TECHNICAL MANUAL

Hydrogen Peroxide (H2O2) Colorimetric Assay Kit

- **SKU CODE:** MAES0089
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure the absorbance value

2. Intended use

This kit can be used to measure the H2O2 content in serum, plasma, urine, tissue and cell samples.

3. Detection principle

Hydrogen peroxide (H2O2) is a metabolic by-product of reactive oxygen species, which is not only a signaling molecule in cells, but also a source of oxidative stress. H2O2 is an important regulatory factor of eukaryotic signal transduction involved in cell proliferation, differentiation and migration. However, abnormal H2O2 can lead to oxidative cell damage and disease, such as cancer, atherosclerosis, osteoporosis and neurodegenerative diseases.

Detection principle: H2O2 reacts with ammonium molybdate reagent to form a yellow complex. The absorbance is measured at 405 nm and is proportional to the H2O2 concentration in the sample.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500Assays)	Storage
Reagent 1	Buffer Solution	6 mL x 1 vial	12 mL x 1 vial	60 mL x 1 vial	2-8 °C, 12 months
Reagent 2	Ammonium Molybdate Reagent	6 mL x 1 vial	12 mL x 1 vial	60 mL x 1 vial	2-8 °C, 12 months

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500Assays)	Storage
Reagent 3	1 mol/L H2O2 Standard	1 mL x 1 vial	1 mL x 2 vials	10 mL x 1 vial	2-8 °C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (402-407 nm, optimum wavelength: 405 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

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

6. Reagent preparation

1. Equilibrate all the reagents to room temperature before use.
2. If precipitation occurs in buffer solution, it can be redissolved by heating in 80 °C water bath before use.
3. **The preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mol/L standard solution 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.

7. Sample preparation

Sample preparation

Serum, plasma and urine: detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C 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 PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4 °C.

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 (MAES0177).

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 300-500 μL PBS (0.01 M, pH 7.4) with an ultrasonic cell disruptor at 4 °C.

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 (MAES0177).

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only). The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

8. The key points of the assay

If the concentration of H2O2 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.

9. Operating steps

1. Add 100 μL of buffer solution to standard well and sample well, 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.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using absolute OD 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) sample and other liquid samples:

H2O2 content (mmol/L) = (ΔA_405 - b) ÷ a × f

2. Tissue and cell samples:

H2O2 content (mmol/gprot) = (ΔA_405 - b) ÷ a ÷ C_{pr} × f

[Note]

ΔA_405: Absolute OD (OD_{Sample} – OD_{Blank}).

f: Dilution factor of sample before test.

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

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	5.50	48.60	102.30
%CV	3.5	3.1	3.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	5.50	48.60	102.30
%CV	3.2	3.6	4.0

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 105%.

Recovery

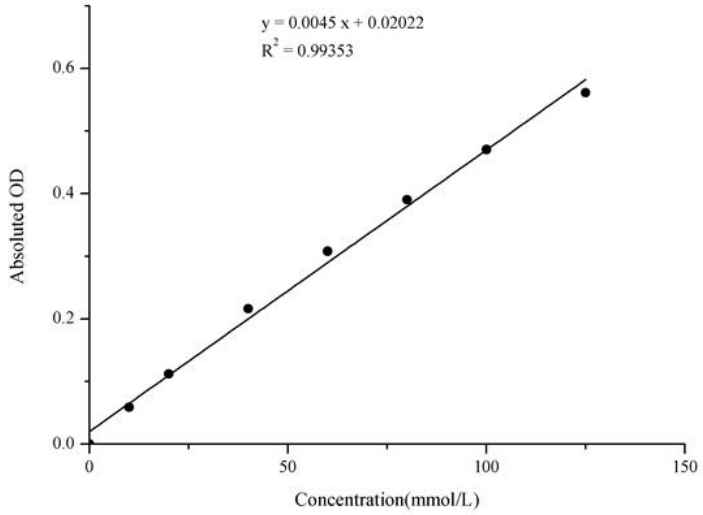
Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	16.5	55.8	95
Observed Conc. (mmol/L)	17.7	58.0	98.8
Recovery rate (%)	107	104	104

Sensitivity

The analytical sensitivity of the assay is 0.41 mmol/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.

Standard curve

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:



Standard curve data

Concentration (mmol/L)	OD value 1	OD value 2	Average OD	Absolute OD
0	0.072	0.076	0.074	0
10	0.132	0.134	0.133	0.059
20	0.188	0.184	0.186	0.112
40	0.287	0.293	0.290	0.216
60	0.380	0.384	0.382	0.308
80	0.468	0.460	0.464	0.390
100	0.543	0.545	0.544	0.470
125	0.636	0.634	0.635	0.561

12. Appendix II Example Analysis

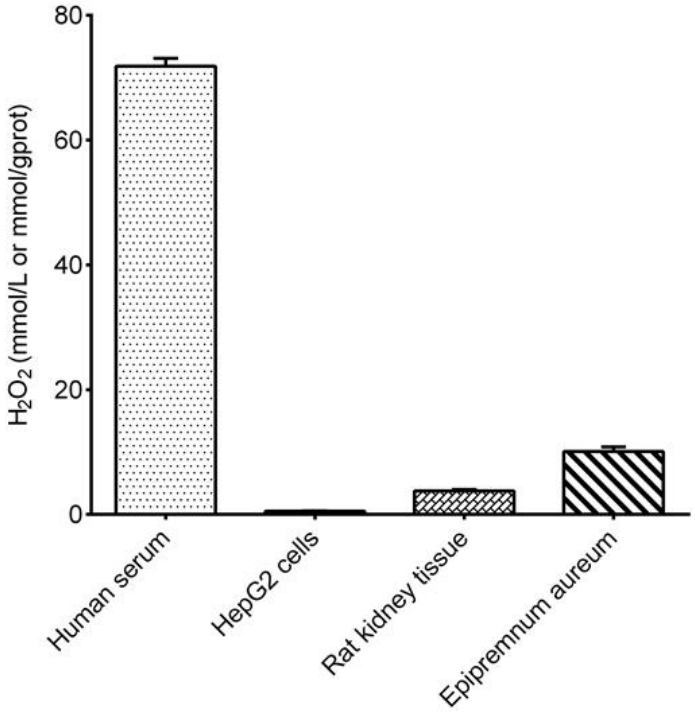
Example analysis:

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

Standard curve: y = 0.0047x + 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:

H2O2 content (mmol/L) = (0.435 - 0.075 - 0.0223) ÷ 0.0047 × 1 = 71.85 (mmol/L)

Detect human serum, HepG2 cell homogenate (the concentration of protein in sample is 5.00 gprot/L), 10% rat kidney tissue homogenate (the concentration of protein in sample is 6.57 gprot/L), 10% epipremnum aureum tissue homogenate (the concentration of protein in sample is 0.99 gprot/L) according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.