TECHNICAL MANUAL

Hydrogen Peroxide (H2O2) Fluorometric Assay Kit

- **SKU CODE:** MAES0001
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

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

2. Intended use

This kit can be used to measure the H_{2O}_2 content in serum, plasma, tissue and cell samples.

3. Detection principle

In the presence of peroxidase, hydrogen peroxide reacts with the fluorescent probe, and the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm is proportional to the hydrogen peroxide concentration.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500 Assays)	Storage
Reagent 1	Buffer Solution	30 mL x 1 vial	60 mL x 1 vial	60 mL x 5 vials	-20 °C, 12 months
Reagent 2	Substrate	0.06 mL x 1 vial	0.12 mL x 1 vial	0.12 mL x 5 vials	-20 °C, 12 months, shading light
Reagent 3	Enzyme Reagent	Powder x 1 vial	Powder x 1 vial	Powder x 5 vials	-20 °C, 12 months, shading light
Reagent 4	1 mol/L H2O2 Standard Stock Solution	0.1 mL x 1 vial	0.1 mL x 1 vial	0.1 mL x 5 vials	-20 °C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500 Assays)	Storage
Reagent 5	Protein Precipitator	10 mL x 1 vial	20 mL x 1 vial	20 mL x 5 vials	2-8 °C, 12 months
Reagent 6	Alkali Reagent	3 mL x 1 vial	6 mL x 1 vial	6 mL x 5 vials	2-8 °C, 12 months
	pH Test Strips	1 bag	1 bag	5 bags	No requirement
	Black Microplate	96 wells	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme application solution:** Dissolve one vial of enzyme reagent with 120 µL of buffer solution. Mix well to dissolve. Store at -20 °C for 1 month protected from light.
3. **Preparation of working solution:** Before testing, prepare sufficient working solution according to the number of test wells. For example, prepare 250 µL of working solution (mix well 240 µL of buffer solution, 5 µL of substrate and 5 µL of enzyme application solution). The working solution should be prepared fresh.
4. **Preparation of 10 mmol/L H2O2 solution:** Dilute 10 µL of 1 mol/L H2O2 solution with 990 µL of buffer solution. Mix well to dissolve.
5. **Preparation of 100 µmol/L H2O2 solution:** Dilute 10 µL of 10 mmol/L H2O2 solution with 990 µL of buffer solution. Mix well to dissolve.
6. **Preparation of 10 µmol/L H2O2 solution:** Dilute 70 µL of 100 µmol/L H2O2 solution with 630 µL of buffer solution. Mix well to dissolve.

7. **Preparation of standard curve:** Dilute 10 μmol/L H2O2 solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 4, 6, 8, 10 μmol/L.

7. Sample preparation

Sample preparation

Serum, plasma and other liquid samples: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C for one month.

Tissue samples:

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 buffer solution with a dounce homogenizer at 4 °C.

4. Centrifuge at 10,000 xg 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 200 μL buffer solution with an ultrasonic cell disruptor at 4 °C.

4. Centrifuge at 12,000 xg 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 samples

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

Human serum: 1-3

Mouse serum: 1-4

Mouse plasma: 1-2

Porcine serum: 1-3

Rat serum: 1-4

HepG2 culture supernatant: 1

10% Mouse liver tissue homogenate: 1-3

10% Mouse brain tissue homogenate: 1-3

10% Rat lung tissue homogenate: 1-3

HepG2 cells homogenate (protein concentration 6.44 g_{prot}/L): 1-2

Note: The diluent is buffer solution. For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. Avoid repeated freezing and thawing of substrate. It is recommended to aliquot the substrate into smaller quantities and store at -20 °C.
2. Because H2O2 is very unstable, prepare the H2O2 standard solution freshly.
3. The prepared working solution must be stored protected from light.
4. The pH of pretreated sample should be 6.5-8.
5. Prevent the formation of bubbles when transferring the supernatant into the microplate.

9. Operating steps

Sample pretreatment

For each well, add 150 μL of sample and 150 μL of protein precipitator, mix well.

Centrifuge at 13,000 xg for 10 min, then take V_1 (e.g., V_1=0.2 mL) of the supernatant, add V_2 (e.g., V_2=0.04 mL) of alkali reagent to adjust the pH to 6.5-8, which is the sample to be tested.

Measurement of samples

1. Standard tube: add 50 μL of standard solution with different concentrations to the wells.

Sample tube: add 50 μL of sample to the wells.

2. Add 50 μL of working solution to each well.

3. Mix fully with microplate reader for 10 s and incubate the plate at room temperature for 10 min protected from light.

4. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

10. Calculation

Standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absolute fluorescence 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 and other liquid samples:

H_{2O}_2 content (μmol/L) = (ΔF - b) ÷ a × 2 × ((V_1 + V_2)/V_1) × f

2. Tissue and cell samples:

H_{2O}_2 content (μmol/g_{prot}) = (ΔF - b) ÷ a × 2 × ((V_1 + V_2)/V_1) × f ÷ C_{pr}

[Note]

ΔF: Absolute fluorescence intensity of sample (F_{sample} – F_{blank})

f: Dilution factor of sample before testing

2: Dilution factor of sample in pretreatment step (V_{sample}:$V_{protein}$ precipitator = 1:1)

V_1: The volume of supernatant in pretreatment step (mL)

V_2: The volume of alkali reagent in pretreatment step (mL)

C_{pr}: Concentration of protein in sample (g_{prot}/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 (μmol/L)	1.20	3.50	8.00
%CV	1.3	1.0	1.0

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 (µmol/L)	1.20	3.50	8.00
%CV	3.4	4.1	3.3

Recovery

Three samples of high, middle and low concentrations were tested with 6 parallel replicates for each concentration to obtain an average recovery rate of 100%.

Recovery

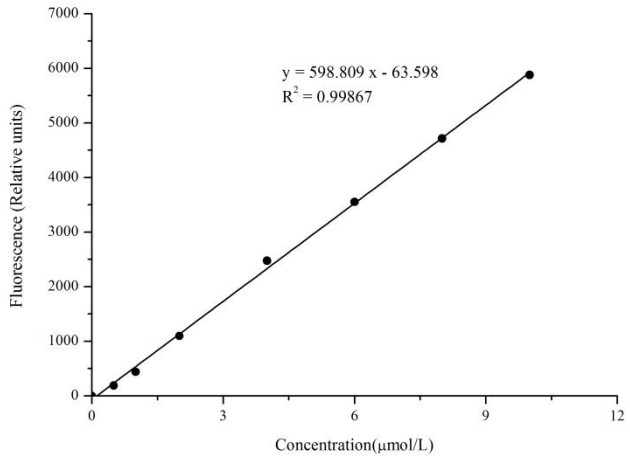
	Standard 1	Standard 2	Standard 3
Expected Conc.(µmol/L)	0.9	4.2	8.5
Observed Conc.(µmol/L)	0.9	4.2	8.8
Recovery rate (%)	96	100	104

Sensitivity

The analytical sensitivity of the assay is 0.02 µmol/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 fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve

Concentration (μmol/L)	Fluorescence value		Average fluorescence value	Absolute fluorescence value
0	119	122	120	0
0.5	311	309	310	190
1	564	557	561	441
2	1226	1210	1218	1098
4	2601	2589	2595	2475
6	3691	3658	3674	3554
8	4873	4800	4836	4716
10	5998	6002	6000	5880

12. Appendix II Example Analysis

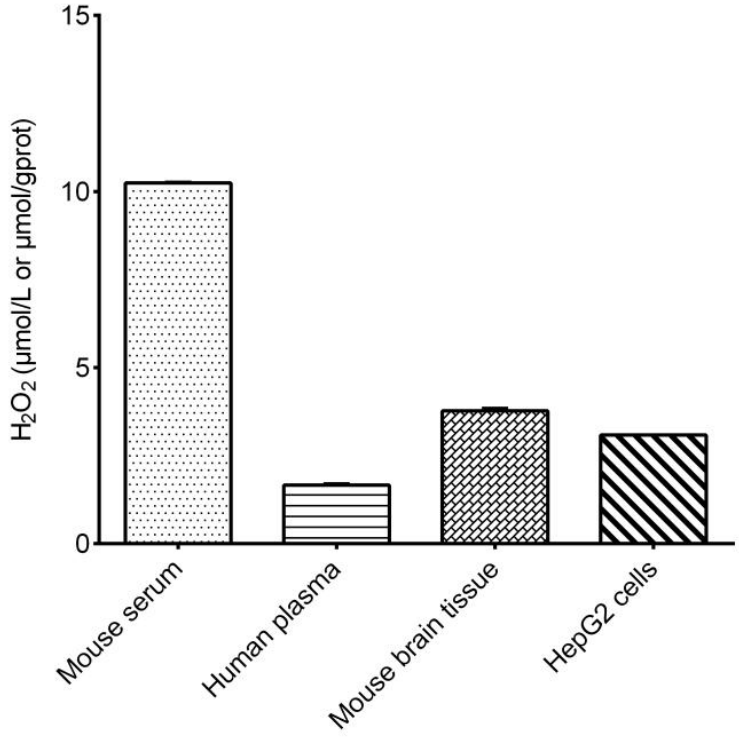
Example analysis:

For 10% mouse brain tissue homogenate, take 50 μL and carry out the assay according to the operating steps. The results are as follows:

Standard curve: y = 556.7x + 11.559, the average fluorescence value of the sample is 2452, the average fluorescence value of the blank is 80, the concentration of protein in sample is 5.4 g_{prot}/L, and the calculation result is:

H_{2O}_2 content (μmol/g_{prot}) = (2452 – 80 – 11.559) ÷ 556.7 × 2 × ((0.2 + 0.04) ÷ 0.2) × 2 ÷ 5.4 = 3.77 μmol/g_{prot}

Detect mouse serum, human plasma, 10% mouse brain tissue homogenate (protein concentration 5.4 g_{prot}/L, diluted 2-fold) and HepG2 cells (protein concentration 6.44 g_{prot}/L) according to the protocol. The results are 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.