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 decreases. The activity of CAT can be calculated according to the rate of change of absorbance.

Kit components

Reagent	Volume	Storage
Extraction Reagent	110mL × 1	4°C
Reagent I	30mL × 1	4°C
Reagent II	110µL × 1	4°C, Spin down before use

Working Reagent
- 96 well UV flat bottom plate: Add 25µL Reagent II to 5mL 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 25µL Reagent II to 6.5mL 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 ×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 ×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 min, 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 190µL Working Reagent and 10µL sample in a micro quartz cuvette/96 well UV flatbottom plate.
- Mix for 5 seconds and immediately measure the absorbance at 240nm (A1)
- Measure absorbance after 1 minute (A2)
- ΔA = A1 – A2

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 1μmol of H_2O_2 reaction system per minute for every milligram of protein.

  CAT (U/mg protein) = $[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^6] \div (Vs \times Cpr) \div T$
  $$= 459 \times \Delta A \div Cpr$$

  ### b) Sample weight:
  Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of 1μmol of H_2O_2 reaction system per minute for every gram of tissue.

  CAT (U/g weight) = $[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^6] \div (W \times Vs \div Vsv) \div T$
  $$= 459 \times \Delta A \div W$$

  ### c) Bacteria/Cells
  Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of 1μmol of H_2O_2 in the reaction system per minute for every 10^4 cells.

  CAT (U/10^4 cells) = $[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^6] \div (500 \times Vs \div Vsv) \div T$
  $$= 0.917 \times \Delta A$$

  ### d) Serum/Plasma
  Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of 1μmol of H_2O_2 in reaction system per minute for every millilitre of serum/plasma.

  CAT (U/ml) = $[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^6] \div Vs \div T$
  $$= 459 \times \Delta A$$

  Vrv : Reaction total volume, 2×10^{-4} L
  ε : Molar extinction coefficient, 43.6 L/mol/cm
  d : Light path of cuvette, 1 cm
  Vs : Sample volume, 0.01 mL
  Vsv : Extraction volume, 1 mL
  T : Reaction time, 1 minute
  Cpr : Sample protein concentration, mg/mL;
  W : Sample weight, g;
  500 : Total number of bacteria and cells, 5 million;
  10^6 : Unit conversion factor, 1 mol=10^6 μmol.

- ## 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 1μmol of H_2O_2 reaction system per minute for every milligram of protein.

  CAT (U/mg protein) = $[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^6] \div (Vs \times Cpr) \div T$
  $$= 764.5 \times \Delta A \div Cpr$$

  ### b) Sample weight:
  Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of 1μmol of H_2O_2 reaction system per minute for every gram of tissue.

  CAT (U/g weight) = $[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^6] \div (W \times Vs \div Vsv) \div T$
  $$= 764.5 \times \Delta A \div W$$

c) *Bacteria/Cells*

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of $1\mu mol$ of H_2O_2 in the reaction system per minute for every 10^4 cells.

$CAT (U/10^4 \text{ cells}) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^6] \div (500 \times Vs \div Vsv) \div T$
$= 1.529 \times \Delta A$

d) *Serum/Plasma*

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the degradation of $1\mu mol$ of H_2O_2 in reaction system per minute for every millilitre of serum/plasma.

$CAT (U/ml) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^6] \div Vs \div T$
$= 764.5 \times \Delta A$

Vrv : Reaction total volume, 2×10^{-4} L
ε : Molar extinction coefficient, 43.6 L/mol/cm
d : Light path of cuvette, 0.6 cm
Vs : Sample volume, 0.01 mL
Vsv : Extraction volume, 1 mL
T : Reaction time, 1 minute
Cpr : Sample protein concentration, mg/mL;
W : Sample weight, g;
500 : Total number of bacteria and cells, 5 million;
10^6 : Unit conversion factor, 1 mol=10^6 μmol.

Note

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