# Technical Manual

# Catalase (CAT) Activity Assay Kit

- **Catalogue Code: MAES0044**
- **Size: 96T**
- **Research Use Only**

# 1. Key features and Sample Types

## Detection method:

Colorimetric method

## Specification:

96T

## Range:

1.12 -150 U/mL

## Sensitivity:

1.12 U/mL

## Storage:

2-8°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 H2O2. 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, cells, cell culture supernatant and tissue homogenate samples.

## 4. Detection Principle

The reaction that catalase (CAT) decomposes $H_2O_2$ can be quickly stopped by ammonium molybdate. The residual $H_2O_2$ reacts with ammonium molybdate to generate a yellowish complex. CAT activity can be calculated by production of the yellowish complex at 405 nm.

## 5. Kit components & storage

| Item | Specification | Storage |
|---|---|---|
| **Buffer Solution** | 24 mL × 1 vial | 2-8°C, 6 months |
| **Substrate** | 1.5 mL × 2 vials | 2-8°C, 6 months |
| **Chromogenic agent** | Lyophilized ×1 vial | 2-8°C, 6 months |
| **Clarificant** | 1.5 mL × 2 vials | 2-8°C, 6 months |
| **$H_2O_2$ standard solution (9.6 mol/L)** | 1.5 mL ×2 vials | 2-8°C, 6 months |
| **Microplate** | 96 wells | No requirement |
| **Plate Sealer** | 2 pieces | |

### Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (400-410 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

## 6. Assay Notes:

1. The reaction time must be accurate when substrate is added.

2. The test tube can be prepared and labelled in advance.

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

4. Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

## 7. Reagent preparation:

1. Incubate buffer solution and substrate at 37°C for 10 min before use.

2. The preparation of chromogenic agent application solution: dissolve a vial of chromogenic agent lyophilized with 24 mL with double-distilled water. (If there is sediment in the bottom, please directly take the supernatant for test, it will not affect the result). The prepared solution can be stored at 4°C for 3 months.

3. Clarificant will be frozen when cold, please warm it in 37°C water-bath until clear.

4. The preparation of $H_2O_2$ standard solution (1 mmol/mL): dilute the standard with double-distilled water at a ratio of 9.6 mol/L $H_2O_2$ standard solution: double-distilled water=5: 43 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. If not detected on the same day, the serum can be stored at -80°C for a month.

**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. If not detected on the same day, the plasma can be stored at -80°C for a month.

**3. Cell culture supernatant:**
Detect directly. If there is turbidity, centrifuge at 3100 g for 10 min. Take the supernatant to preserve it on ice for detection. If not detected on the same day, it can be stored at -80°C for a month.

**4. Cell sample:**
Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number ($2\times10^6$): PBS (0.01 M, pH 7.4) including 1mM EDTA (μL) =1: 300. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

**5. Tissue sample:**
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) including 1mM EDTA (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

## 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 (1.12 -150 U/mL).

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

| Sample Type: | Dilution Factor |
|---|---|
| Human serum | 1 |
| 293T supernatant | 1 |
| 10% Rat heart tissue homogenization | 50-100 |
| 10% Rat liver tissue homogenization | 100-200 |
| 10% Rat spleen tissue homogenization | 50-100 |
| Mouse serum | 1 |
| 10% Epipremnum aureum tissue homogenization | 1-2 |
| 10% Rat lung tissue homogenization | 50-100 |
| 10% Rat kidney tissue homogenization | 50-100 |
| 10% Rat brain tissue homogenization | 20-50 |

**Note:** The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

# 9. Assay Protocol

**Ambient Temperature:** 25-30°C

**Optimum detection wavelength:** 405 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.

# 10. Operation Steps

### The preparation of standard curve

Dilute $H_2O_2$ standard solution ()1 mmol/mL 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.

### The measurement of standard curve

1. Add 20 μL of $H_2O_2$ standard solution with different concentrations to the 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 fully.

3. Stand at room temperature for 10 min and take 200 μL of reaction solution from each tube to the microplate

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

## The measurement of samples

1. **Control tube:** Add 200 μL of buffer solution into the 1.5 mL EP tubes.
   **Sample tube:** Add 20 μL of sample and 200 μL of buffer solution into the 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 1 min accurately.

4. **Sample tube:** Add 200 μL of chromogenic agent application solution and 20 μL of clarificant, mix fully.
   **Control tube:** Add 200 μL of chromogenic agent application solution, 20 μL of clarificant and 20 μL of sample, mix fully.

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

6. Measure the OD value at 405 nm with microplate reader

## Operation Table

### For standard solution

| | Standard well |
|---|---|
| **Standards with different concentrations (μL)** | 20 |
| **Buffer solution (μL)** | 200 |
| **Double distilled water (μL)** | 20 |
| **Chromogenic agent application solution (μL)** | 200 |
| **Clarificant (μL)** | 20 |
| Mix fully and stand at room temperature for 10 min. Take 200 μL of reaction solution to the microplate and measure the OD value at 405 nm with microplate reader | |

### For sample

| | Control tube | Sample tube |
|---|---|---|
| **Sample (μL)** | | 20 |
| **Buffer solution (μL)** | 200 | 200 |
| Incubate at 37°C for 5 min. | | |
| **Substrate (μL)** | 20 | 20 |
| React at 37°C for 1 min accurately**.** | | |
| **Chromogenic agent application solution (μL)** | 200 | 200 |
| **Clarificant (μL)** | 20 | 20 |
| **Sample (μL)** | 20 | |
| Mix fully and stand at room temperature for 10 min. Take 200 μL of reaction solution to the microplate and measure the OD value at 405 nm with microplate reader. | | |



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## 11. Calculations

Plot the standard curve by using OD value 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 OD value of sample. The standard curve is: y= ax + b.

**1. Serum (plasma) sample:**

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

$$\text{CAT activity (U/mL)} = \frac{\Delta A}{a} \times \frac{0.02^*}{1^* \times V} \times f$$

**2. Tissue and cells sample:**

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

$$\text{CAT activity (U/mgprot)} = \frac{\Delta A}{a} \times \frac{0.02^*}{1^* \times V} \times f \div C_{pr}$$

y: $OD_{Standard} - OD_{Blank}$ ($OD_{Blank}$ is the OD value when the standard concentration is 0).
**x:** The concentration of standard.
**a:** The slope of standard curve .
**b:** The intercept of standard curve.
**0.02*:** The volume of standard, 0.02 mL.
**1*:** The reaction time, 1min.
**ΔA:** $OD_{Control} - OD_{Sample}$.
**V:** The volume of sample, mL.
**f:** Dilution factor of sample before test.
**$C_{pr}$:** Concentration of protein in sample, mgprot/mL.

## 12. Performance Characteristics

| | |
|---|---|
| **Detection Range** | 1.12 -150 U/mL |
| **Sensitivity** | 1.12 U/mL |
| **Average recovery rate (%)** | 100 |
| **Average inter-assay CV (%)** | 7.7 |
| **Average intra-assay CV (%)** | 3.9 |

### Analysis

Dilute 10% rat liver tissue homogenate with PBS (0.01 M, pH 7.4) for 100 times, take 0.02 mL of diluted sample, carry the assay according to the operation table.

**The results are as follows:**

Standard curve: y =0.0026 x + 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:

$$\text{CAT activity (U/mgprot)} = (0.612-0.442) \div 0.0026 \times 100 \div 12.38 = 528.15 \text{ U/mgprot}$$



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## Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent 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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