TECHNICAL MANUAL

Catalase (CAT) Activity Assay Kit

- **SKU CODE:** MAES0044
- **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
- Incubation and reaction
- Add chromogenic reagents
- Measure absorbance at 405 nm

2. Intended use

This kit can be used to measure catalase (CAT) activity in serum, plasma, cells, cell culture supernatant, and tissue homogenate samples.

3. Detection principle

Catalase (CAT) can catalyze the decomposition of H_{2O}_2 into oxygen and water, and this reaction is quickly stopped upon the addition of ammonium molybdate. The residual H_{2O}_2 reacts with ammonium molybdate to generate a yellowish complex. CAT activity can be calculated by measuring the production of the yellowish complex at 405 nm.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 assays)	Storage
Reagent 1	Buffer Solution	12 mL × 1 vial	24 mL × 1 vial	60 mL × 2 vials	2-8 °C, 12 months
Reagent 2	Substrate	1.5 mL × 1 vial	1.5 mL × 2 vials	15 mL × 1 vial	2-8 °C, 12 months
Reagent 3	Chromogenic Agent	Powder × 1 vial	Powder × 1 vial	Powder × 5 vials	2-8 °C, 12 months
Reagent 4	Clarificant	1.5 mL × 1 vial	1.5 mL × 2 vials	15 mL × 1 vial	2-8 °C, 12 months

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 assays)	Storage
Reagent 5	9.6 mol/L H_2O_2 Standard Solution	1.5 mL × 1 vial	1.5 mL × 2 vials	15 mL × 1 vial	2-8 °C, 12 months, protect from light
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (400-410 nm), Micropipette, Vortex Mixer, Incubator

Reagents:

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

6. Reagent preparation

1. Incubate buffer solution and substrate at 37 °C for 10 min before use.
2. **Preparation of chromogenic application solution:** Dissolve one vial of chromogenic agent with 24 mL of double distilled water. (If there is sediment at the bottom, directly use the supernatant for testing; it will not affect the result). Store at 4 °C for 3 months.
3. Clarificant will freeze when cold; please warm it in a 37 °C water bath until clear.
4. **Preparation of 1 mmol/mL H_2O_2 standard solution:** Before testing, prepare sufficient 1 mmol/mL H_2O_2 standard solution according to the number of test wells. For example, prepare 384 µL of 1 mmol/mL H_2O_2 standard solution (mix well 40 µL of 9.6 mol/L H_2O_2 standard solution with 344 µL double distilled water).
5. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mmol/mL H_2O_2 standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 50, 60, 100 µmol/mL.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, serum or plasma can be stored at -80 °C for one 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 10,000 × g for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177 is recommended for animal tissue samples; MAES0126 is recommended for plant tissue samples).

Cells:

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

4. Centrifuge at 10,000 × g for 10 min at 4 °C 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):

- Human serum: 1
- 293T supernatant: 1
- 10% Rat heart tissue homogenate: 50-100
- 10% Rat liver tissue homogenate: 100-200
- 10% Rat spleen tissue homogenate: 50-100
- Mouse serum: 1
- 10% Epipremnum aureum tissue homogenate: 1-2
- 10% Rat lung tissue homogenate: 50-100
- 10% Rat kidney tissue homogenate: 50-100

- 10% Rat brain tissue homogenate: 20-50

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. The key points of the assay

1. The reaction time must be accurate when substrate is added.
2. Test tubes can be prepared and labeled in advance.
3. Dilute the samples to the optimal concentration for detection if the CAT activity of samples exceeds the detection range.
4. Prevent the formation of bubbles when transferring the supernatant into the microplate.

9. Operating steps

For standard curve:

1. Add 20 μL of H_{2O}_2 standard solution with different concentrations to 1.5 mL EP tubes respectively.

2. Sequentially add 200 μL of buffer solution, 20 μL of double distilled water, 200 μL of chromogenic agent application solution, and 20 μL of clarificant; mix well.

3. Stand at room temperature for 10 min and transfer 200 μL of reaction solution to the microplate.

4. Measure the OD value at 405 nm with a microplate reader.

For samples:

1. Control tube: add 200 μL of buffer solution into 1.5 mL EP tubes.

Sample tube: add 20 μL of sample and 200 μL of buffer solution into 1.5 mL EP tubes.

2. Incubate at 37 °C for 5 min.

3. Add 20 μL of substrate into each tube, mix fully, and react at 37 °C for exactly 1 min.

4. Control tube: add 200 μL of chromogenic application solution, 20 μL of clarificant, and 20 μL of sample; mix well.

Sample tube: add 200 μL of chromogenic application solution and 20 μL of clarificant; mix well.

5. Stand at room temperature for 10 min and transfer 200 μL of reaction solution to the microplate.

6. Measure the OD value at 405 nm with a 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 corresponding 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:

Definition: The amount of CAT in 1 mL of serum or plasma that decomposes 1 μmol H_{2O}_2 per minute at 37 °C is defined as 1 unit.

CAT activity (U/mL) = (deltaA / a) × 0.02* / 1* × V × f

2. Tissue and cells sample:

Definition: The amount of CAT in 1 mg of tissue protein that decomposes 1 μmol H_{2O}_2 per minute at 37 °C is defined as 1 unit.

CAT activity (U/mgprot) = (deltaA / a) × 0.02* / 1* × V × f ÷ C_{pr}

[Note]

0.02*: The volume of standard, 0.02 mL.

1*: The reaction time, 1 min.

deltaA: $OD_{Control}$ - OD_{Sample}.

V: The volume of sample, mL.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, mgprot/mL.

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 (U/mL)	3.70	26.40	78.60
%CV	4.2	3.8	3.7

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 (U/mL)	3.70	26.40	78.60
%CV	7.4	7.9	7.8

Recovery

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

Recovery

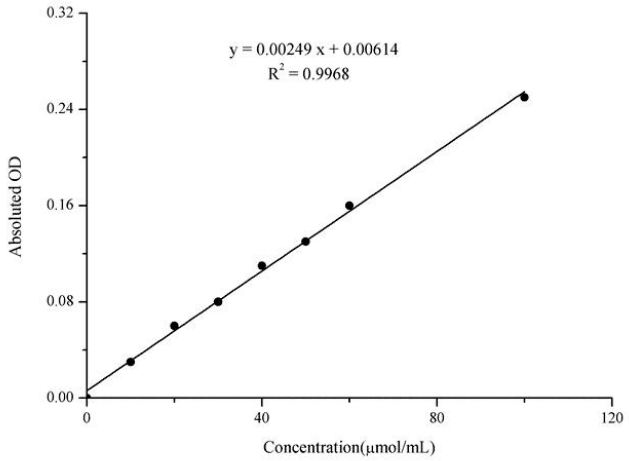
Standard 1	Standard 2	Standard 3
15	36	54
15.2	34.9	55.1
101	97	102

Sensitivity

The analytical sensitivity of the assay is 1.12 U/mL CAT. 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), the standard curve and data are provided below for reference only:



Standard curve

Concentration (μmol/mL)	Average OD	Absolute OD
0	0.062	0.000
10	0.087	0.025
20	0.118	0.056
40	0.143	0.081
50	0.169	0.107
60	0.192	0.130
80	0.219	0.157
100	0.311	0.249

12. Appendix II Example Analysis

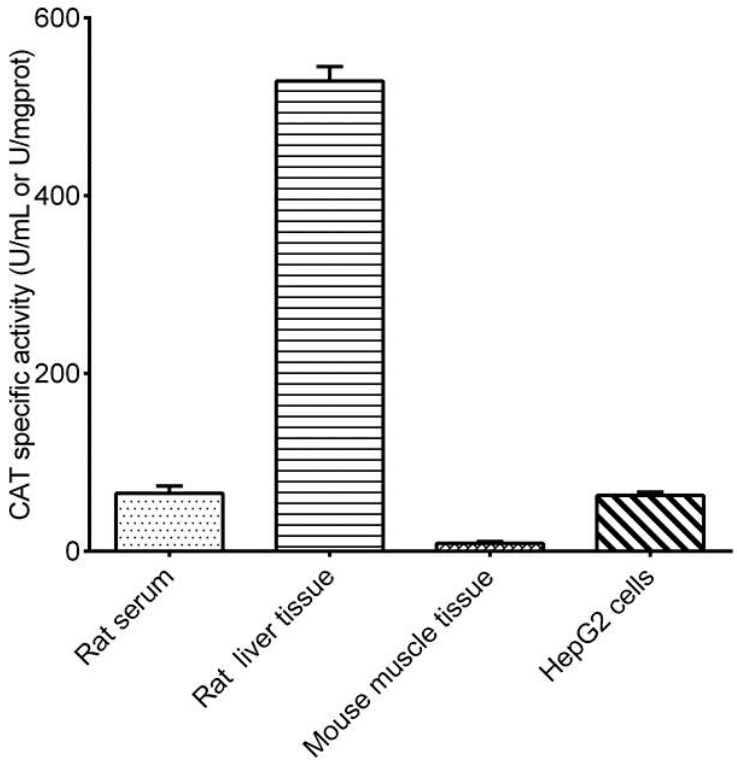
Example analysis:

Dilute 10% rat liver tissue homogenate with PBS (0.01 M, pH 7.4) 100 times, take 0.02 mL of diluted sample and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0026x + 0.0022, the average OD value of the sample is 0.442, the average OD value of the control is 0.612, the concentration of protein in sample is 12.38 gprot/L, and the calculation result is:

CAT activity (U/mgprot) = (0.612 - 0.442) ÷ 0.0026 × 100 ÷ 12.38 = 528.15 U/mgprot

Detect rat serum (dilute 2 times), 10% rat liver tissue homogenate (protein concentration 12.38 gprot/L, dilute 100 times), 10% mouse muscle tissue homogenate (protein concentration 3.10 gprot/L) and HepG2 cells (protein concentration 8.06 gprot/L, dilute 10 times) 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.