Catalase (CAT) Assay Kit

Cat.No: Otto3051

Summary & Explanation

Catalase (CAT) is an important antioxidant enzyme that plays a key role in the body's defense against oxidative stress. It catalyzes the decomposition of hydrogen peroxide (H_2O_2), a reactive oxygen species, into water (H_2O) and molecular oxygen (O_2), thereby preventing the accumulation of hydrogen peroxide and reducing oxidative damage to cells and tissues.

Test Principle

Catalase (CAT) catalyzes the decomposition of hydrogen peroxide (H_2O_2) into water (H_2O) and molecular oxygen (O_2). In this colorimetric assay, catalase present in the sample consumes a defined amount of hydrogen peroxide during the enzymatic reaction.
The residual hydrogen peroxide reacts with a chromogenic reagent to produce a colored compound. Therefore, higher catalase activity results in lower residual hydrogen peroxide and a corresponding change in color intensity.
Catalase activity can be determined by measuring the optical density (OD) at the specified wavelength and calculating the amount of hydrogen peroxide decomposed by catalase relative to the control.

Kit Compenents

Content	Component	Size
Reagent 1	Buffer Solution	30ml
Reagent 2	Chromogen Solution	10ml
Reagent 3	Generator Solution	30ml

Reagent Preparation

R1 Working Reagent

Prepare the R1 Working Reagent by adding 55 µL of R2 to 10 mL of R1.

Mix thoroughly before use.

The prepared R1 Working Reagent is stable for 1 day when stored under the recommended storage conditions. It is recommended to prepare the required amount fresh on the day of the assay.

Storage & Stability

Reagent: Stable up to expiry date when stored capped and at +4°C even after start using

Reactivity

This kit can be used for detection of CAT in serum, plasma, salive, animal and plant tissue samples.

Specimen

Serum: Collect serum using regular sampling tubes. Let the samples clot for 1 hour at room temperature, maintaining a temperature of 2-8°C. Afterward, centrifuge the samples for 10 minutes at 3000×g at 2-8°C. Retrieve the supernatant to perform the assay.
Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge the samples for 10 minutes at 3000×g at 2-8°C, ensuring this is done within 30 minutes of collection.
Tissue homogenates: For general reference, hemolyzed blood may influence the results. Therefore, tissues should be cut into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) or KCl (140 mM) to thoroughly remove any excess blood. The tissue pieces should be weighed and then homogenized in PBS using a glass homogenizer on ice, with a ratio of tissue weight (g) to PBS volume (mL) of 1:9. The homogenate is then centrifuged for 5 minutes at 7000×g at 2-8°C to obtain the supernatant.

Assay Range

5 - 400 U/ml

Refference Range

Each laboratory is recommended to establish their own reference values.

Analytical Performance

Inter Assay Coefficent of Variation (CV) % 5.9
Intra Assay Coefficent of Variation (CV) % 3.2

Manual Assay Protocol

Step	Procedure	Volume / Condition
1	Add R1 Working Reagent (WR1)	150 µL
2	Add sample (S)	10 µL
3	Mix and incubate	15 min at 37°C
4	Add R3	150 µL
5	Incubate	5 min at room temperature
6	Measure absorbance	405 nm, endpoint

Two absorbance measurements are taken. The first is measured immediately before adding the final reagent, and the second is measured at the end of the reaction.

Calibration

Material	Assigned Value
Purified/deionized water	0
Calibrator / Standard	55

Calculation

Catalase activity = [(Delta Water Abs - Delta Sample Abs) / (Delta Water Abs - Delta Standard Abs)] X 55

Where: Water Abs. = absorbance of the water blank/control; Sample Abs. = absorbance of the test sample; Standard Abs. = absorbance of the calibrator/standard; 55 = assigned standard value.

Automated Analyzer Protocol

Parameter	Setting
R1 Working Reagent (WR1)	150 µL
Sample	10 µL
R2 / Second reagent	150 µL
Wavelength	405 nm
Method	Endpoint
Reading point	Use the final permitted endpoint reading of the analyzer reaction cycle

Important Notes

Bring reagents to the required assay temperature before use and mix gently.
Avoid bubbles in the reaction mixture, as they may interfere with absorbance measurements.
Use identical timing conditions for samples, water blank/control, and calibrator.
Analyzer-specific reaction timing and final reading point should be verified during method application and validation.

Warning

For in vitro use only
Do not pipette by mouth
Do not use reagents beyond the expiry date.

References

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