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

Catalog No：E-BC-F001

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

（Ex/Em=535 nm/587 nm）

Detection range：0.02-10 μmol/L

Elabscience®Hydrogen Peroxide (H_2O_2)

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
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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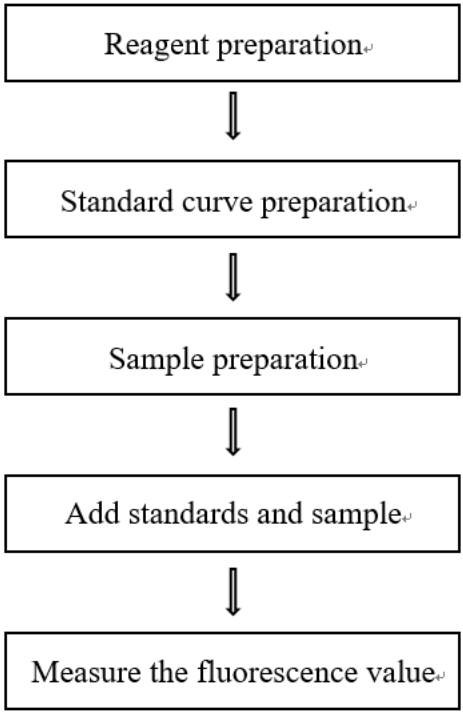
Assay summary



Intended use

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

Detection principle

In the presence of peroxidase, hydrogen peroxide reacts with the fluorescent probe, and the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm is proportional to the hydrogen peroxide concentration.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	30 mL ×1 vial	60 mL ×1 vial	-20℃, 12 month
Reagent 2	Substrate	0.06 mL ×1 vial	0.12 mL ×1 vial	-20℃, 12 month shading light
Reagent 3	Enzyme Reagent	Powder ×1 vial	Powder ×1 vial	-20℃, 12 month shading light
Reagent 4	1 mol/L H_2O_2 Standard Stock Solution	0.1 mL ×1 vial	0.1 mL ×1 vial	-20℃, 12 month
Reagent 5	Protein Precipitator	10 mL ×1 vial	20 mL ×1 vial	2-8℃, 12 month
Reagent 6	Alkali Reagent	3 mL ×1 vial	6 mL ×1 vial	2-8℃, 12 month
	PH test strips	1 bag		
	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), Micropipettor, Incubator, Vortex mixer, Centrifuge

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② 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 ℃ for 1 month protected from light.

③ The preparation of working solution:
Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 250 μL of working solution (mix well 240 μL of buffer solution， 5 μL of substrate and 5 μL of enzyme application solution). The working solution should be prepared on spot.

④ The preparation of 10 mmol/L H_2O_2 solution:
Dilute 10 μL of 1 mol/L H_2O_2 solution with 990 μL of buffer solution. Mix well to dissolve.

⑤ The preparation of 100 μmol/L H_2O_2 solution:
Dilute 10 μL of 10 mmol/L H_2O_2 solution with 990 μL of buffer solution. Mix well to dissolve.

⑥ The preparation of 10 μmol/L H_2O_2 solution:
Dilute 70 μL of 100 μmol/L H_2O_2 solution with 630 μL of buffer solution. Mix well to dissolve.

⑦ The preparation of standard curve：
Dilute 10 μmol/L H_2O_2 solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 4, 6, 8, 10 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**0.5**	**1**	**2**	**4**	**6**	**8**	**10**
10 μmol/L standard (μL)	0	10	20	40	80	120	160	200
Buffer solution (μL)	200	190	180	160	120	80	40	0

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 ℃ 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 ℃.

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).

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 200 μL buffer solution with a ultrasonic cell disruptor at 4 ℃.

④ Centrifuge at 12000×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).

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1-3
Mouse serum	1-4
Mouse plasma	1-2
Porcine serum	1-3
Rat serum	1-4
HepG2 culture supernatant	1
10% Mouse liver tissue homogenate	1-3
10% Mouse brain tissue homogenate	1-3
10% Rat lung tissue homogenate	1-3
HepG2 cells homogenate (the concentration of protein is 6.44 gprot/L)	1-2

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

① Avoid repeated freezing and thawing of substrate, it is recommended to aliquot the substrate into smaller quantities and store at -20 ℃.

② Because H_2O_2 is very unstable, preparae the H_2O_2 standard solution freshly.

③ The prepared working solution must be stored with shading light.

④ The pH of pretreated sample should be 6.5-8.

⑤ Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

Operating steps

The pretreatment of sample

For each well，add 150 μL of sample and 150 μL of protein precipitator, mix well. Centrifuge at 13000×g for 10 min, then take V1 (eg, V1=0.2 mL) of the supernatant, add V2 (eg, V2=0.04 mL) of alkali reagent to adjust the pH to 6.5-8, which is the sample to be tested.

The measurement of samples

① Standard tube: add 50 μL of standard solution with different concentrations to the wells.

Sample tube: add 50 μL of sample to the wells.

② Add 50 μL of working solution to each well.

③ Mix fully with microplate reader for 10 s and incubate the plate at room temperature for 10 min with shading light.

④ 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 and other liquid samples:

$$\begin{matrix}H_2O_2\ \text{content}\\(\mu\text{mol/L})\end{matrix} = (\Delta F - b) \div a \times 2 \times (\frac{V_1 + V_2}{V_1}) \times f$$

2. Tissue and cell samples:

$$\begin{matrix}H_2O_2\ \text{content}\\(\mu\text{mol/gprot})\end{matrix} = (\Delta F - b) \div a \times 2 \times (\frac{V_1 + V_2}{V_1}) \times f \div C_{pr}$$

[Note]

ΔF: Absolute fluorescence intensity of sample ($F_{Sample} - F_{Blank}$)

f: Dilution factor of sample before tested.

2: Dilution factor of sample in pretreatment step (V_{sample}:$V_{protein\ precipitator}$ =1:1).

V_1: The volume of supernatant in pretreatment step (mL).

V_2: The volume of alkali reagent in pretreatment step (mL).

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 (μmol/L)	1.20	3.50	8.00
%CV	1.3	1.0	1.0

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 (μmol/L)	1.20	3.50	8.00
%CV	3.4	4.1	3.3

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/L)	0.9	4.2	8.5
Observed Conc.(μmol/L)	0.9	4.2	8.8
Recovery rate (%)	96	100	104

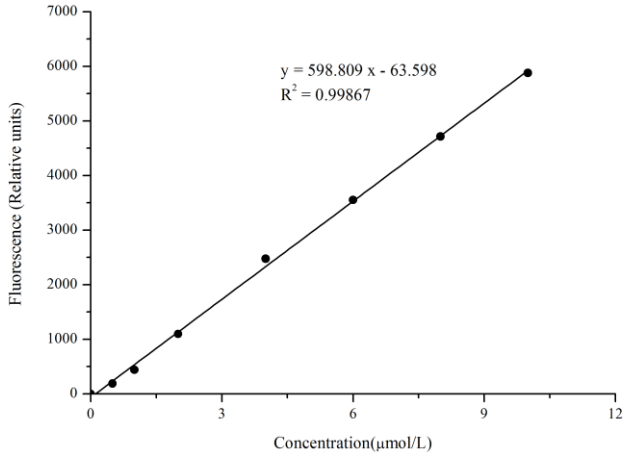
Sensitivity

The analytical sensitivity of the assay is 0.02 μmol/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 fluorescence 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**	**0.5**	**1**	**2**	**4**	**6**	**8**	**10**
Fluorescence value	119	311	564	1226	2601	3691	4873	5998
	122	309	557	1210	2589	3658	4800	6002
Average fluorescence value	120	310	561	1218	2595	3674	4836	6000
Absoluted fluorescence value	0	190	441	1098	2475	3554	4716	5880



Appendix II Example Analysis

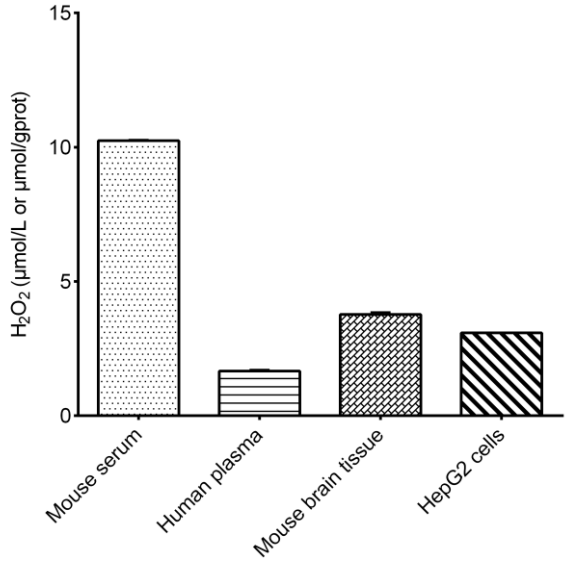
Example analysis:

For 10% mouse brain tissue homogenate, take 50 μL and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 556.7 x + 11.559, the average fluorescence value of the sample is 2452, the average fluorescence value of the blank is 80, the concentration of protein in sample is 5.4 gprot/L, and the calculation result is:

$$\underset{(\mu mol/gprot)}{H_2O_2\ content} = (2452 - 80 - 11.559) \div 556.7 \times 2 \times ((0.2 + 0.04) \div 0.2) \times 2 \div 5.4 = 3.77\ \mu mol/gprot$$

Detect mouse serum, human plasma, 10% mouse brain tissue homogenate (the concentration of protein is 5.4 gprot/L, dilute for 2 times) and HepG2 cells (the concentration of protein is 6.44 gprot/L) 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.