Catalase (CAT) Assay Kit

Catalog No.: abx298827

Size: 96 tests

Detection Range: 1.12 U/ml – 150 U/ml

Sensitivity: 1.12 U/ml

Storage: Store all components at 4°C. Store the Standard (9.6 mol/L) Solution in the dark.

Application: For detection and quantification of Catalase activity in serum, plasma, tissue homogenates, cell lysates, and cell culture supernatants.

Introduction

Catalase (CAT) is an enzyme found in most aerobic and some anaerobic organisms that catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is found in a variety of animal tissues, such as in the liver and kidney. Catalase is produced by some pathogens to prevent the immune system from damaging them with reactive oxygen intermediates. Mutations that result in severely reduced catalase activity cause acatalasia, leading to a propensity towards developing mouth ulcers, and occasionally results in gangrene.

Abbexa's Catalase (CAT) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Catalase activity. In the presence of hydrogen peroxide the Catalase inhibitor ammonium molybdate forms a yellow complex with an absorbance maximum at 405 nm. The change in intensity of the color is proportional to the Catalase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Assay Buffer: 24 ml
3. Substrate: 2 × 1.5 ml
4. Chromogenic Reagent: 1 vial
5. Clarifying Reagent: 2 × 1.5 ml
6. Standard Solution (9.6 mol/L): 2 × 1.5 ml
7. Plate sealer: 2

Materials required but not provided

1. Microplate reader (405 nm)
2. Double-distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

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** and **Plasma:** Serum and plasma samples can be tested directly.
- **Tissue Homogenates:** Carefully weigh out at least 20 mg of tissue, and wash thoroughly in ice-cold PBS (0.01 M, pH 7.4). Add into ice-cold PBS (0.01 M, pH 7.4) in a ratio of 1:9 weight (mg) to volume (µl) (i.e. for 20 mg of tissue, add into 180 µl PBS). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath or at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant and transfer to a fresh tube for detection. Keep on ice and detect immediately.

  Note: To calculate Catalase activity in tissue homogenates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.
- **Cell lysates:** Collect at least 1×10^6 cells for detection, and wash thoroughly in ice-cold PBS (0.01 M, pH 7.4). Add into ice-cold PBS (0.01 M, pH 7.4) in a ratio of 1×10^6 cells : 300 µl PBS. Homogenize manually, using an ultrasonicator, at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant and transfer to a fresh tube for detection. Keep on ice and detect immediately.

  Note: To calculate Catalase activity in cell lysates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor 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
Human serum	1
Mouse serum	1
10% Rat heart tissue homogenate	50 – 100
10% Rat liver tissue homogenate	100 – 200
10% Rat spleen tissue homogenate	50 – 100
10% Rat lung tissue homogenate	50 – 100
10% Rat brain tissue homogenate	20 – 50
10% *Epipremnum aureum* tissue homogenate	1 – 2

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

- **Assay Buffer** and **Substrate**: Preheat the Assay Buffer and Substrate to 37°C, 10 minutes before starting the assay.
- **Working Chromogenic Solution:** Reconstitute the Chromogenic Reagent powder with 24 ml double-distilled water. The reconstituted Working Chromogenic Solution can be stored for up to 3 months at 4°C.
- **Standards:** Prepare a Stock (1 mmol/ml) Standard by adding the Standard Solution (9.6 mol/L) to double-distilled water in a ratio of 1 : 9.6. Prepare only enough Stock (1 mmol/ml) as required for the assay.
- Label 7 tubes with 100 µmol/ml, 60 µmol/ml, 50 µmol/ml, 40 µmol/ml, 30 µmol/ml, 20 µmol/ml, and 10 µmol/ml. Add 100 µl, 60 µl, 50 µl, 40 µl, 30 µl, 20 µl, and 10 µl of Stock (1 mmol/ml) Standard to the 100 µmol/ml, 60 µmol/ml, 50 µmol/ml, 40 µmol/ml, 30 µmol/ml, 20 µmol/ml, and 10 µmol/ml tubes respectively, followed by 900 µl, 940 µl, 950 µl, 960 µl, 970 µl, 980 µl, and 990 µl of double-distilled water, to prepare Standard Dilutions with concentrations 100 µmol/ml, 60 µmol/ml, 50 µmol/ml, 40 µmol/ml, 30 µmol/ml, 20 µmol/ml, and 10 µmol/ml. These volumes are summarized in the following table:

Standard Dilution (µmol/ml)	**10**	**20**	**30**	**40**	**50**	**60**	**100**
Stock (1 mmol/ml) Standard (µl)	10	20	30	40	50	60	100
Double-distilled water (µl)	990	980	970	960	950	940	900

For the blank, or 0 µmol/ml standard, use pure double-distilled water. The volume of each standard will be 1000 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- The Clarifying Reagent may partially solidify at low temperatures. If any solids are present, heat the vial to 37°C in a water bath until the Clarifying Reagent is a completely transparent, homogenous liquid.
- The Working Chromogenic Solution may contain some undissolved material when reconstituted. If this sediment will not dissolve, carefully take the supernatant for use in the assay, without taking up any of the sediment. This will not affect the results of the assay.

B. Assay Procedure

Pre-heat the incubator or water bath and ensure it has reached a stable temperature before use.

1. Mark fresh microcentrifuge tubes for each standard dilution, sample, and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*

Sample and Control Tube Preparation

2. Add 20 µl of sample to the sample tubes.
3. Add 200 µl of Assay Buffer to the sample and control tubes.
4. Incubate the sample and control tubes at 37°C for 5 minutes.
5. Add 20 µl of Substrate into the sample and control tubes, and mix fully.
6. Incubate the tubes at 37°C for 1 minute. *The timing of this incubation must be carefully controlled for all tubes.*
7. Add 200 µl of Working Chromogenic Solution to each tube.
8. Add 20 µl of Clarifying Reagent to each tube.
9. Add 20 µl of sample to the corresponding control tubes.
10. Pipette the contents of each tube gently up and down to mix. Avoid foaming or bubbles.
11. Stand at room temperature for 10 minutes.

Standard Tube Preparation

12. Add 20 µl of each standard dilution to the corresponding standard tubes.
13. Add 200 µl of Assay Buffer to the standard tubes.
14. Add 20 µl of double-distilled water to the standard tubes.
15. Add 200 µl of Working Chromogenic Solution to the standard tubes.
16. Add 20 µl of Clarifying Reagent to the standard tubes.
17. Pipette the contents of each tube gently up and down to mix. Avoid foaming or bubbles.
18. Stand at room temperature for 10 minutes.

Measurement

19. Mark positions on the 96-well microplate for each standard dilution, sample, and control.
20. Transfer 200 µl of solution from each tube to the corresponding wells on the microplate.
21. Measure the OD of each well with a microplate reader at 405 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Catalase in each sample well can be derived with the following formulae:

1. Serum and Plasma samples:

One unit of Catalase activity is defined as the amount required for 1 ml of serum or plasma to catalyze 1 µmol of H_2O_2 per minute at 37°C.

$$\text{Catalase (U/ml)} = \mathrm{F} \times \frac{\mathrm{OD_{Control}} - \mathrm{OD_{Sample}}}{\mathrm{a}} \times \frac{\mathbf{0.02}}{\mathrm{V_{Sample}}}$$

2. Tissue samples:

One unit of Catalase activity is defined as the amount required for 1 mg of total tissue protein to catalyze 1 µmol of H_2O_2 per minute at 37°C.

$$\text{Catalase (U/mg protein)} = \mathrm{F} \times \frac{\mathrm{OD_{Control}} - \mathrm{OD_{Sample}}}{\mathrm{a}} \times \frac{\mathbf{0.02}}{\mathrm{V_{Sample}} \times \mathrm{C_{Protein}}}$$

where:

$\mathrm{OD_{Control}}$	OD value of control
$\mathrm{OD_{Sample}}$	OD value of sample
$\mathrm{V_{Sample}}$	Volume of sample tested (ml)
$\mathrm{C_{Protein}}$	Concentration of protein in sample (mg/ml)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
F	The dilution factor of sample