●TOYOBO ENZYMES●
(Diagnostic Reagent Grade)

UREASE
from Jack bean

Urea amidohydrolase(EC 3.5.1.5)

$(NH_2)_2CO + H_2O \longrightarrow 2NH_3 + CO_2$

PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized
Activity	: Grade Ⅱ(-201) 100U/mg-solid or more
Contaminants	: Asparaginase $\leq 2.0\times10^{-2}$%
	Arginase $\leq 2.0\times10^{-3}$%
	NH_4^+ $\leq 5.0\times10^{-4}$ μg／U
Stabilizers	: EDTA, glutathione, succinate, BSA

PROPERTIES

Stability	: Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	: approx. 480,000 [1]	
Isoelectric point	: 5.0−5.1 [1]	
Michaelis constant	: 1.05×10^{-2}M (Urea) [1]	
Structure	: 8 active sites with SH-groups per the enzyme molecule [2]	
Inhibitors	: Heavy metal ions (Ag^+,Hg^{++},etc.)	
Optimum pH	: 6.0	(Fig.3)
Optimum temperature	: 60℃	(Fig.4)
pH Stability	: pH 5.5−8.5 (30℃, 17hr)	(Fig.5)
Thermal stability	: below 50℃ (pH 8.0, 60min)	(Fig.6)
Effect of various chemicals	: (Table.1)	

APPLICATIONS [3]

This enzyme is useful for enzymatic determination of urea in clinical analysis.

ASSAY

Principle:

$(NH_2)_2CO + H_2O + 2H^+ \xrightarrow{\text{urease}} 2NH_4^+ + CO_2$

$2NH_4^+ + 2\alpha\text{-Ketoglutarate} + 2NADPH \xrightarrow{\text{glutamate dehydrogenase}} 2Glutamate + 2H_2O + 2NADP^+$

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

Unit definition:

One unit causes the formation of two micromoles of ammonia per minute under the conditions described below.

Method:

Reagents

A. A. Urea solution : 6.0M (36g of Urea／100ml of H_2O)(Should be prepared fresh)

B. Tris-HCl buffer, pH 8.0 : 50mM

C. α-Ketoglutarate solution : 0.25M (Dissolve 730mg of α-ketoglutarate in 15 ml of H_2O, adjust pH to 5.0±0.1 with 5N NaOH and fill up to 20ml with H_2O)(Should be prepared fresh)

D. NADPH solution : 15mM [Dissolve 136mg of NADPH・Na_4・$4H_2O$／10ml of H_2O] (Should be prepared fresh)

E. Working solution (Prepare before use and store on ice)

69 ml	Tris-HCl buffer	(B)
0.3ml	α-Ketoglutarate solution	(C)
1.8ml	NADPH solution	(D)
0.9ml	H_2O	

F. GlDH (glutamate dehydrogenase) solution : ca.1,000U/ml [Toyobo GradeⅡ,GTD-209 (Tris-HCl buffer solution, free from ammonia)]

G. Enzyme diluent : 10mM K-phosphate buffer containing 20mM EDTA and 0.2% BSA, pH 7.0

Procedure

1. Prepare the following reaction mixture in a cuvette (d＝1.0cm) and equilibrate at 37℃ for about 5 minutes.

2.40ml	Working solution	(E)
0.05ml	GlDH solution	(F)
0.35ml	H_2O	
0.10ml	Enzyme solution*	

Concentration in assay mixture	
Tris-HCl buffer	38 mM
Urea	200 mM
α-Ketoglutarate	0.83mM
NADPH	0.30mM
EDTA	0.67mM
GlDH	ca.17 U/ml

2. Add 0.10ml of urea solution (A) 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 37℃, 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 is added instead of the enzyme solution.

* Dissolve the enzyme preparation in ice-cold enzyme diluent (G) and dilute to 0.07－0.25U/ml with the same buffer and store on ice.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times Vt \times df}{6.22 \times 2 \times 1.0 \times Vs} = \Delta OD/min \times 2.41 \times df$$

Weight activity (U/mg) ＝ (U/ml)×1/C

Vt : Total volume (3.0ml)

Vs : Sample volume (0.10ml)

6.22 : Millimolar extinction coefficient of NADPH at 340nm (㎠/micromole)

2 : Factor based on the fact that hydrolysis of one mole of urea is equivalent to oxidation of two moles of NADPH

1.0 : Light path length (cm)

df : Dilution factor

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

REFERENCES

1) J.B.Sumner and D.B.Hand; ***J.Am.Chem.Soc., 31,*** 1255 (1929)
2) G.Gorin and C.C.Chin; ***Biochim.Biophys.Acta., 99,*** 418 (1965)
3) ***Rinsho Kagaku Bunseki(Japanese)***, Ⅱ,p18(M.Kitamura,M.Saito and M.Niwa,ed.)Tokyo Kagaku Dojin,Tokyo (1969)
4) H.G.Schlegel and H.Kaltwasser; ***Methods of Enzymatic Analysis,*** Vol.2,p1081 (H.U.Bergmeyer,ed.), Verlag Chemie Weiheim, Acdemic Press, New York-London (1974)

Table 1 Effect of Various Chemicals on Urease

[The enzyme dissolved in 20mM phosphate buffer, pH 7.0 was incubated with each chemical at 30℃ for 1hr.]

Chemical	Concn.(mM)	Residual activity(%)	Chemical	Concn.(mM)	Residual activity(%)
None	—	100	$MnCl_2$	1.0	66
NaCl	10	96	$MgCl_2$	1.0	97
Na_2SO_4	10	104	$CaCl_2$	1.0	105
CH_3COONa	10	108	$ZnCl_2$	1.0	104
Na_2HPO_4	10	100	$FeSO_4$	1.0	94
Citrate-Na_2	10	100	$CuSO_4$	1.0	99
Na_2CO_3	10	100	Ag_2SO_4	0.1	9
Na_3BO_4	10	104	$HgCl_2$	0.1	8
$Na_3S_2O_4$	10	108			

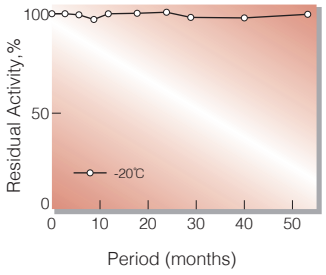

Fig.1. Stability (Powder form)
〔kept under dry conditions〕

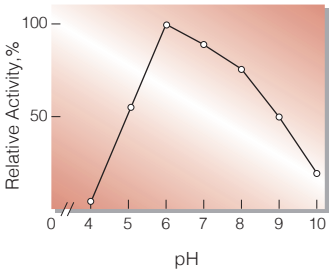

Fig.3. pH-Activity
〔30℃ in 10mM buffer solution: pH3.0-9.0 Veronal-CH_3COONa-HCl; pH9.0-11.0, glycine-NaOH.〕

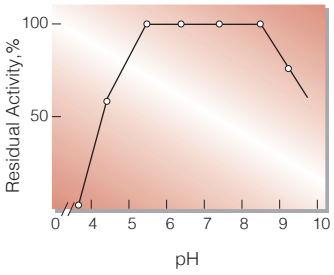

Fig.5. pH-Stability
〔30℃ ,17hr-treatment with 10mM buffer solution: pH 3.0-9.0, Veronal-CH_3COONa-HCl; [pH9.0-11.0: glycine-NaOH〕

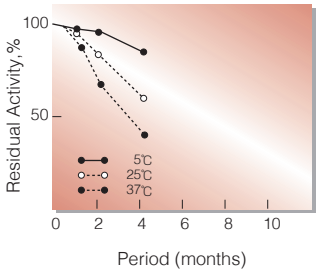

Fig.2. Stability (Powder form)
〔kept under dry conditions〕

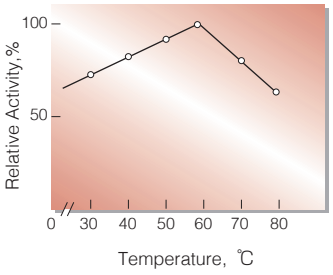

Fig.4. Temperature activity
〔in 20mM phosphate buffer,pH7.0〕

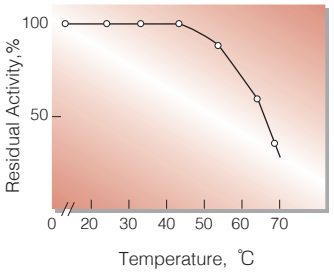

Fig.6. Thermal stability
〔60min-treatment with 20mM phosphate buffer, pH8.0.〕

活性測定法（Japanese）

1.原理

$(NH_2)_2CO + H_2O + 2H^+ \xrightarrow{\text{urease}} 2NH_4^+ + CO_2$

$2NH_4^+ + 2\alpha\text{-Ketoglutarate} + 2NADPH \xrightarrow{\text{glutamate dehydrogenase}} 2Glutamate + 2H_2O + 2NADP^+$

NADPHの減少量を340nmにおける吸光度の変化で測定する。

2.定義

下記条件下で1分間に2マイクロモルのアンモニアを生成する(1マイクロモルの尿素を加水分解する)酵素量を1単位(U)とする。

3.試薬

A. 6.0M 尿素水溶液(36gの尿素を蒸留水に溶解し100mℓとする)(用時調製)
B. 50mM Tris-HCl緩衝液, pH8.0
C. 0.25M α-ケトグルタル酸水溶液(730mgのα-ケトグルタル酸を蒸留水約15mℓに溶解し, 5N NaOHでpH5.0±0.1に調製後, 蒸留水にて20mℓとする)(用時調製)
D. 15mM NADPH水溶液〔136mgのNADPH・Na_4・$4H_2O$を蒸留水10mℓに溶解する〕(用時調製)
E. 試薬混液(使用直前に調製し, 氷冷保存する)

69.0mℓ	Tris-HCl緩衝液	(B)
0.3mℓ	α-ケトグルタル酸水溶液	(C)
1.8mℓ	NADPH水溶液	(D)
0.9mℓ	蒸留水	

F. グルタミン酸脱水素酵素(GIDH)溶液〔東洋紡製, GTD-209(約1,000U/mℓに蒸留水で希釈して使用する)〕

酵素溶液：酵素標品を予め氷冷した20mM EDTAと0.2% BSAを含む10mM K-リン酸緩衝液, pH7.0で溶解し,同緩衝液で0.07～0.25U/mℓ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,37℃で約5分間予備加温する。

2.40mℓ	試薬混液	(E)
0.05mℓ	GIDH溶液	(F)
0.35mℓ	蒸留水	
0.10mℓ	酵素溶液	

②基質溶液(A)0.10mℓを添加し,ゆるやかに混和後,水を対照に37℃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盲検は反応混液①に酵素溶液の代わりに酵素希釈液(20mM EDTAを含む10mM K-リン酸緩衝液, pH7.0)を0.10mℓ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

$$U/m\ell = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times 3.0(m\ell) \times 希釈倍率}{6.22 \times 2 \times 1.0 \times 0.10(m\ell)}$$

$$= \Delta OD/min \times 2.41 \times 希釈倍率$$

U/mg ＝U/mℓ×1/C

6.22 ：NADPHのミリモル分子吸光係数(㎠/micromole)
2 ：酵素反応で1分子の尿素の加水分解に由来するNADPHの酸化は2分子である事による係数
1.0 ：光路長(cm)
C ：溶解時の酵素濃度(c mg/mℓ)