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

Catalog No：E-BC-F006

Specification：96T(40 samples)/ 500Assays(242 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.01-6.51 U/L

Elabscience® Catalase (CAT) Activity

Fluorometric 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
Tel: 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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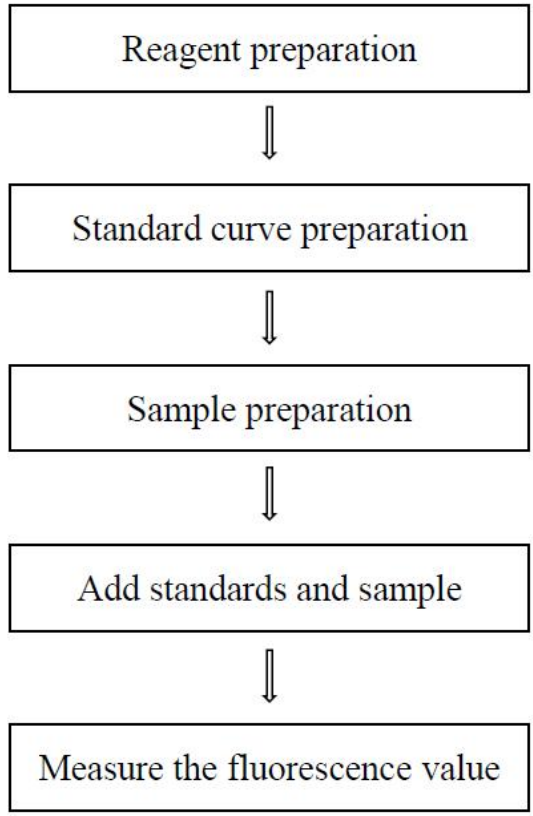
Assay summary



Intended use

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

Detection principle

Catalase can decompose H_2O_2 to generate H_2O and O_2, the residual H_2O_2 in the detection system react with the fluorescent substance, and the content of residual H_2O_2 is proportional to the fluorescence intensity at the excitation wavelength of 535 nm and emission wavelength of 587 nm, the catalase activity is inversely proportional to the fluorescence intensity.

Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500Assays)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	60 mL × 5 vials	-20°C, 12 months
Reagent 2	Substrate	0.1 mL × 1 vial	0.5 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Probe Solution	0.12 mL × 1 vial	0.6 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Enzyme Reagent	Powder × 1 vial	Powder × 5 vials	-20°C, 12 months shading light
Reagent 5	1 mol/L H_2O_2 Standard Solution	0.4 mL × 1 vial	2 mL × 1 vial	-20°C, 12 months shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Vortex mixer, Centrifuge

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate application solution:

Before testing, please prepare sufficient substrate application solution according to the test wells. For example, prepare 10 mL of substrate application solution (mix well 1 μL of substrate and 10 mL of double distilled water). The substrate application solution should be prepared on spot.

③ The preparation of enzyme application solution:

Dissolve one vial of enzyme reagent with 120 μL of buffer solution, mix well to dissolve. Store at -20°C for 1 month protected from light.

④ The preparation of chromogenic agent:

For each well, prepare 50 uL of chromogenic agent (add 48 μL of buffer solution, 1 μL of probe solution and 1 μL of enzyme application solution, mix well.) The chromogenic agent should be prepared on spot and protected from light.

⑤ The preparation of 100 μmol/L H_2O_2 solution:

Dilute 1 μL of 1 mol/L H_2O_2 standard solution with 10 mL of double distilled water, mix well to dissolve.

⑥ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μmol/L H_2O_2 solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 5, 10, 20,

30, 40,50 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**1**	**5**	**10**	**20**	**30**	**40**	**50**
100 μmol/L standard (μL)	0	5	25	50	100	150	200	250
Double distilled water (μL)	500	495	475	450	400	350	300	250

Sample preparation

① Sample preparation

Serum, plasma and other liquid sample: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C 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 buffer solution with a dounce homogenizer at 4°C

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

⑥ 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
Mouse serum	60-80
Mouse plasma	60-80
Rat serum	70-80
Human saliva	30-50
Rat urine	30-50
10% Mouse liver tissue homogenate	2500-3000
10% Mouse lung tissue homogenate	200-400
10% Rat muscle tissue homogenate	100-200
10% Rat brain tissue homogenate	40-50
10% Mouse kidney tissue homogenate	2000-2500

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

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

Operating steps

① Standard well: add 25 μL of standard with different concentrations into the well.

Sample well: add 25 μL of sample into the well.

Control well: add 25 μL of substrate working solution into the well.

② Add 25 μL of double distilled water into standard well. Add 25 μL of substrate application solution into sample well.

③ Mix fully with microplate reader for 10 s and incubate at 37°C for 5 min.

④ Add 50 μL of chromogenic agent into each wells.

⑤ Add 25 μL of sample into control well.

⑥ Mix fully with microplate reader for 10 s and stand at room temperature for 10 min. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted fluorescence 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 L of serum or plasma that decompose 1 μmol H_2O_2 per minute at 37°C is defined as 1 unit.

$$\text{CAT activity (U/L)} = (\Delta F - b) \div a \div 5 \times f$$

2. Tissue sample:

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

$$\text{CAT activity (U/gprot)} = (\Delta F - b) \div a \div 5 \times \div C_{pr}$$

[Note]

ΔF: The absolute fluorescence value of sample, $F_{Control} - F_{Sample}$..

5: the reaction time, 5 min.

f: Dilution factor of sample before tested.

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

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/L)	0.80	3.00	5.40
%CV	4.2	3.5	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/L)	0.80	3.00	5.40
%CV	7.5	6.0	6.9

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 92%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	3	25	38
Observed Conc.(μmol/L)	2.7	23.3	35.3
Recovery rate (%)	90	93	93

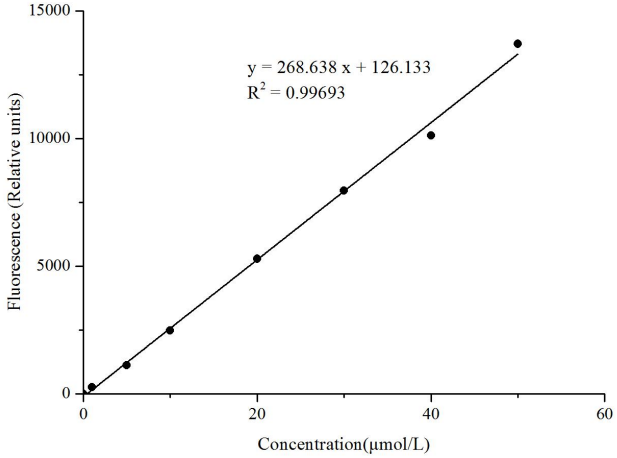
Sensitivity

The analytical sensitivity of the assay is 0.01 U/L. 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/L)	0	1	5	10	20	30	40	50
Fluorescence value	102	351	1209	2542	5341	7984	10023	13847
	101	351	1229	2603	5434	8133	10396	13778
Average fluorescence value	102	351	1219	2572	5387	8058	10209	13812
Absoluted fluorescence value	0	249	1117	2471	5286	7957	10108	13711



Appendix II Example Analysis

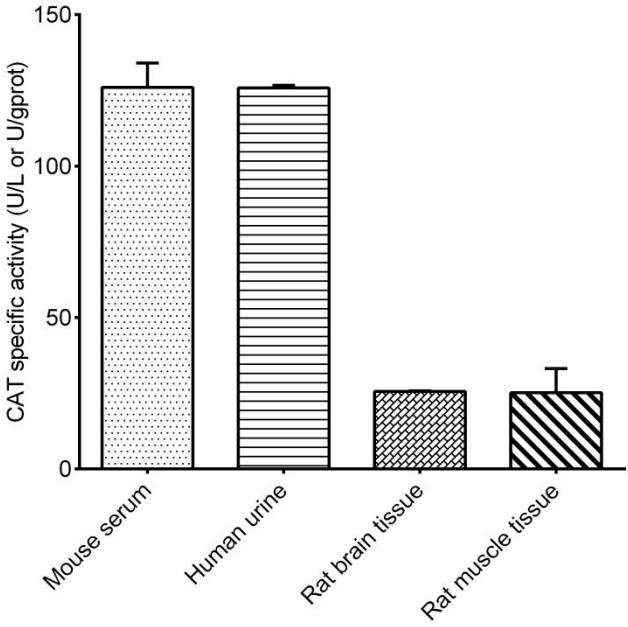
Example analysis：

For Mouse serum, dilute mouse serum with buffer solution for 70 times, take 25 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y =268.77 x -55.205, the average fluorescence value of the sample is 8120, the average fluorescence value of the control is 10483, and the calculation result is:

$$\text{CAT activity (U/L)} = (10483 - 8120 + 55.205) \div 268.77 \div 5 \times 70 = 125.96 \text{ U/L}$$

Detect mouse serum (dilute for 70 times), human urine (dilute for 30 times), 10% rat brain tissue homogenate (the concentration of protein is 6.08 gprot/L dilute for 50 times), 10% rat muscle tissue homogenate (the concentration of protein is 6.73 gprot/L dilute for 400 times) according to the protocol, the result is as follows:



Appendix III Publications

1. Ye H Y, Shang Z Z, Gao X, et al. Dendrobium huoshanense stem polysaccharide exhibits gastroprotective effect via regulating PI3K/AKT, NF-κB and Nrf-2 signaling in high-salt diet-induced gastritis mice[J]. Food Bioscience, 2024, 62: 105309.
2. Wang Y, Zhao X, Wang J, et al. Norisoboldine Reduces Arthritis Severity by Attenuating Inflammation, Oxidative Stress, and Extracellular Matrix Degradation in a Rat Model of Rheumatoid Arthritis[J]. Journal of Inflammation Research, 2024: 8839-8852.
3. Zhang J, Xu L, Zhang K, et al. Synergistic effect of fosfomycin and colistin against KPC-producing Klebsiella pneumonia e: pharmacokinetics-pharmacodynamics combined with transcriptomic approach[J]. BMC microbiology, 2024, 24(1): 430.
4. Chen C, Chen H, Dingda D, et al. The primary studies of epigallocatechin-3-gallate in improving brain injury induced by chronic high-altitude natural environment in rats by 7.0 T high-field MR imaging[J]. Archives of Biochemistry and Biophysics, 2025, 764: 110224.
5. Maleki M, Tabnak P, Golchin A, et al. Resveratrol inhibited colorectal cancer progression by reducing oxidative DNA damage by targeting the JNK signaling pathway[J]. Heliyon, 2024, 10(21).
6. Sandhiutami N M D, Desmiaty Y, Pitaloka P D U, et al. The protective effect of hydroalcoholic Citrus aurantifolia peel extract against doxorubicin-induced nephrotoxicity[J]. Research in Pharmaceutical Sciences, 2024, 19(5): 591-605.

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.