Catalase (CAT) Assay Kit

DESCRIPTION

Catalase is an enzyme present in nearly all living organisms. It protects the cell from oxidative damage caused by reactive oxygen species. Per-chromic acid is formed as an unstable intermediate in this process, which reduces dichromate in acetic acid to chromic acetate when heated in the presence of un-decomposed hydrogen peroxide (H_2O_2). The amount of chromic acetate generated by the reaction is directly correlated with the amount of hydrogen peroxide. The calorimetric measurement of the generated chromic acetate is made at 570 nm.

KEY FEATURES

Sensitive and accurate. 2.5 µmol H_2O_2 Equiv./L

Simple and high-throughput. The procedure involves the addition of a working reagent that can be readily automated as a high-throughput assay for thousands of samples per day.

APPLICATIONS

Direct Assays: serum, plasma, urine, saliva and other biological samples, food and beverages.

Drug Discovery/Pharmacology: effects of drugs on CAT

KIT CONTENTS 100 Reaction Kit

R1- 100 ml R2- 65 ml

Standard: H_2O_2 **1** mL

Prepare 25 µmol /L H_2O_2. Take 1 ml of H2O2 and dilute it with 10 ml distil H_2O. After that take 12.15 µl into 50 ml to prepare 25 µmol/L.

No	25 µmol /L H_2O_2 + Distilled water	Vol (µL)
1	500 µL + 500 µL	1000
2	250 µL + 500 µL	750
3	125 µL + 500µL	625
4	75 µL + 500 µL	575
5	37.5 +500 µL	537.5
6	0+ 500 µL	500

Storage conditions: The kit is shipped at room temperature. Store all components at -20°C-40°C to ensure shelf life of 12 months.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

Sample preparation

Serum sample: Blood should be collected in a gel tube, then centrifuged at 4000 rpm for 5 min, and obtained clear solution on top of the cellular material. Separate it into a new Eppendorf tube and use it for further processing.

Tissue homogenate: Cell lysate is prepared by homogenizing tissue (25mg) or sonicating cells in ice-cold 1 x PBS (1mL) and centrifugation for 10 min at 14,000 rpm to pellet any debris. Use the clearsupernatant for the assay. If not assayed immediately, freeze supernatant at -80°C (stable for 1 month).

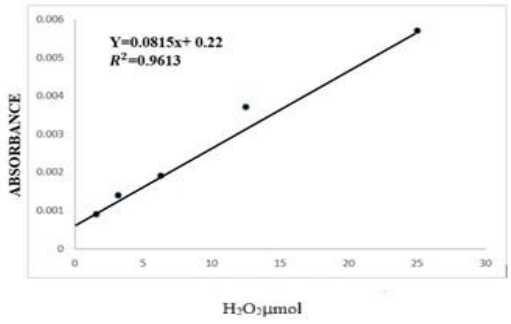
ASSAY PROCEDURE

1. Labeled the tubes as test, control, standard, and blank.
2. Add 50µl of sample 500µl of R1 and 1 ml of R2 to the test tube.
3. Add 50µl of sample 500µl D.water and 1ml of R1 in control tubes.
4. Add 50 µl D.water and 1 ml of R1 and 500 µl of R2 in standard tubes.
5. Add 550µl D.water and 1ml of R1 in a blank tube.
6. Vortex the tubes.
7. Incubate at 37 °C for 3 minutes.
8. Keep the tubes in a boiling water bath for 10 minutes.
9. Centrifuge at 2500 g for 5 minutes.
10. Take absorbance at 570 nm.

Reagents	Test	Control	Standard	Blank
Sample	50 µl	50 µl		
R1	1 ml	1 ml	1 ml	1 ml
R2	500 µl		500 µl	
D.Water		500 µl	50 µl	250 µl

D.water is not given in this kit. Please arrange it before starting your protocol.

Calculation:



$$\text{Catalase Activity of test kU} = \frac{2.303}{t} * \left[\log \frac{S^o}{S-M}\right] * \frac{Vt}{Vs}$$

t: time.
S^o: Absorbance of standard tube.
S: Absorbance of test tube.
M: Absorbance of control test (correction factor).
Vt: Total volume of reagents in test tube.
Vs: Volume of serum.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, plate reader capable of reading optical density at 570 nm, homogenizer or sonicator etc.