Catalase Assay Kit (UV Spectrophotometry)

Description

Catalase (CAT) (EC 1.11.1.6) is widely distributed in animals, plants, microorganisms, and cultured cells. It is the primary hydrogen peroxide(H_2O_2) scavenging enzyme and plays a crucial role in the reactive oxygen species (ROS) scavenging system.

Detection Principle

H_2O_2 exhibits 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. The CAT activity can be calculated based on the rate of absorbance change.

Product Information

Composition and Storage Conditions (50T/48S)

Component	Specification	Storage Condition
CB0044UV-ES	60 mL × 1 bottle	Store at 4°C
CB0044UV-A	60 mL × 1 bottle	Store at 4°C
CB0044UV-B	100 μL × 3 bottles	Store at 4°C
Preparation of CAT Detection Working Solution: Before use, add 20 mL of CB0044UV-A to each bottle of CB0044UV-B, mix thoroughly, and use as the working solution. Unused reagents can be stored at 4°C for one week.		

Note: Before formal measurement, it is recommended to select 2–3 samples with expected large differences for preliminary testing.

Instructions

I. Required Equipment and Materials:

UV spectrophotometer, 1 mL quartz cuvette, benchtop centrifuge, water bath, adjustable pipette, mortar, ice, and double-distilled water.

II. Crude Enzyme Solution Extraction:

1. Bacteria or Cultured Cells

Collect bacteria or cells into centrifuge tubes and centrifuge to remove the supernatant.

Add CB0044UV-ES according to the ratio of bacterial/cell number (10^4 cells): CB0044UV-ES volume (mL) = 500–1000:1. (it is recommended to add 1 mL of CB0044UV-ES per 5 million bacteria or cells.), disrupt the cells via ultrasonication (ice bath, power 20% or 200 W, sonicate for 3 s, interval 10 s, repeat 30 times). Then, centrifuge at 8,000 × g at 4°C for 10 min. Collect the supernatant and keep it on ice for analysis.

2. Tissue Samples

Add extraction solution according to the ratio of tissue mass (g): extraction solution volume (mL) = 1:5–10 (it is recommended to weigh approximately 0.1 g of tissue and add 1 mL of extraction liquid), perform homogenization in an ice bath. Centrifuge at 8000 × g for 10 min at 4°C. Collect the supernatant and keep it on ice for analysis.

3. Serum (plasma) samples: Detect directly.

III. Assay Procedure:

1. Preheat the UV spectrophotometer for at least 30 min, set the wavelength to 240 nm, and zero the instrument with distilled water.
2. Prepare reagents according to the Product Composition and Storage Conditions table.
3. Before measurement, incubate the CAT Detection Working Solution in a water bath at 37°C (for mammals) or 25°C (for other species) for 10 min.
4. Add 1 mL CAT Detection Working Solution into a 1 mL quartz cuvette, then add 35 μL sample and mix thoroughly for 5s. Immediately measure the initial absorbance at 240 nm at room temperature as A1, and measure the absorbance after 1 min as A2. Calculate $\Delta A = A1 - A2$

IV. CAT Activity Calculation:

1. CAT Activity Calculation in Serum (Plasma)

Definition of Unit: One unit (U) of CAT activity is defined as the amount of enzyme in 1 mL of serum (plasma) that catalyzes the degradation of 1 nmol of H_2O_2 per minute in the reaction system.

Calculation Formula: CAT (U/mL) = [ΔA × V_total ÷ (ε×d) × 10^9] ÷ V_sample ÷ T =678 × ΔA

2. CAT Activity Calculation in Tissue, Bacteria, or Cells

(1) Calculation Based on Sample Protein Concentration

Definition of Unit: One unit (U) of CAT activity is defined as the amount of enzyme per mg of tissue protein that catalyzes the degradation of 1 nmol of H_2O_2 per minute in the reaction system.

Calculation Formula: CAT (U/ mg prot) = [ΔA × V_total ÷ (ε×d) × 10^9] ÷ (V_sample × Cpr) ÷ T = 678 × ΔA ÷ Cpr

(2) Calculation Based on Fresh Sample Weight

Definition of Unit: One unit (U) of CAT activity is defined as the amount of enzyme per g of tissue that catalyzes the degradation of 1 nmol of H_2O_2 per minute in the reaction system.

Calculation Formula: CAT (U/g fresh weight) = [ΔA × V_total ÷ (ε×d) × 10^9] ÷ (W × V_sample ÷ V_sample_total) ÷ T = 678 × ΔA ÷ W

(3) Calculation Based on CAT Activity in Bacteria or Cells

Definition of Unit: One unit (U) of CAT activity is defined as the amount of enzyme per 10^4 bacteria or cells that catalyzes the degradation of 1 nmol of H_2O_2 per minute in the reaction system.

Calculation Formula: CAT (U/10^4 cell) = [ΔA × V_total ÷ (ε×d) × 10^9] ÷ (500 × V_sample ÷ V_sample_total) ÷ T = 1.356 × ΔA

Note:

V_total: Total reaction volume, 1.035×10^{-3} L

ε: Molar extinction coefficient of H_2O_2, 4.36×10^4 L/mol/cm

d: Optical path length of cuvette, 1 cm

V_sample: Sample volume added, 0.035 mL

V_sample_total: Volume of CB0044UV-ES added, 1 mL

T: Reaction time, 1 min

W: Sample weight (g)

Cpr: Protein concentration of supernatant, mg/mL

500: Total number of bacteria or cells, 5 million

10^9: Unit conversion factor, 1 mol = 10^9 nmol

Precautions

1. For protein quantification, the TargetMol BCA Protein Quantification Kit (C0050) is recommended.
2. This product is intended for scientific research use only. It must not be used for clinical diagnosis or treatment, food or pharmaceutical applications, and must not be stored in residential environments.
3. For your safety and health, please wear a lab coat and disposable gloves during operation.

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