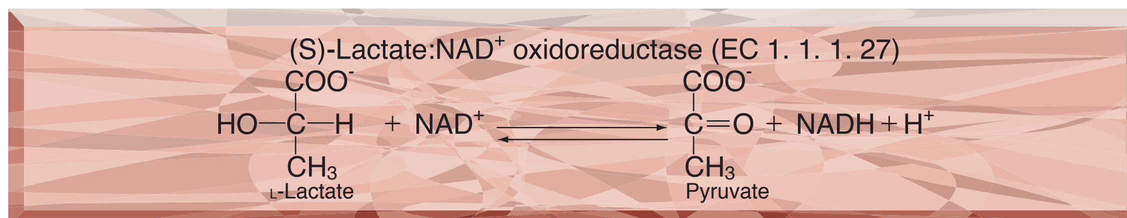
●TOYOBO ENZYMES●
(Diagnostic Reagent Grade)

LACTATE DEHYDROGENASE

from Pig heart



PREPARATION and SPECIFICATION

Appearance	: Crystalline suspension in 1.6M ammonium sulfate solution
Activity	: Grade Ⅱ 2,000U/ml or more
Contaminants	: Malate dehydrogenase $\leq 5.0\times 10^{-2}$%
	Pyruvate kinase $\leq 3.0\times 10^{-2}$%
	GPT $\leq 3.0\times 10^{-2}$%
Stabilizers	: NADH, 2-mercaptoethanol

PROPERTIES [1~3)]

Stability	: Stable at 4℃ for at least one year	(Fig.1)
Molecular weight	: 115,000±6,500	
Michaelis constants	: 2.5×10^{-2}M (Lactate), 1.0×10^{-4}M (Pyruvate)	
Structure	: 4 subunits per enzyme molecule, E^{280nm}_{1cm} (1%)=11.52 (Biuret method)	
Inhibitors	: I^-, Ag^+, Hg^{++}, p-chloromercuribenzoate, LDH inhibitors (formed from NADH)	
Optimum pH	: 6.0−7.4	(Fig.2)
Optimum temperature	: above 60℃	(Fig.3)
pH Stability	: pH 6.0−8.0 (23℃, 22hr)	(Fig.4)
Thermal stability	: below 50℃ (pH 7.4, 10min)	(Fig.5)

APPLICATIONS [4)]

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^+ —(lactate dehydrogenase)→ L-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 sodium pyruvate (Should be prepared fresh)
B. K-Phosphate buffer, pH 7.4	: 1.0M
C. NADH solution	: 1.0mM (Should be prepared fresh)
D. Enzyme diluent	: 0.1M K-phosphate buffer, pH 7.4 contg. 0.1% of BSA

Procedure

1. Immediately before use prepare the following working solution (10 tests) in a brownish bottle 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	

Concentration in assay mixture		
K-Phosphate buffer	67	mM
Pyruvate	0.50	mM
NADH	0.10	mM
BSA	3.3	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 :

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

Vt	: Total volume (3.05ml)
Vs	: Sample volume (0.05ml)
6.22	: Millimolar extinction coefficient of NADH (cm^2/micromole)
1.0	: Light path length (cm)
df	: Dilution factor

REFERENCES

1) R.Jaenicke and G.Pfleiderer; *Biochim.Biophys.Acta, 60,* 615 (1962).
2) J.J.Holbrook et al; *The Enzymes, 11,* 191 (1975).
3) J.Everse and N.O.Kaplan; *Adv.in Enzymol., 37,* 61 (1973).
4) 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).

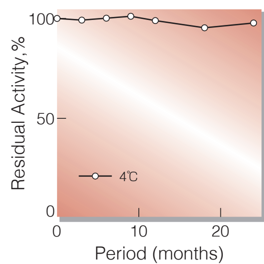


Fig.1. Stability (Liquid form at 4℃)

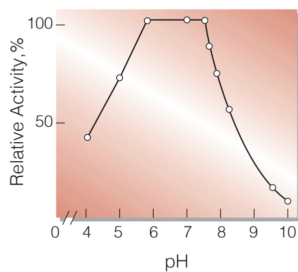


Fig.2. pH-Activity

〔25℃,in 0.1M buffer slution: pH4.0-5.0, acetate; pH6.0-7.0,Veronal-CH_3COONa-HCl; pH7.4,phosphate;pH8.0-9.0, Tris-HCl;pH 9.5-10 glycine-NaOH〕

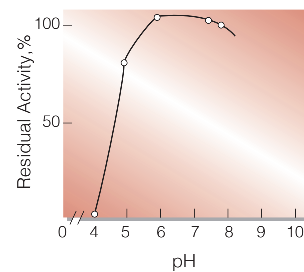


Fig.4. pH-Stability

〔23℃,22hr-treatment with 0.1M buffer: pH4.0-6.0, aceate; pH6.0-7.0,Veronal-CH_3COONa-HCl; pH7.4,phosphate;pH8.0,Tris-HCl〕

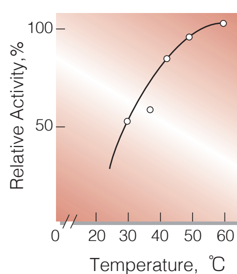


Fig.3. Temperature activity

〔in 67mM phosphate buffer,pH7.4〕

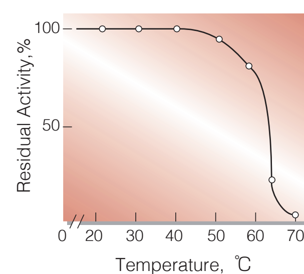
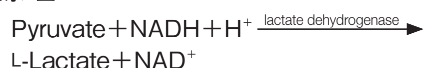


Fig.5. Thermal stability

〔10min-treatment with 0.1M phosphate buffer,pH7.4〕

活性測定法（Japanese）

1.原理

Pyruvate＋NADH＋H^+ 

L-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%牛血清アルブミンを含む0.1M K-リン酸緩衝液, pH7.4で0.2～1.0 U/mℓ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)。

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

②反応混液3.0mℓ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0.05mℓ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①に酵素溶液の代りに酵素希釈液(0.1%牛血清アルブミンを含む0.1M K-リン酸緩衝液, pH 7.4)を0.05mℓ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

$$U/m\ell = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times 3.05(m\ell) \times 希釈倍率}{6.22 \times 1.0 \times 0.05(m\ell)}$$

$$= \Delta OD/min \times 9.81 \times 希釈倍率$$

6.22　：NADHのミリモル分子吸光係数 (cm^2/micromole)
1.0　：光路長(cm)