homogenization	50-100
10% Rat brain tissue homogenization	20-50

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① The reaction time must be accurate when substrate is added.
② The test tube can be prepared and labeled in advance.
③ Dilute the samples to the optimal concentration for detection if the CAT activity of samples exceed the detection range.
④ Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

Operating steps

For standard curve:

① Add 20 μL of H_2O_2 standard solution with different concentrations to the 1.5 mL EP tubes respectively.

② Sequentially add 200 μL of buffer solution, 20 μL of double distilled water, 200 μL of chromogenic agent application solution and 20 μL of clarificant, mix well.

③ Stand at room temperature for 10 min and take 200 μL of reaction solution to the microplate.

④ Measure the OD value at 405 nm with microplate reader.

For samples:

① Control tube: add 200 μL of buffer solution into the 1.5 mL EP tubes.
Sample tube: 20 μL of sample and 200 μL of buffer solution into the 1.5 mL EP tubes.

② Incubate at 37℃ for 5 min.

③ Add 20 μL of substrate into each tube, mix fully and react at 37℃ for 1 min accurately.

④ Control tube: add 200 μL of chromogenic application solution, 20 μL of clarificant and 20 μL of sample, mix well.
Sample tube: 200 μL of chromogenic application solution and 20 μL of clarificant, mix well.

⑤ Stand at room temperature for 10 min and take 200 μL of reaction solution to the microplate.

⑥ Measure the OD value at 405 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

Definition: The amount of CAT in 1 mL of serum or plasma that decompose 1 μmol H_2O_2 per minute at 37℃ is defined as 1 unit.

$$\text{CAT activity (U/mL)} = \frac{\Delta A}{a} \times \frac{0.02^*}{1^* \times V} \times f$$

2. Tissue and cells sample:

Definition: The amount of CAT in 1 mg of tissue protein that decompose 1 μmol H_2O_2 per minute at 37℃ is defined as 1 unit.

$$\text{CAT activity (U/mgprot)} = \frac{\Delta A}{a} \times \frac{0.02^*}{1^* \times V} \times f \div C_{pr}$$

[Note]

0.02*: The volume of standard, 0.02 mL.

1*: The reaction time, 1 min.

ΔA: $OD_{Control}$ - OD_{Sample}.

V: The volume of sample, mL.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, mgprot/mL.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	3.70	26.40	78.60
%CV	4.2	3.8	3.7

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	3.70	26.40	78.60
%CV	7.4	7.9	7.8

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/mL)	15	36	54
Observed Conc. (μmol/mL)	15.2	34.9	55.1
Recovery rate (%)	101	97	102

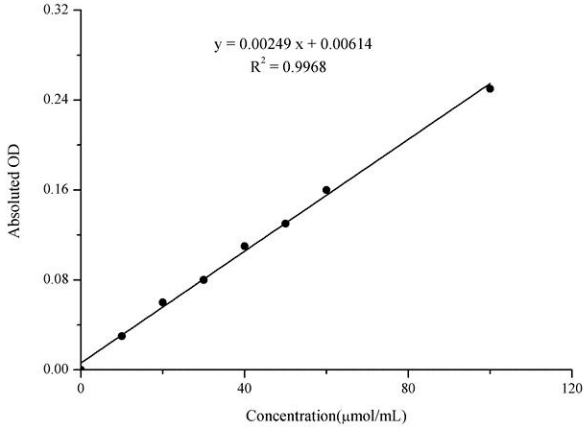
Sensitivity

The analytical sensitivity of the assay is 1.12 U/mL CAT. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μmol/mL)	**0**	**10**	**20**	**40**	**50**	**60**	**80**	**100**
Average OD	0.062	0.087	0.118	0.143	0.169	0.192	0.219	0.311
Absoluted OD	0.000	0.025	0.056	0.081	0.107	0.130	0.157	0.249



Appendix Π Example Analysis

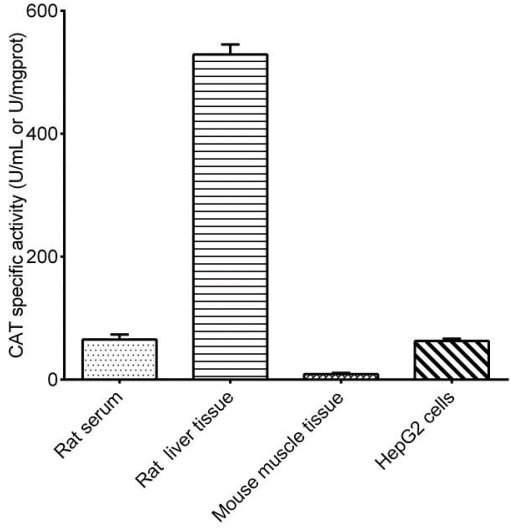
Example analysis：

Dilute 10% rat liver tissue homogenate with PBS (0.01 M, pH 7.4) for 100 times, take 0.02 mL of diluted sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y =0.0026 x + 0.0022, the average OD value of the sample is 0.442, the average OD value of the control is 0.612, the concentration of protein in sample is 12.38 gprot/L, and the calculation result is:

$$\text{CAT activity (U/mgprot)} = (0.612 - 0.442) \div 0.0026 \times 100 \div 12.38 = 528.15 \text{ U/mgprot}$$

Detect rat serum (dilute for 2 times), 10% rat liver tissue homogenate (the concentration of protein in sample is 12.38 gprot/L, dilute for 100 times), 10% mouse muscle tissue homogenate (the concentration of protein in sample is 3.10 gprot/L) and HepG2 cells (the concentration of protein in sample is 8.06 gprot/L, dilute for 10 times) according to the protocol, the result is as follows：



Appendix III Publications

1. Zhang H , Feng Y , Si Y ,et al. Corrigendum to "Shank3 ameliorates neuronal injury after cerebral ischemia/reperfusion via inhibiting oxidative stress and inflammation". 2024, 69 102983. Redox Biology, 2024, 78: 103432. DOI: 10.1016/j.redox.2024.103432.
2. Hou B , Wang D , Yan F ,et al. Fhb7-GST catalyzed glutathionylation effectively detoxifies the trichothecene family [J]. Food Chemistry, 2024(May 1): 439. DOI: 10.1016/j.foodchem.2023.138057.
3. Ma L ,Li Ma Li MaSchool of Design, Shanghai Jiao Tong University, Shanghai , People's Republic of ChinaR&D Center for Aromatic Plants, Shanghai Jiao Tong University, Shanghai , People's Republic of ChinaMore by Li Ma,,et al.Artemisia sieversiana Ehrhart ex Willd. Essential Oil and Its Main Component, Chamazulene: Their Photoprotective Effect against UVB-Induced Cellular Damage and Potential as Novel Natural Sunscreen Additives[J].ACS Sustainable Chemistry & Engineering, 2023(50):11.
4. Iannetta A , Zugaro S , Massimini M ,et al.Combined effects of glyphosate and chemical hypoxia in zebrafish: A new toxicological point of view[J].Chemosphere, 2024, 366.DOI:10.1016/j.chemosphere.2024.143484.
5. Fu M , Jiang X H , Wang M ,et al.Catalase catalyzed tannic acid-Fe3+ network coating: A theranostic strategy for intestinal barrier restoration[J].International Journal of Biological Macromolecules: Structure, Function and Interactions, 2024(Pt.2):274.DOI:10.1016/j.ijbiomac.2024.133304.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and not be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.