Catalase (CAT)

from bovine liver

Hydrogen-peroxide : hydrogen-peroxide oxidoreductase, EC 1.11.1.6

\[ 2 \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}_2\text{O} \]

**SPECIFICATION**

- **Appearance**: yellowish green lyophilizate
- **Activity**: ≥12,000 U/mg lyophilizate
- **Stabilizer**: lactitol
- **Storage**: at −20°C

**PROPERTIES**

- **Molecular weight**: ca. 240 kDa (gel filtration)
- **Structure**: 4 identical tetrahedrally arranged subunits of ca. 60 kDa each, 4 ferriprotoporphyrin groups per mole of enzyme
- **pH Optimum**: 6.5–8.0 (Fig. 1)
- **pH Stability**: 4.0–9.5 (Fig. 2)
- **Optimum temperature**: ca. 30°C (Fig. 3)
- **Thermal stability**: below 55°C (Fig. 4)
- **Inhibitors**: azide, cyanide, cyanogenbromide, hydroxylamine, ascorbate, nitrite, fluoride, acetate, formate, ethanol, methanol
ASSAY PROCEDURE

Principle

\[
2 \text{H}_2\text{O}_2 \xrightarrow{\text{catalase}} \text{O}_2 + 2 \text{H}_2\text{O}
\]

The disappearance of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) is measured spectrophotometrically at 240 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which decomposes 1 \(\mu\text{mol}\) of H\textsubscript{2}O\textsubscript{2} per min at 25°C and pH 7.0 under the conditions described below.

Reagents

A. Phosphate buffer, 50 mM; pH 7.0: mix 50 mM Na\textsubscript{2}HPO\textsubscript{4} solution and 50 mM KH\textsubscript{2}PO\textsubscript{4} solution to make a pH 7.0 solution.

B. Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) solution: add about 0.75 ml of 30% H\textsubscript{2}O\textsubscript{2} to 100 ml of phosphate buffer (Reagent A).

(Prepare freshly before measurement and store at 4°C)

Measure the absorbance of the mixture (2.0 ml of phosphate buffer (Reagent A) and 1.0 ml of H\textsubscript{2}O\textsubscript{2} solution) at 240 nm in 1 cm light path versus phosphate buffer (Reagent A) and check the absorbance of 0.85 (±0.02).

Otherwise add more 30% H\textsubscript{2}O\textsubscript{2} or phosphate buffer (Reagent A) to the H\textsubscript{2}O\textsubscript{2} solution and repeat the same check.

Sample: dissolve the lyophilized enzyme to a concentration of 5.0 mg/ml in ice-cold phosphate buffer (Reagent A) and dilute to a volume activity of 0.3–0.6 U/ml with ice-cold phosphate buffer (Reagent A) immediately before measurement.

Procedure

1. Pipette 2.0 ml of sample into a cuvette (light path: 1 cm).
2. Equilibrate at 25°C for about 5 min.
3. Add 1.0 ml of H\textsubscript{2}O\textsubscript{2} solution (Reagent B).
4. Record the decrease of absorbance at 240 nm in a spectrophotometer thermostated at 25°C, and calculate the \(\Delta A\) per min using the linear portion of the curve \((\Delta A_0)\).

The blank solution is prepared by adding phosphate buffer (Reagent A) instead of sample \((\Delta A_0)\).
Calculation

Activity can be calculated by using the following formula:

\[
\text{Volume activity (U/ml)} = \frac{(\Delta A_b - \Delta A_a) \times 3.0 \text{ (ml)} \times df}{0.0436 \times 2.0 \text{ (ml)}} = \Delta A \times 34.4 \times df
\]

\[
\text{Weight activity (U/mg)} = (U/ml) \times \frac{1}{C}
\]

0.0436 : Millimolar extinction coefficient of hydrogen peroxide at 240 nm (cm²/µmol)
\(df\) : Dilution factor
\(C\) : Content of catalase preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for removal of hydrogen peroxide in clinical analysis.

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2 \text{ H}_2\text{O}_2 \overset{\text{catalase}}{\longrightarrow} \text{O}_2 + 2 \text{ H}_2\text{O}
\]

REFERENCES

EXPERIMENTAL DATA

Fig. 1 pH Optimum

- △: 50 mM acetate buffer
- □: 50 mM MES-NaOH buffer
- ◦: 50 mM phosphate buffer
- ●: 50 mM Tris-HCl buffer
- ▲: 50 mM CHES-NaOH buffer
- ▼: 50 mM CAPS-NaOH buffer

Fig. 2 pH Stability

Treatment: 30°C, 30 min

- ▲: 50 mM glycine-HCl buffer
- △: 50 mM acetate buffer
- □: 50 mM MES-NaOH buffer
- ◦: 50 mM phosphate buffer
- ●: 50 mM Tris-HCl buffer
- ▲: 50 mM CHES-NaOH buffer
- ▼: 50 mM CAPS-NaOH buffer

Fig. 3 Optimum temperature

Buffer: 50 mM phosphate buffer, pH 7.0

Fig. 4 Thermal stability

Treatment: 50 mM phosphate buffer, pH 7.0, 10 min