

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 H₂O₂-scavenging enzyme and plays a key role in the reactive oxygen species scavenging system.

H₂O₂ has a characteristic absorption peak at 240 nm. CAT decomposes H₂O₂, 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°C. 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°C 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°C 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°C for mammals or 25°C 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 H₂O₂ per minute in each mL of serum or plasma in the reaction system is defined as one enzyme activity unit.

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

2. Tissue

2.1 Calculated by Sample Protein Concentration

Unit definition: The amount of enzyme that catalyzes the degradation of 1 µmol H₂O₂ per minute in each mg of tissue protein in the reaction system is defined as one enzyme activity unit.

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

2.2 Calculated by Sample Fresh Weight

Unit definition: The amount of enzyme that catalyzes the degradation of 1 µmol H₂O₂ per minute in each g of tissue in the reaction system is defined as one enzyme activity unit.

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

3. Bacteria or Cells

Unit definition: The amount of enzyme that catalyzes the degradation of 1 µmol H₂O₂ per minute in each 10,000 bacteria or cells in the reaction system is defined as one enzyme activity unit.

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

Formula Parameters

$V_{\text{total reaction}}$	Total volume of the reaction system, 0.21×10^{-3} L
ϵ	H ₂ O 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
C _{pr}	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