Catalase (CAT) Activity Assay Kit (Ammonium Molybdate Method)

Note: Take two or three different samples for prediction before test.

Detection equipment: Spectrophotometer/ Microplate reader

Cat No: BC4785

Size: 100T/48S

Components:

Extract Solution： Liquid 75 mL×1, store at 2-8℃.

Reagent I: Liquid 15 mL×1, store at 2-8℃.

Reagent II: Powder×2, store at 2-8℃; Before use, take 1 bottle and add 12.5mL of H_2O to fully dissolve it. Unused reagents can be stored in aliquots at 2-8°C for one week.

Standard: Liquid 0.5 mL×1, store at 2-8℃. 1 mmol/mL H_2O_2 standard.

Preparation of 30μmol/mL standard: The standard is placed in the EP tube in the reagent bottle and should be centrifuged before use. Befire use, prepared according to the ratio of 1 mmol/mL standard: reagent I=15μL:485μL (500μL, can be used for 5 Standard tubes or 5 test tubes) according to the sample volume, and then mixed thoroughly, that is, 30μmol/mL standard.

Description:

CAT (EC 1.11.1.6) is widely present in animals, plants, microorganisms and cultured cells. It is the most important H_2O_2 scavenging enzyme and plays an important role in the active oxygen scavenging system.

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

需自备的仪器和用品：

Spectrophotometer/ Microplate reader, centrifuge, adjustable pipette, micro glass cuvette/96-well plate, water bath/incubator, mortar/homogenizer/sonicator, ice, distilled water.

操作步骤：

一、样本处理（可适当调整待测样本量，具体比例可以参考文献）

1. Tissue sample:

according to the proportion of tissue weight (g): extraction solution volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of extraction solution and fully homogenized on ice bath. Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

2. Bacteria or cells:

Collecting bacteria or cells into the centrifuge tube, suggested 5 million with 1 mL of extraction solution. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 200w, working time 3 seconds, interval 7 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.

3. Serum: Detect directly.

二、测定步骤

II. Determination procedure：

1. Preheat spectrophotometer/ Microplate reader for 30 minutes, adjust wavelength to 405 nm, set zero with distilled water.

2. Before determination, 30μmol/mL standard solution and reagent 1 are bathed in water at 25 °C for more than 10 min.

3. Add reagents with the following list:

Reagent（μL）	Test tube (A_T)	Contrast tube (A_C)	Blank tube (A_B)	Standard tube (A_S)
Sample	20	20	-	-
Extract Solution	-	-	20	20
Standard Solution	100	-	-	100
Reagent I	-	100	100	-
Mix thoroughly. 25℃ water bath for 10 minutes.				
Reagent II	180	180	180	180
Mix thoroughly, react for 10 minutes at RT. Take 200 μL into cuvette or 96-well plate, measure the absorbance at 405 nm, and record them as A_T, A_C, A_B, and A_S. Calculate $\Delta A= A_T - A_C$, $\Delta As= A_S - A_B$，$\Delta A=\Delta As-\Delta A_T$。Blank tube and Standard tube only need to test 1~2 times.				

III. Calculations：

1、 Serum (plasma)：

Unit definition: One unit of enzyme activity is defined as the amount of enzyme decompose 1 μmol H_2O_2 per minute per milliliters.

CAT (U/mL)=（ΔA÷ΔAs）×30×V_S÷V÷T×F=15×（ΔA÷ΔAs）×F

2、 Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme decompose 1 μmol H_2O_2 per minute per milligram protein.

CAT (U/mg prot)=（ΔA÷ΔAs）×30×V_S÷（Cpr×V）÷T×F=15×（ΔA÷ΔAs）÷Cpr×F

3、 Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme decompose 1 μmol H_2O_2 per minute every gram tissue.

CAT (U/g weight)=（ΔA÷ΔAs）×30×V_S÷（V÷V_E×W）÷T×F=15×（ΔA÷ΔAs）÷W×F

4、 Cell amount：

Unit definition: One unit of enzyme activity is defined as the amount of enzyme decompose 1 μmol H_2O_2 per minute every 10^4 bacteria or cells.

CAT (U/10^4 cell)=（ΔA÷ΔAs）×30×V_S÷（V÷V_E× cells（10^4）÷T×F=15×（ΔA÷ΔAs）÷W×F

20: standard concentration，30μmol/mL；

V_S：Add standard volume，0.1mL；

V：Add sample volume, 0.02mL；

T：Reaction time, 10min；

F：Dilution ratio;

Cpr: Protein concentration of sample, mg/mL;

V_E：Extract solution volume, 1 mL；

W：Sample weight, g；

Note:

1. This kit gives an extra 25mL of extract for sample dilution.
2. If there are a large number of bubbles in the reaction solution, the sample is diluted with the extract and then measured.
3. In order to ensure the accuracy of the reaction time (25℃, 10min), it is recommended to be divided into several batches of testing if there have large number of samples, each batch of testing needs to be equipped with 1-2 blank tubes and standard tubes.
4. When the A_T is less than 0.12 or approximately equal to the A_C, it is recommended that the sample be diluted with the extract solution and then determined.
5. Animal tissues such as liver, kidney and other samples with high enzyme activity, pre-experiment suggests that the samples should be tested after multiple high-fold dilutions with the extract solution (such as 25 times, 50 times, 100 times, etc.).

Experimental example:

1. Take 0.1 g of rat liver tissue, add 1 mL extract solution, and homogenize in ice bath. Centrifuge at 12000 g, 4℃ for 10 min. Take the supernatant with 200-fold dilution for test. Following the measurement procedure. Calculate $\Delta As= A_S - A_B$ =0.939-0.104=0.835. $\Delta A= A_T - A_C$=0.179-0.104=0.075, $\Delta A=\Delta As-\Delta A_T$ =0.835-0.075=0.760. Calculate the activity according to the formula:

CAT (U/g weight) =15×（ΔA÷ΔAs）÷W×F15×（0.76÷0.835)×200÷0.1=27305.39 U/g weight。

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