NIPRO ENZYMES

SUPEROXIDE DISMUTASE (SOD)

[EC 1.15.1.1]

from *Bacillus stearothermophilus*

$$O_2^- + O_2^- + 2H^+ \leftrightarrow O_2 + H_2O_2$$

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 9,000 U/mg protein	
Contaminants	:	(as SOD activity = 100 %)	
		Catalase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 50,000	
Subunit molecular weight	:	ca. 25,000	
Metal content	:	1.5 g atoms of Mn per mole of enzyme	
Optimum pH	:	9.5	(Fig. 1)
pH stability	:	6.0 - 9.0	(Fig. 2)
Isoelectric point	:	4.5	
Thermal stability	:	No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for medicine, cosmetic material and nutrition or antioxidant.

ASSAY

Principle

To determine the enzyme activity of cytochrome c reduction is measured by the following reactions.

$$\text{Xanthine} + O_2 \xrightarrow{\text{Xanthine oxidase}} \text{Urate} + O_2^- + H_2O_2$$

$$O_2^- \xrightarrow{\text{cytochrome c}} \text{cytochrome c (red.)}$$

$$O_2^- \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

Unit Definition

One unit of activity is defined as the amount of SOD required to inhibit the rate of reduction of cytochrome C by 50 % at 30 °C.

Solutions

Ⅰ Buffer solution ; 75 mM Potassium phosphate buffer, pH 7.8

Ⅱ Xanthine solution ; 0.75 mM (0.010 g xanthine/50 mL N/250 NaOH)

Ⅲ Cytochrome c solution ; 0.15 mM (0.019 g cytochrome c/10 mL distilled water, Sigma-Aldrich Co., No. C-2506, from horse heart)

Ⅳ EDTA solution ; 1.5 mM (0.028 g EDTA disodium salt·$2H_2O$/50 mL distilled water)

Ⅴ Xanthine oxidase (XOD) ; (from buttermilk, Sigma-Aldrich Co., No. X-1875) suspension in 2.3 M $(NH_4)_2SO_4$ solution is diluted to 0.04 U/mL with distilled water. (prepare freshly)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to approx. 600 U/mL with 50 mM potassium phosphate buffer, pH 7.5.

Procedure

1. Prepare the following reaction mixture and pipette 2.80 mL of reaction mixture and 0.005 mL of enzyme solution into a cuvette.

Solution Ⅰ	22.00 mL	Solution Ⅲ	2.00 mL
Solution Ⅱ	2.00 mL	Solution Ⅳ	2.00 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.20 mL of Solution Ⅴ into the cuvette and mix.
4. Read absorbance change at 550 nm per minute for the linear portion of curve (ΔAbs•test)*.
5. Add 0.005 mL of Solution Ⅰ in place of enzyme solution and measure the same above 4 (ΔAbs•blank).

*Dilute enzyme solution with 50 mM potassium phosphate buffer, pH 7.5, because the decrease in the initial rate should not fall outside the range of 40 to 60 % for the results to be valid.

Calculation

$$\text{Volume activity (U/mL)} = \left[\frac{(\Delta\text{Abs•blank})}{(\Delta\text{Abs•test})} - 1 \right] \times \frac{601}{1} \times \text{d.f.}$$

$$\text{Specific activity (U/mg protein)} = \frac{\text{Volume activity (U/mL)}}{\text{protein concentration (mg/mL)*}}$$

d.f. ; dilution factor

*Protein concentration ; determined by Bradford's method

REFERENCE

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4. Auffret, A.D., Blake, T,J., and Williams, D.H.; *Eur. J. Biochem.*, **113**, 333 (1981)
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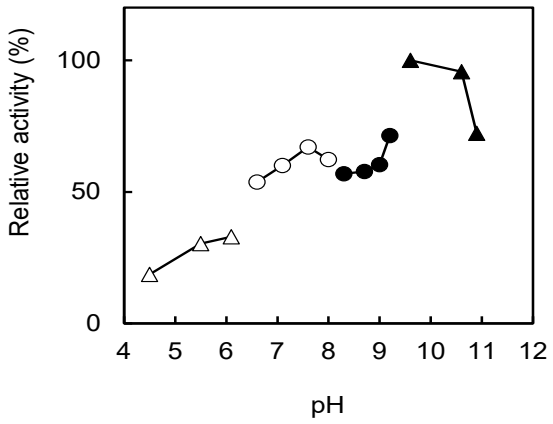

Fig. 1 pH profile

△ acetate, ○ phosphate,
● Tris-HCl, ▲ carbonate

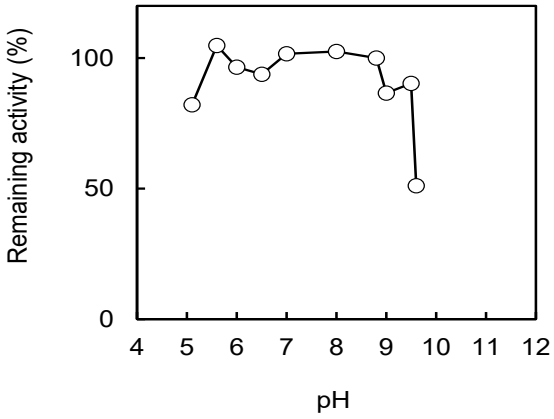

Fig. 2 pH stability

treated for 24 hr at 4 ℃ in the Britton-Robinson buffer

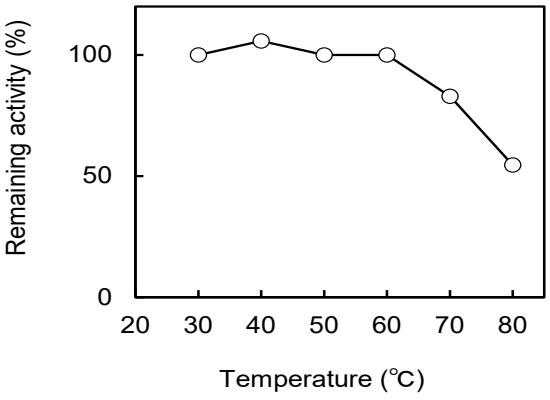

Fig. 3 Thermal stability

treated for 15 min in 0.1 M potassium phosphate buffer, pH 7.5

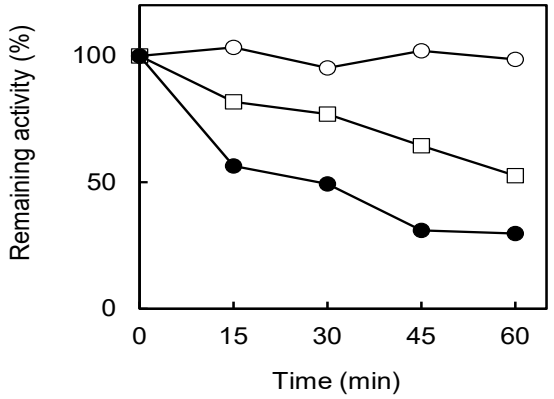

Fig. 4 Thermal stability

treated in 0.1 M potassium phosphate buffer, pH 7.5
○ 60 ℃, □ 70 ℃, ● 80 ℃