Catalase (CAT) Activity Assay Kit

Catalogue No.: K094

Size: 100T

Kit component:

Item	Specifications
Catalase assay buffer	60 mL
Hydrogen peroxide (about 1M)	5 ml
Catalase reaction stop solution	50 ml
Substrate	20 ml
Peroxidase	20 μl
Product Description	1 copy

Storage:

The kit should be stored at -20° C for one year.

Principle of the Assay:

Catalase (CAT) Activity Assay Kit is a simple and easy kit that detects Catalase activity in cells, tissues or other samples through color reaction. In the case of relatively sufficient hydrogen peroxide, catalase can catalyze hydrogen peroxide to produce water and oxygen. The residual hydrogen peroxide can oxidize the chromogenic substrate under the catalysis of Peroxidase to produce a red product (N-(4-antipyryl) -3-chloro-5-sulfonate-P benzoquinonemonoimine). The maximum absorption wavelength was 520nm. Using the hydrogen peroxide standard, the standard curve was made, so that the catalase in the sample could be calculated how much hydrogen peroxide was converted to water and oxygen per unit time per unit volume, and the enzyme activity of the catalase in the sample could be calculated.

Assay Procedur (For reference):

1. Preparation of the kit:

A 250mM hydrogen peroxide solution was prepared. The concentration of hydrogen peroxide provided by this kit was approximately 1M. Because hydrogen peroxide is not very stable, the actual concentration of hydrogen peroxide should be determined by yourself before use. Hydrogen peroxide with a concentration of about 1M was diluted 100 times with the catalase detection buffer provided by this kit, so that the concentration of hydrogen peroxide was about 10mM. A_{240} was determined. It is necessary to set an equal volume of double distilled water as a blank control during the determination, and this blank control should be subtracted from the calculation.

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

Therefore, the concentration of hydrogen peroxide (mM)=$22.94 \times A_{240}/b$

2. Preparation of samples:

Cells or tissues were lysed with the appropriate lysate. Samples were diluted with catalase assay buffer provided with this kit. The lysed samples were diluted by adding at least an equal volume of catalase detection buffer.

3. Determination of Standard CURVE:

0, 12.5, 25, 50, or 75 µl of prepared 5mM hydrogen peroxide solution were taken to 1.5ml or 0.5ml plastic centrifuge, and catalase detection buffer was added to a final volume of 100 µl, respectively. Mix well. At this time, the concentration of hydrogen peroxide solution was 0, 0.625, 1.25, 2.5 and 3.75mM, respectively. If necessary, a higher concentration of hydrogen peroxide standard solution can be set.

4 µl of each were added to one well in a 96-well plate. 200µl of the color development working solution was added. A_{520} was determined after at least 15 min of incubation at 25° C, but the incubation time should not exceed 45 min. (Note: This step can be performed at the same time as the last step in the sample determination step)

4. Determination of samples:

	blank	sample
Volume of sample	0 µl	x µl
Catalase assay buffer	40 µl	40-x µl
250mM hydrogen peroxide solution	10 µl	10 µl

a. Refer to the table above, take x µl (0-40 µl) sample into 1.5ml plastic centrifuge tube. Add the catalase detection buffer to a volume of 40 µl (add 40-x µl catalase detection buffer), and mix. An additional 10 µl of 250 mM hydrogen peroxide solution were added and quickly mixed using a pipettor. Reactions were performed at 25° C for 1-5 minutes.

b. Four 450 µl of catalase reaction stop solution were added and mixed upside down or by Vortex to terminate the reaction. The following two steps should be completed within 15 minutes after termination of the reaction. Steps c and d below should be completed within 15 minutes after termination of the reaction.

c. Add 40 µl of catalase detection buffer to a clean plastic centrifuge tube, and add 10 µl of the reaction system that has been terminated and mixed, and mix. Ten microliters from the 50 microliter system in the previous step were added to one well in a 96-well plate. Two hundred microliters of color development working solution were added.

d. A_{520} was determined after at least 15 min of incubation at 25° C, but the incubation time should not exceed 45 min.

5. Calculation of catalase enzyme activity in samples

a. A standard curve was calculated. A_{520} = k[micromoles of hydrogen peroxide]+b, and the values of k and b were calculated from the standard curve.

b. The number of micromoles of hydrogen peroxide remaining in the sample was calculated.

Micromoles of residual hydrogen peroxide = $(A_{520}-b)/k$

c. Definition of catalase enzyme activity unit: one enzyme activity unit (1 unit) can catalyze the decomposition of 1 micromole of hydrogen peroxide in 1 minute at 25° C, pH7.0.

d1. Catalase activity calculation for cell or tissue samples:

[samples of catalase enzyme activity] = [hydrogen peroxide consumption micro moles] x [diluted times] / [[reaction minutes] x [sample volume] x [protein concentration])

[samples of catalase enzyme activity] is expressed in units/mg protein

[hydrogen peroxide consumption micro moles] = [micromoles of hydrogen peroxide residual in blank control]-[micromoles of hydrogen peroxide residual in sample]

[diluted times]=250

[reaction minutes] is the actual reaction minutes

[Sample volume] is X μl in above table, expressed X/1000ml.

[Protein concentration] is the concentration of protein in the sample in mg/ml when the X μl sample is taken.

d2. Calculation of catalase activity for liquid samples such as plasma:

[samples of catalase enzyme activity] = [hydrogen peroxide consumption micro moles] x [diluted times]/([reaction minutes] x [sample volume])

[samples of catalase enzyme activity] is expressed in units/mg protein

Notes:

1. The catalase samples to be tested, whether pure catalase or cell or tissue cleavage products, can usually be stored for 1 week at 4ºC and for a long time at -70ºC, but the activity of catalase decreases significantly after storage at -20ºC.

2. The color reaction should be started within 15 minutes after the addition of catalase reaction termination solution during the detection process.

3. Hydrogen peroxide is not very stable, and the precise hydrogen peroxide concentration should be determined according to the method in the instructions.

4. For accurate quantification of catalase activity in samples, self-contained protein concentration assay kits are required.

5. This product is limited to the scientific research of professionals, not for clinical diagnosis or treatment, not for food or medicine, not stored in ordinary residential.

6. For your safety and health, please wear a lab coat and wear disposable gloves to operate.