Hydrogen Peroxide (H_2O_2)
Colorimetric Assay Kit

Catalog No: E-BC-K102-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 1.5 mmol/L

Detection range: 1.5-150 mmol/L

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

General information

▲Intended use

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

▲Background

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.

▲Detection principle

Hydrogen peroxide can react with ammonium molybdate to form a yellow complex which has a maximum absorption peak at 405 nm. H_2O_2 content can be calculated by measuring the absorbance value at 405 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 2 vials	2-8℃ , 6 months
Reagent 2	Ammonium Molybdate Reagent	60 mL × 2 vials	2-8℃ , 6 months
Reagent 3	1 mol/L H_2O_2 Standard	12 mL × 1 vial	2-8℃ , 6 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.			

▲Materials prepared by users

Instruments

Spectrophotometer (405 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

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

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. Preheat the reagent 1 at 37 °C for 10 minutes. 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.

Pre-assay preparation

▲Reagent preparation

1. Preheat reagent 1 in 37°C for 10 min before use.
2. Reagent 2 is supersaturated solution. Incubate it in 60°C water bath if crystallized.
3. Preparation of 60 mmol/L H_2O_2 standard solution
   Prepare fresh solution by diluting the 1 mol/L H_2O_2 standard stock solution with double distilled water at a ratio of 3:47 before use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (1.5-150 mmol/L).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	405 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

1. Add 1 mL of reagent 1 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_2O_2 standard solution to the tube.
   Sample tube: Add 0.1 mL of sample to the tube.
3. Add 1 mL of reagent 2 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.

▲Operation table

	Blank tube	Standard tube	Sample tube
Reagent 1 (mL)	1	1	1
Preheat at 37°C for 10 min			
Double distilled water (mL)	0.1		
60 mmol/L H_2O_2 standard solution (mL)		0.1	
Sample (mL)			0.1
Reagent 2 (mL)	1	1	1
Mix fully, 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

1. Serum (plasma) and other liquid sample:

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

2. Tissue and cells sample:

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

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

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	1.5-150 mmol/L	Average intra-assay CV (%)	1.3
Sensitivity	1.5 mmol/L	Average inter-assay CV (%)	2.7
Average recovery rate (%)	98		

▲Inter-assay CV

Take three kits of different batches to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 3 times (n=3) parallelly with each kit. Then calculate the corresponding values according to the following formula. The average Inter-assay CV is 2.7%.

$$\bar{x}_T = \frac{\bar{x}_1 + \bar{x}_2 + \bar{x}_3}{3}$$

$$R = \frac{\bar{x}_{max} - \bar{x}_{min}}{\bar{x}_T} \times 100\%$$

$$\bar{R} = \frac{R_1 + R_2 + R_3}{3} \quad (n=3)$$

$\bar{x}_{max}$--- The max values of $\bar{x}_i$

$\bar{x}_{min}$--- The min values of $\bar{x}_i$

$\bar{x}_T$---The average values of $\bar{x}_i$

R_i---The value of each batch number kit

▲Intra-assay CV

Take one kit to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 6 times (n=6) parallelly, and the average Intra-assay CV is 1.3%, which was calculated according to the following formula.

$$CV = \frac{S}{\bar{x}} \times 100\%$$

S--- Standard deviation

▲Sensitivity

OD values of standard curve and 20 blank samples were measured according to the operation table. Plot the standard curve and calculate the standard deviation of blank, three standard deviations divided by the slope is the sensitivity (1.5 mmol/L) according to the formula of IUPAC.

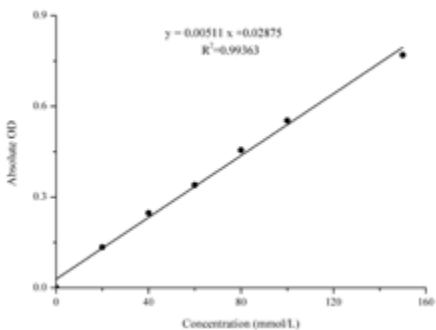
▲Recovery rate

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 3 times parallelly to get the average recovery rate of 98%.

▲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
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



▲Example analysis

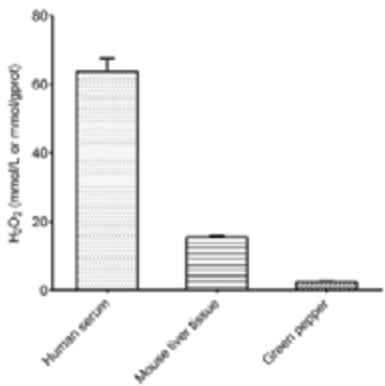
Take 0.1 mL of human serum, carry the assay according to the operation table.

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:

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

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 II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲Cell culture supernatant

Detect directly. If there is turbidity, centrifuge at 3100 g for 10 min. Take the supernatant to preserve it on ice for detection. If not detected on the same day, it can be stored at -80℃ for a month.

▲Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲Cells

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) including 0.1 mM EDTA.
2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

▲Note for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.

Appendix III References

1. Neill S J, Desikan R, Clarke A, et al. Hydrogen peroxide and nitric oxide as signalling molecules in plants. Journal of Experimental Botany, 2002, 53(372): 1237-1247.
2. Veal E A, Day A M, Morgan B A. Hydrogen peroxide sensing and signaling. Molecular Cell, 2007, 26(1): 1-14.
3. Marinho H S, Real C, Cyrne L, et al. Hydrogen peroxide sensing, signaling and regulation of transcription factors. Redox Biology, 2014, 2(2): 535-562.
4. Carnevale R, Nocella C, Pignatelli P, et al. Blood hydrogen peroxide breakdown activity in healthy subjects and in patients at risk of cardiovascular events. Atherosclerosis, 2018, 274: 29-34.
5. Moloney J N, Cotter T G. ROS signalling in the biology of cancer. Seminars in Cell & Developmental Biology, 2018, 80: 50-64.