IMMUNOLOGICAL SCIENCES

Product Data Sheet

CO-K031-M Catalase (CAT) Activity Assay Kit

Size: 96T (Can detect 40 samples without duplication)

Method: Colorimetric method

Instrument: Microplate reader

Sensitivity: 1.12 U/mL

Detection range: 1.12 -150 U/mL

Average intra-assay CV (%): 3.9

Average inter-assay CV (%): 7.7

Average recovery rate (%): 100

- ▲ 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 catalase (CAT) activity in serum, plasma, cells, cell culture supernatant and tissue homogenate samples.

Background

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	24 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Substrate	1.5 mL × 2 vials	2-8℃ , 12 months
Reagent 3	Chromogenic Agent	Powder ×1 vial	2-8℃ , 12 months
Reagent 4	Clarificant	1.5 mL × 2 vials	2-8℃ , 12 months
Reagent 5	9.6 mol/L H_2O_2 Standard	1.5 mL × 2 vials	2-8℃ , 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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

Microplate reader(400-410 nm), Micropipettor, Vortex Mixer, Incubator

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 reaction time must be accurate when reagent 2 is added.
2. The test tube can be prepared and labeled in advance.
3. Dilute the samples to the optimal concentration for detection if the CAT activity of samples exceed the detection range.
4. Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

Pre-assay preparation

▲Reagent preparation

1. Incubate reagent 1 and reagent 2 at 37℃ for 10 min before use.
2. The preparation of reagent 3 application solution: dissolve a vial of powder with 24 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 can be stored at 4℃ for 3 months.
3. Reagent 4 will be frozen when cold, please warm it in 37℃ water-bath until clear.
4. The preparation of 1 mmol/mL H_2O_2 standard solution: dilute the reagent 5 with double-distilled water at a ratio of reagent 5: double-distilled water=5: 43 and mix fully.

▲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 (1.12 -150 U/mL).

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

Sample type	Dilution factor
Human serum	1
293T supernatant	1
10% Rat heart tissue homogenization	50-100
10% Rat liver tissue homogenization	100-200
10% Rat spleen tissue homogenization	50-100
Mouse serum	1
10% Epipremnum aureum tissue homogenization	1-2
10% Rat lung tissue homogenization	50-100
10% Rat kidney tissue homogenization	50-100
10% Rat brain tissue homogenization	20-50

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

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 1 mmol/mL H_2O_2 standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 50, 60, 100 μmol/mL. Reference is as follows:

Number	Standard concentrations (μmol/mL)	1 mmol/mL H_2O_2 standard solution (μL)	Double distilled water (μL)
A	0	0	1000
B	10	10	990
C	20	20	980
D	30	30	970
E	40	40	960
F	50	50	950
G	60	60	940
H	100	100	900

2. The measurement

For standard curve:

(1) Add 20 μL of H_2O_2 standard solution with different concentrations to the 1.5 mL EP tubes respectively.

(2) Sequentially add 200 μL of reagent 1, 20 μL of double distilled water, 200 μL of reagent 3 application solution and 20 μL of reagent 4, mix fully.

(3) Stand at room temperature for 10 min and take 200 μL of reaction solution to the microplate.

(4) Measure the OD value at 405 nm with microplate reader.

For samples:

(1) Control tube: Add 200 μL of reagent 1 into the 1.5 mL EP tubes.

Sample tube: Add 20 μL of sample and 200 μL of reagent 1 into the 1.5 mL EP tubes.

(2) Incubate at 37℃ for 5 min.

(3) Add 20 μL of reagent 2 into each tube, mix fully and react at 37℃ for 1 min accurately.

(4) Sample tube: Add 200 μL of reagent 3 application solution and 20 μL of reagent 4, mix fully.
Control tube: Add 200 μL of reagent 3 application solution, 20 μL of reagent 4 and 20 μL of sample, mix fully.

(5) Stand at room temperature for 10 min and take 200 μL of reaction solution to the microplate.

(6) Measure the OD value at 405 nm with microplate reader.

▲Summary operation table

1. For standard curve

	Standard tube
H_2O_2 standard solution with different concentrations (μL)	20
Reagent 1 (μL)	200
Double distilled water (μL)	20
Reagent 3 application solution (μL)	200
Reagent 4 (μL)	20
Mix fully and stand at room temperature for 10 min. Take 200 μL of reaction solution and measure the OD value.	

2. For samples

	Control tube	Sample tube
Sample (μL)		20
Reagent 1 (μL)	200	200
Incubate at 37℃ for 5 min.		
Reagent 2 (μL)	20	20
React at 37℃ for 1 min accurately.		
Reagent 3 application solution (μL)	200	200
Reagent 4 (μL)	20	20
Sample (μL)	20	
Mix fully and stand at room temperature for 10 min. Take 200 μL of reaction solution and measure the OD value.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: y= ax + b.

1. 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.

$$\text{CAT activity (U/mL)} = \frac{\Delta A}{a} \times \frac{0.02^*}{1^* \times V} \times f$$

2. 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.

$$\text{CAT activity (U/mgprot)} = \frac{\Delta A}{a} \times \frac{0.02^*}{1^* \times V} \times f \div C_{pr}$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve.

0.02*: The volume of standard, 0.02 mL.

1*: The reaction time, 1 min.

ΔA: $OD_{Control} - OD_{Sample}$.

V: The volume of sample, mL.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, mgprot/mL.

Appendix I Data

▲Example analysis

Dilute 10% rat liver tissue homogenate with PBS (0.01 M, pH 7.4) for 100 times, take 0.02 mL of diluted sample and carry the assay according to the operation table. The results are as follows:

standard curve: y =0.0026 x + 0.0022, the average OD value of the sample is 0.442, the average OD value of the control is 0.612, the concentration of protein in sample is 12.38 gprot/L, and the calculation result is:

CAT activity (U/mgprot)=(0.612-0.442)÷0.0026×100÷12.38
=528.15 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

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5. Nicholls P, Fita I, Loewen P C. Enzymology and structure of catalases. Advances in Inorganic Chemistry, 2000, 51(1): 51-106.