

● **TOYOBO ENZYMES** ●
(Diagnostic Reagent Grade)

LACTATE OXIDASE

from Microorganism

L-Lactate:oxygen oxidoreductase (EC 1. 1. 3. 2)



PREPARATION and SPECIFICATION

Appearance	: Yellowish amorphous powder, lyophilized
Activity	: Grade III 80U/mg-solid or more
Contaminants	: Pyruvate oxidase $\leq 1.0 \times 10^{-3}\%$
	: Cholesterol oxidase $\leq 1.0 \times 10^{-3}\%$
	: Uricase $\leq 1.0 \times 10^{-3}\%$
	: Glucose oxidase $\leq 1.0 \times 10^{-3}\%$

PROPERTIES

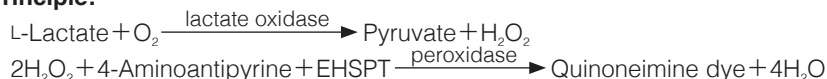
Stability	: Stable at -20°C for at least one year	(Fig.1)
Molecular weight	: approx. 160,000 (by gel filtration)	
Isoelectric point	: 4.3 ± 0.2	
Michaelis constant	: $1.0 \times 10^{-3}\text{M}$ (L-Lactate)	
Inhibitors	: Fe^{+++} , SDS	
Optimum pH	: 7.5	(Fig.2)
Optimum temperature	: $35-40^\circ\text{C}$	(Fig.3)
pH Stability	: 4.0—9.8 (25°C , 16hr)	(Fig.4)
Thermal stability	: below 50°C (pH 7.0, 10min)	(Fig.5)
Effect of various chemicals	: (Table 1)	

APPLICATIONS

This enzyme is useful for enzymatic determination of L-lactate.

ASSAY

Principle:



The appearance of quinoneimine dye is measured at 555nm by spectrophotometry.

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. DL-Lactate solution : 0.125M [240mg of DL-lithium lactate (MW=96.01)/20ml of 50mM K-PB pH7.5]
(Should be prepared fresh)
- B. 4-AA solution : 0.5% (500mg of 4-aminoantipyrine/100ml of H₂O) (Store at 4°C in a brownish bottle)
- C. EHSPT(TOOS) solution : 20mM [591mg
N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine
(MW=295.3)/100ml of H₂O] (Store at 4°C in a brownish bottle)
- D. Peroxidase solution : 25U/ml [ca. 23mg of horseradish peroxidase (Toyobo GradeIII, 110 purpurogallin
units/mg)/100 ml of H₂O]
- E. SDS solution : 0.25% (500mg sodium dodecyl sulfate/200ml of H₂O)
- F. Enzyme diluent : 20mM K-PB, pH7.0 containing 0.1%(w/v) sodium cholate

Procedure

1. Prepare the following working solution (20 tests) in a brownish bottle, and store on ice.

8.0ml	DL-Lactate solution	(A)
1.2ml	4-AA solution	(B)
0.8ml	EHSPT solution	(C)
2.0ml	Peroxidase solution	(D)
8.0ml	distilled water	

Concentration in assay mixture		
K-phosphate buffer	20	mM
DL-Lactate	48	mM
4-Aminoantipyrine	1.2	mM
EHSPT	0.76	mM
Peroxidase	2.4	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 15minutes at 37°C, add 2.0ml of SDS solution (E) to stop the reaction and measure the optical density at 555nm against water (ODtest).

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

- * Dissolve the enzyme preparation in ice-cold 20mM ACES-NaOH pH7.0 containing 1mM EDTA and 0.5%(w/v) sodium cholate, and dilute to 0.04–0.1 U/ml with the enzyme diluent (F) immediately before assay.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta \text{OD (OD test - OD blank)} \times V_t \times d_f}{34.3 \times 1/2 \times t \times 1.0 \times V_s} = \Delta \text{OD} \times 0.237 \times d_f$$

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

V_t : Total volume (3.05ml)

V_s : Sample volume (0.05ml)

34.3 : 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 (15minutes)

1.0 : Light path length (cm)

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

REFERENCES

- 1) A. Toda, and Y. Nishiya; *J. Ferment. Technol.*, **85**, 507 (1998)

Table 1. Effect of Various Chemicals on Lactate oxidase

[The enzyme solution dissolved in 20mM ACES-NaOH, pH7.0 (50U/ml) was incubated with each chemical at 25°C for 1hr.]

Chemical	Concn.(mM)	Residual activity(%)	Chemical	Concn.(mM)	Residual activity(%)
None	—	100	IAA	2.0	90
Metal salt	2.0		Hydroxylamine	2.0	99
MgCl ₂		100	EDTA	5.0	94
CaCl ₂		101	o-Phenanthroline	2.0	100
Ba(OAc) ₂		101	α, α' -Dipyridyl	2.0	94
FeCl ₃		5	Borate	50.0	97
CoCl ₂		100	NaF	2.0	99
MnCl ₂		100	NaN ₃	2.0	100
ZnCl ₂		94	Triton X-100	0.10%	98
Cd(OAc) ₂		91	Brij 35	0.10%	86
NiCl ₂		99	Tween 20	0.10%	81
CuSO ₄		94	Span 20	0.10%	96
AgNO ₃		54	Na-cholate	0.10%	101
MIA	2.0	94	DAC	0.05%	76
NEM	2.0	99	SDS	0.05%	0

Ac, CH₃CO; MIA, Monoiodoacetate; NEM, N-ethylmaleimide; IAA, Iodoacetate; EDTA, Ethylenediaminetetraacetate; SDS, Sodium dodecyl sulfate; DAC, Dimethylbenzylalkylammonium chloride.

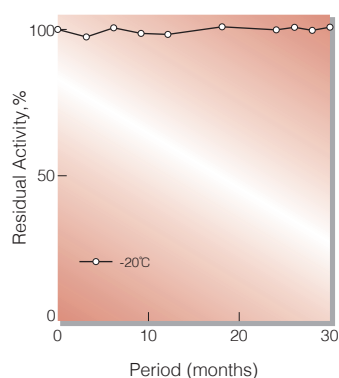


Fig.1. Stability (Powder form)
[kept under dry conditions]

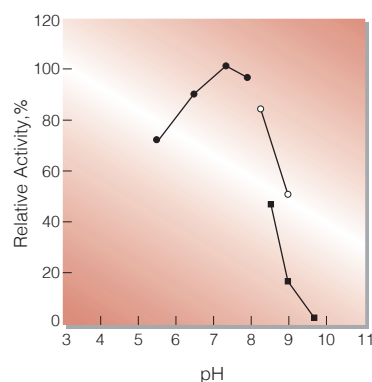


Fig.2. pH-Activity

[37°C, in 20mM buffer solution]
●, pH5.7-7.9 K-phosphate;
○, pH8.2-9.0 borate;
■, pH8.6-9.6 glycine-NaOH

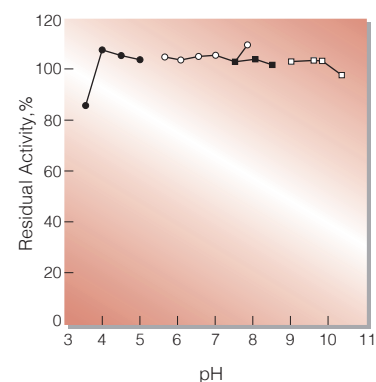


Fig.4. pH-Stability

[25°C, 16hr-treatment with 50mM buffer solution:
●, pH3.6-5.0 acetate;
○, pH5.6-7.8 K-phosphate;
■, pH7.5-8.5 Tris-HCl;
□, pH8.9-10.2 glycine-NaOH

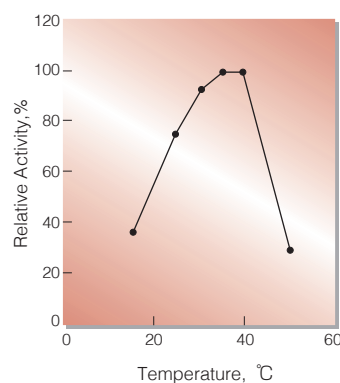


Fig.3. Temperature activity
[in 20mM K-phosphate, pH7.5]

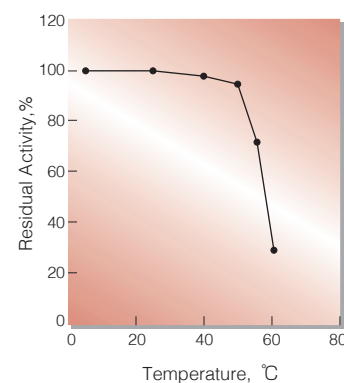


Fig.5. Thermal stability

[10min-treatment with 20mM ACES-NaOH, pH7.0.
Enzyme concentration: 100U/ml]

活性測定法 (Japanese)

1.原理

$$\text{L-Lactate} + \text{O}_2 \xrightarrow{\text{lactate oxidase}} \text{Pyruvate} + \text{H}_2\text{O}_2$$

$$2\text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} + \text{EHSPT} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4\text{H}_2\text{O}$$
 4-AminoantipyrineとEHSPTの酸化縮合物であるQuinoneimine色素を555nm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

- 0.125M DL-乳酸溶液〔240mgのDL-乳酸リチウム(MW=96.01)を50mM K-PB pH7.5に溶解し、最終液量を20mlとする。〕(用時調製)
- 0.5% 4-AA水溶液 (500mgの4-アミノアンチピリンを蒸留水に溶解し、最終液量を100mlとする。)(褐色瓶中で4℃保存)
- 20mM EHSPT(TOOS)水溶液〔591mgのEHSPT (MW=295.3)を蒸留水に溶解し、最終液量を100mlとする。〕(褐色瓶中で4℃保存)
- 25U/ml POD水溶液〔約23mgの西洋ワサビ由来ペルオキシダーゼ(東洋紡製, GradeⅢ) (110プルプロガリン単位/mg)を冷蒸留水に溶解し、最終液量を100mlとする。〕
- 0.25% SDS水溶液〔500mgのドデシル硫酸ナトリウム(SDS)を蒸留水に溶解し、最終液量を200mlとする。〕

酵素溶液：酵素標品を予め氷冷した1.0mM EDTA, 0.5%(w/v)コール酸ナトリウムを含む20mM ACES-NaOH, pH7.0で溶解し、分析直前に0.1%(w/v)コール酸ナトリウムを含む20mM K-リン酸緩衝液, pH7.0で希釈する。

4.手順

- 下記反応混液(20テスト分)を調製する(褐色瓶にて氷冷保存)

8.0ml	DL-乳酸溶液	(A)
1.2ml	4-AA水溶液	(B)
0.8ml	EHSPT水溶液	(C)
2.0ml	POD水溶液	(D)
8.0ml	蒸留水	
- 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。
- 酵素溶液0.05mlを加え、反応を開始する。
- 37℃で正確に15分反応させた後、SDS水溶液(E) 2.0mlを加えて反応を停止させる。この液につき水を対照に555nmにおける吸光度を測定する(ODtest)。
- 盲検は酵素溶液の代わりに酵素希釈液〔0.1%(w/v)コール酸ナトリウムを含む20mM K-リン酸緩衝液, pH7.0〕を用い、上記同様に操作を行って吸光度を測定する(ODblank)。

5.計算式

$$\begin{aligned}
 \text{U/ml} &= \frac{\Delta \text{OD} (\text{OD test} - \text{OD blank}) \times 3.05(\text{ml}) \times \text{希釈倍率}}{34.3 \times 1/2 \times 15(\text{分}) \times 1.0 \times 0.05(\text{ml})} \\
 &= \Delta \text{OD} \times 0.237 \times \text{希釈倍率}
 \end{aligned}$$

$$\text{U/mg} = \text{U/ml} \times 1/\text{C}$$

34.3 : Quinoneimine色素の上記測定条件下でのミリモル分子吸光係数($\text{cm}^2/\text{micromole}$)

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分子であることによる係数

1.0 : 光路長(cm)

C : 溶解時の酵素濃度(c mg/ml)