●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

MALATE DEHYDROGENASE

from Microorganism

L-Malate:NAD^+oxidoreductase (EC 1. 1. 1. 37)

L-Malate + NAD^+ ⇄ Oxaloacetate + NADH + H^+

PREPARATION and SPECIFICATION

Appearance	: Slightly yellowish amorphous powder, lyophilized	
Activity	: Grade Ⅱ 40U/mg-solid or more	
Contaminants	: Glutamate oxaloacetate transaminase	≤1.0×10^{-3}%
	Lactate dehydrogenase	≤1.0×10^{-3}%
	NADH oxidase	≤1.0×10^{-3}%

PROPERTIES

Stability	: Stable at −20℃ for at least one year	(Fig.1)
Molecular weight [1]	: approx. 140,000	
Isoelectric point [2]	: pH 4.8±0.1	
Michaelis constants [3]	: 5.4×10^{-5}M (L-Malate), 5.0×10^{-6}M (Oxaloacetate), 8.1×10^{-6}M (NADH)	
Structure	: 4 subunits per enzyme molecule	
Inhibitors	: Hg^{++}	
Optimum pH	: 8.0	(Fig.2)
Optimum temperature	: 70℃	(Fig.3)
pH Stability	: pH 3.0−9.0 (25℃, 20hr)	(Fig.4)
Thermal stability	: below 70℃ (pH 7.5, 15min)	(Fig.5)
Effect of various chemicals	: (Table 1)	

APPLICATIONS

This enzyme is useful for enzymatic determination of L-malate and of glutamate oxaloacetate transaminase (GOT) in clinical analysis.

ASSAY

Principle:

Oxaloacetate + NADH + H^+ $\xrightarrow{\text{malate dehydrogenase}}$ L-Malate + 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. K-phosphate buffer, pH 7.5 : 0.1M

B. Oxaloacetate solution : 15mM [2.0mg oxaloacetic acid (MW=132.1)/ml of ice-cold K-phosphate buffer (A). This reagent is rather unstable and should be stored in an ice-bath during use] (Should be prepared fresh)

C. NADH solution : 6.0mM [4.25mg NADH · Na_2 (ORIENTAL YEAST, MW=709.4)/ml of H_2O] (Should be prepared fresh)

D. Enzyme diluent : 0.1M K-phosphate buffer, pH 7.5 contg. 0.2% BSA

Procedure

1. Prepare the following reaction mixture in a cuvette (d=1.0cm) and equilibrate at 30℃ for about 5 minutes.

2.80ml	K-phosphate buffer, pH 7.5	(A)
0.10ml	Oxaloacetate solution	(B)
0.10ml	NADH solution	(C)

Concentration in assay mixture	
K-phosphate buffer	97 mM
Oxaloacetate	0.49mM
NADH	0.20mM

2. Add 0.05ml of the enzyme solution* and mix by gentle inversion.
3. Record the decrease in optical density at 340nm against water for 3 to 4 minutes in a spectrophotometer thermostated at 30℃, 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 expect that the enzyme diluent is added instead of the enzyme solution.

* Dissolve the enzyme preparation in ice-cold enzyme diluent (D), dilute to 0.05−0.5U/ml with the same buffer and store on ice.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta\text{OD/min (}\Delta\text{OD test} - \Delta\text{OD blank)} \times \text{Vt} \times \text{df}}{6.22 \times 1.0 \times \text{Vs}} = \Delta\text{OD/min} \times 9.807 \times \text{df}$$

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 under the assay condition (㎠/micromole)

1.0 : Light path length (cm)

df : Dilution factor

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

REFERENCES

1) C.J.R.Thorne and N.O.Kaplan; ***J.Biol.Chem., 238,*** 1861 (1963).

2) R.G.Wolfe and J.B.Neilands; ***J.Biol.Chem., 221,*** 61 (1956).

3) C.J.R.Thorne; ***Biochim, Biophys, Acta., 59,*** 624 (1962).

4) D.J.Blonde et al; ***Can.J.Biochem., 45,*** 641 (1967).

Table 1. Effect of Various Chemicals on Malate dehydrogenase

[The enzyme solution dissolved in 0.1M K-phosphate buffer, pH 7.5 contg. 0.2% of BSA (17U/ml) was incubated with each chemical at 25℃ for 1hr.]

Chemical	Concn.(mM)	Residual activity(%)
None	—	100
Metal salt	2.0	
$MgCl_2$		100
$CaCl_2$		100
$Ba(OAc)_2$		101
$FeCl_3$		102
$CoCl_2$		100
$MnCl_2$		102
$ZnSO_4$		99
$Cd(OAc)_2$		94
$NiCl_2$		100
$CuSO_4$		99
$Pb(OAc)_2$		99
$AgNO_3$		98
$HgCl_2$		0
NEM	2.0	100
MIA	2.0	99

Chemical	Concn.(mM)	Residual activity(%)
2-Mercaptoethanol	2.0	102
PCMB	0.1	100
IAA	2.0	99
Hydroxylamine	2.0	98
EDTA	5.0	99
o-Phenanthroline	2.0	99
α,α'-Dipyridyl	2.0	100
Borate	5.0	99
NaF	2.0	98
NaN_3	2.0	98
Triton X-100	0.10%	99
Brij 35	0.10%	98
Tween 20	0.10%	98
Span 20	0.10%	97
Na-cholate	0.1%	98
SDS	0.05%	95
DAC	0.05%	96

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

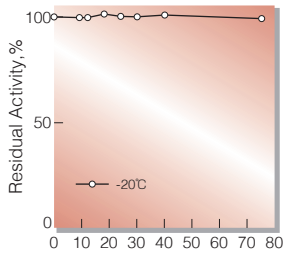

Fig.1. Stability (Powder form)

〔kept under dry conditions〕

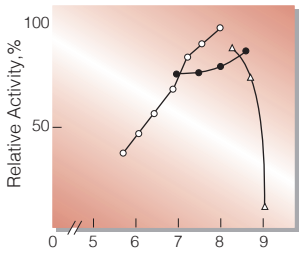

Fig.2. PH-Activity

〔30℃, in 0.1M buffer solution:
pH5.5-8.0, K-phosphate:pH7.0-8.5, Tris-HCl;
pH8.0-9.0, Borate〕

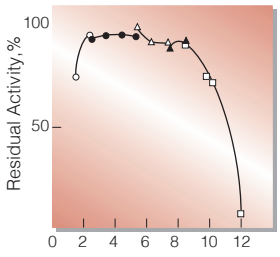

Fig.4. pH-Stability

〔25℃,20hr-treatment with 0.1M buffer solution:
pH2.0-3.5,glycine-HCl; pH3.0-6.0,acetate;
pH6.0-8.0,K-phosphate; pH8.0-9.0, Tris-HCl;
pH8.5-12.0,borate〕

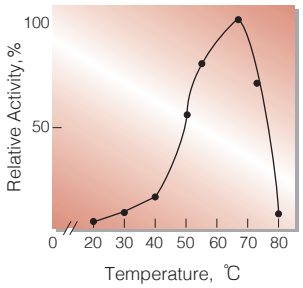

Fig.3. Temperature activity

〔in 0.1mM K-phosphate buffer, pH7.5〕

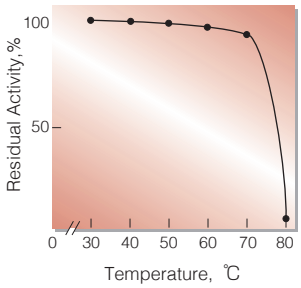

Fig.5. Thermal stability

〔15min-treatment with 0.1m K-phosphate buffer,pH7.5
enzyme concentration:4.0U/ml〕

活性測定法（Japanese）

1.原理

Oxaloacetate＋NADH＋H^+ $\xrightarrow{\text{malate dehydrogenase}}$
L-Malate＋NAD^+
NADHの減少を340nmにおける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を酸化する酵素量を1単位(U)とする。

3.試薬

A. 0.1M K-リン酸緩衝液, pH7.5
B. 15mMオキサロ酢酸溶液〔2.0㎎のオキサロ酢酸(MW＝132.1)を予め氷冷したK-リン酸緩衝液(A)1.0㎖に溶解する〕(この試薬はかなり不安定である故,使用時も氷冷保存する)
C. 6.0mM NADH水溶液〔4.25㎎のNADH・Na_2(オリエンタル酵母製,MW＝709.4)を蒸留水1.0㎖に溶解する〕(用時調製)

酵素溶液：酵素標品を予め氷冷した0.2%牛血清アルブミンを含む50mM K-リン酸緩衝液,pH7.5で溶解し,同緩衝液で0.05～0.5U/㎖に希釈して氷冷保存する。

4.手順

①下記反応混液をキュベット(d＝1.0cm)に調製し,30℃で約5分間予備加温する。

2.80㎖	K-リン酸緩衝液	(A)
0.10㎖	オキサロ酢酸溶液	(B)
0.10㎖	NADH水溶液	(C)

②酵素溶液0.05㎖を添加し,ゆるやかに混和後,水を対照に30℃に制御された分光光度計で340nmの吸光度変化を3～4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)。
③盲検は反応混液①に酵素溶液の代りに酵素希釈液(0.2%牛血清アルブミンを含む50mM K-リン酸緩衝液,pH 7.5)を0.05㎖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(ΔOD blank)。

5.計算式

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

$$= \Delta OD/min \times 9.807 \times 希釈倍率$$

U/mg ＝U/㎖×1/C
6.22 ：NADHのミリモル分子吸光係数(cm^2/micromole)
1.0 ：光路長(cm)
C ：溶解時の酵素濃度(c ㎎/㎖)