Catalase Assay Kit

Catalog No.: abx294001

Size: 100 assays

Detection Range: 0.27 U/ml – 155.4 U/ml

Sensitivity: 0.27 U/ml

Storage: Store all components at 4°C for up to 12 months.

Application: For detection and quantification of CAT activity in serum, plasma, tissue homogenates, cell lysates and other biological fluids.

Introduction

Catalase (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.

Abbexa's CAT Assay Kit is a quick, convenient, and sensitive method for measuring and calculating CAT activity. The absorbance should be measured at 405 nm. The intensity of the color is proportional to the activity of the CAT enzyme(s), which can then be calculated.

Kit components

1. Buffer Solution: 2 x 60 ml
2. Substrate: 12 ml
3. Chromogenic Reagent: 2 vials
4. Clarificant: 12 ml

Materials Required But Not Provided

1. Microplate reader/spectrophotometer (405 nm)
2. 37°C incubator
3. Distilled water
4. Micropipette and disposable micropipette tips
5. Vortex mixer
6. Sonicator
7. PBS (0.01 M, pH 7.4)

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using EDTA as the anticoagulant. Centrifuge for 10 mins at 1000 - 2000 x g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml homogenization medium. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.
- **Cell Lysates:** Collect cells into a centrifuge tube and wash with PBS. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl homogenization medium per 1 × 106 cells, then sonicate in an ice water bath. Centrifuge at 1500 × g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor and volume of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor	Volume of Sample (µl)
10% Rat liver tissue homogenate	25 – 50	50
10% Rat kidney tissue homogenate	10 – 25	50
10% Rat brain tissue homogenate	5 – 10	50
Human serum	1	100
HEK-293T supernatant	1	100

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Buffer Solution and Substrate:** Incubate at 37°C for 10 minutes prior to use in the assay.
- **Chromogenic Reagent working solution:** Dissolve each vial of Chromogenic Reagent with 60 ml of distilled water to generate the Chromogenic Reagent working solution. If sediment appears at the bottom, take the supernatant. Prepared Chromogenic Reagent working solution can be stored at 4°C for up to 3 months.
- **Clarifying Reagent**: Incubate at 37°C until the solution is clear.

B. Assay Procedure

1. Detection of CAT activity in serum/plasma:

1.1. Set the sample tubes and control tubes.
1.2. Add 100 µl of sample to each sample tube.
1.3. Add 1 ml of Buffer Solution to the sample and control tubes.
1.4. Mix thoroughly and incubate at 37°C for 5 minutes.
1.5. Add 100 µl of Substrate to the sample and control tubes.
1.6. Mix thoroughly and incubate at 37°C for 1 minute.
1.7. Add 1 ml of Chromogenic Reagent working solution to the sample and control tubes.
1.8. Add 100 µl of Clarifying Reagent to the sample and control tubes.
1.9. Add 100 µl of sample to each control tube.
1.10. For each tube, mix thoroughly and incubate at room temperature for 10 minutes and measure the OD values at 405 nm with a microplate reader.

2. Detection of CAT activity in cell lysates and tissue homogenates:

2.1. Set the sample tubes and control tubes.
2.2. Add 50 µl of sample to each sample tube.
2.3. Add 1 ml of Buffer Solution to the sample and control tubes.
2.4. Mix thoroughly and incubate at 37°C for 5 minutes.
2.5. Add 100 µl of Substrate to the sample and control tubes.
2.6. Mix thoroughly and incubate at 37°C for 1 minute.
2.7. Add 1 ml of Chromogenic Reagent working solution to the sample and control tubes.
2.8. Add 100 µl of Clarifying Reagent to the sample and control tubes.
2.9. Add 50 µl of sample to each control tube.
2.10. For each tube, mix thoroughly and incubate at room temperature for 10 minutes and measure the OD values at 405 nm with a microplate reader.

C. Calculation of Results

1. Serum and plasma samples:

One unit of CAT activity is defined as the quantity of CAT in 1 ml of sample that catalyzes the consumption of 1 μmol/L H_2O_2 per minute at 37°C.

$$\mathbf{CAT\ (U/ml)} = \mathbf{F} \times \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}) \times \mathbf{32.5}}{\mathbf{V_{SAM}}}$$

2. Tissues and cell lysate samples:

One unit of CAT activity is defined as the quantity of CAT in 1 mg of sample that catalyzes the consumption of 1 μmol/L H_2O_2 per minute at 37°C.

$$\mathbf{CAT\ (U/ml)} = \mathbf{F} \times \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}) \times \mathbf{32.5}}{\mathbf{V_{SAM}} \times \mathbf{C_{Protein}}}$$

where:

OD_{Sample}	OD value of sample
$OD_{Control}$	OD value of control
V_{SAM}	Volume of sample (ml)
$C_{Protein}$	Concentration of protein in sample (mg/ml)
F	The dilution factor of sample