PRODUCT INFORMATION & MANUAL

Catalase Activity Assay Kit (Colorimetric)

NBP3-24477

For research use only.
Not for diagnostic or therapeutic procedures.

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Catalase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24477

Method: Colorimetric method

Specification: 100 Assays (Can detect 50 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.27 U/mL

Detection range: 0.27-155.4 U/mL

Average intra-assay CV (%): 3.1

Average inter-assay CV (%): 5.1

Average recovery rate (%): 96

▲ This kit is for research use only.
▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure CAT activity in animal serum, plasma and tissue homogenate samples.

▲Background

Catalase (CAT) is an enzyme in organism that can efficiently and specifically decompose hydrogen peroxide and is a binding enzyme with iron porphyrin as an auxiliary group. CAT clears hydrogen peroxide in the body and protects cells from the toxicity of H_2O_2. CAT can also oxidize certain cytotoxic substances, such as formaldehyde, formic acid, phenol and ethanol. According to the difference of catalytic center structure, CAT can be divided into two types, one is iron porphyrin structure, also known as iron porphyrin enzyme, the other contain manganese ion, also known as manganese catalase. CAT is common in breathing organisms. It is mainly found in chloroplasts, mitochondria, endoplasmic reticulum, liver and red blood cells of animals.

▲Detection principle

The reaction that catalase (CAT) decomposes H_2O_2 can be quickly stopped by ammonium molybdate. The residual H_2O_2 reacts with ammonium molybdate to generate a yellowish complex. CAT activity can be calculated by production of the yellowish complex at 405 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 2 vials	2-8℃ , 12 months
Reagent 2	Substrate	12 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Chromogenic Agent	Powder × 2 vials	2-8℃ , 12 months
Reagent 4	Clarificant	12 mL × 1 vial	2-8℃ , 12 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer (405 nm), Micropipettor, Vortex mixer, Incubator or Water bath

Reagents

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

The key points of the assay

1. The test tube can be prepared and labeled in advance. After incubating at 37 ℃ for 10 min, add samples and reagent 1, then incubate the test tube at 37 ℃ for 5 min.
2. Dilute the samples to the optimal concentration for detection if the CAT activity of samples exceed the detection range.
3. The reaction time must be accurate when reagent 2 is added.

Pre-assay preparation

▲Reagent preparation

1. Reagent 1 and reagent 2 should be incubated at 37°C for 10 min before use.
2. Preparation of reagent 3 application solution:

   Dissolve the powder to 60 mL with double-distilled water. (If there is sediment in the bottom, please directly take the supernatant for test, it will not affect the result). The prepared reagent 3 application solution can be stored at 2-8℃ for 3 months.
3. Reagent 4 will be frozen when cold, please warm it in 37℃ water bath until clear.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

SDS, NP-40 and other detergents should not be added to the samples.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.27-155.4 U/mL).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor	The volume of sample
10% Rat liver tissue homogenization	25-50	50 μL
10% Rat kidney tissue homogenization	10-25	50 μL
10% Rat brain tissue homogenization	5-10	50 μL
Human serum	1	100 μL
293T supernatant	1	100 μL

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol

▲Detailed operation steps

(1) Control tube: Add 1 mL of reagent 1 into the 5 mL EP tubes.
Sample tube: Add a* mL of sample and 1 mL of Reagent 1 into the 5 mL EP tubes.

(2) Incubate at 37℃ for 5 min.

(3) Add 0.1 mL of reagent 2 into each tube, mix fully and react at 37℃ for 1 min accurately.

(4) Sample tube: Add 1 mL of reagent 3 application solution and 0.1 mL of reagent 4, mix fully.
Control tube: Add 1 mL of reagent 3 application solution, 0.1 mL of reagent 4 and a* mL of sample, mix fully.

(5) Stand for 10 min at room temperature. Set to zero with double distilled water and measure the OD values of each tube at 405 nm with 0.5 cm optical path cuvette.

[Note]: For serum or plasma samples, a* is 0.1 mL. For the cell homogenate and tissue homogenate, a* is 0.05 mL.

▲Summary operation table

	Control tube	Sample tube
Sample (mL)		a*
Reagent 1 (mL)	1.0	1.0
Mix fully and incubate at 37℃ for 5 min.		
Reagent 2 (mL)	0.1	0.1
Mix fully and react accurately at 37℃ for 1 min.		
Reagent 3 application solution (mL)	1.0	1.0
Reagent 4 (mL)	0.1	0.1
Sample (mL)	a*	
Mix thoroughly, stand for 10 min at room temperature. Set to zero and measure the OD values of each tube.		

▲Calculation

Serum (plasma) sample:

Definition: The amount of CAT in 1 mL of serum or plasma that decompose 1 μmol H_2O_2 per minute at 37℃ is defined as 1 unit.

$$\text{CAT activity (U/mL)} = \frac{\Delta A \times 32.5^*}{1^* \times V} \times f$$

Tissue and cells sample:

Definition: The amount of CAT in 1 mg of tissue protein that decompose 1 μmol H_2O_2 per minute at 37℃ is defined as 1 unit.

$$\text{CAT activity (U/mL)} = \frac{\Delta A \times 32.5^*}{1^* \times V} \times f \div C_{pr}$$

Note:

*32.5: reciprocal of slope

1: Reaction time

ΔA: Absolute OD ($OD_{Control}$ – OD_{Sample}).

V: Volume of sample, mL.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

Appendix I Data

▲Example analysis

Take 0.05 mL of 10% cabbage leaves tissue homogenate, carry the assay according to the operation table. The results are as follows:

The average OD value of the sample is 0.351, the average OD value of the control is 0.602, the concentration of protein in sample is 1.64 mgprot/mL, and the calculation result is:

$$\text{CAT activity(U/mgprot)} = \frac{(0.602-0.351)\times 32.5}{1\times 0.05} \div 1.64$$

$$= 99.44 \text{ U/mgprot}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (EDTA-2K is used as anticoagulant), centrifuge at 1000-2000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲ Cells

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 1500 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) including 1mM EDTA.
2. Homogenized method:

   (1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.
   Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

   (2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

   (3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/ time, interval for 3 s, the total time is 10 min).

Appendix III References

1. Sepasi H T, Moosavimovahedi A A. Catalase and its mysteries[J]. Progress in Biophysics & Molecular Biology, 2018.
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4. Glorieux C, Calderon P B. Catalase, a remarkable enzyme: targeting the oldest antioxidant enzyme to find a new cancer treatment approach. Biological Chemistry, 2017, 398(10): 1095-1108.
5. Nicholls P, Fita I, Loewen P C. Enzymology and structure of catalases. Advances in Inorganic Chemistry, 2000, 51(1): 51-106.