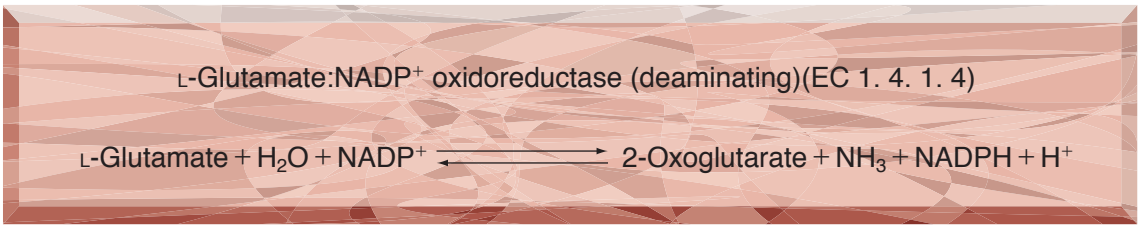
●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

GLUTAMATE DEHYDROGENASE (NADP-dependent)

from Proteus sp.

L-Glutamate:$NADP^+$ oxidoreductase (deaminating)(EC 1. 4. 1. 4)



PREPARATION and SPECIFICATION

Appearance	: Solution with 50mM Tris-HCl buffer containing 0.05% NaN_3 and 5.0mM EDTA, pH 7.8
Activity	: Grade Ⅱ・Ⅲ 300U/mg-protein or more (9,000U/ml or more)
Contaminants	: NADPH oxidase ≤1.0×10^{-2}% Glutathione reductase ≤1.0×10^{-2}%(Grade Ⅱ-209) ≤1.0×10^{-1}%(Grade Ⅲ-309)
Stabilizer	: Ethylenediaminetetraacetic acid (EDTA)

PROPERTIES

Stability	: Stable at 4℃ for at least one year (Fig.1,2)
Molecular weight	: approx. 300,000
Isoelectric point	: 4.6
Michaelis constants	: 1.1×10^{-3}M (NH_3), 3.4×10^{-4}M(α-Ketoglutarate) 1.2×10^{-3}M (L-Glutamate), 1.4×10^{-5}M(NADPH), 1.5×10^{-5}M($NADP^+$)
Structure	: 6 subunits (M.W.50,000) per enzyme molecule
Inhibitors	: Hg^{++}, Cd^{++}, p-chloromercuribenzoate, pyridine, 4-4'-dithiopyridine, 2,2'-dithiopyridine
Optimum pH	: 8.5 (α-KG→L-Glu) 9.8 (L-Glu→α-KG) (Fig.5)
Optimum temperature	: 45℃ (α-KG→L-Glu) 45–55°C (L-Glu→α-KG) (Fig.6)
pH Stability	: pH 6.0－8.5 (25℃, 20hr) (Fig.7)
Thermal stability	: below 50℃ (pH 7.4, 10min) (Fig.8)
Substrate specificity	: (Table 1)

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 + NADPH + H^+ $\xrightarrow{\text{glutamate dehydrogenase}}$ L-Glutamate + $NADP^+$ + H_2O

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

Unit definition:

One unit causes the oxidation of one micromole of NADPH 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 prepared fresh)
D. NADPH solution : 7.5mM (Should be prepared fresh)
E. Enzyme diluent : 50mM K-Phosphate buffer, pH 6.6 containing 0.2% BSA and 50mM EDTA

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	NADPH solution	(D)

Concentration in assay mixture	
Tris-HCl buffer	85 mM
α-Ketoglutarate	7.6 mM
NH_4Cl	0.22 M
NADPH	0.25mM
EDTA	0.85mM

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 spectro-photometer thermostated at 30℃ and calculate the ΔOD per minute from the 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.

* Dilute the enzyme preparation to 0.4−0.9U/ml with ice-cold enzyme diluent (E), immediately before the assay.

Calculation

Activity can be calculated by using the following formula :

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

Vt : Total volume (2.95ml)
Vs : Sample volume (0.05ml)
6.22 : Millimolar extinction coefficient of NADPH (㎤/micromole)
1.0 : Light path length (cm)
df : Dilution factor

REFERENCES

1) H.Shimizu, T.Kuratsu and F.Hirata; ***J.Ferment.Technol., 57,*** 428 (1979).

Table 1. Substrate Specificity of Glutamate dehydrogenase

Substrate (50mM)	Relative activity(%)	Substrate (50mM)	Relative activity(%)
L-Glutamate	100	L-Glutamine	< 0.01
L-Norvaline	0.39	L-Aspartate	< 0.01
L-α-Aminobutyrate	0.19	L-Asparagine	< 0.01
L-Norleucine	0.04	L-Valine	< 0.01
D,L-Homocysteine	0.03	L-Leucine	< 0.01
L-Isoleucine	0.02	L-Alanine	< 0.01
		L-Methionine	< 0.01

Glutamate dehydrogenase:18U/ml of 0.1M glycine-NaOH buffer, pH 9.0 $NADP^+$: 0.3mM

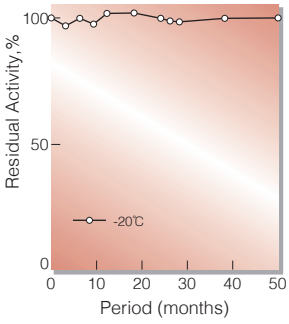


Fig.1. Stability (GTD-209) (Solution)

50% glycerol solution in 25mM Tris-HCl buffer contg. 2.5mM EDTA, pH7.8
enyzme concentration: 5,000U/ml

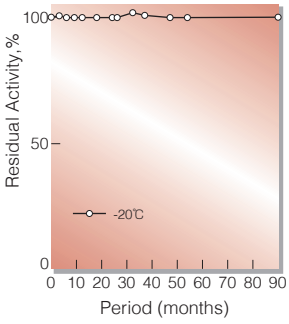


Fig.2. Stability (GTD-309) (Solution)

50% glycerol solution in 25mM Tris-HCl buffer contg. 2.5mM EDTA, pH7.8
enyzme concentration: 5,000U/ml

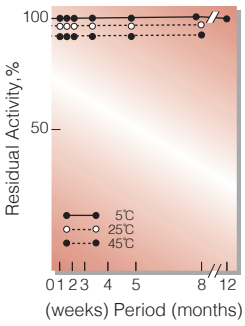


Fig.3. Stability (Solution)

50% glycerol solution in 25mM Tris-HCl buffer contg. 2.5mM EDTA, pH7.8
enyzme concentration: 5,000U/ml

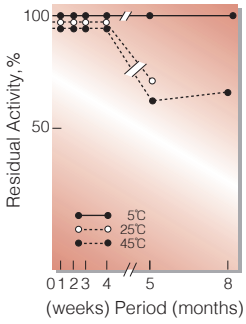


Fig.4. Stability (Suspension)

3.0M ammonium sulfate suspension in 50mM Tris-HCl buffer containing 5mM EDTA. pH7.8
enzyme concentration : 10,000U/ml

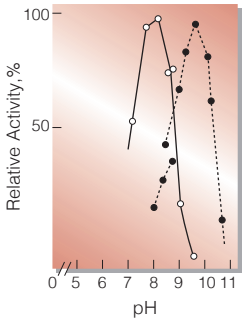


Fig.5. pH-Activity

○—○, α-KG →L-Glu;●---●,L-Glu→ α-KG in 0.1M buffer solution:pH7.4-8.8, Tris-HCl;pH8.7-10.7,glycine-NaOH

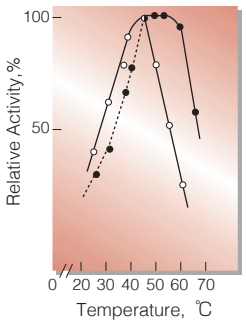


Fig.6. Temperature activity

○—○, α-KG→L-Glu: 0.1M Tris-HCl buffer, pH8.3;●---● , L-Glu→ α-KG:0.1M glycine-NaOH buffer,pH10.0

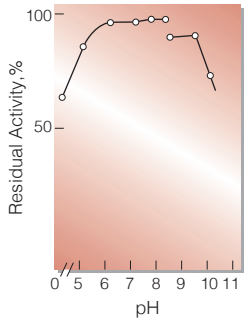


Fig.7. pH-Stability

25℃, 20hr-treatment with 0.1M buffer solution: pH4.4-6.2, acetate; pH6.2-8.4, phosphate; pH8.8-10.2, glycine-NaOH

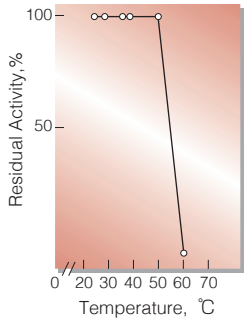


Fig.8. Thermal stability

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

活性測定法（Japanese）

1.原理

α-Ketoglutarate＋NH_3＋NADPH＋H^+
$\xrightarrow{\text{glutamate dehydrogenase}}$ L-Glutamate＋$NADP^+$＋H_2O
NADPH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NADPH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 NADPH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0.2% BSAと50mM EDTAを含む50mM K-リン酸緩衝液, pH6.6で0.4～0.9U/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	NADPH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(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%BSAと50mM EDTAを含むK-リン酸緩衝液, pH6.6)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

$$U/mℓ = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times 2.95(mℓ) \times 希釈倍率}{6.22 \times 1.0 \times 0.05(mℓ)}$$
$$= \Delta OD/min \times 9.486 \times 希釈倍率$$

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