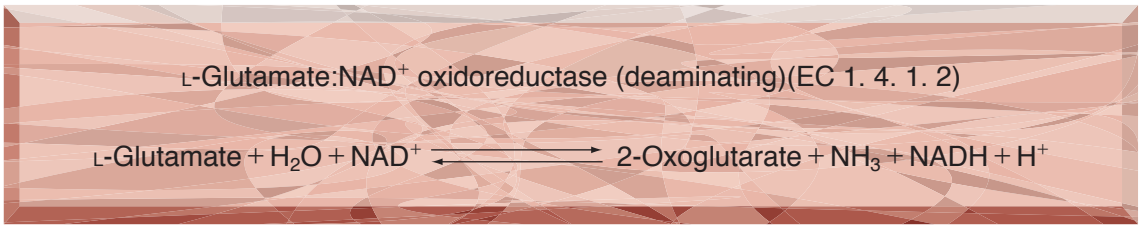
●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

GLUTAMATE DEHYDROGENASE (NAD-dependent)

from Microorganism

L-Glutamate:NAD^+ oxidoreductase (deaminating)(EC 1. 4. 1. 2)



PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized
Activity	: Grade Ⅱ 100 U/mg-solid or more
Contaminants	: NAD oxidase ≤1.0×10^{-2}%

PROPERTIES

Stability	: Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	: approx. 260,000	
Isoelectric point	: 5.6	
Michaelis constants	: 9.21×10^{-3}M (NH_3), 4.80×10^{-3}M(α-Ketoglutarate) 7.8×10^{-5}M (L-Glutamate), 1.29×10^{-4}M(NADH), 5.89×10^{-4}M(NAD^+)	
Structure	: 6 subunits per enzyme molecule	
Inhibitors	: Heavy metals, PCMB, IAA	
Optimum pH	: 7.5−8.0 (α-KG→L-Glu) 9.0 (L-Glu→α-KG)	(Fig.2)
Optimum temperature	: 55℃ (α-KG→L-Glu) 50°C (L-Glu→α-KG)	(Fig.3)
pH Stability	: pH 5.0−10.0 (25℃, 20hr)	(Fig.4)
Thermal stability	: below 50℃ (pH 8.3, 10min)	(Fig.5)
Substrate specificity	: (Table 1)	
Effect of various chemicals	: (Table 2)	

APPLICATIONS

This enzyme is useful for enzymatic determination of NH_3, α-ketoglutaric acid and L-glutamic acid, and for assay of leucine aminopeptidase and urease. This enzyme is also used for enzymatic determination of urea when coupled with urease (URH-201) in clinical analysis.

ASSAY

Principle:

α-Ketoglutarate + NH_3 + NADH + H^+ $\xrightarrow{\text{glutamate dehydrogenase}}$ L-Glutamate + NAD^+ + H_2O

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 8.3
B. NH_4Cl solution : 3.3M
C. α-Ketoglutarate solution : 0.225M (adjust the pH to 7.0−9.0 with NaOH)(Should be phepared fresh)
D. NADH solution : 7.5mM (Should be prepared fresh)
E. Enzyme diluent : 0.1M Tris-HCl buffer, pH 8.3

Procedure

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

2.5ml	Buffer solution	(A)
0.2ml	NH_4Cl solution	(B)
0.1ml	α-Ketoglutarate solution	(C)
0.1ml	NADH solution	(D)

Concentration in assay mixture	
Tris-HCl buffer	86 mM
α-Ketoglutarate	7.6 mM
NH_4Cl	0.22 M
NADH	0.25mM

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 2 to 3 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 except that the enzyme diluent (E) is added instead of the enzyme solution.

* Dissolve the enzyme preparation to 0.1−0.8U/ml with ice-cold diluent (E), immediately before assay.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta\text{OD/min (}\Delta\text{OD test} - \Delta\text{OD blank)} \times \text{Vt} \times \text{df}}{6.22 \times 1.0 \times \text{Vs}} = \Delta\text{OD/min} \times 9.486 \times \text{df}$$

Weight activity (U/mg) = (U/ml)×1/C

Vt : Total volume (2.95ml)
Vs : Sample volume (0.05ml)
6.22 : Millimolar extinction coefficient of NADH at 340nm (㎤/micromole)
1.0 : Light path length (cm)
df : Dilution factor
C : Enzyme concentration in dissolution (c mg/ml)

Table 1. Substrate Specificity of Glutamate dehydrogenase

Substrate (2mM)	Relative activity(%)	Substrate (2mM)	Relative activity(%)
L-Glutamate	100	L-Glutamine	0.05
L-Norvaline	0.35	L-Aspartate	0.07
L-α-Aminobutyrate	0.16	L-Asparagine	0.11
L-Norleucine	0	L-Valine	0.09
D,L-Homocysteine	0.06	L-Leucine	0.03
L-Isoleucine	0.09	L-Alanine	0.07
		L-Methionine	0.06

Glutamate dehydrogenase : 0.3U/ml of 0.1M Tris-HCl buffer, pH 9.0 NAD^+:12mM

Table 2. Effect of Various Chemicals on Glutamate dehydrogenase

[The enzyme dissolved in 0.1M Tris-HCl buffer, pH 8.3 was incubated with each chemical at 25℃ for 1hr.]

Chemical	Concn.(mM)	Residual activity(%)	Chemical	Concn.(mM)	Residual activity(%)
None	—	100	NaF	2.0	100
Metal salt	2.0		NaN_3	20	102
$MgCl_2$		97	EDTA	5.0	102
$CaCl_2$		99	o-Phenanthroline	2.0	101
$Ba(OAc)_2$		101	α,α′-Dipyridyl	2.0	102
$FeCl_3$		1.8	Borate		102
$CoCl_2$		97	IAA	2.0	0.2
$MnCl_2$		78	NEM	2.0	96
$ZnSO_4$		6.9	Hydroxylamine	2.0	100
$Cd(OAc)_2$		58	Triton X-100	0.10%	102
$NiCl_2$		100	Brij 35	0.10%	103
$CuSO_4$		0.3	Tween 20	0.10%	101
$Pb(OAc)_2$		0.01	Span 20	0.10%	107
$AgNO_3$		1.6	Na-cholate	0.10%	103
$HgCl_2$		0	SDS	0.05%	0.1
PCMB	2.0	0.6	DAC	0.05%	0.2
MIA	2.0	98			

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

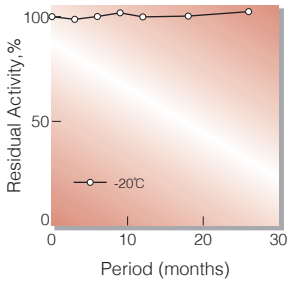


Fig.1. Stability (Powder form)

〔kept under dry conditions〕

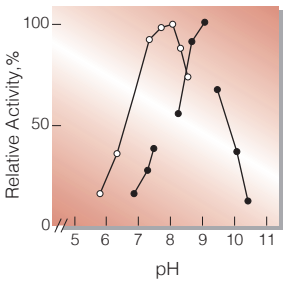


Fig.2. pH-Activity

〔○—○, α-KG →L-Glu;●—●L-Glu → α-KG in 0.1M buffer solution:pH5.7-7.6 K-phosphate,pH7.8-9.0,Tris-HCl; pH9.4-10.3, glycine-NaOH〕

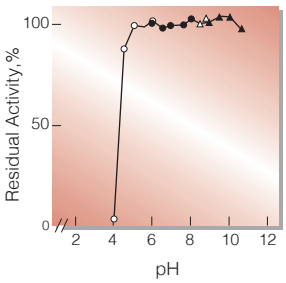


Fig.4. pH-Stability

〔25℃, 20hr-treatment with 0.1M buffer solution: ○—○,acetate;●—●,K-phosphate,△—△Tris-HCl; ▲—▲glycine-NaOH〕

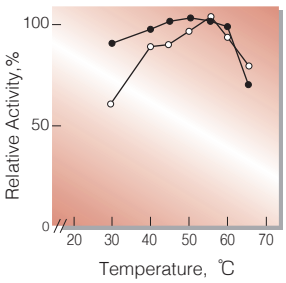


Fig.3. Temperature activity

〔○—○, α-KG →L-Glu;0.1M Tris-HCl buffer pH8.3;●—●,L-Glu → α-KG:0.1M Tris-HCl buffer,pH9.0〕

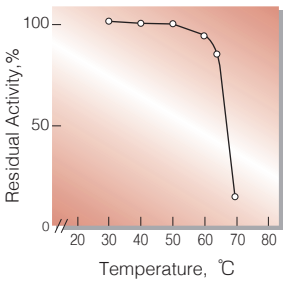


Fig.5. Thermal stability

〔10min-treatment with 0.1M Tris-HCl buffer, pH8.3〕

活性測定法（Japanese）

1.原理

α-Ketoglutarate＋NH_3＋NADPH＋H^+
$\xrightarrow{\text{glutamate dehydrogenase}}$ L-Glutamate＋NAD^+＋H_2O

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. 0.1M Tris-HCl緩衝液, pH8.3
B. 3.3M NH_4Cl水溶液
C. 0.225M α-ケトグルタル酸水溶液(NaOHでpHを7.0～9.0に調整)(用時調製)
D. 7.5mM 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0.1M Tris-HCl緩衝液,pH8.3で0.1～0.8U/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。

2.5mℓ	Tris-HCl緩衝液	(A)
0.2mℓ	NH_4Cl水溶液	(B)
0.1mℓ	α-ケトグルタル酸水溶液	(C)
0.1mℓ	NADH水溶液	(D)

②酵素溶液を0.05mℓ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(ΔODtest)。

③盲検は反応混液①に酵素溶液の代りに酵素希釈液(0.1M Tris-HCl緩衝液, pH8.3)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

$$U/mℓ = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times 2.95(mℓ) \times 希釈倍率}{6.22 \times 1.0 \times 0.05(mℓ)}$$

$$= \Delta OD/min \times 9.486 \times 希釈倍率$$

$U/mg = U/mℓ \times 1/C$

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 mg/mℓ)