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

Catalog No：E-BC-K031-S

Specification：50 Assays(25 samples)/ 100 Assays(50 samples)

Measuring instrument：Spectrophotometer(405 nm)

Detection range：0.27-155.4 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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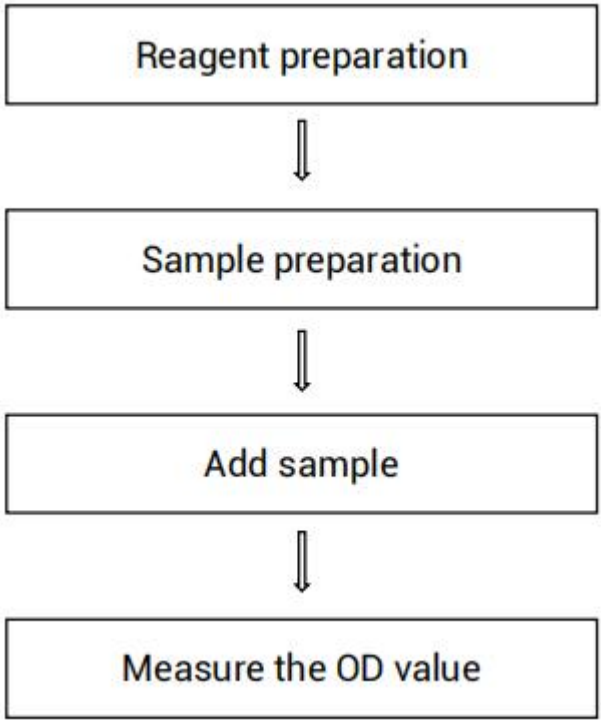
Assay summary



Intended use

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

Detection principle

The reaction that catalase (CAT) decomposes H_2O_2 can be quickly stopped by 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 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	60 mL × 2 vials	2-8℃, 12 months
Reagent 2	Substrate	12 mL × 1 vial	12 mL × 1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months
Reagent 4	Clarificant	6 mL × 1 vial	12 mL × 1 vial	2-8℃, 12 months

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:

Spectrophotometer (405 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 60 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 2-8°C for 3 months.

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

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).

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 300 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	The volume of sample
10% Rat liver tissue homogenization	25-50	50 μL
10% Rat kidney tissue homogenization	10-25	50 μL
10% Rat brain tissue homogenization	5-10	50 μL
Human serum	1	100 μL
293T supernatant	1	100 μL

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 test tube can be prepared and labeled in advance. After incubating at 37 ℃ for 10 min, add samples and buffer solution, then incubate the test tube at 37 ℃ for 5 min.

② Dilute the samples to the optimal concentration for detection if the CAT activity of samples exceed the detection range.

③ The reaction time must be accurate when substrate is added.

Operating steps

① Control tube: Add 1 mL of buffer solution into the 5 mL EP tubes.
Sample tube: Add a* mL of sample and 1 mL of buffer solution into the 5 mL EP tubes.

② Incubate at 37℃ for 5 min.

③ Add 0.1 mL of substrate into each tube, mix well and react at 37℃ for 1 min accurately.

④ Control tube: Add 1 mL of chromogenic application solution, 0.1 mL of clarificant and a* mL of sample, mix well.
Sample tube: Add 1 mL of chromogenic application solution and 0.1 mL of clarificant, mix well

⑤ Stand for 10 min at room temperature. Set to zero with double distilled water and measure the OD values of each tube at 405 nm with 0.5 cm optical path cuvette.

[Note]: For serum or plasma samples, a* is 0.1 mL. For the cell homogenate and tissue homogenate, a* is 0.05 mL.

Calculation

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.

$$\text{CAT activity (U/mL)} = \frac{\Delta A \times 32.5^*}{1^* \times V} \times f$$

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.

$$\text{CAT activity (U/mgprot)} = \frac{\Delta A \times 32.5^*}{1^* \times V} \times f \div C_{pr}$$

[Note]

*32.5: reciprocal of slope

1: Reaction time

ΔA: Absolute OD ($OD_{Control}$ – OD_{Sample}).

V: Volume of sample, mL.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L

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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)	3.50	34.50	88.50
%CV	3.6	3.2	2.5

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)	3.50	34.50	88.50
%CV	5.7	4.9	4.7

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 96%.

	Sample 1	Sample 2	Sample 3
Expected Conc.(U/mL)	19	77	106
Observed Conc. (U/mL)	18.4	73.2	101.8
Recovery rate (%)	97	95	96

Sensitivity

The analytical sensitivity of the assay is 0.27 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.

Appendix Π Example Analysis

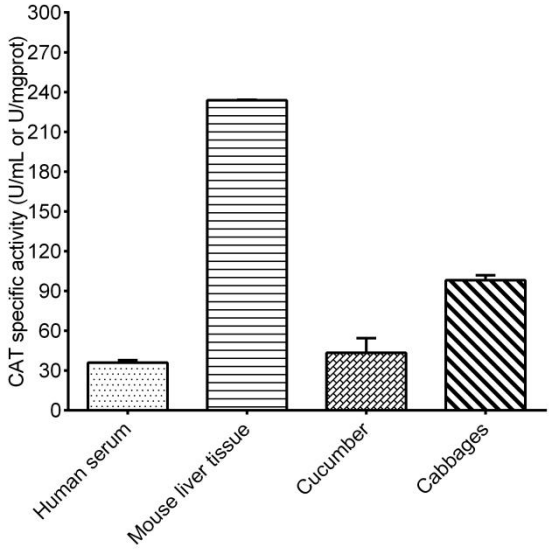
Example analysis：

Take 0.05 mL of 10% cabbage leaves tissue homogenate, carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.351, the average OD value of the control is 0.602, the concentration of protein in sample is 1.64 mgprot/mL, and the calculation result is:

$$\text{CAT activity (U/mgprot)} = \frac{(0.602 - 0.351) \times 32.5}{1 \times 0.05} \div 1.64 = 99.44 \text{ U/mgprot}$$

Detect human serum (V=0.1 mL), 2% mouse liver tissue homogenate (the concentration of protein in sample is 1.82 mgprot/mL, V=0.05 mL), 20% cucumber tissue homogenate (the concentration of protein in sample is 0.91 mgprot/mL, V=0.05 mL) and 10% cabbage tissue homogenate (the concentration of protein in sample is 1.64 mgprot/mL, V=0.05 mL) according to the protocol, the result is as follows：



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.