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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

Cat No: AK0580

Size: 50T/48S

Components:

Extraction reagent: Liquid 60 mL×1. Storage at 4℃;

Reagent I: Liquid 60 mL×1. Storage at 4℃ .

Reagent Ⅱ: Liquid 320 μL×1. Storage at 4℃ . Centrifuge before use.

Working solution: Add 100 μL of Reagent Ⅱ to 20 mL of Reagent I before use, mix thoroughly as Working solution (about 20T). Or according to the proportion of preparation, the reagent should be prepared just before use.

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.

Reagents and Equipment Required but Not Provided:

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

Procedure:

I. Sample preparation:

1. 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℃ to remove insoluble materials and take the supernatant on ice for test.

2. 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℃ 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. Preheat CAT working reagent in water bath at 37℃(mammals) or 25℃ (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:

1. 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) = [ΔA×Vrv÷(ε×d)×10^6]÷Vs÷T=678×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 1 μmol 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=678×ΔA÷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) =[ΔA×Vrv÷(ε×d)×10^6]÷(W×Vs÷Vsv)÷T=678×ΔA÷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/10^4cell) = [ΔA×Vrv÷(ε×d)×10^6]÷(500×Vs÷Vsv)÷T=1.356×ΔA

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.

Recent Product Citations:

[1] Zhang Z, Liu H, Sun C, et al. A C2H2 zinc-finger protein OsZFP213 interacts with OsMAPK3 to enhance salt tolerance in rice[J]. Journal of plant physiology, 2018, 229: 100- 110.

[2] Yin Y J, Chen C J, Guo S W, et al. The fight against Panax notoginseng root-rot disease using zingiberaceae essential oils as potential weapons[J]. Frontiers in plant science, 2018, 9: 1346.

[3] Yang Y, Li J, Wei C, et al. Amelioration of nonalcoholic fatty liver disease by swertiamarin in fructose-fed mice[J]. Phytomedicine, 2019, 59: 152782.

[4] Chen G, Jia Z, Wang L, et al. Effect of acute exposure of saxitoxin on development of zebrafish embryos (Danio rerio) [J]. Environmental Research, 2020: 109432.

References:

[1] Catalase in vitro. [J]. Methods Enzymol, 105:121- 126.

[2] Johansson L H, Borg L A H. A spectrophotometric method for determination of catalase activity in small tissue samples[J]. Analytical biochemistry, 1988, 174(1): 331-336.

Related Products:

AK0293/AK0292 Polyphenol Oxidase (PPO) Activity Assay Kit

AK0578/AK0577 Phenylalnine Ammonialyase (PAL) Activity Assay Kit

AK0584/AK0583 Superoxide Dismutase (SOD) Activity Assay Kit

AK0598/AK0597 Peroxidase (POD) Activity Assay Kit