Technical Manual

Hydrogen Peroxide (H_2O_2) Colorimetric Assay Kit

- Catalogue Code: MAES0089
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.41-125 mmol/L

Sensitivity:

0.41 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, urine, tissue and cells samples.

4. Detection Principle

Hydrogen peroxide can react with ammonium molybdate to form a yellow complex which has a maximum absorption peak at 405nm. H2O2 content can be calculated by measuring the absorbance value at 405 nm.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	12 mL × 1 vial	2-8°C, 6 months
Ammonium Molybdate Reagent	12 mL × 1 vial	2-8°C, 6 months
H_2O_2 Standard (1 mol/L)	1 mL × 2 vials	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (402-407 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. Assay Notes:

1. Prevent the formulation of bubbles when measuring the OD value.

2. Prepare the fresh standards with different concentrations before use

7. Reagent preparation:

Bring all reagents to room temperature before use.

8. 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. Urine:
Collect fresh urine and centrifuge at 10000 g for 15 min at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine 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: 200. 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 (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);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 405 nm

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.

10. Operation Steps

The preparation of standard curve

Dilute H_2O_2 standard (1 mol/L) 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.

The measurement of samples

1. Add 100 µL of buffer solution to each well and preheat at 37°C 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 ammonium molybdate reagent 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.

Operation Table

	Standard well	Sample well
Buffer solution (μL)	100	100
Preheat at 37°C for 10 min		
Standards with different concentrations (μL)	15	
Sample (μL)		15
Ammonium molybdate reagent (μL)	100	100
Mix for 5 s with microplate reader and stand for 10 min at room temperature. Measure the OD values of each well at 405 nm with microplate reader.		

11. Calculations

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. The standard curve is: y= ax + b.

1. Serum (plasma) sample:

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

2. Tissue and cells sample:

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

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: $OD_{Sample.} - OD_{Blank}$
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, gprot/L.

12. Performance Characteristics

Detection Range	0.41-125 mmol/L
Sensitivity	0.41 mmol/L
Average recovery rate (%)	105
Average inter-assay CV (%)	3.6
Average intra-assay CV (%)	3.2

Analysis

Take 15 µL of human serum, 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:

$$\text{H}_2\text{O}_2 \text{ content (mmol/L)} = (0.435 - 0.075 - 0.0223) \div 0.0047 \times 1$$

$$= 71.85 \text{ (mmol/L)}$$

Email: info@assaygenie.com **Web:** www.assaygenie.com

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent 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.

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com