(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Catalase (CAT) Activity Assay Kit

Catalog No.: BC00005

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎Tel: 0086-27-87002838

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

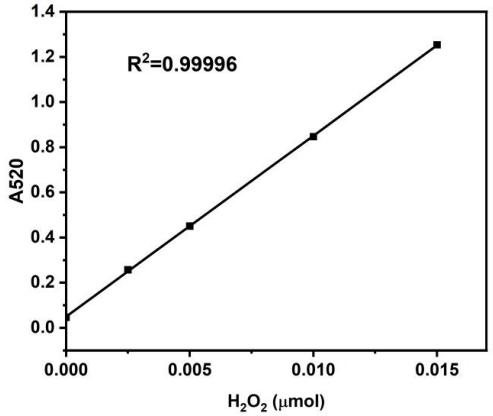
Product Name	Catalase (CAT) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, cells
Assay Type	Enzyme activity
Detection Instrument	Microplate reader (240 nm, 520 nm)
Range	0.1-1U/mL
Sensitivity	0.1U/mL

Product Introduction

Detecting the activity of catalase in cells, tissues, or other samples through a colorimetric reaction. Catalase is a common enzyme that plays an important role in redox reactions within organisms and is widely distributed in the liver, kidneys, and red blood cells.

Principle

Under conditions where hydrogen peroxide is relatively abundant, catalase can catalyze the decomposition of hydrogen peroxide to produce water and oxygen. Unreacted residual hydrogen peroxide can oxidize a colorimetric substrate under the catalysis of peroxidase, resulting in a red product with a maximum absorption wavelength of 520nm. By measuring A520 and comparing it with a standard curve made from hydrogen peroxide standards, the amount of hydrogen peroxide consumed by catalase in the sample can be calculated, and thus the enzyme activity of catalase in the sample can be determined.
The figure below shows the A520 readings for hydrogen peroxide standards detected by this kit.



Components

Components	Size (100T)	Storage
Catalase Assay Buffer	60ml	-20℃, store at 2-8℃ after opening.
Hydrogen Peroxide (approximately 1M)	5ml	-20℃
Catalase Reaction Stop Solution	50ml	-20℃, store at 2-8℃ after opening.
Chromogenic Substrate	20ml	-20℃
Peroxidase	20μl	-20℃
Microplate	1 plate	RT
Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20°C for 12 months.

Preparation

- **Sample handling**

Lyse cells or tissues with an appropriate lysis solution. Dilute the lysed samples with the catalase assay buffer provided in this kit, and at least an equal volume of catalase assay buffer should be added to dilute the lysed samples. The specific dilution factor can refer to the table below.

Sample name	Protein concentration (mg/ml)	Amount of sample used for testing (μl)	Reaction time (min)	A520 (Blank control - sample)
Blank (measured value)				1.20-1.32

Human red blood cell lysate	0.2	2–6	2	0.21–0.71
Rat liver lysate	0.3	5–10	1	0.36–0.78
Rat spleen lysate	0.3	5–10	3	0.11–0.21
Rat kidney lysate	0.3	5–10	3	0.28–0.57
Rat brain lysate	0.7	10–20	3	0.014–0.028
Jurkat lysate	2.0	2–4	3	0.28–0.64
HepG2 lysate	2.0	2–4	3	0.21–0.43

The data in the table are for reference only. Actual measured values may differ from the reference values by 20-50% due to differences in the detection system. For samples not listed in the table, similar samples in the table can be referred to for testing, and then the protein concentration, sample usage, or reaction time can be appropriately adjusted based on the test results. For completely unknown samples, the samples can be diluted by 1, 10, 20, and 50 times, and then 10μl of each can be taken for a 1-minute reaction. The concentration of the diluted sample should ideally reduce the hydrogen peroxide in the reaction system by about 30-50% after 1-5 minutes of reaction for more accurate test results.

- **Preparation of the kit**

1. Prepare 250mM hydrogen peroxide solution

The hydrogen peroxide provided with this kit has an approximate concentration of 1M. Due to the poor stability of hydrogen peroxide, its actual concentration should be determined before use. Dilute the approximately 1M hydrogen peroxide with the catalase assay buffer provided in this kit by 100 times to achieve a hydrogen peroxide concentration of about 10mM. Measure A240. The determination of A240 can be done using either of the following methods:

a. Ordinary UV spectrophotometer method: Use UV spectrophotometer with cuvette holder, NanoDrop 2000C, NanoDrop One^{c}, QuickDrop and other instruments, with quartz cuvettes. Determine the path length of the cuvette, which is generally 1 cm. The concentration of hydrogen peroxide detected by the cuvette is closest to the actual concentration.
b. Micro-volume UV spectrophotometer method: such as NanoDrop 2000, NanoDrop One, QuickDrop, Varioskan with ultra-micro detection plate μDrop Plate and other instruments. Determine the path length: For NanoDrop 2000, NanoDrop One, etc., it is

necessary to cancel the "automated path length", and the path length is generally 0.1 cm; the path length of Varioskan's ultra-micro detection plate μDrop Plate is generally 0.05 cm. For the specific path length of the micro-volume UV spectrophotometer, please refer to the instrument parameters.

c. 96-well UV microplate reader method (capable of detecting 240nm wavelength): Determine the optical path length based on the parameters of the 96-well plate, which is generally 0.552 cm for a 200-microliter sample (sample volume divided by the cross-sectional area of a single well in a 96-well plate). It is generally recommended to use a dedicated 96-well UV detection plate (such as a 96-well UV plate). If a UV detection plate is not available, a regular 96-well plate can be used, but since it is not specifically designed for UV detection, it will have a very high UV absorption signal. Therefore, it is necessary to set up wells with an equal amount of double-distilled water as a blank control (generally, 200 μl of water in such a 96-well plate has an A240 of around 3.8), and this blank control must be subtracted during calculation. When using a non-UV detection plate, due to the limited detection range of the 96-well microplate reader at 240 nm, it is recommended to dilute the hydrogen peroxide to about 10 mM before measuring the concentration.

Note: All of the above methods require setting up an equal amount of double-distilled water as a blank control, and this blank control must be subtracted during the calculation.

The formula for calculating the concentration of hydrogen peroxide is: $c=A/(\varepsilon \times b)$. Where: c is the sample concentration (in units of mol/L or M); A is the absorbance value; ε is the wavelength-dependent molar extinction coefficient (in units of $L \times mol^{-1} \times cm^{-1}$ or $M^{-1} \times cm^{-1}$), and the molar extinction coefficient of hydrogen peroxide is $43.6 M^{-1} cm^{-1}$; b is the path length (in units of cm).

Therefore: Hydrogen peroxide concentration (M) = A240/(43.6×b); that is: Hydrogen peroxide concentration (mM) = 22.94×A240/b, which allows us to calculate the actual concentration of hydrogen peroxide provided by this kit. Then, based on the actual concentration of hydrogen peroxide, dilute and prepare a 250mM hydrogen peroxide solution with the catalase assay buffer.

2. Prepare 5mM hydrogen peroxide solution

Dilute the catalase assay buffer to prepare 5mM hydrogen peroxide solution based on the actual hydrogen peroxide concentration obtained from measurement.

3. Prepare the chromogenic working solution

Dissolve the chromogenic substrate in an ice bath, aliquot as needed before use, and avoid repeated freezing and thawing. Keep other reagents on ice for standby. Take an appropriate amount of peroxidase and dilute it with the chromogenic substrate at a ratio of 1:1000 to prepare the chromogenic working solution. For example, take 5μl of peroxidase and add it to 5ml of chromogenic substrate, mix well to obtain 5ml of chromogenic working solution.

Operation process

1. Determination of the Standard Curve

Take 0, 12.5, 25, 50, or 75 microliters of the prepared 5mM hydrogen peroxide solution into 1.5ml or 0.5ml plastic centrifuge tubes, and add catalase assay buffer to a final volume of 100 microliters, mix well. At this point, the concentrations of hydrogen peroxide solutions are 0, 0.625, 1.25, 2.5, and 3.75mM, respectively. If necessary, hydrogen peroxide standard solutions of higher concentrations can be set up.

Take 4 microliters each and add them to the respective wells of a 96-well plate. Add 200μl of chromogenic working solution. Incubate at 25ºC for at least 15 minutes and measure A520, but the incubation time should not exceed 45 minutes. This step can be performed concurrently with the final step of the sample measurement. The operation table is as follows:

	Standard well	Measurement well
Hydrogen peroxide standards of different concentrations (μL)	4	--
Sample to be tested (μL)	--	4
Chromogenic working solution (μL)	200	200
Incubate at 25ºC for at least 15 minutes before measuring A520, but do not exceed an incubation time of 45 minutes.		

2. Determination of samples

Reagent	Blank control (μL)	Sample (μL)
Sample volume	0	x
Catalase assay buffer	40	40-x

250 mM hydrogen peroxide solution	10	10

(1) Refer to the table above, take x microliters (0-40 μL) of the sample into a 1.5 mL plastic centrifuge tube, add catalase assay buffer to a volume of 40 μL (i.e., add 40-x μL of catalase assay buffer), and mix well. Then add 10 μL of 250 mM hydrogen peroxide solution and mix quickly with a pipette. Prepare the sample and react at 25ºC for 1-5 minutes according to the table.
(2) Add 450 μL of catalase reaction stop solution, invert and mix well or vortex to terminate the reaction. Steps (3) and (4) below must be completed within 15 minutes after terminating the reaction.
(3) In a clean plastic centrifuge tube, add 40 μL of catalase assay buffer, then add 10 μL of the terminated and mixed reaction system mentioned above, and mix well.
(4) Take 10 μL from the 50 μL system of the previous step and add it to a well in a 96-well plate. Add 200 μL of chromogenic working solution.
(5) Incubate at 25ºC for at least 15 minutes before measuring A520, but the incubation time should not exceed 45 minutes.

Calculation

1. Use the standards to plot the standard curve and calculate the relationship between hydrogen peroxide micromoles (x) and A520 (y), obtaining the calculation formula, y = kx + b. Here, k is the slope of the linear fitting curve, and b is the intercept.
2. Utilize the above formula to calculate the micromoles of hydrogen peroxide involved in the sample based on the measured A520 value of the sample.
3. The definition of catalase enzyme activity units: 1 enzyme activity unit (1 unit) can catalyze the decomposition of 1 micromole of hydrogen peroxide at 25ºC, pH 7.0, in 1 minute.
4. For the calculation of catalase enzyme activity in cell or tissue samples:
   [Sample Catalase Activity] = [Consumed Hydrogen Peroxide Micromoles] × [Dilution Factor] / ([Reaction Minutes] × [Sample Volume] × [Protein Concentration])
   The unit of [Sample Catalase Activity] is units/mg protein
   [Consumed Hydrogen Peroxide Micromoles] = [Blank Control Residual Hydrogen Peroxide Micromoles] - [Sample Residual Hydrogen Peroxide Micromoles]
   [Dilution Factor] = 250
   [Reaction Minutes] is the actual reaction time in minutes
   [Sample Volume] is the X microliters from Table 2, which is X/1000 milliliters when expressed in milliliters.
   [Protein Concentration] is the protein concentration in the sample when taking X microliters, with the unit of mg/ml.

5. For the calculation of catalase enzyme activity in plasma and other liquid samples:
   [Sample Catalase Activity] = [Consumed Hydrogen Peroxide Micromoles] × [Dilution Factor] / ([Reaction Minutes] × [Sample Volume])
   The unit of [Sample Catalase Activity] is units/ml of sample.

Notes

1. The catalase sample to be tested, whether it is pure catalase or cell or tissue lysate products, can usually be stored for 1 week at 4ºC, and can be stored for a long term at -70ºC, but the activity of catalase will significantly decrease after storage at -20ºC.
2. The color development reaction must begin within 15 minutes after the addition of the catalase reaction stop solution during the detection process.
3. Hydrogen peroxide is unstable, and the precise concentration of hydrogen peroxide should be determined by the method described in this manual.
4. If precise quantification of catalase activity in the sample is required, a protein concentration assay kit needs to be prepared, such as a BCA protein concentration assay kit to determine the protein concentration of the sample.
5. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.