Technical Manual

Catalase (CAT) Activity Assay Kit

- **Catalogue Code: MAES0003**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Fluorimetric method

Specification:

96T

Range:

0.01-6.51 U/L

Sensitivity:

0.01 U/L

Storage:

-20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

CAT is an enzyme in organism that can efficiently and specifically decompose hydrogen peroxide and is a binding enzyme with iron porphyrin as an auxiliary group. CAT clears hydrogen peroxide in the body and protects cells from the toxicity of H_2O_2. CAT can also oxidize certain cytotoxic substances, such as formaldehyde, formic acid, phenol and ethanol. According to the difference of catalytic center structure, CAT can be divided into two types, one is iron porphyrin structure, also known as iron porphyrin enzyme, the other contain manganese ion, also known as manganese catalase. CAT is common in breathing organisms. It is mainly found in chloroplasts, mitochondria, endoplasmic reticulum, liver and red blood cells of animals.

3. Intended Use

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

4. Detection Principle

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

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	60 mL × 1 vial	-20°C, 6 months
Substrate	0.1 mL × 1 vial	-20°C, 6 months, away from direct sunlight
Probe Solution	0.12 mL × 1 vial	-20°C, 6 months, away from direct sunlight
Enzyme Reagent	1 vial Lyophilized	-20°C, 6 months, away from direct sunlight
H_2O_2 Standard Solution (1 mol/L)	0.4 mL × 1 vial	-20°C, 6 months, away from direct sunlight
Black Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Centrifuge
- Fluorescence microplate reader (Ex/Em=535 nm/587 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

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

7. Reagent Preparation

1. The preparation of **substrate application solution:** Dilute the substrate for 10000 times with double distilled water and mix fully. Prepare the fresh solution before use.

2. The preparation of **enzyme application solution:** Dissolve a vial of enzyme reagent with 0.12 mL of buffer solution and mix fully. It can be stored at -20˚C for 30 days away from direct sunlight.

3. The preparation of **chromogenic agent:** Mix the buffer solution, probe solution and enzyme application solution at a ratio of 48:1:1. Prepare fresh solution before use and store away from direct sunlight.

4. The preparation of **100 µmol/L H_2O_2 standard solution:** Dilute 1 mol/L H_2O_2 Standard for 10000 times with double distilled water and mix fully.

8. Sample Preparation

1. **Serum sample:**
   Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection.

2. **Plasma sample:**
   Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection.

3. **Urine:**
   Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80°C for a month.
4. **Tissue sample:**
   Weigh the tissue sample, add 9 times the volume of Buffer Solution according to the ratio of Weight (g): Volume (mL) =1:9. Mechanically homogenate the sample in an ice water bath. Centrifuge at 1500 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

Homogenized Method:
Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), put the tissue pieces into a glass homogenized tube. Add homogenized medium into the homogenized tube and place the tube into an ice bath. Using a glass tamping rod, grind up and down for 6-8 min. Alternatively, place the tissue into a mortar and add liquid nitrogen to grind fully. Then, add the homogenized medium.
Mechanical Homogenate: Weigh the tissue in an EP tube. Add the homogenized medium to homogenize the tissue with an homogenizer instrument (60 Hz, 90s) in an ice bath. (For skin, muscle and plant tissue samples, prolong homogenization time accordingly).
Ultrasonication: Treat the cells with an ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, total time is 5 min).

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.01-6.51 U/L).

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

Sample Type:	Dilution Factor:
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.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: Ex/Em=535 nm/587 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'- S40', control wells.

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10. Operation Steps

The preparation of standard curve

Dilute 100μmol/L H_2O_2 standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 50, 40, 30, 20, 10, 5, 1, 0μmol/L.

The measurement of samples

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 application solution into the well.

2. Add 25 μL of double distilled water into standard well. Add 25 μL of substrate application solution into sample well.

3. Mix fully with microplate reader for 10 s and incubate at 37°C for 5 min.

4. Add 50 μL of chromogenic agent into each wells.

5. Add 25 μL of sample into control well.

6. Mix fully with microplate reader for 10 s and stand at room temperature for 10 min. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Operation Table

	Standard well	Sample well	Control well
Standard with different concentrations (μL)	25		
Samples (μL)		25	
Double distilled water (μL)	25		
Substrate application solution (μL)		25	25
Mix fully with microplate reader for 10 s and incubate at 37°C for 5 min			
Chromogenic Agent (μL)	50	50	50
Samples (μL)			25
Mix fully with microplate reader for 10 s and stand at room temperature for 10 min. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm with fluorescence microplate reader.			

11. Calculations

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the F value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

Definition: The amount of CAT in 1 L of serum or plasma that decompose 1 μmol H_2O_2 per minute at 37°C is defined as 1 unit.

$$\text{CAT activity (U/L)} = (\Delta F - b) \div a \div 5 \times f$$

2. **Tissue sample:**

Definition: The amount of CAT in 1 g of tissue protein that decompose 1 μmol H_2O_2 per minute at 37°C is defined as 1 unit.

$$\text{CAT activity (U/gprot)} = (\Delta F - b) \div a \div 5 \times f \div C_{pr}$$

y: The absolute fluorescence value of standard, $F_{Standard} - F_{Blank}$ (F_{Blank} is the F value when the standard concentration is 0)

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

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

5: The reaction time, 5 min

f: Dilution factor of sample before tested

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

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12. Performance Characteristics

Detection Range	0.01-6.51 U/L
Sensitivity	0.01 U/L
Average recovery rate (%)	92
Average inter-assay CV (%)	6.8
Average intra-assay CV (%)	3.4

Analysis

For Mouse serum, dilute mouse serum with buffer solution for 70 times, take 25 μL of diluted sample, carry the assay according to the operation table. The results are as follow.

The results are as follows:

standard curve: y =268.77 x -55.205, the average fluorescence value of the sample is 8120, the average fluorescence value of the control is 10483, and the calculation result is:

$$\boldsymbol{CAT\ activity}\left(\frac{\boldsymbol{U}}{\boldsymbol{L}}\right) = (\mathbf{10483} - \mathbf{8120} + \mathbf{55.205}) \div \mathbf{268.77} \div \mathbf{5} \times \mathbf{70}$$

$$= \mathbf{125.96}\ \boldsymbol{U/L}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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