

Ver.240105

Catalase (CAT) Activity Assay Kit

BC1102-02 (100 Tests/96 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

CAT is an enzyme found broadly in animals, plants, microorganisms and cultured cells. It is the main enzyme of clearing H_2O_2 , which plays an important role in the active oxygen scavenging system. H_2O_2 has characteristic absorption peak at 240nm. It can be decomposed into water and oxygen by CAT which makes the absorbance at 240nm to decrease. The activity of CAT can be calculated according to the rate of change of absorbance.

Kit components

Reagent	Volume	Storage
Extraction Reagent	110 mL \times 1	4°C
Reagent I	30 mL \times 1	4°C
Reagent II	200 μL \times 1	4°C, Spin down before use
Working Reagent		
<ul style="list-style-type: none">96 well UV flat bottom plate: Add 40 μL Reagent II to 5.1 mL Reagent I and mix thoroughly. This will be sufficient for about 26 tests. Prepare Working Reagent according to the number of sample to be assayed, as per the ratio mentioned above.Micro quartz cuvette: Add 40 μL Reagent II to 6.8 mL Reagent I and mix thoroughly. This will be sufficient for about 34 tests. Prepare working solution according to the number of sample to be assayed, as per the ratio mentioned above.		

Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, microplate reader, micro quartz cuvette / 96-well UV-flat bottom plates, mortar/homogenizer and distilled water.

Protocol

I. Sample preparation

Tissue: Add 1mL Extraction Reagent to 0.1g tissue. Homogenate on ice and centrifuge at 8000 \times g at 4°C for 10 minutes. Take the supernatant and place it on ice for assay.

Bacteria or cells: Add 1mL Extraction Reagent to 5 million cells. Subject to ultrasonication while keeping the samples in an ice bath (power 200W, sonication 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 8000 \times g at 4°C for 10 minutes. Take the supernatant and place it on ice for assay. (If the supernatant is not clear, centrifuge for 3 minutes more).

Serum/Plasma: Use directly.

II. Assay procedure

- Preheat the spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 240nm and set zero with distilled water.
- Pre heat the Working Reagent in a water bath at 37°C (mammals) 25°C (other species) for 10 minutes.
- Add 190 μL Working Reagent and 10 μL sample in a micro quartz cuvette/96 well UV flatbottom plate.
- Mix for 5 seconds and immediately measure the absorbance at 240nm (A_1)
- Measure absorbance after 1 minute (A_2)
- $\Delta A = A_1 - A_2$

Calculation

- **Micro quartz cuvette**

- a) **Protein concentration**

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of 1 μ mol of H₂O₂ reaction system per minute for every milligram of protein.

$$\text{CAT (U/mg protein)} = [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^6] \div (V_s \times C_{pr}) \div T \\ = 459 \times \Delta A \div C_{pr}$$

- b) **Sample weight:**

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of 1 μ mol of H₂O₂ reaction system per minute for every gram of tissue.

$$\text{CAT (U/g weight)} = [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^6] \div (W \times V_s \div V_{sv}) \div T \\ = 459 \times \Delta A \div W$$

- c) **Bacteria/Cells**

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of 1 μ mol of H₂O₂ in the reaction system per minute for every 10⁴ cells.

$$\text{CAT (U/10}^4 \text{ cells)} = [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^6] \div (500 \times V_s \div V_{sv}) \div T \\ = 0.917 \times \Delta A$$

- d) **Serum/Plasma**

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of 1 μ mol of H₂O₂ in reaction system per minute for every millilitre of serum/plasma.

$$\text{CAT (U/mL)} = [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^6] \div V_s \div T \\ = 459 \times \Delta A$$

V_{rv} : Reaction total volume, 2 \times 10⁻⁴ L

ϵ : Molar extinction coefficient, 43.6 L/mol/cm

d : Light path of cuvette, 1 cm

V_s : Sample volume, 0.01 mL

V_{sv} : Extraction volume, 1 mL

T : Reaction time, 1 minute

C_{pr} : Sample protein concentration, mg/mL;

W : Sample weight, g;

500 : Total number of bacteria and cells, 5 million;

10⁶ : Unit conversion factor, 1 mol=10⁶ μ mol.

- **96 well UV flat-bottom plate**

- a) **Protein concentration**

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of 1 μ mol of H₂O₂ reaction system per minute for every milligram of protein.

$$\text{CAT (U/mg protein)} = [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^6] \div (V_s \times C_{pr}) \div T \\ = 764.5 \times \Delta A \div C_{pr}$$

- b) **Sample weight:**

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of 1 μ mol of H₂O₂ reaction system per minute for every gram of tissue.

$$\text{CAT (U/g weight)} = [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^6] \div (W \times V_s \div V_{sv}) \div T \\ = 764.5 \times \Delta A \div W$$

c) *Bacteria/Cells*

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of 1 μ mol of H₂O₂ in the reaction system per minute for every 10⁴ cells.

$$\text{CAT (U/10}^4 \text{ cells)} = [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^6] \div (500 \times V_s \div V_{sv}) \div T \\ = 1.529 \times \Delta A$$

d) *Serum/Plasma*

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of 1 μ mol of H₂O₂ in reaction system per minute for every millilitre of serum/plasma.

$$\text{CAT (U/mL)} = [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^6] \div V_s \div T \\ = 764.5 \times \Delta A$$

V_{rv} : Reaction total volume, 2 \times 10⁻⁴ L

ϵ : Molar extinction coefficient, 43.6 L/mol/cm

d : Light path of cuvette, 0.6 cm

V_s : Sample volume, 0.01 mL

V_{sv} : Extraction volume, 1 mL

T : Reaction time, 1 minute

C_{pr} : Sample protein concentration, mg/mL;

W : Sample weight, g;

500 : Total number of bacteria and cells, 5 million;

10⁶ : Unit conversion factor, 1 mol=10⁶ μ mol.

Note

- If there are a lot of bubbles in the reaction solution, dilute the sample with distilled water before assay.