Ver. 240802

Catalase (CAT) Activity Assay Kit

BC1102-01 (50 Tests/48 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

CAT is an enzyme found broadly in animals, plants, microorganisms and cultured cells. It is the main enzyme of clearing H_2O_2, which plays an important role in the active oxygen scavenging system. H_2O_2 has characteristic absorption peak at 240 nm. It can be decomposed into water and oxygen by CAT which makes the absorbance of reagent at 240 nm decreases. The activity of CAT can be calculated according to the change rate of absorbance.

Kit components

Reagent	Volume	Storage
Extraction Solution	60 mL × 1	2-8°C
Reagent I	60 mL × 1	2-8°C
Reagent II	300 μL × 1	2-8°C

Solution Preparation:

Reagent II:

The liquid is placed in an EP tube inside the bottle and needs to be centrifuged before use.

Preparation of working liquid:

Before use, the working liquid was prepared according to the sample size in the ratio of : Reagent II: Reagent I =50 μL: 13mL (13.05 mL, 13T)

Reagents and Equipment Required but Not Provided

Ultraviolet spectrophotometer, refrigerated centrifuge, transferpettor,1 mL quartz cuvette, mortar/homogenizer, ice and distilled water.

Protocol

I.   Sample preparation
   Bacteria or cells:
   Collect bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. Accordance ratio bacteria or cell amount (10^4): Extraction reagent volume (mL)=500~1000:1. It is suggested that add 1 mL of Extraction reagent to 5 million of bacteria or cells. Use ultrasonication to split bacteria and cell (place on ice, ultrasonic power 200W, working time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 8000×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice for test.
   Tissue
   Accordance ratio tissue weight (g): Extraction reagent volume (mL)=1:5~10. It is suggested that add 1 mL of Extraction reagent to 0.1 g of tissue, and fully homogenize on ice bath. Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials, and take the supernatant on ice for test.

   Serum (plasma) sample: Detect sample directly

II. Determination procedure

1. Preheat the spectrophotometer more than 30 minutes, adjust the wavelength to 240 nm, set zero with distilled water.
2. Preheat CAT working reagent in water bath at 37°C(mammals) or 25°C (other species) for 10 minutes.
3. Add 1 mL of CAT working reagent and 35 µL of sample in 1 mL quartz cuvette, mix for 5 seconds. Immediately detect the absorbance at 240 nm at the initial time (A1) and the absorbance after reaction for 1 minute (A2), calculate ΔA=A1-A2.

III. Calculation:

a) Serum (plasma) sample

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the degradation of 1 μmol of H_2O_2 in the reaction system per minute every milliliter serum (plasma).

$$CAT(U/mL) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^6] \div Vs \div T$$
$$= 678 \times \Delta A$$

b) Tissue, bacteria or cells

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the degradation of 1 μmol of H_2O_2 in the reaction system per minute every milligram protein.

$$CAT (U/mg\ prot) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^6] \div (Vs \times Cpr) \div T$$
$$= 678 \times \Delta A \div Cpr$$

2. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the degradation of 1 μmol of H_2O_2 in the reaction system per minute every gram tissue sample.

$$CAT (U/g\ weight) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^6] \div (W \times Vs \div Vsv) \div T$$
$$= 678 \times \Delta A \div W$$

3. Bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the degradation of 1 μmol of H_2O_2 in the reaction system per minute every 10^4 bacteria or cells.

$$CAT(U/10^4 cell) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^6] \div (500 \times Vs \div Vsv) \div T$$
$$= 1.356 \times \Delta A$$

Vrv: Reaction total volume, 1.035×10^{-3} L
ε: Molar extinction coefficient, 43.6 L/mol/cm
d: Light path of cuvette, 1 cm
Vs: Sample volume, 0.035 mL
Vsv: Extraction volume, 1 mL
T: Reaction time, 1 minute
Cpr: Sample protein concentration, mg/mL
W: Sample weight, g
500: Total number of bacteria and cells, 5 million
10^6: Unit conversion factor, 1 mol=10^6 μmol.

Note

If there are a lot of bubbles in the reaction solution, dilute the sample with distilled water before determination.