SUNLONG

SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Catalase (CAT) Activity Assay Kit (Ammonium molybdate method)

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

Operation Equipment: Spectrophotometer/ Microplate reader

Cat No: AK0017

Size: 100T/48S

Components:

Extracting solution: Liquid 75 mL×1. Storage at 4℃ .

Reagent I: Liquid 15 mL×1. Storage at 4℃ .

Reagent Ⅱ: Powder×2. Storage at 4℃ . Before use, take a bottle of Reagent Ⅱ and add 12.5 mL of distilled water to dissolve it completely. Keep the unused reagent at 4℃ for one week.

Standard solution: Liquid 0.5 mL×1. Storage at 4℃ . Preparation of 30 μmol/mL standard solution: the standard solution provided by this kit is 1 mmol/mL H_2O_2 standard solution. Before use, take 0.15 mL of standard solution and add 4.85 mL of Reagent Ⅰ to dilute it or prepare it according to the proportion of sample size, and mix it well. That is 30 μmol/mL standard solution, prepare when the solution will be used.

Product Description:

CAT (EC 1. 11. 1.6) widely exists in animals, plants, microorganisms and cultured cells. It is the most important H_2O_2 scavenging enzyme and plays an important role in the scavenging system of reactive oxygen species.

H_2O_2 can react with ammonium molybdate to form a stable yellow complex with a strong absorption peak at 405 nm, and its absorption value is proportional to the concentration of hydrogen peroxide. By measuring the amount of residual H_2O_2 in the reaction system, the amount of H_2O_2 catalysed by CAT is obtained, which reflected the activity of CAT.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, desktop centrifuge, adjustable pipette, constant temperature water bath, micro glass cuvette/96 well plate, mortar/homogenizer, ice and distilled water.

Procedure:

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

1. Preparation of bacterial, cellular or tissue samples:

A 、Bacteria or cultured cells: collect bacteria or cells into a centrifuge tube and discard the supernatant after centrifu gation. The ratio of the number of cells (10^4): the volume of Extracting solution (mL) is 500- 1000:1 (it is recommended to add 1 mL of Extracting solution to 5 million cells), the cells are broken by ice bath ultrasonic wave (power 200W, ultrasonic time 3s, interval 9s, repeat 30 times); then the cells are

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centrifuged at 4C, 8000g for 10 min, and the supernatant is put on ice for testing.

B 、Tissue: The ratio of mass (g): volume of Extracting solution (mL) is 1:5- 10 (it is recommended to weigh about 0.1 g and add 1 mL of Extracting solution), homogenize on ice, centrifuge at 4℃, 8000 g for 10 min, and place the supernatant on ice for testing.

3. Liquid: directly detect.

II. Determination procedure:

1. Preheat the Spectrophotometer/Microplate reader for 30 minutes, adjust the wavelength to 405 nm, set zero with distilled water.
2. Before the determination, 30 μmol/mL standard solution and Reagent Ⅰ are bath in water at 25℃ for more than 10 minutes.
3. Sampling table:

Reagent	Test tube (T)	Control tube (C)	Blank tube (B)	Standard tube (S)
Sample (μL)	20	20	-	-
Extracting solution (μL)	-	-	20	20
30μmol/mL standard solution (μL)	100	-	-	100
Reagent Ⅰ (μL)	-	100	100	-
Mix well and react accurately in 25℃ water bath for 10 min.				
Reagent Ⅱ (μL)	180	180	180	180
Mix well, stand at room temperature for 10 min, take 200 μL to measure the absorbance value at 405 nm in a micro glass cuvette/96 well plate, and record them as A_T, A_C, A_B and A_S respectively. Calculate $\Delta A_T=A_T-A_C$, $\Delta A_S= A_S-A_B$, $\Delta A= \Delta A_S- \Delta A_T$. Blank tube and standard tube need to test once or twice.				

III. Calculation of CAT activity:

1. Calculated according to volume of liquid:

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the degradation of 1 μmol H_2O_2 per minute every milliliter serum (plasma) at 25℃ .

CAT(U/mL) = $(\Delta A\div\Delta A_S)\times30\times V_{STA}\div V_S\div T\times F=15\times(\Delta A\div\Delta A_S)\times F$

2. Calculation of CAT activity in tissues, bacteria or cells:

(1) Calculated according to protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the degradation of 1 μmol H_2O_2 per minute every milligram tissue protein at 25℃ .

CAT (U/mg prot) = $(\Delta A\div\Delta A_S)\times30\times V_{STA}\div(Cpr\times V_S)\div T\times F=15\times(\Delta A\div\Delta A_S)\div Cpr\times F$

(2) Calculated by sample mass

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the degradation of 1 μmol H_2O_2 per minute every gram tissue at 25℃ .

CAT (U/g mass) = $(\Delta A\div\Delta A_S)\times30\times V_{STA}\div(V_S\div V_{ST}\times W)\div T\times F=15\times(\Delta A\div\Delta A_S)\div W\times F$

(3) Calculated by cell number

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the degradation of 1μmol H_2O_2 per minute every 10^4 cells at 25℃ .

CAT (U/g mass) = $(\Delta A \div \Delta A_S) \times 30 \times V_{STA} \div (V_S \div V_{ST} \times 500) \div T \times F = 0.03 \times (\Delta A \div \Delta A_S) \div W \times F$ 30:

standard concentration, 30 μmol/mL;

V: volume of standard solution added, 0.1 mL;

V: sample volume, 0.02 mL;

T: reaction time, 10 min;

F: sample dilution ratio;

Cpr: sample protein concentration, mg/mL;

V: volume of Extracting solution added, 1 mL;

W: sample mass, g;

500: total number of cells or bacteria, 5 million.

Note:

1. This kit provides more than 25 mL Extracting solution for sample dilution.
2. If there are a lot of bubbles in the reaction solution, dilute the sample with the Extracting solution before determination.
3. If the sample size is too large, in order to ensure the accuracy of the reaction time (25℃, 10 min), It is recommended to test in batches, and each batch should be equipped with 1-2 blank tubes and standard tubes.
4. When A_T is less than 0.12 or $A_T \approx A_C$, it is recommended to dilute the sample with Extracting solution before determination.
5. Animal tissues such as liver, kidney and other samples enzyme activity are too high, it is suggested that the samples should be diluted with multiple times of the Extracting solution (such as 25 times, 50 times, 100 times, 200 times, etc.) before testing.

Experimental example:

1. Take 0. 1g of mouse liver, and add 1 mL of Extracting solution for homogenate grinding. Take the supernatant and operate according to the determination steps. Take the supernatant and dilute it 200 times with the Extracting solution, and follow the measurement procedure Calculate with 96 well plate the $\Delta A_S = A_S - A_B = 0.939 - 0.104 = 0.835$, $\Delta A_T = A_T - A_C = 0.179 - 0.104 = 0.075$, $\Delta A = \Delta A_S - \Delta A_T = 0.835 - 0.075 = 0.760$, calculate the enzyme activity according to the sample quality:

CAT activity (U/g mass) $= 15 \times (\Delta A \div \Delta A_S) \div W \times F = 15 \times (0.76 \div 0.835) \times 200 \div 0.1 = 27305.39$ U/g mass.

Related products:

AK0293/AK0292	Polyphenol Oxidase (PPO) Activity Assay Kit
AK0578/AK0577	Phenylalanine Ammonia Lyase (PAL) Activity Assay Kit
AK0584/AK0583	Superoxide Dismutase (SOD) Activity Assay Kit
AK0598/AK0597	Peroxidase (POD) Activity Assay Kit