PRODUCT INFORMATION & MANUAL

Hydrogen Peroxide Assay Kit (Colorimetric)

NBP3-24505

For research use only.
Not for diagnostic or therapeutic procedures.

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Hydrogen Peroxide Assay Kit (Colorimetric)

Catalog No: NBP3-24505

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.41 mmol/L

Detection range: 0.41-125 mmol/L

Average intra-assay CV (%): 3.2

Average inter-assay CV (%): 3.6

Average recovery rate (%): 105

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ 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, urine, tissue and cells 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 405nm. 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	12 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Ammonium Molybdate Reagent	12 mL × 1 vial	2-8℃ , 12 months
Reagent 3	1 mol/L H_2O_2 Standard	1 mL × 2 vials	2-8℃ , 12 months
	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.			

▲Materials prepared by users

Instruments

Microplate reader (402-407 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

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. 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

▲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 (0.41-125 mmol/L).

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

Sample type	Dilution factor
Human serum	1
Rat serum	1
Mouse serum	1
Mouse plasma	1
Porcine serum	1
Human urine	1
Cell homogenate	1
10% Plant tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat liver tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1.The preparation of standard curve

Dilute 1 mol/L H_2O_2 standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 60, 80, 100, 125 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	1 mol/L H_2O_2 standard (μL)	Double distilled water (μL)
A	0	0	1000
B	10	10	990
C	20	20	980
D	40	40	960
E	60	60	940
F	80	80	920
G	100	100	900
H	125	125	875

2.The measurement of samples

1) Add 100 μL of reagent 1 to each well and preheat at 37℃ for 10 min.
2) Standard well: add 15 μL of standards with different concentrations to the corresponding wells.

   Sample well: add 15 μL of sample to the corresponding wells.
3) Add 100 μL of reagent 2 and mix fully.
4) Mix for 5 s with microplate reader and stand for 10 min at room temperature.
5) Measure the OD values of each well at 405 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Reagent 1 (μL)	100	100
Preheat at 37℃ for 10 min		
Standards with different concentrations (μL)	15	
Sample (μL)		15
Reagent 2 (μL)	100	100
Mix and stand for 10 min at room temperature. Measure the OD values of each well.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

1.For serum (plasma), urine sample

$$H_2O_2 \text{ content } (\text{mmol/L}) = (\Delta A_{405} - b) \div a \times f$$

2.For tissue and cells sample

$$H_2O_2 \text{ content } (\text{mmol/gprot}) = (\Delta A_{405} - b) \div a \div C_{pr} \times f$$

Note:

y: $OD_{Standard} - OD_{Blank}$. ($OD_{Blank}$ is the OD value when the standard concentration is 0)

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve.

ΔA_{405}: Absolute OD ($OD_{Sample} - OD_{Blank}$).

f: Dilution factor of sample before test.

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

Appendix I Data

▲Example analysis

Take 15 μL of human serum sample and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.0047 x + 0.0223, the average OD value of the sample is 0.435, the average OD value of the blank is 0.075, and the calculation result is:

$$H_2O_2 \text{ content (mmol/L)} = \frac{0.435 - 0.075 - 0.0223}{0.0047} \times 1 = 71.85 \text{ (mmol/L)}$$

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.

▲ Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine 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 °C . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. 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. If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ 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).

Appendix III References

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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.