TECHNICAL MANUAL

Catalase (CAT) Activity Assay Kit

- **SKU CODE:** MAES0343
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add samples and buffer solution
- Add substrate and react
- Add chromogenic reagents
- Measure absorbance

2. Intended use

This kit can be used to measure catalase (CAT) activity in serum, plasma and tissue 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 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months
Reagent 2	Substrate	12 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months
Reagent 3	Chromogenic Agent	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months
Reagent 4	Clarificant	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months

5. Materials prepared by users

Instruments:

Spectrophotometer (405 nm), micropipettor, 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 60 mL of double distilled water. (If there is sediment at the bottom, directly use the supernatant for testing, as this will not affect the result). Store at 2-8°C for 3 months.
3. Clarificant will freeze when cold; please warm it in a 37°C water bath until clear.

7. Sample preparation

Sample preparation

Serum and plasma: Detect directly. If not analyzed 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 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 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):

8. Sample dilution recommendations

Sample type	Dilution factor	Volume of sample
10% Rat liver tissue homogenate	25-50	50 µL
10% Rat kidney tissue homogenate	10-25	50 µL
10% Rat brain tissue homogenate	5-10	50 µL
Human serum	1	100 µL
293T supernatant	1	100 µL

9. The key points of the assay

1. Test tubes can be prepared and labeled in advance. After incubating at 37°C for 10 min, add samples and buffer solution, then incubate the test tube at 37°C for 5 min.
2. Dilute samples to the optimal concentration for detection if the CAT activity of samples exceeds the detection range.
3. The reaction time must be accurate when substrate is added.

10. Operating steps

1. Control tube: Add 1 mL of buffer solution into 5 mL EP tubes. Sample tube: Add a* mL of sample and 1 mL of buffer solution into 5 mL EP tubes.
2. Incubate at 37°C for 5 min.
3. Add 0.1 mL of substrate into each tube, mix well and react at 37°C for exactly 1 min.
4. Control tube: Add 1 mL of chromogenic application solution, 0.1 mL of clarificant and a* mL of sample, mix well. Sample tube: Add 1 mL of chromogenic application solution and 0.1 mL of clarificant, mix well.
5. Stand for 10 min at room temperature. Set to zero with double distilled water and measure the OD values of each tube at 405 nm with 0.5 cm optical path cuvette.

[Note]: For serum or plasma samples, a* is 0.1 mL. For cell homogenate and tissue homogenate, a* is 0.05 mL.

11. Calculation

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) = ΔA × 32.5* / (1* × V) × f

2. Tissue and cell 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) = ΔA × 32.5* / (1* × V) × f ÷ C_{pr}

[Note]

*32.5: reciprocal of slope

1: Reaction time

ΔA: Absolute OD ($OD_{Control}$ – OD_{Sample})

V: Volume of sample, mL

f: Dilution factor of sample before test

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

12. 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).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	3.50	34.50	88.50
%CV	3.6	3.2	2.5

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	3.50	34.50	88.50
%CV	5.7	4.9	4.7

Recovery

Three samples of high, medium, and low concentrations were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 96%.

Sample	Expected Conc.(U/mL)	Observed Conc. (U/mL)	Recovery rate (%)
Sample 1	19	18.4	97
Sample 2	77	73.2	95
Sample 3	106	101.8	96

Sensitivity

The analytical sensitivity of the assay is 0.27 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.

13. Appendix II Example Analysis

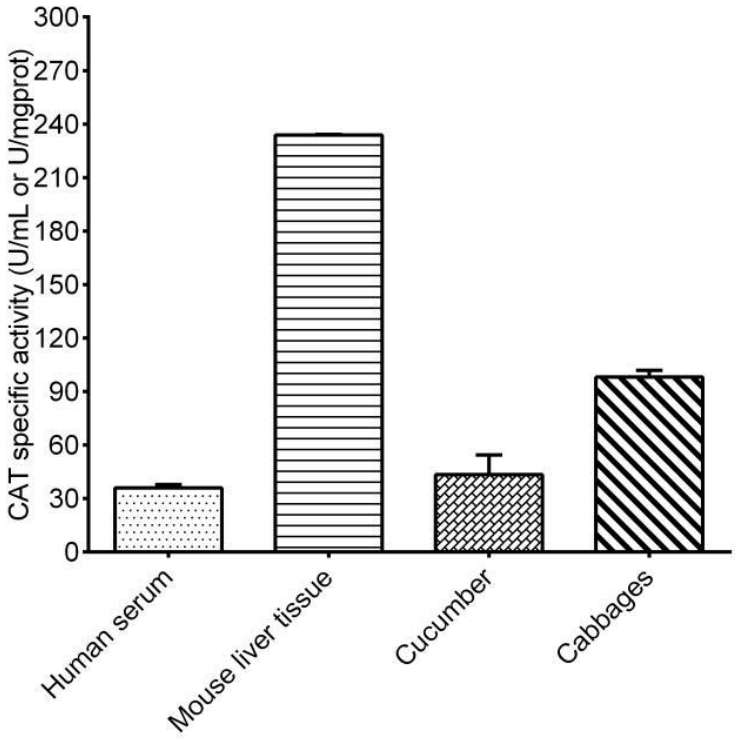
Example analysis:

Take 0.05 mL of 10% cabbage leaves tissue homogenate and carry out the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.351, the average OD value of the control is 0.602, the concentration of protein in sample is 1.64 mgprot/mL, and the calculation result is:

CAT activity (U/mgprot) = (0.602 - 0.351) × 32.5 / (1 × 0.05) ÷ 1.64 = 99.44 U/mgprot

Detect human serum (V=0.1 mL), 2% mouse liver tissue homogenate (protein concentration: 1.82 mgprot/mL, V=0.05 mL), 20% cucumber tissue homogenate (protein concentration: 0.91 mgprot/mL, V=0.05 mL) and 10% cabbage tissue homogenate (protein concentration: 1.64 mgprot/mL, V=0.05 mL) according to the protocol. The results are as follows:



14. 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.

Notes:

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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.