

## ● TOYOBO ENZYMES ●

(Diagnostic Reagent Grade)

# GLUTAMATE DEHYDROGENASE (NADP-dependent)

*from Proteus sp.*L-Glutamate : NADP<sup>+</sup> oxidoreductase (deaminating)(EC 1. 4. 1. 4)**PREPARATION and SPECIFICATION**

Appearance	: Solution with 50mM Tris-HCl buffer containing 0.05% NaN <sub>3</sub> 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 <sup>-2</sup> % Glutathione reductase ≤1.0 × 10 <sup>-2</sup> %(Grade II -209) ≤1.0 × 10 <sup>-1</sup> %(Grade III -309)
Stabilizer	: Ethylenediaminetetraacetic acid (EDTA)

**PROPERTIES**

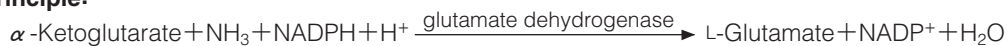
Stability	: Stable at 5°C for at least 6 months	(Fig.1,2)
Molecular weight	: approx. 300,000	
Isoelectric point	: 4.6	
Michaelis constants	: 1.1 × 10 <sup>-3</sup> M (NH <sub>3</sub> ), 3.4 × 10 <sup>-4</sup> M (α-Ketoglutarate) 1.2 × 10 <sup>-3</sup> M (L-Glutamate), 1.4 × 10 <sup>-5</sup> M (NADPH),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

**APPLICATIONS**

This enzyme is useful for enzymatic determination of NH<sub>3</sub>, 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:



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<sub>4</sub>Cl solution : 3.3M  
 C.  $\alpha$ -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°C for about 5 minutes.

2.5ml	Buffer solution	(A)
0.2ml	NH <sub>4</sub> Cl solution	(B)
0.1ml	$\alpha$ -Ketoglutarate solution	(C)
0.1ml	NADPH solution	(D)

Concentration in assay mixture	
Tris-HCl buffer	85 mM
$\alpha$ -Ketoglutarate	7.6 mM
NH <sub>4</sub> Cl	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°C and calculate the  $\Delta$ OD per minute from the linear portion of the curve ( $\Delta$ OD test).  
 At the same time, measure the blank rate ( $\Delta$ OD 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 :

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

- V<sub>t</sub> : Total volume (2.95ml)  
 V<sub>s</sub> : Sample volume (0.05ml)  
 6.22 : Millimolar extinction coefficient of NADPH (cm<sup>2</sup>/micromole)  
 1.0 : Light path length (cm)  
 df : Dilution factor

## REFERENCE

- 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- $\alpha$ -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<sup>+</sup>: 0.3mM

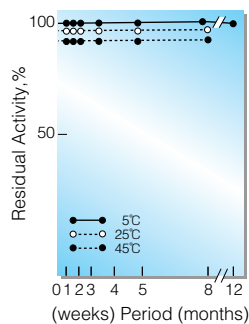


Fig. 1. Stability (Solution)

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

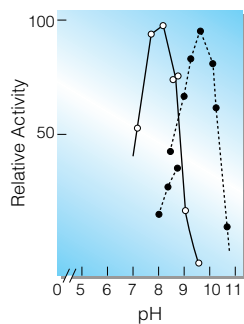


Fig. 3. pH-Activity

[○—○,  $\alpha$  KG  $\rightarrow$  L-Glu; ●—● L-Glu  $\rightarrow$   $\alpha$  -KG  
in 0.1M buffer solution: pH7.4-8.8,  
Tris-HCl: pH8.7-10.7, glycine-NaOH]

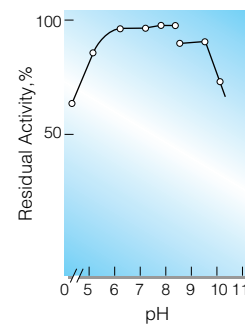


Fig. 5. pH-Stability

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

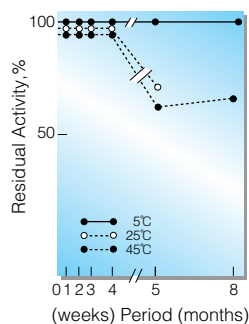


Fig. 2. Stability (Suspension)

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

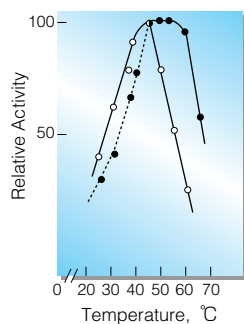


Fig. 4. Temperature activity

[○—○,  $\alpha$  KG  $\rightarrow$  L-Glu: 0.1M Tris-HCl buffer,  
pH8.3; ●—●, L-Glu  $\rightarrow$   $\alpha$  -KG: 0.1M glycine-  
NaOH buffer, pH10.0]

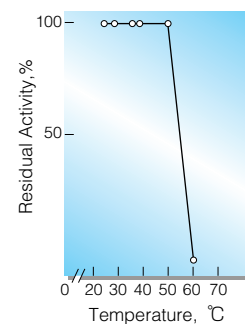
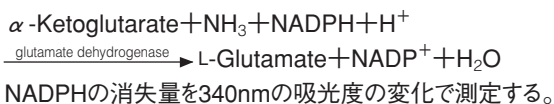


Fig. 6. Thermal stability

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

## 活性測定法 (Japanese)

### 1.原理



### 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<sub>4</sub>Cl水溶液
  - C. 0.225M  $\alpha$ -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 <sub>4</sub> Cl水溶液	(B)
0.1ml	$\alpha$ -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( $\Delta$ OD test)。
- ③ 盲検は反応混液①に酵素溶液の代わりに酵素希釈液(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( $\Delta$ ODblank)。

### 5.計算式

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

6.22 : NADHのミリモル分子吸光係数 (cm<sup>2</sup>/micromole)

1.0 : 光路長(cm)