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

Catalog No：E-BC-K102-S

Specification：50Assays(48 samples)/100Assays(96 samples)

Measuring instrument：Spectrophotometer（402-407 nm）

Detection range：1.5-150 mmol/L

Elabscience® Hydrogen Peroxide (H_2O_2) Colorimetric 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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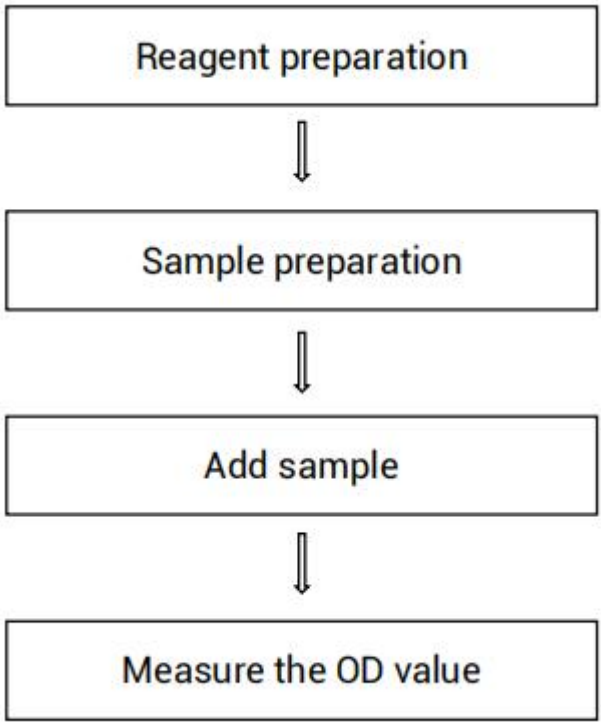
Assay summary



Intended use

This kit can be used to measure the H_2O_2 content in serum, plasma, urine, tissue and cells samples.

Detection principle

Hydrogen peroxide (H_2O_2) is a metabolic by-product of reactive oxygen species, which is not only a signal molecule in cells, but also a source of oxidative stress. H_2O_2 is an important regulatory factor of eukaryotic signal transduction involved in cell proliferation, differentiation and migration. However, abnormal H_2O_2 can lead to oxidative cell damage and disease, such as cancer, atherosclerosis, osteoporosis and neurodegenerative diseases.

Kit components & storage

Item	Component	Size 1 (50Assays)	Size 2 (100Assays)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months
Reagent 2	Ammonium Molybdate Reagent	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months
Reagent 3	1 mol/L H_2O_2 Standard	12 mL × 1 vial	12 mL ×1 vial	2-8°C, 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 (402-407 nm, optimum wavelength: 405 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Reagents:

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

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② If precipitation occurs in buffer solution. it can be redissolved by heating in 80°C water bath before use.

③ The preparation of 60 mmol/L H_2O_2 standard solution:

For each well, prepare 100 μL of 60 mmol/L H_2O_2 standard solution (mix well 6 μL of 1 mol/L H_2O_2 standard and 94 μL of double distilled water).

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°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 PBS (0.01 M, pH 7.4) 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.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-M, E-BC-K165-S).

Cell (adherent or suspension) samples:

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 300-500 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 min 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, E-BC-K168-M, E-BC-K165-S).

② Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenization	5-10
10% Green pepper tissue homogenization	1
Human serum	1
Rat serum	1
Mouse serum	1

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 Preheat the reagent 1 at 37°C for 10 min. Dissolve the reagent 2 in 60°C water bath if crystallized.

② If the concentration of H_2O_2 in the sample is too high, please dilute the samples appropriately. If the concentration is too low, the sampling volume of the sample should be increased, and the sampling volume of standard and double distilled water should be increased equally at the same time.

Operating steps

① Add 1 mL of buffer solution to 5 mL EP tubes and incubate the tubes in 37°C for 10 min.

② Blank tube: Add 0.1 mL of double distilled water to the tube.

Standard tube: Add 0.1 mL of 60 mmol/L H_2 O_2 standard solution to the tube.

Sample tube: Add 0.1 mL of sample to the tube.

③ Add 1 mL of ammonium molybdate reagent to each tube of Step 2 and mix fully.

④ Set the spectrophotometer to zero with double-distilled water, then measure the OD value of each tube at 405 nm with 1 cm optical path quartz cuvette.

Calculation

The sample:

1. Serum (plasma) sample and other liquid samples:

$$\frac{H_2O_2\,\text{content}}{(\text{mmol/L})} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

2. Tissue and cell samples:

$$\frac{H_2O_2\,\text{content}}{(\text{mmol/ gprot})} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: The concentration of H_2O_2 standard, 60 mmol/L.

f: The dilution factor of sample before test.

C_{pr}: The 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 (mmol/L)	8.50	66.40	98.50
%CV	1.5	1.2	1.2

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 (mmol/L)	8.50	66.40	98.50
%CV	2.5	3.0	2.6

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 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	18.4	86.1	134.5
Observed Conc. (mmol/L)	18.2	85.2	129.1
Recovery rate (%)	99	99	96

Sensitivity

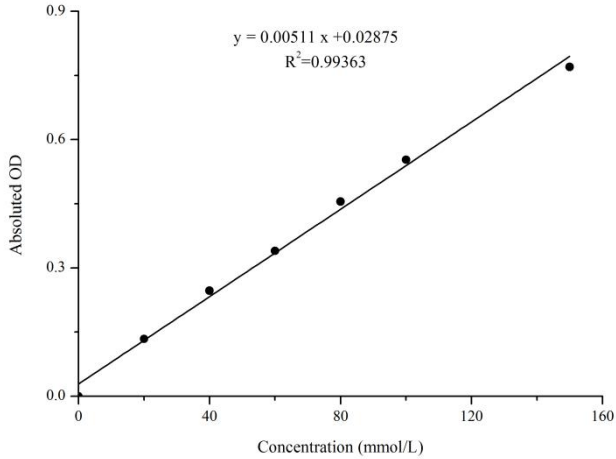
The analytical sensitivity of the assay is 1.5 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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 (mmol/L)	0	20	40	60	80	100	150
OD value	0.055	0.190	0.304	0.399	0.511	0.608	0.822
	0.059	0.192	0.304	0.395	0.513	0.612	0.832
Average OD	0.057	0.191	0.304	0.397	0.512	0.610	0.827
Absoluted OD	0	0.134	0.247	0.340	0.455	0.553	0.770



Appendix Π Example Analysis

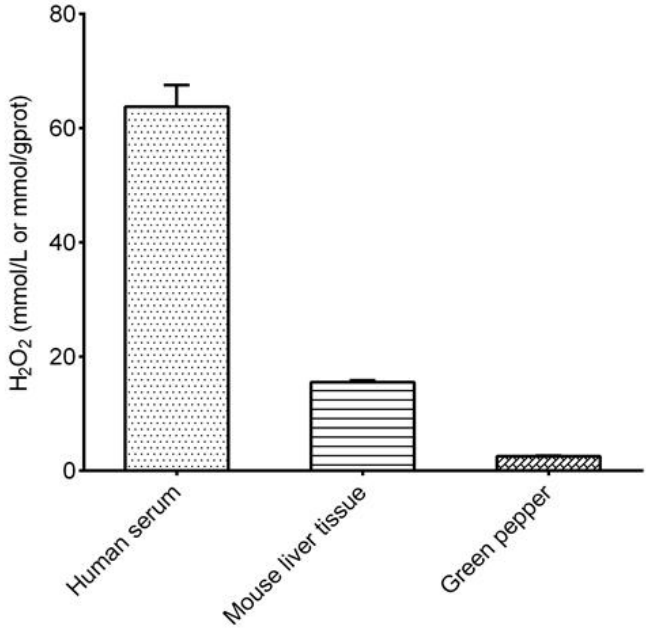
Example analysis：

Take 0.1 mL of human serum, carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.445, the average OD value of the blank is 0.051, the average OD value of the standard is 0.422, and the calculation result is:

$$\frac{H_2O_2 \text{ content}}{(\text{mmol/L})} = \frac{0.445 - 0.051}{0.422 - 0.051} \times 60 \times 1 = 63.72 \ (\text{mmol/L})$$

Detect human serum, 2% mouse liver tissue homogenate (the concentration of protein in sample is 1.82 gprot/L), 10% green pepper tissue homogenate (the concentration of protein in sample is 1.99 gprot/L) according to the protocol, the result is as follows：



Appendix III Publications

1. Liu H , Ji M , Bi Y ,et al.Integration of MyD88 inhibitor into mesoporous cerium oxide nanozymes-based targeted delivery platform for enhancing treatment of ulcerative colitis[J].Journal of Controlled Release: Official Journal of the Controlled Release Society, 2023:361.DOI:10.1016/j.jconrel.2023.08.015.
2. Chagas T Q , Freitas T N , Montalvo M F ,et al.Multiple ENDPOINTS of polylactic acid biomicroplastic toxicity in adult zebrafish (Danio rerio)[J].Chemosphere, 2021, 277(12):130279.DOI:10.1016/j.chemosphere.2021.130279.
3. Liu J , Yang Z , Yan Z ,et al.Chemical Micromotors Move Faster at Oil钬 掃ater Interfaces[J].
4. Li L , Wang C , Wang W ,et al.Uncovering the mechanisms of how corn steep liquor and microbial communities minimize cadmium translocation in Chinese cabbage[J].Environmental Science & Pollution Research, 2024, 31(15).DOI:10.1007/s11356-024-32579-5.
5. Alzharani M M , Almuqri E A , Ahmed M M ,et al.Exogenous Melatonin Supplement Contributes as Antioxidant to Attenuate the Oxidative Stress Induced by Cadmium Toxicity in Male Wistar Rats[J].Biomedical & Biotechnology Research Journal, 2024, 8(2).DOI:10.4103/bbrj.bbrj_54_24.

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.