# Catalase(CAT) Activity Assay Kit(UV spectrophotometry)(UV spectrophotometry)



**Catalase (CAT) Activity Assay Kit - Microplate Method (Ultraviolet Absorption Method)**

Product code: 112869

## Product Introduction

Catalase (CAT, EC 1.11.1.6) is widely present in animals, plants, microorganisms, and cultured cells. It is an important $H_2O_2$-scavenging enzyme and plays a key role in the reactive oxygen species scavenging system.

$H_2O_2$has a characteristic absorption peak at 240 nm. CAT decomposes $H_2O_2$, causing the absorbance of the reaction solution at 240 nm to decrease over time. CAT activity is calculated from the rate of absorbance change.

Reference image note: The sample shown was 500-fold diluted pig liver. Actual readings may vary depending on test conditions and the instrument used. Data shown in the source figure are for reference only.

## Product Packing List

| Size | Code | Component | Quantity |
|---|---|---|---|
| 100T | 112869.1 | Reagent I | 24 mL |
| 100T | 112869.2 | Reagent II | 55 μL × 2 |
| 100T | 112869.3 | Extraction Solution | 110 mL |
| 100T | 112869.m | Instruction Manual | 1 copy |

## Quality Standards and Safety Information

| Raw Material or Packaging Name | Quality Standard | Main Toxicity |
|---|---|---|
| Reagent I | -- | -- |
| Reagent II | -- | -- |
| Extraction Solution | -- | -- |

## Transportation and Storage

| | |
|---|---|
| Transportation | Transport with ice packs. |
| Storage | Store at 2-8℃. Shelf life is 180 days. |

## Instructions for Use

### 1. Sample Preparation

### 1.1 Bacteria or Cells

1. Collect bacteria or cells into a centrifuge tube.
2. Centrifuge and discard the supernatant.
3. Add 400 μL extraction solution for every 2,000,000 bacteria or cells.
4. Disrupt the bacteria or cells by ultrasonication at 20% power: ultrasonication for 3 s, interval for 10 s, repeat 30 times.
5. Centrifuge at 8000 g and 4℃ for 10 min.
6. Collect the supernatant and keep it on ice for testing.

### 1.2 Tissue

1. Weigh approximately 0.1 g tissue.
2. Add 1 mL extraction solution.
3. Homogenize in an ice bath.
4. Centrifuge at 8000 g and 4℃ for 10 min.
5. Collect the supernatant and keep it on ice for testing.

### 1.3 Serum or Plasma

Test serum or plasma samples directly.

### 2. Assay Procedure

1. Before use, add 11 mL Reagent I to each vial of Reagent II and mix thoroughly to prepare the working solution.
2. Before measurement, place the CAT assay working solution in a water bath at 37℃ for mammals or 25℃ for other species for 10 min.
3. Add 200 μL CAT assay working solution to a 96-well UV plate.
4. Add 10 μL crude enzyme solution and mix thoroughly.
5. Immediately measure the initial absorbance at 240 nm at room temperature as $A_1$.
6. Measure the absorbance after 1 min as $A_2$.
7. Calculate $\Delta A = A_1 - A_2$.

## CAT Activity Calculation

### 1. Serum or Plasma

Unit definition: The amount of enzyme that catalyzes the degradation of 1 μmol $H_2O_2$ per minute in each mL of serum or plasma in the reaction system is defined as one enzyme activity unit.

$$CAT\ (U/mL) = [\Delta A \times V_{total\ reaction} \div (\varepsilon \times d) \times 10^6] \div V_{sample} \div T = 802.751 \times \Delta A$$

### 2. Tissue

### 2.1 Calculated by Sample Protein Concentration

Unit definition: The amount of enzyme that catalyzes the degradation of 1 μmol $H_2O_2$ per minute in each mg of tissue protein in the reaction system is defined as one enzyme activity unit.

$$CAT\ (U/mg\ prot) = [\Delta A \times V_{total\ reaction} \div (\varepsilon \times d) \times 10^6] \div (V_{sample} \times Cpr) \div T = 802.751 \times \Delta A \div Cpr$$

### 2.2 Calculated by Sample Fresh Weight

Unit definition: The amount of enzyme that catalyzes the degradation of 1 μmol $H_2O_2$ per minute in each g of tissue in the reaction system is defined as one enzyme activity unit.

$$CAT\ (U/g\ fresh\ weight) = [\Delta A \times V_{total\ reaction} \div (\varepsilon \times d) \times 10^6] \div (W \times V_{sample} \div V_{total\ sample}) \div T = 802.751 \times \Delta A \div W$$

### 3. Bacteria or Cells

Unit definition: The amount of enzyme that catalyzes the degradation of 1 μmol $H_2O_2$ per minute in each 10,000 bacteria or cells in the reaction system is defined as one enzyme activity unit.

$$CAT\ (U/10^4 cells) = [\Delta A \times V_{total\ reaction} \div (\varepsilon \times d) \times 10^6] \div (500 \times V_{sample} \div V_{total\ sample}) \div T = 1.6055 \times \Delta A$$

### Formula Parameters

| | |
|---|---|
| $V_{total\ reaction}$ | Total volume of the reaction system, $0.21 \times 10^{-3}$ L |
| ε | $H_2O_2$ molar extinction coefficient, 43.6 L/mol/cm |
| | |

| | |
|---|---|
| d | 1 mL optical path length of the quartz cuvette, 0.6 cm |
| $V_{sample}$ | Volume of sample added, 0.01 mL |
| $V_{total\ sample}$ | Volume of extraction solution added, 1 mL |
| T | Reaction time, 1 min |
| W | Sample mass, g |
| Cpr | Supernatant protein concentration, mg/mL |
| 500 | Total number of cells or bacteria, 5,000,000 |
| $10^6$ | Unit conversion factor, 1 mol = $10^6$μmol |

## Precautions

1. Before formal measurement, select 2-3 samples with large expected differences for preliminary testing. This 100T kit can test 96 samples.
2. Required instruments and supplies are not provided: benchtop centrifuge, microplate reader, 96-well UV plate, adjustable pipette, mortar or homogenizer, ice, and distilled water.
3. If a large number of bubbles form in the reaction solution, the enzyme activity of the sample is too high. Dilute the sample with distilled water before measurement.

## Visual Reference