●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

D-LACTATE DEHYDROGENASE

from Microorganism

(R)-Lactate:NAD^+ oxidoreductase (EC 1. 1. 1. 28)

PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized	
Activity	: Grade Ⅱ 400U/mg-solid or more	
Contaminants	: NADH oxidase	≤1.0×10^{-3}%
	Malate dehydrogenase	≤1.0×10^{-2}%
	GOT	≤5.0×10^{-3}%
	GPT	≤5.0×10^{-3}%
	Myokinase	≤1.0×10^{-2}%
	Pyruvate kinase	≤1.0×10^{-3}%

PROPERTIES

Stability	: Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	: approx. 140,000 (by gel filtration)	
Isoelectric point	: 4.0	
Michaelis constant	: 1.6×10^{-4}M (pyruvate, pH 7.0)	
Inhibitors	: Ag^+, Hg^{++}, SH-reagents	
Optimum pH	: 6.0−7.0	(Fig.3)
Optimum temperature	: 35−40℃	(Fig.4)
pH Stability	: pH 5.0−9.0 (25℃, 48hr)	(Fig.5)
Thermal stability	: below 45℃ (pH 7.0, 15min)	(Fig.6)
Effect of various chemicals	: (Table 1)	

APPLICATIONS

This enzyme is useful for enzymatic determination of numerous metabolites, e.g.ATP, ADP, glucose, creatinine, pyruvate, lactate and glycerol, and of enzyme activities, e.g.GPT, PK and CPK when coupled with the related enzymes.

ASSAY

Principle:

Pyruvate + NADH + H^+ $\xrightarrow{\text{D-lactate dehydrogenase}}$ D-Lactate + NAD^+

The disappearance of NADH is measured at 340nm by spectrophotometry.

Unit definition:

One unit causes the oxidation of one micromole of NADH per minute under the conditions described below.

Method:

Reagents

A. Pyruvate solution : 5.0mM [5.50mg sodium pyruvate (MW=110)/10ml of H_2O] (Should be prepared fresh)

B. K-Phosphate buffer, pH 7.4 : 1.0M

C. NADH solution : 1.0mM [7.63mg NADH · 2Na (MW=763)/10ml of H_2O] (Should be prepared fresh)

D. Enzyme diluent : 0.1M K-phosphate buffer, pH 7.4 contg. 0.1% of BSA

Procedure

1. Prepare the following working solution (10 tests) in a brownish bottle, immediately before use and store on ice.

3.0ml	Substrate solution	(A)
2.0ml	K-Phosphate buffer, pH 7.4	(B)
3.0ml	NADH solution	(C)
22.0ml	H_2O	(D)

Concentration in assay mixture		
K-Phosphate buffer	67	mM
Pyruvate	0.49	mM
NADH	0.098	mM
BSA	16.4	μg/mM

2. Pipette 3.0ml of working solution into a cuvette (d=1.0cm) and equilibrate at 25℃ for about 5 minutes.
3. Add 0.05ml of the enzyme solution* and mix by gentle inversion.
4. Record the decrease in optical density at 340nm against water for 2 to 3 minutes in a spectrophotometer thermostated at 25℃, and calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test).

   At the same time, measure the blank rate (ΔOD blank) by using the same method as the test except that the enzyme diluent is added instead of the enzyme solution.

* Dilute the enzyme preparation to 0.2−1.0U/ml with ice-cold enzyme diluent (D), immediately before assay.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times Vt \times df}{6.22 \times 1.0 \times Vs} = \Delta OD/min \times 9.81 \times df$$

Weight activity (U/mg) = (U/ml) × 1/C

Vt : Total volume (3.05ml)

Vs : Sample volume (0.05ml)

6.22 : Millimolar extinction coefficient of NADH (㎠/micromole)

1.0 : Light path length (cm)

df : Dilution factor

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

REFERENCES

1) C.A.Loshon, R.B.McComb, L.W.Bond, G.N.Bowers, Jr.W.H.Coleman and R.H.Gwynn; *Clin.Chem., 23,* 1576 (1977).

2) H.Taguchi, M.Machida, H.Matsuzawa and T.Ohta; *Agric.Biol.Chem., 49* (2), 359 (1985).

3) F.Gasser, M.Doudoroff, and R.Contopoulos; *J.Gen.Microbiol. 62,* 241 (1970).

Table 1. Effect of Various Chemicals on D-Lactate dehydrogenase

[The enzyme dissolved in 0.1M K-Phosphate buffer, pH 7.4 (20U/ml)was incubated with each chemical at 25℃ for 1hr.]

Chemical	Concn.(mM)	Residual activity(%)
None	—	100
Metal salt	2.0	
$MgCl_2$		93.0
$CaCl_2$		99.8
$Ba(OAc)_2$		98.1
$FeCl_2$		87.9
$CoCl_2$		91.5
$MnCl_2$		92.2
$ZnSO_4$		89.6
$Cd(OAc)_2$		91.3
$NiCl_2$		92.6
$CuSO_4$		93.8
$Pb(OAc)_2$		93.2
$AgNO_3$		73.0
$HgCl_2$		0

Chemical	Concn.(mM)	Residual activity(%)
PCMB	2.0	89.3
MIA	2.0	0.1
NaF	2.0	98.3
NaN_3	20	94.6
EDTA	5.0	99.2
o-Phenanthroline	2.0	95.3
α,α'-Dipyridyl	1.0	93.7
Borate	50	95.6
IAA	2.0	33.6
NEM	2.0	97.7
Hydroxylamine	2.0	95.6
Triton X-100	0.10%	121
Brij 35	0.10%	116
Tween 20	0.10%	117
Span 20	0.10%	105
Na-cholate	0.10%	112
SDS	0.05%	109
DAC	0.05%	52.0

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

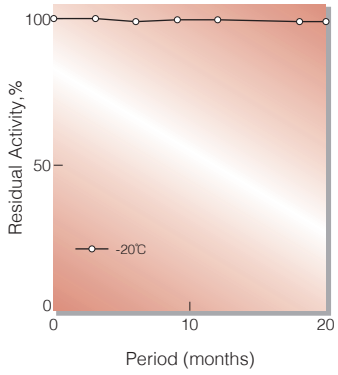

Fig.1. Stability (Powder form)

〔kept under dry conditions〕

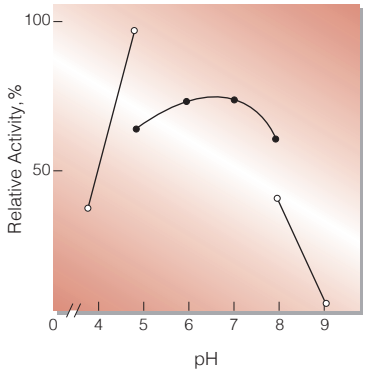

Fig.3. pH-Activity

〔in 67mM buffer solution; pH 4-5, acetate;pH5-8,K-phosphate;pH8-9 Tris-HCl〕

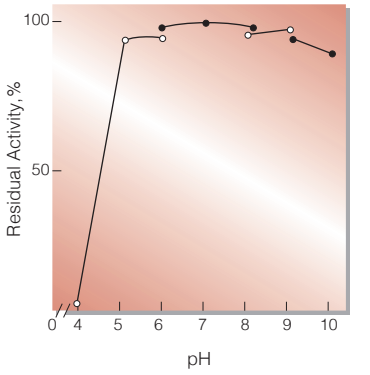

Fig.5. pH-Stability

〔25℃,48hr-treatment with 0.1M buffer solution: pH 4-6, dimethylgutaric acid-NaOH;pH6-8, K-phosphate; pH 8-9,Tris-HCl; pH9-10,glycine-NaOH. enzyme concn.: 10U/ml〕

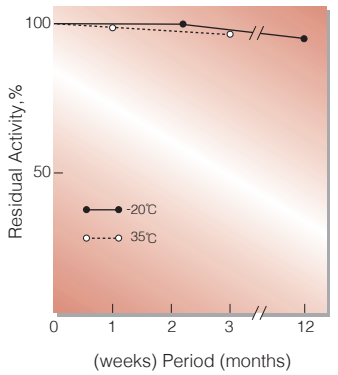

Fig.2. Stability (Powder form)

〔kept under dry conditions〕

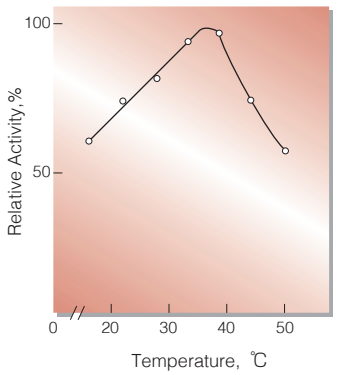

Fig.4. Temperature activity

〔in 67mM K-phosphate buffer, pH7.4〕

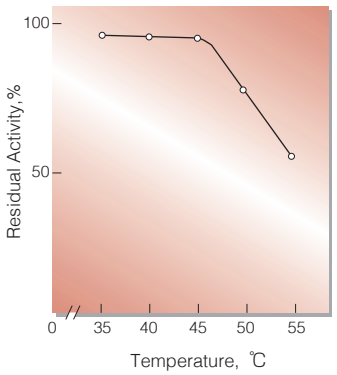

Fig.6. Temperature stability

〔15 min-treatment with 50mM K-phosphate buffer,pH7.0.enzyme concn.: 10U/ml〕

活性測定法（Japanese）

1.原理

Pyruvate＋NADH＋H^+ $\xrightarrow{\text{D-lactate dehydrogenase}}$
D-Lactate＋NAD^+
NADHの消失量を340nmの吸光度の変化で測定する。

2.定義

下記条件下で1分間に1マイクロモルのNADHが酸化される酵素量を1単位(U)とする。

3.試薬

A.　5.0mMピルビン酸ナトリウム水溶液
(用時調製)
B.　1.0M K-リン酸緩衝液，pH7.4
C.　1.0mM NADH水溶液(用時調製)
酵素溶液：分析直前に酵素標品を予め氷冷した0.1% BSAを含む0.1M K-リン酸緩衝液,pH7.4で溶解(約1㎎/㎖)し,分析直前に同緩衝液で0.2～1.0U/㎖に希釈する。

4.手順

①下記反応混液を使用直前に調製する。
(褐色瓶にて氷冷保存)

3.0㎖	基質溶液	(A)
2.0㎖	K-リン酸緩衝液	(B)
3.0㎖	NADH水溶液	(C)
22.0㎖	H_2O	

②反応混液3.0㎖をキュベット(d=1.0cm)に採り,25℃で約5分間予備加温する。
③酵素溶液0.05㎖を添加し,ゆるやかに混和後,水を対照に25℃に制御された分光光度計で340nmの吸光度変化を2～3分間記録し,その初期直線部分から1分間当りの吸光度変化を求める(ΔOD test)。
④盲検は反応混液①3.0㎖に酵素溶液の代りに酵素希釈液(0.1% BSAを含む0.1M K-リン酸緩衝液，pH7.4)を0.05㎖を加え,上記同様に操作を行って,1分間当りの吸光度変化を求める(ΔODblank)。

5.計算式

$$U/㎖ = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times 3.05(㎖) \times 希釈倍率}{6.22 \times 1.0 \times 0.05(㎖)}$$
$$= \Delta OD/min \times 9.81 \times 希釈倍率$$
$$U/mg = U/㎖ \times 1/C$$

6.22　：NADHのミリモル分子吸光係数
($㎠$/micromole)
1.0　：光路長(cm)
C　：溶解時の酵素濃度(c ㎎/㎖)