TECHNICAL MANUAL

Catalase (CAT) Activity Fluorometric Assay Kit

- **SKU CODE:** MAES0003
- **SIZE:** 96 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
- Incubate with substrate
- Add chromogenic agent
- Measure fluorescence intensity

2. Intended use

This kit can be used to measure Catalase (CAT) activity in serum, plasma, and tissue samples.

3. Detection principle

Catalase can decompose H_2O_2 to generate H_2O and O_2. The residual H_2O_2 in the detection system reacts with the fluorescent substance, and the content of residual H_2O_2 is proportional to the fluorescence intensity at an excitation wavelength of 535 nm and emission wavelength of 587 nm. The catalase activity is inversely proportional to the fluorescence intensity.

4. Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500Assays)	Storage
Reagent 1	Buffer Solution	60 mL x 1 vial	60 mL x 5 vials	-20 °C, 12 months
Reagent 2	Substrate	0.1 mL x 1 vial	0.5 mL x 1 vial	-20 °C, 12 months, protect from light
Reagent 3	Probe Solution	0.12 mL x 1 vial	0.6 mL x 1 vial	-20 °C, 12 months, protect from light
Reagent 4	Enzyme Reagent	Powder x 1 vial	Powder x 5 vials	-20 °C, 12 months, protect from light

Item	Component	Size 1 (96 T)	Size 2 (500Assays)	Storage
Reagent 5	1 mol/L H_2O_2 Standard Solution	0.4 mL x 1 vial	2 mL x 1 vial	-20 °C, 12 months, protect from light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate working solution:** Before testing, prepare sufficient substrate working solution according to the number of test wells. For example, to prepare 10 mL of substrate working solution, thoroughly mix 1 µL of substrate with 10 mL of double distilled water. The substrate working solution should be prepared fresh.
3. **Preparation of enzyme working solution:** Dissolve one vial of enzyme reagent with 120 µL of buffer solution and mix well to dissolve completely. Store at -20 °C protected from light and use within 1 month.
4. **Preparation of chromogenic agent:** For each well, prepare 50 µL of chromogenic agent by adding 48 µL of buffer solution, 1 µL of probe solution, and 1 µL of enzyme working solution. Mix well. The chromogenic agent should be prepared fresh and protected from light.
5. **Preparation of 100 µmol/L H_2O_2 solution:** Dilute 1 µL of 1 mol/L H_2O_2 standard solution with 10 mL of double distilled water and mix well to dissolve.
6. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 100 µmol/L H_2O_2 solution with double distilled water to serial concentrations. The recommended dilution gradient is as follows: 0, 1, 5, 10, 20, 30, 40, 50 µmol/L.

7. Sample preparation

Sample preparation:

Serum, plasma and other liquid samples: 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 buffer solution with a dounce homogenizer at 4 °C.

4. Centrifuge at 10,000 x 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):

Mouse serum: 60-80

Mouse plasma: 60-80

Rat serum: 70-80

Human saliva: 30-50

Rat urine: 30-50

10% Mouse liver tissue homogenate: 2500-3000

10% Mouse lung tissue homogenate: 200-400

10% Rat muscle tissue homogenate: 100-200

10% Rat brain tissue homogenate: 40-50

10% Mouse kidney tissue homogenate: 2000-2500

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

8. The key points of the assay

Dilute the samples to the optimal concentration for detection if the CAT activity of samples exceeds the detection range.

9. Operating steps

1. Standard well: Add 25 µL of standard with different concentrations into the well. Sample well: Add 25 µL of sample into the well. Control well: Add 25 µL of substrate working solution into the well.
2. Add 25 µL of double distilled water into standard wells. Add 25 µL of substrate working solution into sample wells.
3. Mix thoroughly with microplate reader for 10 s and incubate at 37 °C for 5 min.
4. Add 50 µL of chromogenic agent into each well.
5. Add 25 µL of sample into control wells.
6. Mix thoroughly with microplate reader for 10 s and incubate at room temperature for 10 min. Measure the fluorescence intensity at an excitation wavelength of 535 nm and emission wavelength of 587 nm.

10. Calculation

The standard curve:

1. Average the duplicate readings 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 using the absolute fluorescence value of standards 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 L of serum or plasma that decomposes 1 µmol H_{2O}_2 per minute at 37 °C is defined as 1 unit.

CAT activity (U/L) = (deltaF - b) ÷ a ÷ 5 × f

2. Tissue sample:

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

CAT activity (U/g prot) = (deltaF - b) ÷ a ÷ 5 × f ÷ C_{pr}

[Note]

deltaF: The absolute fluorescence value of sample, $F_{Control}$ - F_{Sample}

5: The reaction time, 5 min

f: Dilution factor of sample before testing

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 Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.80	3.00	5.40
%CV	4.2	3.5	2.5

Inter-assay Precision

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

Inter-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.80	3.00	5.40
%CV	7.5	6.0	6.9

Recovery

Three samples of high, middle, and low concentrations were tested with 6 replicates each to obtain an average recovery rate of 92%.

Recovery Results

	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	3	25	38
Observed Conc. (µmol/L)	2.7	23.3	35.3

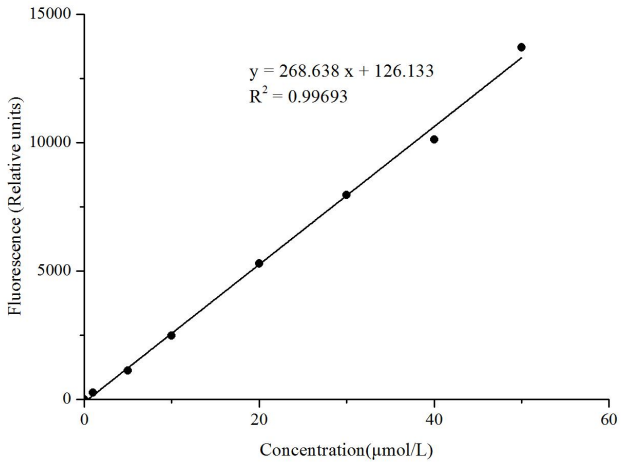
	Standard 1	Standard 2	Standard 3
Recovery rate (%)	90	93	93

Sensitivity

The analytical sensitivity of the assay is 0.01 U/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 data

Concentration (μmol/L)	Fluorescence value 1	Fluorescence value 2	Average fluorescence value	Absolute fluorescence value
0	102	101	102	0
1	351	351	351	249
5	1209	1229	1219	1117
10	2542	2603	2572	2471

Concentration (μmol/L)	Fluorescence value 1	Fluorescence value 2	Average fluorescence value	Absolute fluorescence value
20	5341	5434	5387	5286
30	7984	8133	8058	7957
40	10023	10396	10209	10108
50	13847	13778	13812	13711

12. Appendix II Example Analysis

Example analysis:

For mouse serum, dilute mouse serum with buffer solution 70-fold. Take 25 μL of diluted sample and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 268.77x - 55.205

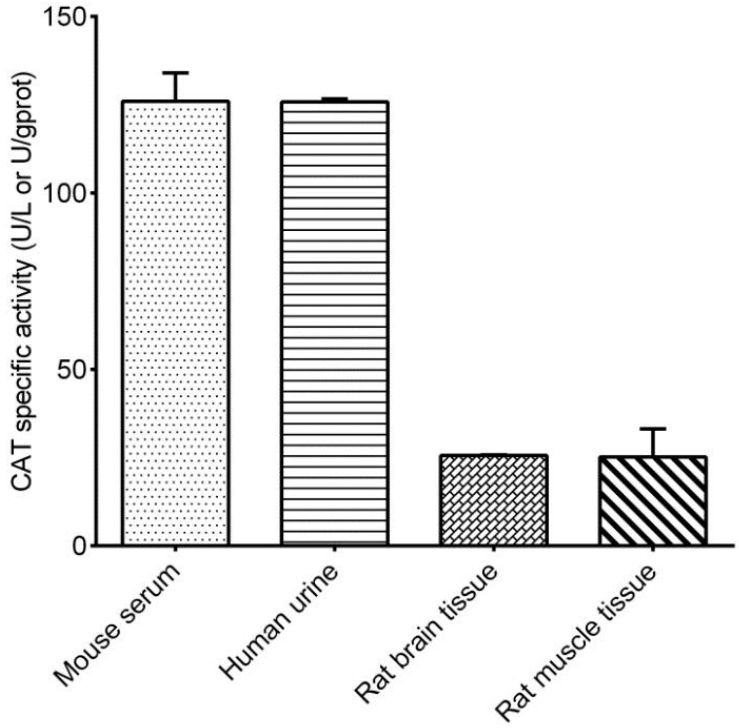
Average fluorescence value of sample: 8120

Average fluorescence value of control: 10483

Calculation result:

CAT activity (U/L) = (10483 - 8120 + 55.205) ÷ 268.77 ÷ 5 × 70 = 125.96 U/L

Detect mouse serum (diluted 70-fold), human urine (diluted 30-fold), 10% rat brain tissue homogenate (protein concentration 6.08 g prot/L, diluted 50-fold), and 10% rat muscle tissue homogenate (protein concentration 6.73 g prot/L, diluted 400-fold) 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 pretest 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.