

● TOYOBO ENZYMES ●
(Diagnostic Reagent Grade)

SARCOSINE OXIDASE

from Microorganism

Sarcosine:oxygen oxidoreductase(demethylating)(EC 1.5.3.1)



PREPARATION and SPECIFICATION

Appearance	: Yellowish amorphous powder, lyophilized
Activity	: Grade III 8.0U/mg-solid or more
Contaminants	: Catalase ≤1.0%
Stabilizers	: Potassium chloride

PROPERTIES

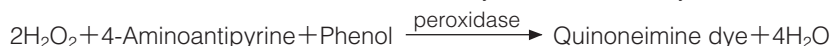
Stability	: Stable at -20°C for at least 6 months	
Molecular weight	: approx.43,000 (by SDS-PAGE)	
Isoelectric point	: 4.8±0.1	
Michaelis constant	: 2.8×10 ⁻³ M	
Inhibitors	: Cu ⁺⁺ , Ag ⁺ , Hg ⁺⁺ , p-chloromercuribenzoate, N-ethylmaleimide, SDS	
Optimum pH	: 7.5-8.5	(Fig.1)
Optimum temperature	: 55-60°C	(Fig.2)
pH Stability	: 6.0-9.5 (25°C, 20hr)	(Fig.3)
Thermal stability	: below 60°C (pH 7.5, 30min)	(Fig.4)
Effect of various chemicals	: (Table.1)	

APPLICATIONS

This enzyme is useful for enzymatic determination of creatinine, creatine, and sarcosine when coupled with creatinine amidohydrolase (CNH-211, CNH-311) and creatine amidinohydrolase (CRH-211, CRH-221).

ASSAY

Principle:



Unit definition:

One unit causes the formation of one micromole of hydrogen peroxide (half a micromole of quinoneimine dye) per minute under the conditions described below.

Method:

Reagents

- A. Sarcosine solution : 0.2M [Weight 1.78g of sarcosine (MW=89.09), dissolve in 80ml of 0.125M Tris-HCl buffer, pH8.0 containing 0.125% of Triton X-100 and, after adjusting pH 8.0 at 25°C with 1.0N NaOH or 1.0N HCl, fill up to 100ml with H₂O.] (Stable for one week if stored at 0–5°C)
- B. 4-AA solution : 0.1% (100mg of 4-aminoantipyrine/100ml of H₂O)(Store at 4°C in a brownish bottle)
- C. Phenol solution : 0.1%(100mg of phenol/100ml of H₂O)(Store at 4°C in a brownish bottle)
- D. Peroxidase solution : 0.025% [25mg of peroxidase (110 purpurogallin units/mg)/100ml of H₂O] (Should be prepared fresh)
- E. SDS solution : 0.25%(1.25g of sodium dodecyl sulfate/500ml of H₂O)
- F. Enzyme diluent : 20mM Tris-HCl buffer, pH8.0 containing 2.0mM EDTA

Procedure

1. Prepare the following working solution(100 tests) in a brownish bottle and store on ice.

50ml	Sarcosine solution	(A)
10ml	4-AA solution	(B)
20ml	Phenol solution	(C)
20ml	Peroxidase solution	(D)

Concentration in assay mixture	
Tris-HCl buffer	48 mM
Sarcosine	95 mM
4-Aminoantipyrine	0.47mM
Phenol	2.0 mM
Triton X-100	0.045 %
POD	ca.5.2 U/ml

2. Pipette 1.0ml of working solution into a test tube and equilibrate at 37°C for about 5 minutes.
3. Add 0.05ml of the enzyme solution*and mix.
4. After exactly 10 minutes at 37°C, add 2.0ml of SDS solution (E) to stop the reaction and measure the optical density at 500nm against water (OD test).

At the same time, prepare the blank by using the same method as the test except that the enzyme diluent is used instead of the enzyme solution (OD blank).

- * Dissolve the enzyme preparation in ice-cold enzyme diluent (F) and dilute to 0.07–0.17U/ml with the same buffer, immediately before assay.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta \text{OD (OD test - OD blank)} \times \text{Vt} \times \text{df}}{13.3 \times 1/2 \times 1.0 \times \text{t} \times \text{Vs}} = \Delta \text{OD} \times 0.917 \times \text{df}$$

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

Vt : Total volume (3.05ml)

Vs : Sample volume (0.05ml)

13.3 : Millimolar extinction coefficient of quinoneimine dye under the assay condition (cm²/micromole)

1/2 : Factor based on the fact that one mole of H₂O₂ produced half a mole of quinoneimine dye

t : Reaction time (10 minutes)

1.0 : Light path length (cm)

C : Enzyme concentration in dissolution (c mg/ml)

REFERENCES

- 1) N.Mori, M.Sato, Y.Tani and Y.Yamada; *Agric.Biol.Chem.*, **44**, 1391 (1980).
- 2) M.Suzuki; *J. Biochem.*, **89**, 599 (1981).
- 3) M.Suzuki and M.Yoshida; *Proceedings of the Symposium on Chemical Physiology and Pathology* (Kyoto), Vol16, p.220 (1976).
- 4) T.Kinoshita and Y.Hiraga; *Chem.Pharm.Bull.*, **28**, 3501 (1980).
- 5) Y.Nishiya, S.Zuihara and T.Imanaka; *APPLIED AND ENVIRONMENTAL MICROBIOLOGY.*, **61**, 367 (1995).

Table 1. Effect of Various Chemicals on Sarcosine oxidase

[The enzyme dissolved in 50mM K-phosphate buffer, pH 7.5 (10U/ml) was incubated at 30°C for 30minutes.]

Chemical	Concn.(mM)	Residual activity	Chemical	Concn.(mM)	Residual activity
None	-	100%	NAF	2.0	99
Metal salt	2.0		NaN ₃	20.0	90
MgCl ₂		99	EDTA	5.0	97
CaCl ₂		99	o-Phenanthroline	2.0	99
Ba(OAc) ₂		98	α,α' -Dipyridyl	2.0	97
FeCl ₃		99	Borate	50.0	98
CoCl ₂		98	IAA	2.0	97
MnCl ₂		99	NEM	2.0	74
Zn(OAc) ₂		98	Hydroxylamine	2.0	97
Cd(OAc) ₂		99	Triton X-100	0.10%	99
NiCl ₂		96	Brij 35	0.10%	99
CuSO ₄		43	Tween 20	0.10%	97
Pb(OAc) ₂		98	Span 20	0.10%	101
AgNO ₃		0.4	Na-cholate	0.10%	99
HgCl ₂		0.4	SDS	0.05%	68
PCMB	2.0	36	DAC	0.05%	97
MIA	2.0	101			

Ac; CH₃CO, PCMB; p-Chloromercuribenzoate, MI; Monoiodoacetate, EDTA; Ethylenediaminetetraacetate, IAA; Iodoacetate, NEM; N-Ethylmaleimide, SDS; Sodium dodecyl sulfate, DAC; Dimethyl-benzyl-alkyl-ammonium chloride.

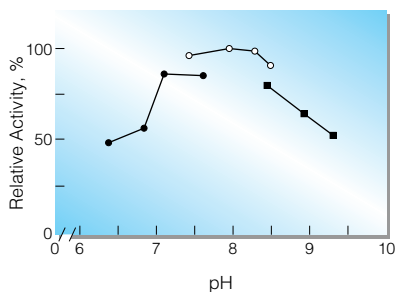


Fig.1. pH-Activity

[37°C in 0.1M buffer solution :
pH6.5-7.5 K-phosphate.;pH7.5-8.5
Tris-HCl;pH8.5-9.5 Glycine-NaOH]

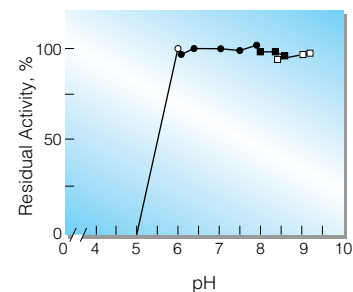


Fig.3. pH-Stability

[25°C,24hr-treatment with 100mM
buffer solution:pH5-6 Acetate buffer,
;pH6-8 K-phosphate.;pH8-9 Tris-HCl,
;pH8.5-9.5 Glycine-NaOH]

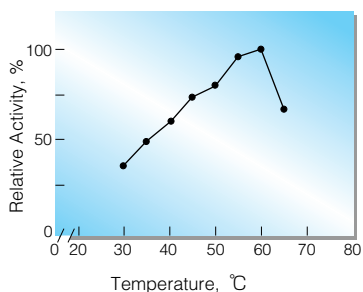


Fig.2. Temperature activity

(in 0.1M Tris-HCl buffer : pH8.0)

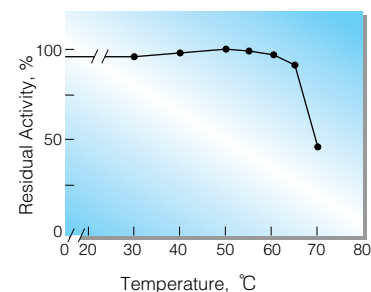
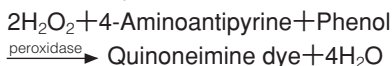
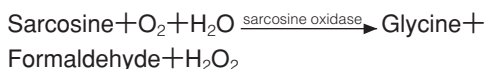


Fig.4. Thermal stability

[30min-treatment with 50mM
K-phosphate pH7.5
(contg. 100mM NaCl)
enzyme concn. 5U/ml]

活性測定法 (Japanese)

1.原理



4-AminoantipyrineとPhenolの酸化縮合物であるQuinoneimine色素を500nmで測定し、上記反応で生成したH₂O₂量を定量する。

2.定義

下記条件下で1分間に1マイクロモルのH₂O₂を生成する酵素量を1単位(U)とする。

3.試薬

- 0.2Mサルコシン溶液 [1.78gのサルコシン(MW=89.09)を80mlの0.125%Triton X-100を含む0.125M Tris-HCl緩衝液, pH8.0に溶解後,1.0NのNaOHあるいはHClでpHを8.0に調整(25°C)し,蒸留水で100mlとする)(0~5°C保存で1週間は使用可能)
 - 0.1%4-AA水溶液(100mgの4-アミノアンチピリンを100mlの蒸留水に溶解する)(褐色瓶中で4°C保存)
 - 0.1%フェノール水溶液(100mgのフェノールを100mlの蒸留水に溶解する)(褐色瓶中で4°C保存)
 - POD溶液 [25mgのペルオキシダーゼ(POD)(110プルプロガリン単位/mg)を蒸留水100mlに溶解する](用時調製)
 - 0.25%SDS水溶液 [1.25gのsodium dodecyl sulfate(SDS)を500mlの蒸留水に溶解する]
- 酵素溶液：酵素標品を予め氷冷した2.0mM EDTAを含む20mM Tris-HCl緩衝液, pH8.0で溶解し,分析直前に同緩衝液で0.07-0.17U/mlに希釈する。

4.手順

- 下記反応混液を調製する(褐色瓶にて氷冷保存)。

50ml	サルコシン溶液	(A)
10ml	4-AA水溶液	(B)
20ml	フェノール水溶液	(C)
20ml	POD水溶液	(D)
- 反応混液1.0mlを試験管に採り,37°Cで約5分間予備加温する。
- 酵素溶液0.05mlを加え,反応を開始する。
- 37°Cで正確に10分間反応させた後,SDS水溶液(E)2.0mlを加えて反応を停止させる。この液につき500nmにおける吸光度を測定する(OD test)。
- 盲検は酵素溶液の代わりに酵素希釈液(2.0mM EDTAを含む20mM Tris-HCl緩衝液, pH8.0)を用い,上記同様に操作を行って吸光度を測定する(OD blank)。

5.計算式

$$\begin{aligned} \text{U/ml} &= \frac{\Delta \text{OD}(\text{OD test} - \text{OD blank}) \times 3.05(\text{ml}) \times \text{希釈倍率}}{13.3 \times 1/2 \times 1.0 \times 10(\text{分}) \times 0.05(\text{ml})} \\ &= \Delta \text{OD} \times 0.917 \times \text{希釈倍率} \\ \text{U/mg} &= \text{U/ml} \times 1 / \text{C} \\ 13.3 &: \text{Quinoneimine色素の上記測定条件下での} \\ &\quad \text{ミリモル分子吸光係数}(\text{cm}^2/\text{micromole}) \\ 1/2 &: \text{酵素反応で生成したH}_2\text{O}_2\text{の1分子から形成} \\ &\quad \text{するQuinoneimine色素は1/2分子である事} \\ &\quad \text{による係数} \\ 1.0 &: \text{光路長}(\text{cm}) \\ \text{C} &: \text{溶解時の酵素濃度}(\text{c mg/ml}) \end{aligned}$$