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Catalase (CAT) Activity Assay Kit

100T/96S

Catalogue Number:AK0612-100T-96S

Store all reagents at 4℃

Validity Period: six months

Operation Equipment: Spectrophotometer/Microplate reader

For samples:

Serum, plasma, cell, Bacteria, fungj, Tissue homogenate.

FOR RESEARCH USE ONLY！

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS！

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING！

Catalase (CAT) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

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.

Components:

Extraction reagent: Liquid 100 mL×1. Storage at 4℃;
Reagent I: Liquid 30 mL×1. Storage at 4℃.
Reagent Ⅱ: Liquid 125 μL×1. Storage at 4℃.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, refrigerated centrifuge, transferpettor, micro quartz cuvette/96 well UV flat-bottom plate, mortar/ homogenizer, ice and distilled water.

Procedure:

I. Sample preparation:

1. Bacteria or cells: Collecting bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. It is suggested that add 1 mL of Extraction reagent to 5 million of bacteria or cells. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 20%, working time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing.
2. Tissue: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℃ to remove insoluble materials, and take the supernatant on ice for test.
3. 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. CAT working reagent: Add 25 mL ofReagent I to Reagent Ⅱ before use, mix thoroughly.
3. Preheat CAT working reagent in water bath at 37℃(mammas) or 25℃ (other species) for 10 minutes.
4. Add 190 μL of CAT working reagent and 10 μL of sample in micro quartz cuvette/96 well UV flat-

bottom plate. Immediately mixing and timing, 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. micro quartz cuvette

1. Serum (plasma) sample

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the degradation of 1μmol ofH_2O_2 in the reaction system per minute every milliliter serum (plasma).

CAT (U/mL) =[ΔA×Vrv÷(ε×d)×10^6]÷Vs÷T=459×ΔA

2. 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 1μmol ofH_2O_2 in the reaction system per minute every milligram protein.

CAT (U/mg prot) = [ΔA×Vrv÷(ε×d)×10^6]÷(Vs×Cpr)÷T=459×ΔA÷Cpr

2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the degradation of 1μmol ofH_2O_2 in the reaction system per minute every gram tissue sample.

CAT (U/g weight)=[ΔA×Vrv÷(ε×d)×10^6]÷(W×Vs÷Vsv)÷T=459×ΔA÷W

3) Bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the degradation of 1μmol ofH_2O_2 in the reaction system per minute every 10^4 bacteria or cells.

CAT (U/10^4cell) = [ΔA×Vrv÷(ε×d)×10^6]÷(500×Vs÷Vsv)÷T=0.917×ΔA

Vrv: Reaction total volume, 2×10^{-4} L;

ε: Molar extinction coefficient, 43.6 L/mol/cm;

d: Light path of cuvette, 1 cm;

Vs: Sample volume,0.01 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;

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

B. 96 well UV flat-bottom plate

1. Serum (plasma) sample

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the degradation of 1μmol ofH_2O_2 in the reaction system per minute every milliliter serum (plasma).

CAT (U/mL) =[ΔA×Vrv÷(ε×d)×10^6]÷Vs÷T=764.5×ΔA

2. 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 1μmol of H_2O_2 in the reaction system per minute every milligram protein.

CAT (U/mg prot) = [ΔA×Vrv÷(ε×d)×10^6]÷(Vs×Cpr)÷T=764.5×ΔA÷Cpr

2) Sample weight

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

CAT (U/g) =[ΔA×Vrv÷(ε×d)×10^6]÷(W×Vs÷Vsv)÷T=764.5×ΔA÷W

3) Bacteria or cells

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

CAT (U/10^4cell) = [ΔA×Vrv÷(ε×d)×10^6]÷(500×Vs÷Vsv)÷T=1.529×ΔA

Vrv: Reaction total volume, $2×10^{-4}$ L;

ε: Molar extinction coefficient, 43.6 L/mol/cm;

d: light path of 96 well plate, 0.6 cm;

Vs: Sample volume,0.01 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;

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