●TOYOBO ENZYMES●
(Diagnostic Reagent Grade)

SARCOSINE OXIDASE

from Microorganism

Sarcosine:oxygen oxidoreductase(demethylating) (EC 1.5.3.1)

Sarcosine + O_2 + H_2O ⟶ Glycine + Formaldehyde + H_2O_2

PREPARATION and SPECIFICATION

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

PROPERTIES

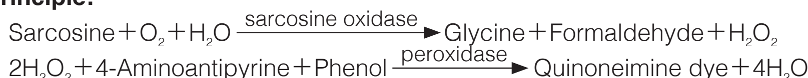
Stability	: Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	: approx.43,000 (by SDS-PAGE)	
Isoelectric point	: 4.8±0.1	
Michaelis constant	: 2.8×10^{-3}M	
Inhibitors	: Cu^{++}, Ag^{+}, Hg^{++}, p-chloromercuribenzoate, N-ethylmaleimide, SDS	
Optimum pH	: 7.5−8.5	(Fig.2)
Optimum temperature	: 55−60℃	(Fig.3)
pH Stability	: 6.0−9.5 (25℃, 20hr)	(Fig.4)
Thermal stability	: below 60℃ (pH 7.5, 30min)	(Fig.5)
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, CRH-229).

ASSAY

Principle:



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

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

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℃ with 1.0N NaOH or 1.0N HCl, fill up to 100ml with H_2O.] (Stable for one week if stored at 0−5℃)
B. 4-AA solution	: 0.1% (100mg of 4-aminoantipyrine/100ml of H_20)(Store at 4℃ in a brownish bottle)
C. Phenol solution	: 0.1%(100mg of phenol/100ml of H_2O)(Store at 4℃ in a brownish bottle)
D. Peroxidase solution	: 0.025% [25mg of peroxidase (110 purpurogallin units/mg)/100ml of H_2O] (Should be prepared fresh)
E. SDS solution	: 0.25%(1.25g of sodium dodecyl sulfate/500ml of H_2O)
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 soluton into a test tube and equilibrate at 37℃ for about 5 minutes.
3. Add 0.05ml of the enzyme solution* and mix.
4. After exactly 10 minutes at 37℃, 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 :

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

Weight activity (U/mg) = (U/ml) × 1/C

Vt	: Total volume (3.05ml)
Vs	: Sample volume (0.05ml)
13.3	: Millimolar extinction coefficient of quinoneimine dye under the assay condition (㎠/micromole)
1/2	: Factor based on the fact that one mole of H_2O_2 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 Physiolosy* 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 ENVIROMENTAL 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℃ for 30minutes.]

Chemical	Concn.(mM)	Residual activity(%)
None	—	100
Metal salt	2.0	
$MgCl_2$		99
$CaCl_2$		99
$Ba(OAc)_2$		98
$FeCl_3$		99
$CoCl_2$		98
$MnCl_2$		99
$Zn(OAc)_2$		98
$Cd(OAc)_2$		99
$NiCl_2$		96
$CuSO_4$		43
$Pb(OAc)_2$		98
$AgNO_3$		0.4
$HgCl_2$		0.4
PCMB	2.0	36
MIA	2.0	101

Chemical	Concn.(mM)	Residual activity(%)
NAF	2.0	99
NaN_3	20.0	90
EDTA	5.0	97
o-Phenanthroline	2.0	99
α,α'-Dipyridyl	2.0	97
Borate	50	98
IAA	2.0	97
NEM	2.0	74
Hydroxylamine	2.0	97
Triton X-100	0.10%	99
Brij 35	0.10%	99
Tween 20	0.10%	97
Span 20	0.10%	101
Na-cholate	0.10%	99
SDS	0.05%	68
DAC	0.05%	97

Ac, CH_3CO; PCMB, p-Chloromercuribenzoate; MIA, Monoiodoacetate; EDTA, Ethylenediaminetetraacetate;
IAA, Iodoacetate; NEM, N-Ethylmaleimide; SDS, Sodium dodecyl sulfate; DAC, Dimethylbenzylalkylammonium chloride.

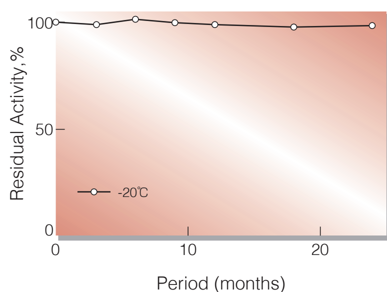


Fig.1. Stability (Powder form)
(kept under dry conditions)

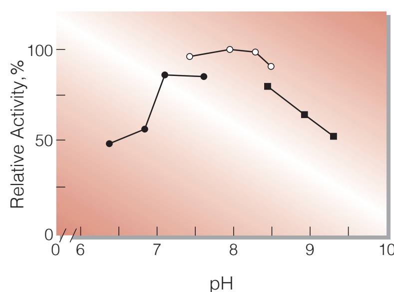


Fig.2. pH-Activity
[37℃ in 0.1M buffer solution : pH6.5-7.5 K-phosphate,:pH7.5-8.5 Tris-HCl:pH8.5-9.5 Glycine-NaOH]

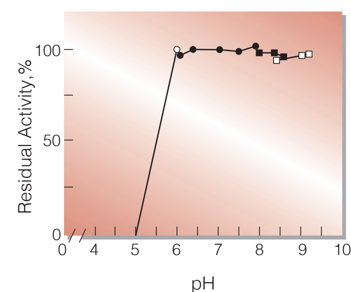


Fig.4. pH-Stability
[25℃,24hr-treatment with 100mM buffer solution:pH5-6 Acetate buffer, :pH6-8 K-phosphate,:pH8-9 Tris-HCl, :pH8.5-9.5 Glycine-NaOH]

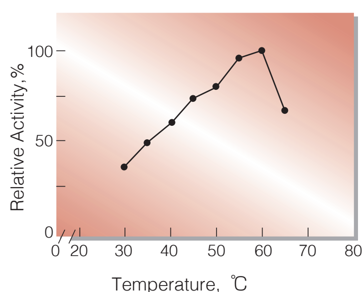


Fig.3. Temperature activity
(in 0.1M Tris-HCl buffer : pH8.0)

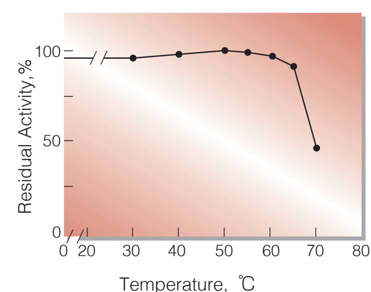


Fig.5. Thermal stability
[30min-treatment with 50mM K-phosphate pH7.5 (contg. 100mM NaCl) enzyme concn. 5U/ml]

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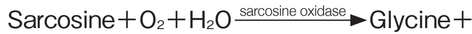
活性測定法（Japanese）

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H_2O_2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H_2O_2を生成する酵素量を1単位(U)とする。

3.試薬

A.　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℃)し,蒸留水で100mℓとする〕(0～5℃保存で1週間は使用可能)

B.　0.1%4-AA水溶液(100mgの4ー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℃保存)

C.　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℃保存)

D.　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)

E.　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

①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)

②反応混液1.0mℓを試験管に採り,37℃で約5分間予備加温する。

③酵素溶液0.05mℓを加え,反応を開始する。

④37℃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)。

⑤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

$$U/m\ell = \frac{\Delta OD\ (OD\ test - OD\ blank) \times 3.05(m\ell) \times 希釈倍率}{13.3 \times 1/2 \times 1.0 \times 10(分) \times 0.05(m\ell)}$$

$$= \Delta OD \times 0.917 \times 希釈倍率$$

$$U/mg = U/m\ell \times 1/C$$

13.3　：Quinoneimine色素の上記測定条件下でのミリモル分子吸光係(㎠/micromole)

1/2　：酵素反応で生成したH_2O_2の1分子から形成するQuinoneimine色素は1/2分子である事による係数

1.0　：光路長(cm)

C　：溶解時の酵素濃度(c mg/mℓ)