Catalase (CAT)

from bovine liver

Hydrogen-peroxide : hydrogen-peroxide oxidoreductase, EC 1.11.1.6

$$2\ H_2O_2 \longrightarrow O_2 + 2\ H_2O$$

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

$$2\ H_2O_2 \xrightarrow{\text{catalase}} O_2 + 2\ H_2O$$

The disappearance of hydrogen peroxide (H_2O_2) is measured spectrophotometrically at 240 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which decomposes 1 μmol of H_2O_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_2HPO_4 solution and 50 mM KH_2PO_4 solution to make a pH 7.0 solution.

B. Hydrogen peroxide (H_2O_2) solution: add about 0.75 ml of 30% H_2O_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_2O_2 solution) at 240 nm in 1 cm light path versus phosphate buffer (Reagent A) and check the absorbance of 0.85 ($\pm$0.02).
Otherwise add more 30% H_2O_2 or phosphate buffer (Reagent A) to the H_2O_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_2O_2 solution (Reagent B).
4. Record the decrease of absorbance at 240 nm in a spectrophotometer thermostated at 25°C, and calculate the ΔA per min using the linear portion of the curve (ΔA_S).
The blank solution is prepared by adding phosphate buffer (Reagent A) instead of sample (ΔA_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_S - \Delta A_0) \times 3.0\,(\text{ml}) \times df}{0.0436 \times 2.0\,(\text{ml})} = \Delta A \times 34.4 \times df$$

Weight activity (U/mg) = (U/ml) × 1/*C*

0.0436 : Millimolar extinction coefficient of hydrogen peroxide at 240 nm ($cm^2/\mu mol$)
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.

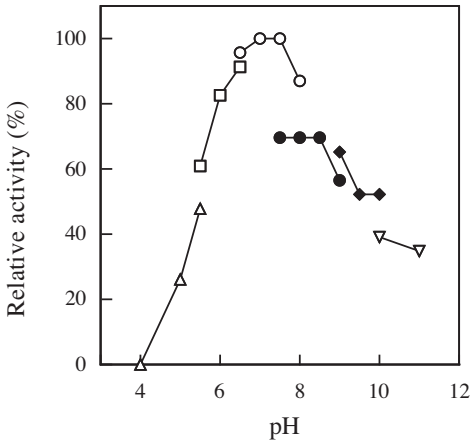
$$2\ H_2O_2 \xrightarrow{\text{catalase}} O_2 + 2\ H_2O$$

REFERENCES

Nicholls, P. and Schonbaum, G. R., "The Enzyme," Vol. 8 (2nd ed.), Academic Press, New York and London, 1963, pp. 147–225.
Bergmeyer, H. U., "Methods of Enzymatic Analysis," Vol. 3 (3rd ed.), Verlag Chemie, Weinheim, Germany, 1983, pp. 273–286.

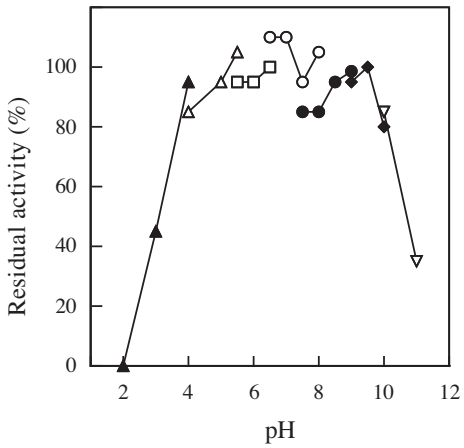
EXPERIMENTAL DATA

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

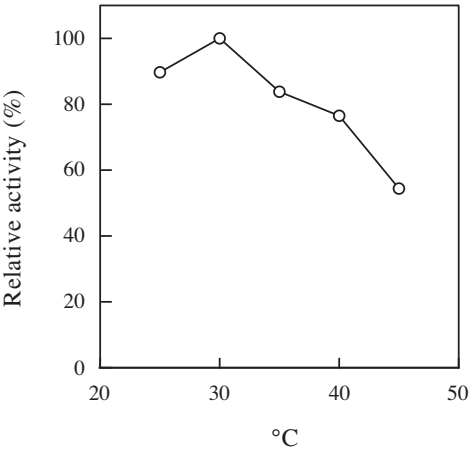
Fig. 2 pH Stability



Treatment: 30°C, 30 min

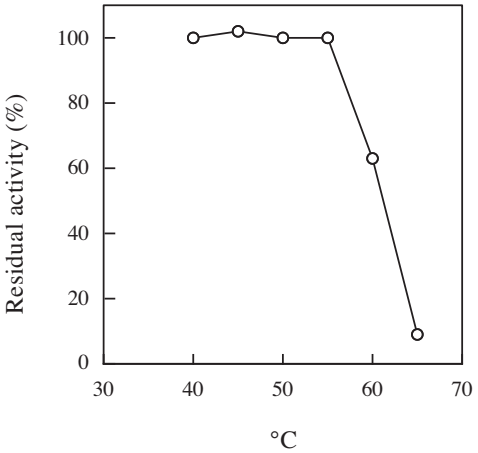
▲: 50 mM glycine-HCl buffer
△: 50 mM acetate buffer
□: 50 mM MES-NaOH buffer
○: 50 mM phosphate buffer
●: 50 mM Tris-HCl buffer
◆: 50 mM CHES-NaOH buffer
▽: 50 mM CAPS-NaOH buffer

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