Catalase (CAT) Activity Assay Kit

Note: The reagents of this product have changed, please pay attention to and strictly follow the instructions.

Operation Equipment: Spectrophotometer

Cat No: BC0200

Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 60 mL×1	2-8℃
Reagent II	Liquid 300μL×1	2-8℃

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

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:

Ultraviolet 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 solution 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] Saikumar S, Mani R, Ganesan M, Dhinakarasamy I, Palanisami T, Gopal D. Trophic transfer and their impact of microplastics on estuarine food chain model. J Hazard Mater. 2024 Feb 15; 464:132927. doi: 10.1016/j.jhazmat.2023. 132927. Epub 2023 Nov 4. PMID: 37984149.

[2] Wang L, Lu H, Zhang X, He Y, Zhang J, Guo X, Fu H, Ye G, Shu Q. Disruption of serotonin biosynthesis increases resistance to striped stem borer without changing innate defense response in rice. J Pineal Res. 2023 Sep;75(2): e12895. doi: 10.1111/jpi.12895. Epub 2023 Jul 10. PMID: 37392131.

[3] Liu Z, Chen B, Xiang S, Hu S. Self-immolative nanocapsules precisely regulate depressive neuronal microenvironment for synergistic antidepression therapy. J Nanobiotechnology. 2023 Aug 17;21(1):274. doi: 10.1186/s12951-023-02008-9. PMID: 37592281; PMCID: PMC10433581.

[4] Xiao S, Song W, Xing J, Su A, Zhao Y, Li C, Shi Z, Li Z, Wang S, Zhang R, Pei Y, Chen H, Zhao J. ORF355 confers enhanced salinity stress adaptability to S-type cytoplasmic male sterility maize by modulating the mitochondrial metabolic homeostasis. J Integr Plant Biol. 2023 Mar;65(3):656-673. doi: 10.1111/jipb.13382. Epub 2023 Jan 3. PMID: 36223073.

[5] Liang L, Zhang G, Dai X, Li W. The removal of antibiotic resistant bacteria and antibiotic resistance genes by sulfidated nanoscale zero-valent iron activating periodate: Efficacy and mechanism. Environ Res. 2023 Nov 1;236(Pt 2):116829. doi: 10.1016/j.envres.2023.116829. Epub 2023 Aug 5. PMID: 37544470.

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:

BC0190/BC0195	Polyphenol Oxidase (PPO) Activity Assay Kit
BC0210/BC0215	Phenylalnine Ammonialyase (PAL) Activity Assay Kit
BC0170/BC0175	Superoxide Dismutase (SOD) Activity Assay Kit
BC0090/BC0095	Peroxidase (POD) Activity Assay Kit