Ver.P261001

Catalase (CAT) Activity Assay Kit

BC1102-02 (100 Tests/48 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

CAT 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.

Kit components

Reagent	Volume	Storage
Extract solution	110mL	2-8°C
Reagent I	15mL	2-8°C
Reagent II	Powder×2	2-8°C
Before use, take 1 bottle and add 20mL of H_2O to fully dissolve it. Unused reagents can be stored in aliquots at 2-8°C for one week		
Standard	1mL	2-8°C
Working standard preparation: Mix the Standard and Reagent I in the ratio Standard: Reagent I= 80μL:920μL, and mix properly. Concentration of Working Standard is 80μmol/mL standard. This must always be freshly prepared.		

Reagents and Equipment Required but Not Provided

Visible Spectrophotometer/enzymometer, benchtop centrifuge, thermostatic water bath, adjustable pipette, micro-glass cuvette/96-well plate, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Protocol

I. Sample Extraction

1. **Tissue:** According to the proportion of tissue mass (g): extract solution volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1mL of extract solution and fully homogenized on ice bath. Centrifuge 8000 g for 10 minutes at 4°C 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 1mL of extraction solution. Use ultrasonication to splitting bacteria and cells (placed on ice, power 200w, working time 3 seconds, interval 9 seconds, repeat for 30 times). Centrifuge at 8000×g for 10 minutes at 4°C to remove insoluble materials, and take the supernatant on ice for testing.
3. **Serum or plasma:** Use directly.

II. Determination procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 405nm, set zero with distilled water.
2. Before determination, 80μmol/mL standard solution and Reagent I are bathed in water at 25 °C for more than 10 minutes.
3. Add reagents according to the following table.
   Note: Each sample requires a corresponding sample control to be run in parallel

Reagent (μL)	Test tube (A_T)	Control tube (A_C)	Blank tube (A_B)	Standard tube (A_S)
Sample	20	20	-	-
Extract Solution	-	-	20	20
Working Standard	100	-	-	100
Reagent I	-	100	100	-
Mix thoroughly and incubate at 25°C 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 $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$, $\Delta A = \Delta A_S - \Delta A_T$. Blank tube and Standard tube only need to be tested 1-2 times.

Calculations

1. **Serum or plasma:**
   Unit definition: One unit of enzyme activity is defined as the amount of enzyme required to decompose 1μmol H_2O_2 per minute per milliliter.
   $$\mathbf{CAT\ (U/mL) = (\Delta A \div \Delta A_S) \times 80 \times V_S \div V \div T \times F}$$
   $$\mathbf{= 40 \times (\Delta A \div \Delta A_S) \times F}$$
2. **Protein concentration:**
   Unit definition: One unit of enzyme activity is defined as the amount of enzyme required to decompose 1μmol H_2O_2 per minute per milligram protein.
   $$\mathbf{CAT\ (U/mg\ prot) = (\Delta A \div \Delta A_S) \times 80 \times V_S \div (Cpr \times V) \div T \times F}$$
   $$\mathbf{= 40 \times (\Delta A \div \Delta A_S) \div Cpr \times F}$$
3. **Sample mass:**
   Unit definition: One unit of enzyme activity is defined as the amount of enzyme required to decompose 1μmol H_2O_2 per minute every gram tissue.
   $$\mathbf{CAT\ (U/g\ mass) = (\Delta A \div \Delta A_S) \times 80 \times V_S \div (V \div V_E \times W) \div T \times F}$$
   $$\mathbf{= 40 \times (\Delta A \div \Delta A_S) \div W \times F}$$
4. **Cell amount:**
   Unit definition: One unit of enzyme activity is defined as the amount of enzyme required to decompose 1μmolH_2O_2 per minute every 10^4 bacteria or cells.
   $$\mathbf{CAT\ (U/10^4 cell) = (\Delta A \div \Delta A_S) \times 80 \times V_S \div (V \div V_E \times 500) \div T \times F}$$
   $$\mathbf{= 0.08 \times (\Delta A \div \Delta A_S) \times F}$$

80 : Standard concentration, 80μmol/mL
V_S : Volume of Standard, 0.1mL
V : Volume of sample, 0.02mL
T : Reaction time, 10 minutes
F : Dilution ratio
Cpr: Protein concentration of sample, mg/mL
V_E : Extract solution volume, 1 mL
W : Sample mass, g:
500: Total number of bacteria or cells, 10^4.

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°C, 10 minutes), it is recommended to be divided the total number of samples 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-told dilutions with the extract solution (such as 25 times, 50 times, 100 times, etc.).