PRODUCT INFORMATION & MANUAL

Catalase Activity Assay Kit (Fluorometric)

NBP3-24478

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

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Catalase Activity Assay Kit (Fluorometric)

Catalog No: NBP3-24478

Method: Fluorimetric method

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

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 0.01 U/L

Detection range: 0.01-6.51 U/L

Average intera-assay CV (%) : 3.4

Average inter-assay CV (%): 6.8

Average recovery rate (%): 92

▲ 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, and tissue 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

Catalase can decompose H_2O_2 to generate H_2O and O_2, the residual H_2O_2 in the detection system react with the fluorescent substance, and the content of residual H_2O_2 is proportional to the fluorescence intensity at the excitation wavelength of 535 nm and emission wavelength of 587 nm, the catalase activity is inversely proportional to the fluorescence intensity.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	-20℃ , 12 months
Reagent 2	Substrate	0.1 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 3	Probe Solution	0.12 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 4	Enzyme Reagent	Powder ×1 vial	-20℃ , 12 months, shading light
Reagent 5	1 mol/L H_2O_2 Standard Solution	0.4 mL × 1 vial	-20℃ , 12 months, shading light
	Black 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

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Vortex mixer, Centrifuge

▲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 point of the assay

Dilute the samples to the optimal concentration for detection if the CAT activity of samples exceed the detection range.

Pre-assay preparation

▲Reagent preparation

1. The preparation of reagent 2 application solution:
   Dilute the reagent 2 for 10000 times with double distilled water and mix fully. Prepare the fresh solution before use.
2. The preparation of reagent 4 application solution:
   Dissolve a vial of reagent 4 with 0.12 mL of reagent 1 and mix fully. It can be stored at -20℃ for 30 days with shading light.
3. The preparation of chromogenic agent:
   Mix the reagent 1, reagent 3 and reagent 4 application solution at a ratio of 48:1:1. Prepare the fresh solution before use and stored with shading light.
4. The preparation of 100 μmol/L H_2O_2 standard solution:
   Dilute the reagent 5 for 10000 times with double distilled water and mix fully.

▲Sample preparation

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

▲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 (0.01-6.51 U/L).

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

Sample type	Dilution factor
Mouse serum	60-80
Mouse plasma	60-80
Rat serum	70-80
Human saliva	30-50
Rat urine	30-50
10% Mouse liver tissue homogenate	2500-3000
10% Mouse lung tissue homogenate	200-400
10% Rat muscle tissue homogenate	100-200
10% Rat brain tissue homogenate	40-50
10% Mouse kidney tissue homogenate	2000-2500

Note: The diluent is reagent 1.

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

The preparation of standard curve

Dilute 100 μmol/L H_2O_2 standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 50, 40, 30, 20, 10, 5, 1, 0 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	100 μmol/L H_2O_2 standard solution (μL)	Double distilled water (μL)
A	0	0	500
B	1	5	495
C	5	25	475
D	10	50	450
E	20	100	400
F	30	150	350
G	40	200	300
H	50	250	250

The measurement of samples

1) Standard well: Add 25 μL of standard with different concentrations into the well.

   Sample well: Add 25 μL of sample into the well.

   Control well: Add 25 μL of reagent 2 application solution into the well.

2) Add 25 μL of double distilled water into standard well. Add 25 μL of reagent 2 application solution into sample well.

3) Mix fully with microplate reader for 10 s and incubate at 37℃ for 5 min.

4) Add 50 μL of chromogenic agent into each wells.

5) Add 25 μL of sample into control well.

6) Mix fully with microplate reader for 10 s and stand at room temperature for 10 min. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

▲Summary operation table

	Standard well	Sample well	Control well
Standard with different concentrations (μL)	25		
Samples (μL)		25	
Double distilled water (μL)	25		
Reagent 2 application solution (μL)		25	25
Mix fully and incubate at 37℃ for 5 min.			
Chromogenic Agent (μL)	50	50	50
Samples (μL)			25
Mix fully and stand at room temperature for 10 min. Measure the fluorescence intensity.			

▲Calculation

Plot the standard curve by using fluorescence value (F) 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 F value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) sample:

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

$$\text{CAT activity (U/L)} = (\Delta F - b) \div a \div 5 \times f$$

2. Tissue sample:

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

$$\text{CAT activity (U/gprot)} = (\Delta F - b) \div a \div 5 \times f \div C_{pr}$$

Note:

y: The absolute fluorescence value of standard, $F_{Standard} - F_{Blank.}$ (F_{Blank} is the F value when the standard concentration is 0)

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔF: The absolute fluorescence value of sample, $F_{Control} - F_{Sample.}$

5: the reaction time, 5 min.

f: Dilution factor of sample before tested.

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

Appendix I Data

▲Example analysis

For Mouse serum, dilute mouse serum with reagent 1 for 70 times, take 25 μL of diluted sample and carry the assay according to the operation table. The results are as follows:

standard curve: y =268.77 x -55.205, the average fluorescence value of the sample is 8120, the average fluorescence value of the control is 10483, and the calculation result is:

$$\text{CAT activity (U/L)} = \frac{(10483 - 8120 + 55.205)}{268.77} \div 5 \times 70 = 125.96 \text{ U/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Fresh blood was collected and placed at 25℃ for 30 min to clot the blood. Centrifuge the sample at 4℃ for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection.

▲ Plasma

The fresh blood was added into the test tube containing anticoagulant and mixed upside down. Centrifuge the sample at 4℃ for 10 min at 700~1000 g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed. Place the plasma on ice for detection.

▲Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

▲ Tissue sample

Accurately weigh the tissue sample, add 9 times the volume of reagent 1 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample 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.

Note:

1. Homogenized medium: Reagent 1.

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.)

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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