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/Microplate reader

Cat No: BC0205

Size: 100T/96S

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 110 mL×1	2-8℃
Reagent I	Liquid 30 mL×1	2-8℃
Reagent II	Liquid 110μL×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 liquid:

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 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/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:

Collect 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 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 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 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. Serum (plasma) sample

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

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 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=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;

10^6: 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] 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.

[2] Hu XH, Shen S, Wu JL, Liu J, Wang H, He JX, Yao ZL, Bai YF, Zhang X, Zhu Y, Li GB, Zhao JH, You X, Xu J, Ji YP, Li DQ, Pu M, Zhao ZX, Zhou SX, Zhang JW, Huang YY, Li Y, Ning Y, Lu Y, Huang F, Wang WM, Fan J. A natural allele of proteasome maturation factor improves rice resistance to multiple pathogens. Nat Plants. 2023 Feb;9(2):228-237. doi: 10.1038/s41477-022-01327-3. Epub 2023 Jan 16. PMID: 36646829.

[3] Xu X, Li G, Zhang D, Zhu H, Liu GH, Zhang Z. Gut Microbiota is Associated with Aging-Related Processes of a Small Mammal Species under High-Density Crowding Stress. Adv Sci (Weinh). 2023 May;10(14): e2205346. doi: 10.1002/advs.202205346. Epub 2023 Mar 25. PMID: 36965140; PMCID: PMC10190659.

[4] Liu YS, Tao Y, Yang XZ, Liu YN, Shen RF, Zhu XF. Gibberellic acid alleviates cadmium toxicity in rice by regulating NO accumulation and cell wall fixation capacity of cadmium. J Hazard Mater. 2022 Oct 5; 439:129597. doi: 10.1016/j.jhazmat.2022.129597. Epub 2022 Jul 16. PMID: 35868086.

[5] Zhang S, Wang H, Liu M, Yu H, Peng J, Cao X, Wang C, Liu R, Kamali M, Qu J. Press perturbations of microplastics and antibiotics on freshwater micro-ecosystem: Case study for the ecological restoration of submerged plants. Water Res. 2022 Nov 1; 226:119248. doi: 10.1016/j.watres.2022.119248. Epub 2022 Oct 14. PMID: 36323200.

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