Technical Manual

Hydrogen Peroxide (H_2O_2) Colorimetric Assay Kit

- Catalogue Code: MAES0090
- Size: 100 Assays
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

1.5-150 mmol/L

Sensitivity:

1.5 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	60 mL × 2 vials	2-8˚C, 6 months
Ammonium Molybdate Reagent	60 mL × 2 vials	2-8˚C, 6 months
H_2O_2 Standard (1 mol/L)	12 mL × 1 vial	2-8˚C, 6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (405 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preheat buffer solution in 37˚C for 10 min before use.

3. Preparation of 60 mmol/L H_2O_2 standard solution: Prepare fresh solution by diluting the 1 mol/L H_2O_2 standard with double-distilled water at a ratio of 3:47 before use.

7. Sample Preparation

1. **Serum sample:**
   Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. 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°C for a month.

2. **Plasma sample:**
   Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. 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°C for a month.

3. **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°C for a month.

4. **Cell sample:**
   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 (2×10^6): PBS (0.01 M, pH 7.4) including 0.1 mM EDTA (μL) =1: 300. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

5. **Tissue sample:**
   Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) including 0.1 mM EDTA (2-8°C) (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. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (1.5-150 mmol/L).

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

Sample Type:	Dilution Factor:
10% Mouse liver tissue homogenization	5-10
Human serum	1
10% Green pepper tissue homogenization	1
Rat serum	1
Mouse serum	1

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

8. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 405 nm

9. Operation 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_2O_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.

Operation Table

	Blank tube	Standard tube	Sample tube
Buffer solution (mL)	1	1	1
Preheat at 37°C for 10 min			
Double distilled water (mL)	0.1		
H_2O_2 standard solution (60 mmol/L) (mL)		0.1	
Sample (mL)			0.1
Ammonium molybdate reagent (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.			

10. Calculations

1. Serum (plasma) and other liquid sample:

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

2. Tissue and cell sample:

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

ΔA_1: OD_{sample}-OD_{blank}

ΔA_2: $OD_{standard}$-OD_{blank}

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

f: Dilution factor of sample before tested

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

11. Performance Characteristics

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

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:

$$\boldsymbol{H2O2\ content\ (mmol/L)} = \boldsymbol{(0.445 - 0.051)/(0.422 - 0.051) \times 60 \times 1}$$
$$= \boldsymbol{63.72\ (mmol/L)}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching 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.

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

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