Sarcosine Oxidase (SOD-TE)

from recombinant *E. coli*

Sarcosine : oxygen oxidoreductase (demethylating), EC 1.5.3.1

$$\text{Sarcosine} + H_2O + O_2 \longrightarrow \text{Glycine} + \text{Formaldehyde} + H_2O_2$$

SPECIFICATION

Appearance	yellow lyophilizate	
Activity	≧15 U/mg lyophilizate	
Contaminants	catalase	≦0.5%
	glucose oxidase	$\leqq 1.0\times10^{-5}$%
Stabilizer	sucrose	
Storage	at −20°C	

PROPERTIES

Molecular weight	*ca*. 49 kDa (gel filtration)	
Structure	monomer of 43 kDa (SDS-PAGE)	
	one mole of FAD per mole of enzyme	
Isoelectric point	5.3	
Michaelis constant	4.7×10^{-3} M (sarcosine)	
pH Optimum	6.7–9.5	(Fig. 1)
pH Stability	6.5–10.5	(Fig. 2)
Optimum temperature	50°C	(Fig. 3)
Thermal stability	below 55°C	(Fig. 4)
Stability (liquid form)	stable at 37°C for at least two weeks	
		(Fig. 5)
Stability (powder form)	stable at 30°C for at least one month	
		(Fig. 6)
Inhibitors	Zn^{2+}, Cu^{2+}, Hg^{2+}, Ag^{+}	

ASSAY PROCEDURE

Principle

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{sarcosine oxidase}} \text{Glycine} + \text{Formaldehyde} + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4\ H_2O$$

The appearance of quinoneimine dye is measured spectrophotometrically at 495 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 μmol of hydrogen peroxide per min at 37°C and pH 7.7 under the conditions described below.

Reagents

A. Tris-HCl buffer, 125 mM; pH 7.7: dissolve 15.1 g of Tris(hydroxymethyl)aminomethane in 900 ml of distilled water, adjust to pH 7.7 with 5 N HCl and dilute with distilled water to 1000 ml.
B. Sarcosine solution, 0.2 M: dissolve 1.78 g of sarcosine in 80 ml of Tris-HCl buffer (Reagent A) containing 0.125% of Triton X-100 and 2.5 mM KCl, adjust to pH 7.7 with 1 N NaOH and dilute with distilled water to 100 ml.
C. Phenol solution, 0.1%: 100 mg of phenol/100 ml of distilled water.
D. 4-Aminoantipyrine (4-AA) solution, 0.2%: 200 mg of 4-AA/100 ml of distilled water.
E. Peroxidase (POD) solution, 80 U/ml; 4 mg of POD (200 guaiacol U/mg)/10 ml of distilled water.
F. Sodium dodecyl sulfate (SDS) solution, 0.3%: 1.5 g of SDS/500 ml of distilled water.
G. Enzyme dilution buffer: 20 mM Tris-HCl buffer, pH 7.7, containg 1.0 mM KCl and 0.2% bovine serum albumin (BSA).

Sample: dissolve the lyophilized enzyme to a volume activity of 0.06–0.09 U/ml in ice-cold enzyme dilution buffer (Reagent G) immediately before measurement.

Procedure

1. Prepare the following reaction mixture immediately before use and store on ice in a brownish bottle.

50 ml	Sarcosine solution	(Reagent B)
20 ml	Phenol solution	(Reagent C)
10 ml	4-AA solution	(Reagent D)
10 ml	POD solution	(Reagent E)
10 ml	Distilled water	

2. Pipette 0.95 ml of the reaction mixture into a cuvette (light path: 1 cm).
3. Equilibrate at 37°C for about 5 min.
4. Add 0.05 ml of sample and incubate for 10 min at 37°C.
5. Add 2.0 ml of SDS solution (Reagent F) to stop the reaction.
6. Read the absorbance at 495 nm in a cuvette (light path: 1 cm) (A_S).
   The blank solution is prepared by adding enzyme dilution buffer (Reagent G) instead of sample (A_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(A_S - A_0) \times 3.0(\text{ml}) \times df}{15.5 \times 1/2 \times 0.05(\text{ml}) \times 10(\text{min})} = \Delta A \times 0.774 \times df$$

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

15.5 : Millimolar extinction coefficient of quinoneimine dye under the assay conditions ($cm^2/\mu mol$)
1/2 : Factor based on the fact that 1 mol of hydrogen peroxide produces 1/2 mol of quinoneimine dye
df : Dilution factor
C : Content of sarcosine oxidase preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for the determination of creatinine and creatine in clinical analysis.

$$\text{Creatinine} + H_2O \xrightarrow{\text{creatininase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{creatinase}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{sarcosine oxidase}} \text{Glycine} + \text{Formaldehyde} + H_2O_2$$

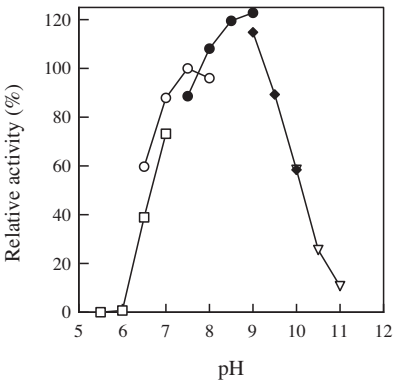
$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4\ H_2O$$

REFERENCES

Suzuki, M., *Medical Technology*, **7**, 945–950 (1979).
Suzuki, M. and Yoshida, M., *Clin. Chim. Acta*, **140**, 289–294 (1984).
Suzuki, M. and Yoshida, M., *Clin. Chim. Acta*, **143**, 147–155 (1984).
Koyama, Y. *et al*., *Agric. Biol. Chem*., **55**, 1259–1263 (1991).

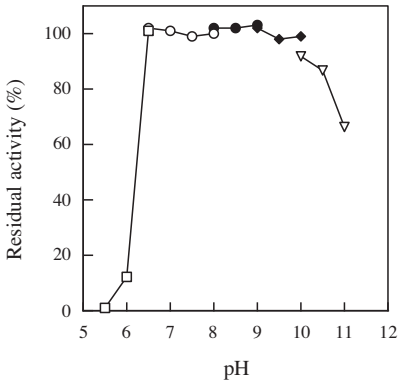
EXPERIMENTAL DATA

Fig. 1 pH Optimum



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

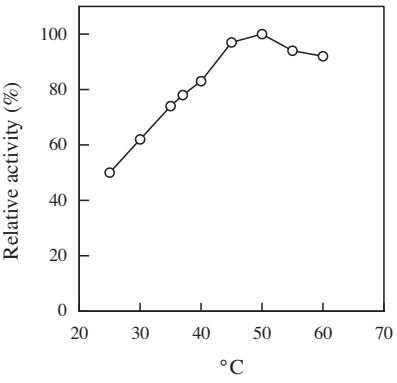
Fig. 2 pH Stability



Treatment: 20°C, 5 h

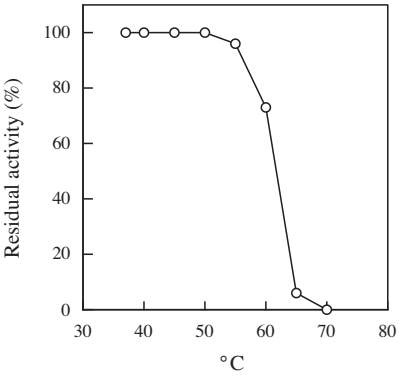
□: 50 mM MES-NaOH buffer
○: 50 mM phosphate buffer
●: 50 mM Tris-HCl buffer
◆: 50 mM CHES-NaOH buffer
▽: 50 mM CAPS-NaOH buffer

Fig. 3 Optimum temperature



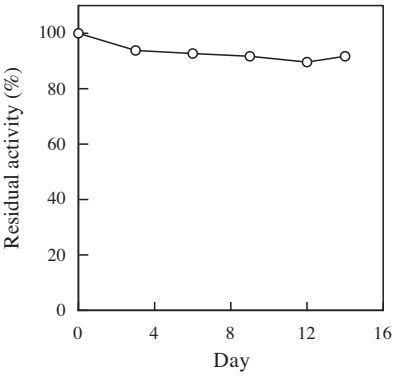
Buffer: 60 mM phosphate buffer, pH 7.7

Fig. 4 Thermal stability



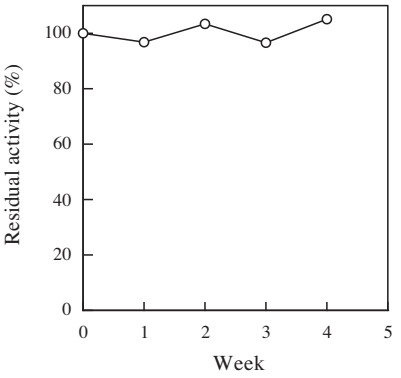
Treatment: 0.3 M phosphate buffer, pH 7.7, 10 min

Fig. 5 Stability (liquid form) at 37°C



[Kept in 10 mM potassium phosphate buffer, pH 8.0, containing 3.5% sucrose and 10 mM KCl]

Fig. 6 Stability (powder form) at 30°C



(Kept under dry conditions)