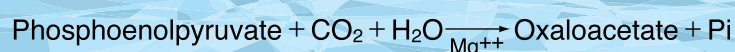


● TOYOBO ENZYMES ●
(Diagnostic Reagent Grade)

PHOSPHOENOLPYRUVATE CARBOXYLASE

from Microorganism

Orthophosphate:oxaloacetate carboxy-lyase(Phosphorylating)(EC 4.1.1.31)



PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized
Activity	: Grade III 5.0U/mg-solid or more
Contaminants	: Lactate dehydrogenase $\leq 1.0 \times 10^{-3}\%$ Pyruvate kinase $\leq 0.5\%$
Stabilizers	: BSA, sugar alcohols

PROPERTIES

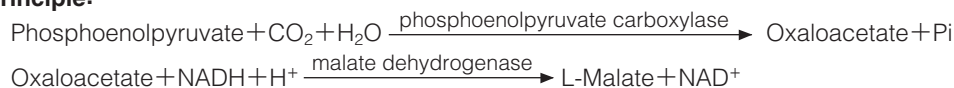
Stability	: Stable at -20°C for at least one year	(Fig.1)
Molecular weight	: approx. 390,000 (by gel filtration)	
Isoelectric point	: 6.0 ± 0.1	
Structure	: 4 Subunits (M.W.100,000) per mole of enzyme	
Michaelis constant	: $1.9 \times 10^{-4}\text{M}$ (Phosphoenolpyruvate)	
Optimum pH	: 7.5–8.0	(Fig.2)
Optimum temperature	: 60°C	(Fig.3)
pH Stability	: pH 5.0–8.0 (25°C, 24hr)	(Fig.4)
Thermal stability	: below 40°C (pH 7.0, 15min)	(Fig.5)

APPLICATIONS

This enzyme is useful for enzymatic determination of carbon dioxide when coupled with malate dehydrogenase ([MAD-201](#), [MAD-211](#)) in clinical analysis.

ASSAY

Principle:



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. Buffer solution : 0.1M Tris-HCl Buffer, pH 8.0
 B. Na₂CO₃ solution : 0.1M [Dissolve 1.06g of Na₂CO₃(MW=105.99)/100ml of H₂O]
 C. K-Phosphoenolpyruvate solution : 32mM [Dissolve 33.0mg of PEP · K(MW=206.1)/5ml of H₂O] (Should be prepared fresh)
 D. MgSO₄ solution : 1M [Dissolve 4.93g of MgSO₄ · 7H₂O(MW=246.48)/20 ml of H₂O]
 E. NADH solution : 1.4mM [Dissolve 5.34mg of NADH · 3H₂O(MW=763)/5ml of H₂O]
 F. MDH solution : ca.100U/ml [Dissolve malate dehydrogenase (TOYOBO Grade II) to ca.100U/ml with 20mM Tris-HCl Buffer,pH 8.0] (Should be prepared fresh)
 G. Enzyme diluent : 20mM K-phosphate buffer, pH 7.0

Procedure

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

Concentration in assay mixture	
K-Phosphoenolpyruvate	3.1 mM
Tris-HCl	57 mM
Na ₂ CO ₃	9.7 mM
MgSO ₄	9.7 mM
NADH	0.14mM
MDH	ca.9.7 U/ml
K-Phosphate	0.65mM

1.77ml	Buffer solution	(A)
0.3 ml	Na ₂ CO ₃ solution	(B)
0.3 ml	K-Phosphoenolpyruvate solution	(C)
0.03ml	MgSO ₄ solution	(D)
0.3 ml	NADH solution	(E)
0.3 ml	MDH solution	(F)
 - Add 0.1ml of the enzyme solution* and mix by gentle inversion.
 - Record the decrease in optical density at 340nm against water for 3 to 4 minutes in a spectrophotometer thermostated at 30°C, and calculate the ΔOD per minute from 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 (G) is added instead of the enzyme solution.
- * Dissolve the enzyme preparation in ice-cold enzyme diluent (G) and dilute to 0.2–0.7U/ml with the same buffer and store on ice.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta \text{OD}/\text{min} (\Delta \text{OD test} - \Delta \text{OD blank}) \times V_t \times \text{df}}{6.22 \times 1.0 \times V_s} = \Delta \text{OD}/\text{min} \times 4.98 \times \text{df}$$

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

V_t : Total volume (3.1ml)

V_s : Sample volume (0.1ml)

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

1.0 : Light path length (cm)

df : Dilution factor

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

REFERENCES

- W.Wilson, P.Jesky, R.Rand and R.D.Bevill; *Clin.Chem.*, **19**, 640(1973)
- R.L.Forrester, L.J.Wataji, D.A.Silverman and K.J.Pierre; *Clin.Chem.*, **22**, 243(1976)

Table 1. Effect of Various Chemicals on Phosphoenolpyruvate carboxylase

[The enzyme solution dissolved in 20mM K-phosphate buffer, pH 7.0 (20U/ml) was incubated with each chemical at 25°C for 1hr.]

Chemical	Concn.(mM)	Residual activity	Chemical	Concn.(mM)	Residual activity
None	—	100%	PCMB	0.1	80
Metal salt	2.0		NEM	2.0	87
MgCl ₂		105	IAA	2.0	90
CaCl ₂		105	Hydroxylamine	2.0	95
Ba(OAc) ₂		103	EDTA	5.0	100
FeCl ₃		92	o-Phenanthroline	2.0	103
CoCl ₂		106	α,α'-Dipyridyl	2.0	109
MnCl ₂		107	Borate	5.0	103
ZnSO ₄		103	NaF	2.0	106
Cd(OAc) ₂		104	NaN ₃	2.0	106
NiCl ₂		0	Triton X-100	0.10%	111
CuSO ₄		0	Brij 35	0.10%	110
Pb(OAc) ₂		105	Tween 20	0.10%	112
AgNO ₃		0	Span 20	0.10%	109
HgCl ₂		0	Na-cholate	0.10%	108
MIA	2.0	60	SDS	0.05%	1
2-Mercaptoethanol	2.0	101	DAC	0.05%	99

Ac, CH₃CO; PCMB, p-Chloromercuribenzoate; MIA, Monoiodoacetate; EDTA, Ethylenediaminetetraacetate; IAA, Iodoacetamide; NEM, N-Ethylmaleimide; SDS, Sodium dodecyl sulfate; DAC, Dimethyl-benzyl-alkyl-ammonium chloride.

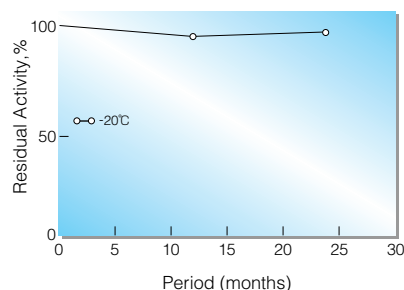


Fig.1. Stability (Powder form)
[kept under dry conditions]

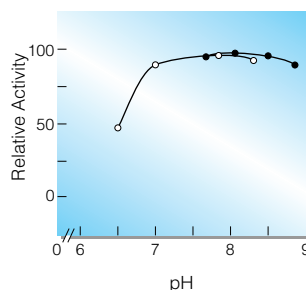


Fig.2. pH-Activity
[30°C, in 50mM buffer solution:
pH6.0-8.5, MES: pH7.5-9.0, Tris-HCl]

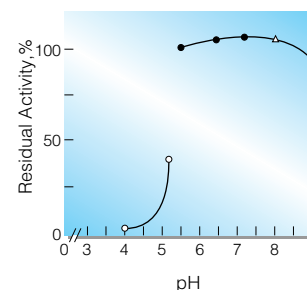


Fig.4. pH-Stability
[25°C, 24hr-treatment with 50mM
buffer solution contg. 10mM
MgSO₄: pH3.0-5.0, Acetate; pH5.0-8.0,
K-phosphate; pH8.0-9.0, Tris-HCl]

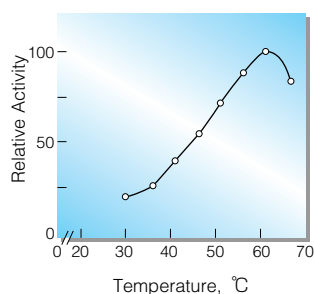


Fig.3. Temperature activity
[in 20mM K-phosphate buffer, pH7.0]

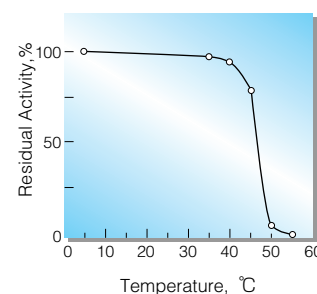


Fig.5. Thermal stability
[15min-treatment with 20mM
K-phosphate buffer, pH7.0
enzyme concn.: 2.0U/ml]

活性測定法 (Japanese)

1.原理



NADHの減少量を340nmにおける吸光度の変化で測定する。

2.定義

下記条件で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 Tris-HCl緩衝液,pH8.0
- B. 0.1M Na₂CO₃水溶液 [1.06gの無水炭酸ナトリウム(MW=105.99)を蒸留水100mlに溶解する。]
- C. 32.0mM K-Phosphoenolpyruvate水溶液 [33.0mgのK-phosphoenolpyruvate(MW=206.1)を蒸留水5.0mlに溶解する。](用時調製)
- D. 1.0M MgSO₄水溶液 [4.93gのMgSO₄·7H₂O(MW=246.48)を蒸留水20mlに溶解する。]
- E. 1.4mM NADH水溶液 [5.34mgのNADH·Na₂(MW=763)を蒸留水5.0mlに溶解する。]
- F. 100U/ml Malate dehydrogenase溶液 [リンゴ酸脱水素酵素(東洋紡製 GradeII)を20mM Tris-HCl pH8.0で溶解する。](用時調製)

酵素溶液：酵素標品を予め氷冷した20mM K-リン酸緩衝液,pH7.0で溶解し,同緩衝液で0.2~0.7U/mlに希釈する。

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。

1.77ml	Tris-HCl緩衝液	(A)
0.30ml	Na ₂ CO ₃ 水溶液	(B)
0.30ml	K-Phosphoenolpyruvate水溶液	(C)
0.03ml	MgSO ₄ 水溶液	(D)
0.30ml	NADH水溶液	(E)
0.30ml	Malate dehydrogenase溶液	(F)
- ② 酵素溶液0.1ml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(ΔODtest)。
- ③ 盲検は,酵素溶液の代わりに酵素希釈液を0.1ml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

$$\begin{aligned} \text{U/ml} &= \frac{\Delta \text{OD}/\text{min} (\Delta \text{OD test} - \Delta \text{OD blank}) \times 3.1(\text{ml}) \times \text{希釈倍率}}{6.22 \times 1.0 \times 0.1(\text{ml})} \\ &= \Delta \text{OD}/\text{min} \times 4.984 \times \text{希釈倍率} \\ \text{U/mg} &= \text{U/ml} \times 1 / C \\ 6.22 &: \text{NADHのミリモル分子吸光係数} \\ & \quad (\text{cm}^2/\text{micromole}) \\ 1.0 &: \text{光路長(cm)} \\ C &: \text{溶解時の酵素濃度(c mg/ml)} \end{aligned}$$