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Catalase (CAT) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: AK0579-100T-96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent name	100T Size	200T Size	Preservation Condition
Extract solution	Liquid 110 mL×1	Liquid 110 mL×2	2-8℃
Reagent I	Liquid 30 mL×1	Liquid 45 mL×1	2-8℃
Reagent II	Liquid 110μL×1	Liquid 210μL×1	2-8℃

100T/200T Solution Preparation:

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

2. Preparation of working liquid(One 15 mL empty bottle is provided):

A. 96 well UV flat-bottom plate： add 25 μL of Reagent II to 5 mL of Reagent I before use, mix thoroughly as Working solution (about 26T). Or according to the proportion of preparation, the reagent should be prepared just before use.

B. micro quartz cuvette： add 25 μL of Reagent II to 6.5 mL of Reagent I before use, mix thoroughly as Working solution (about 34T). Or according to the proportion of preparation, the reagent should be prepared just before use.

Product Description:

CAT(EC 1.11.1.6)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.

Reagents and Equipment Required but Not Provided:

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

Operation procedure

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Bacteria or cells:

Collect bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. It is suggested that add 1 mL of Extract solution reagent to 5 million of bacteria or cells. Use ultrasonication to split bacteria and cells (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℃ to remove insoluble materials and take the supernatant on ice for testing.

2. Tissue:

It is suggested that add 1 mL of Extract solution 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.

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℃(mammals) or 25℃ (other species) for 10 minutes.

3. Add 190 μL of CAT working reagent and 10 μL of sample in micro quartz cuvette/96 well UV flat-bottom plate. Immediately mix and 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. Calculate by liquid volume:

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 milliliter serum (plasma).

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

2. Calculate by sample 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×F=459×ΔA÷Cpr×F

3. Calculate by sample mass:

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 mass) =[ΔA×Vrv÷(ε×d)×10^6]÷(W×Vs÷Vsv)÷T×F=459×ΔA÷W×F

4. Calculate by the number of 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]÷(N×Vs÷Vsv)÷T×F=459×ΔA÷N×F

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 mass, g;
N: Total number of bacteria and cells, count by 10^4;
10^6: Unit conversion factor, 1 mol=10^6 μmol;
F: Dilution factor.

B. 96 well UV flat-bottom plate

1. Calculate by liquid volume:

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 milliliter serum (plasma).

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

2. Calculate by sample 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×F=764.5×ΔA÷Cpr×F

3. Calculate by sample mass:

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×F=764.5×ΔA÷W×F

4. Calculate by the number of 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×F=1.529×ΔA×F

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 mass, g;
500: Total number of bacteria and cells, 5 million;

10^6: Unit conversion factor, 1 mol=10^6 μmol.

F: Dilution factor.

Note:

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

Recent Product Citations:

[1] Xu J, Chu T, Yu T, Li N, Wang C, Li C, Zhang Y, Meng H, Nie G. Design of Diselenide-Bridged Hyaluronic Acid Nano-antioxidant for Efficient ROS Scavenging to Relieve Colitis. ACS Nano. 2022 Aug 23;16(8):13037-13048. doi: 10.1021/acsnano.2c05558. Epub 2022 Jul 21. PMID: 35861614.